

Greenpeace critique of Monsanto's Roundup Ready Oilseed Rape, GT73¹

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Introduction

The dossier and subsequent information submitted by Monsanto contain many inadequacies, conflicting statements and unexplained differences between the GE (genetically engineered) OSR (oilseed rape), GT73 and the parental line. Original material has not been disclosed, contravening EU legislation. Despite the numerous irregularities in the dossier, many member States have been limited in their critical scrutiny and the EFSA (European Food Safety Authority) has given GT73 a positive opinion.

Here, we document the irregularities of the feeding trials, the failings of EFSA's evaluation, the lack of consideration for the environmental consequences of accidental releases, concerns regarding the secrecy of fundamental studies and question the ability of EU regulatory authorities to competently regulate GE foodstuffs in a manner that assures food and environmental safety. It is arranged in three parts: 1) The bad science of GT73 feeding trials, 2) Technical critique of EFSA's opinion on GT73 and 3) Concerns about regulatory authorities' handling of the application. Finally, we conclude that the application for the import and processing of GT73 should be rejected.

1. The bad science of GT73 feeding trials

Introduction

To support their application for the import of Roundup Ready oilseed rape, GT73, Monsanto submitted 3 feeding trials on rats, 2 on trout and 2 on quails. However, all are flawed.

Rat studies

It is not clear from the dossier what experimental protocol was followed. Since standard protocols do exist, the protocol used should be stated.

Rat study no. 1, (Naylor, 1994)

This first study showed a 10% loss in bodyweight, but was rejected because the GE seed had got mixed up with another GE variety: "*As a consequence of co-mingling of the seed, a second study was undertaken*". This raises the question of how, if Monsanto can't even separate the seed adequately for its own feed trials, are GE and non-GE OSR expected to be kept separate at commercial scales?

Rat Study no. 2 (Naylor, 1995)

In the second rat study, only processed GE OSR is used because the effects of unprocessed OSR according to Monsanto has "*little practical relevance to commercial practices since unprocessed ground oilseed rape seed is not fed to animals*".

Significant differences were found: *“relative liver weights were increased approximately 12-16% for males and females fed the 15 % (but not the 5%) GTOSR diet compared to parental line controls.”*

Substantially equivalent or not?: Monsanto first tried to explain the differences as a product of two factors: 1) increased glucosinolate levels in the GE OSR and 2) the processing of oilseed rape.

Monsanto claim in the SNIF (3c) that *“There are no specific differences when GT73 is compared to conventional oilseed rape except for its tolerance to glyphosate. GT73 has been shown to be substantially equivalent, with exception of the introduced trait...”*. But, then Monsanto informs that the level of total glucosinolate in the GT73 OSR-meal was approximately 0.6 gm/kg, twice as high as in the parental Westar line. This is not consistent with Monsanto’s claim that herbicide tolerance is the only difference between GT73 and its non-GM parental line. To use the differences in the glucosinolate levels as an explanation for differences in the feeding trials contradicts with the claim of substantial equivalence.

Subsequently, Monsanto decides that glucosinolate levels are not the cause of the differences. In the additional information supplied on 18th November 1999, Monsanto decide *“our hypothesis that differences in glucosinolate levels accounted for the observed differences in liver weight is not supportable based on the available data.”* This implies that the differences seen were either due to the processing or the GE product itself. A further experiment using unprocessed OSR could determine the differences are due to processing or the GE product. Monsanto did not conduct this key study but instead conducted a 3rd rat study using wider controls. Nevertheless, without offering any justification, the EFSA in its opinion simply considers that the enlarged livers as *“an incidental finding”*.²

Rat study no. 3 (Naylor, 1996)

After the original submission for marketing of GT73, Monsanto then conducted a third rat study! *“The purpose of this study was to determine whether the liver and kidney weights of rats fed processed meal from the RU3 fall within the range of liver and kidney weights of rat fed processed meal from nine commercial varieties of canola from Canada and oilseed rape from Europe.”* In other words, this third study was conducted with controls from as wide as range as possible, even from different continents. This third study seems, therefore, to be deliberately designed to mask any differences that occurred in the second rat study when GT73-fed rats were compared only to rats fed the parental line, Westar. Such a study design does not appear to follow any acceptable protocol. Predictably, no significant differences were observed. To go to this effort to refute the findings of the previous study, but yet not perform a key experiment testing the hypothesis that differences are due to processing rather than the GE product itself is absurd. This does not give any reassurances regarding feed safety.

Monsanto conclude: no more evidence necessary!

In their additional information, 18th November 1999, Monsanto state: *“While we do not have a scientific explanation for the observed differences in liver weight in the second rat feeding study, the fact that this finding was not observed in the first and third feeding studies suggest the finding is not reproducible and probably not biologically significant.”*

Monsanto further conclude: *“While we do not have further information to understand the differences in weight gain in the first feeding studies, and the increased liver weight in the*

second rat feeding study, when the processing of canola meal is carefully controlled (as done in the third feeding study), we find no differences in weight gain, food composition, or organ weights (liver and kidney) between rats fed RR canola and the parental line and commercial lines. Thus, the earlier findings do not appear to be biologically meaningful and further evidence to support earlier hypotheses is no longer needed.”

This conclusion is based wholly on the third study. The reason for the differences in the previous study has still not been examined.

Trout feeding studies

The first trout study also suffers from a co-mingling of seed and was repeated. In the second study³, significant differences were noted, but were considered positive attributes so of no concern! *“The only significant differences detected in the second experiment were PR [protein retention] and whole body moisture, protein, and fat concentrations. In each case, fish fed GT73 exhibited improvements in the three variables as compared to fish fed Westar. Increased retention of dietary protein is a positive attribute of a feed, and the higher protein, lower fat concentrations should also be considered a positive attribute of the fish produced.”* However, the long-term effects of these so-called “positive attributes” are not known. It’s quite possible that these could become adverse effects in the long term. These short-term feeding trials are so limited that any significant differences, even if they appear positive, should be investigated thoroughly.

Quail studies

Quail were selected because they may feed on OSR seeds left in the field after harvest. However, Quail were fed GE OSR for only 5 DAYS! This is a wholly unacceptable timescale for testing of wholesomeness of GE OSR. Again, the seed was co-mingled and the experiment was repeated but again for only FIVE days. Despite the 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). Feed consumption was comparable for all groups”* (EFSA Journal (2004) 29, 1-19 (p14 4.2.3). Apparently, the GT73 quails suffered weight loss during the time they were fed GT73, and only recovered the weight loss in the subsequent three days when they were no longer fed GT73. Yet the findings are ignored.

UK regulatory authorities are concerned with the feeding studies

Two of the UK advisory committees on GE crops, ACRE (Advisory Committee on Releases to the Environment) and ACAF (Advisory Committee on Animal Feedingstuffs), issued advice in September 2003⁴ that includes concerns over the feeding trials:

“ACAF considered the animal feed studies provided in the dossier. No significant differences in growth were observed between rats fed GT73 or Westar parental variety oilseed rape (Naylor, M. W. 1995; MSL-14164). However, the relative liver weights for the rats fed 15% processed GT73 oilseed rape were found to be significantly higher than controls. This was attributed by the notifiers to higher glucosinolate concentration in the GM diets compared with the corresponding control diets. This adverse effect was not observed in a subsequent rat experiment (Naylor, M. W. 1996; MSL-14778), ACAF is not satisfied that the subsequent feeding study satisfactorily supports this hypothesis as the two studies are not equally comparative. Therefore ACAF remains of the view that the adverse response in rats to a diet containing 15% GT73 requires further explanation.”

“ACRE and ACAF are not satisfied that the notifiers have supported the hypothesis that increased liver weight in rats fed GT73 compared with controls is attributed to higher glucosinolate content levels in the test material. A satisfactory explanation for this potentially adverse response observed in the rat feeding study is required.”

Conclusion on GT73 feeding trials

Monsanto is picking and choosing studies as it pleases. After the second rat study showed significant differences, the experiment was repeated with a wide range of canola. Why?

From their conclusion of the rat studies, it is evident that Monsanto are simply trying to not perform additional experiments, or indeed determine the reason behind these significant differences. Instead the feeding trials have been repeated in such a way as to mask any real differences. These differences could turn out to be important because these feeding trails are very short. Significant differences could be indicative of longer-term problems. It is vital that the underlying reason for the significant differences should be satisfactorily determined.

2. Critique of EFSA's opinion on GT73

Introduction

The GMO panel of the EFSA was set up to contribute to an improved risk assessment of GE crops in the EU. However, analysis of the GT73 assessment made by EFSA demonstrates that the EFSA has not contributed to a higher level of consumer and environmental protection from GE crops and foodstuffs.

Molecular Data

When describing the GE constructs in GT73, (Section 2.2.2) EFSA states “*The sequencing of 3' and 5' flanking regions revealed that 40 base pairs (bp) of parental (Westar) DNA is absent from GT73, and that GT73 contains 22 bp of DNA adjacent to the 5' insert/plant junction which is not present in Westar*”. This means that there has been a small deletion of plant DNA in the GE plant and small extra fragment of DNA (it is not clear where this extra fragment originates from). The significance of these irregularities has not been examined in depth. EFSA should have initiated further studies, but instead it simply states that there are no similarities between these sequences and known toxins and allergens. But these are not the only concerns from molecular irregularities. Are these fragments functional? What has been deleted from the plant, was a gene or regulatory sequence? These questions remain unanswered.

Compositional analysis – pooled data and significant differences

The EFSA detail (Section 3.2.2) the analysis performed “*Kernels from oilseed rape (GT73, Westar and other commercial varieties) were obtained from field trials in Canada (1992 [7 sites], 1993 [5 sites]), 1997 [4-19 sites per variety]) and Europe (1994 [3 sites], 1995 [3 sites]).*” But, in the dossier and further information, these data are pooled from the different locations. This is exactly one of the major criticisms of poor quality data that was made prior to the initiation of the EFSA. Pooling of data can mask any variations present. Therefore, such data cannot be submitted in support of compositional sameness.

Even though the data was pooled significant differences were noted: the level of linolenic acid was lower and, importantly, glucosinolate levels were higher in the GE oilseed rape. Why these levels are different has not been investigated further, the EFSA has simply accepted two possible explanations for the difference in glucosinolate levels from Monsanto as “*reasonable explanations*”. These explanations include variation within the original cultivar and variation induced by tissue cultivation. This is not scientifically rigorous, the EFSA should have rejected the GE crop at this point, or at least asked for further studies to determine exactly why these differences exist. It is certainly not in keeping with the philosophy of substantial equivalence as a starting point, i.e. where significances are noted they should be investigated.

The differences in glucosinolate levels could be important

The glucosinolate levels are important because they are known antinutrients. As the EFSA states, the EC maximum allowable for this type of oilseed rape is 25 $\mu\text{mol/g}$ seeds (9 % moisture content). But Monsanto haven't even given the concentration of glucosinolate in seeds, they have only estimated that they are below the threshold by converting this maximum into alkyl glucosinolates/g of defatted meal, which have been measured. However, no indication of moisture content is given and the estimates are just rough calculations. Much of the analysis on these glucosinolate levels is from samples pooled from different locations and most of the data are from GE oilseed rape that hasn't even been sprayed with Roundup (the

herbicide that would be used with the GE oilseed rape). Analysing for glucosinolate levels in seeds is routine analysis – if would be easy for EFSA to ask for further studies on the glucosinolate levels but instead, they simply conclude “*The glucosinolate levels reported are thus clearly below the maximum content set by the European Commission*”.

No environmental considerations of GT73 oilseed rape imports

No reasons are given why EFSA and many Member States authorities do not consider the environmental implications of imports of oilseed rape, as environmental considerations are supposed to be part of their remit. Import of GT73 will result in feral GE oil seed rape populations. Most recently, a study conducted by the Japan Wildlife research Centre revealed that around the port of Kashima (and possibly also Kobe port) imported oil seed rape (GE and non-GE) is growing wild in 25 out of 48 checking points at locations within 5 km radius of the port.⁵ There was possible GE canola reseeding at 17 out of the 23 confirmed locations. Thus, GE feral oilseed populations are likely to occur at sites of importation and distribution.

Whilst the application is for the import of GT73 grain for food/feed purposes only, seeds will escape into the EU environment, e.g. during processing and transport. As feral populations of oilseed rape are widespread in Europe, it is highly likely, that escaped seed will germinate, flower and potentially cross-pollinate with oilseed rape crops, feral populations or wild relatives in Europe. The assumption that populations of oilseed rape that result from losses during transport, storage and processing (the so called “volunteers”) “*are easily displaced by other weeds*” cannot be made, as the results of several studies have shown⁶. Pessel et al. (2001), Pessel and Lecomte (2002)⁷ Relict plants of oil seed rape persisted for at least 8 years after their last cultivation, most probably in the soil seed bank, that feral populations persisted also by local recruitment, and that the spatial distribution of feral populations is partially related to the transport traffic at harvest from the field to the silo. Eastham & Sweet (2002)⁸ reported feral rape populations existing for at least 10 years. Feral (non cultivated) populations of oil seed rape and GE oilseed rape volunteers have been found from trials in the UK⁹ producing populations for up to the three years (the entire length of the study). Control of feral populations, e.g. with herbicides, could adversely affect biodiversity in hedgerows, roadside areas, railway banks which may also be a haven for wildlife.

Also the proposition that oilseed rape (OSR) roadside populations are “*often prevented from reaching maturity by mowing or by chemical treatment*” is highly questionable. This most certainly would not apply to railway lines. Who is going to control these volunteer plants? And how? Control of feral populations, e.g. with herbicides, could adversely affect biodiversity in hedgerows, roadside areas, railway banks which may also be a haven for wildlife.

There is the potential for gene transfer from GT73 to other Brassica species. For example, it has now been demonstrated that *B. napus* can hybridize and pass on the GE trait to *B. rapa*, a wild relative of *B. napus* occurring in Europe and which readily produces hybrids¹⁰.

The costs associated with herbicide resistance in *Brassica napus* are probably negligible¹¹. Even if the gene is neutral (as opposed to conferring a benefit), evolutionary theory predicts that the gene frequency will persist. It is a misconception that only beneficial genes will persist¹².

Such populations may also act as a reservoir of GE constructs for feral or planted conventional oilseed rape, which would compromise co-existence requirements. In addition,

there is the possibility of gene stacking, as has happened in Canada, where escapes have led to multiple herbicide resistance, leading to the increased use of other, less benign herbicides as reported by English Nature¹³.

ACRE is also concerned with the possibility that spillages of oilseed rape could lead to feral populations of GE oilseed rape¹⁴: *“ACRE still has concerns regarding seed spill and remains of the opinion that the post market monitoring plan should include active monitoring for spillage of GT73 seed during import, transportation and processing and include tests for the establishment of feral populations of GT73 oilseed rape. The plan should also include appropriate emergency plans should such populations be identified.”*

In summary, spillage of GE herbicide tolerant oilseed rape grain could result in feral populations, which could compromise cultivation of conventional oil seed rape. The persistence of oilseed rape seeds and feral populations is problematic for the control of any volunteers arising from a spillage, and raises the possibility of adverse effects on wildlife through herbicide applications on non cultivated land. There are clear issues of liability, which are not currently addressed.

Conclusions on EFSA’s Opinion on GT73

- **Irregularities in the molecular characterisation have not been studied further.**
- **Compositional analysis has been performed on samples pooled from different sites.**
- **Significant differences in composition have been found, but have not been investigated further.**
- **There is no environmental consideration of oilseed rape imports, in particular no consideration of the fact that import of GT73 can result in feral GE oil seed rape populations**

3. Concerns about regulatory authorities' handling of the GT73 application

The three-tier risk assessment and public scrutiny of the application material set out in Directive 2001/18/EC is wholly dependent on the proper handling of the application by the country that received the notification, which in the case of GT73 is the Netherlands. However, the Dutch authorities have not met their obligations under the Directive, and this has severely hindered the entire subsequent risk assessment process. Also the subsequent “scrutiny” by Member States and by the EFSA deserves to be strongly criticised. Public scrutiny on the other hand has been largely impossible, due to large sections of the material illegally being kept secret.

According to article 13.1 of the Directive, “The competent authority [in the Member State that receives the application] shall without delay examine whether the notification is in accordance with paragraph 2 and shall, if necessary, ask the notifier for additional information.” The exact requirements to the application material are set out in Annex II, Annex III and Annex IV of the Directive.

Application material both incomplete and of unacceptable quality

Despite this responsibility, the Dutch authorities accepted a clearly incomplete and not updated application for GT73. Although several Member States subsequently pointed out that the application was incomplete, the application process was still not suspended. The risk assessment cannot work as intended by Directive 2001/18/EC when incomplete applications are being considered. As highlighted by the example above (Compositional analysis – pooled data and significant differences) and by the highly questionable feeding trials, the application material received for GT73 was also of a generally poor quality.

Material has illegally been kept secret preventing public scrutiny

According to article 25.3 of Directive 2001/18/EC, “The competent authority shall, after consultation with the notifier, decide which information will be kept confidential and shall inform the notifier of its decisions”. Article 25.2 of the Directive clearly states that only information, “the disclosure of which might harm [the notifier’s] competitive position” can be treated as confidential and that “verifiable justification must be given in such cases”. However the Dutch authorities allowed a large part of the application to be kept confidential. This confidentiality is supposed to be applied only to material obtained through innovative research. However, much of the material in the GT73 application, that the Dutch authorities allowed to be stamped confidential, do not qualify for secrecy under Directive 2001/18/EC - no matter how unfavourable it is to the GMO.

The vast confidential section of the GT73 application even includes basic animal wholesomeness studies (Naylor 1994, Naylor 1995, Naylor 1996), which show abnormalities in rats fed with GT73. Data from such feed trials involve absolutely no material of interest to Monsanto’s competitors, and thus do not constitute a justifiable harm to the applicant’s competitive position. The material do therefore not qualify for confidentiality under article 25.2. Furthermore, feeding trials are part of the food safety assessment required by the Directive, which in turn is part of the environmental risk assessment detailed in Annex II. For the environmental risk assessment, and thus these feed trials, it is specifically stated in article 25.4 of Directive 2001/18/EC that this information “in no case may [...] be kept confidential”. The Dutch authorities have thus in clear breach of both articles 25.2 and 25.4 of the Directive

impeded due public scrutiny of the application. To a large extent, other Member States have maintained this illegal secrecy.

Lack of critical scrutiny by the authorities

Although greatly hindered by the illegal secrecy, Greenpeace has, from other sources, obtained basic information about the questionable feed trials conducted with GT73. These feed trials illustrate very well that the authorities' scrutiny of the application has been far from satisfactory. Many member states did not even react when presented with material of an unacceptable poor scientific standard.

Greenpeace can fully understand why Monsanto designed and conducted the feeding trials as they did, and can fully understand why Monsanto would argue that the negative effects revealed in the feeding trials should simply be ignored. It is, however, totally incomprehensible why many regulatory authorities, including EFSA, have decided to accept this material. Having revealed significant changes also in the second feeding trial, Monsanto conducts a third trial clearly designed to mask rather than to further investigate the significant differences revealed in the earlier trials. The fact that many Member States and the EFSA, despite this, are ready to approve GT73 illustrates clearly - as did MON863 application- that the risk assessment performed according to Directive 2001/18/EC fails to address even the most obvious risks.

Conclusion on the regulatory authorities' handling of the GT73 application
Directive 2001/18/EC is based on the precautionary principle. Article 4 regarding the General obligations reads: “Member States shall, in accordance with the precautionary principle, ensure that all appropriate measures are taken to avoid adverse effects on human health and the environment which might arise from the deliberate release or the placing on the market of GMOs”. This obligation cannot be met when:

- **Incomplete material of an unacceptable quality has been accepted as a basis for the assessment.**
- **Secrecy has illegally been applied to large parts of the application material, thus hindering public scrutiny.**
- **Many competent authorities (including the EFSA) have chosen to simply disregard data showing potential adverse effects, which as a minimum should have been investigated further.**
- **The environmental risk assessment has not addressed the obvious risk that the marketing approval will result in feral GE oil seed rape populations.**

4. Overall Conclusion

Significant differences have been found in the feeding trials. These should have been thoroughly investigated. Instead, various factors have been offered as hypotheses and the experiment was repeated with a range of controls to mask any significant differences. In their opinion on GT73, EFSA do not require further analysis but accept Monsanto's hypotheses. This is not adequately rigorous. The environmental consequences of accidental or unintentional releases of GT73 seeds during the import, transport or processing, have never been properly assessed. The authorities handling of the application could be improved on immensely, and should be if the public is to have trust in the regulators. The withholding of fundamental studies is contrary to an open and transparent regulation of GE foodstuffs and the Commission and Member States should take action to ensure the transparency of the evaluation process. For GT73, there are many irregularities and causes for concern. The application for the import and processing of GT73 should be rejected.

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¹ EFSA (2004) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/NL/98/11) for the placing on the market of glyphosate-tolerant oilseed rape event GT73, for import and processing, under Part C of Directive 2001/18/EC from Monsanto, *The EFSA Journal* (2004) 29, 1-19.

² Ibid

³ Brown, P.B., Wilson, K.A., Jonker, Y. & Nickson, T.E. (2003) Glyphosate tolerant canola meal is equivalent to the parental line in diets fed to rainbow trout. *Journal of Agricultural and Food Chemistry*, 51, 4268-4272.

⁴ ACRE (2004) *Advice on a notification for marketing of herbicide tolerant GM oilseed rape*
http://www.defra.gov.uk/environment/acre/advice/pdf/acre_advice36.pdf

⁵ Bio Journal (August 2004) <http://www5d.biglobe.ne.jp/~cbic/english/2004/journal0408.html>

⁶ Pascher K, Macalka-Kampfer S & Reiner H (2000) Vegetationsökologische und genetische Grundlagen für die Risikobeurteilung von Freisetzungen von transgenem Raps und Vorschläge für ein Monitoring. Bundesministerium f. soziale Sicherheit und Generationen, Forschungsberichte 7/2000.

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⁷ Pessel F, Lecomte J, Emeriau V, Krouti M, Messean A & Gouyon P (2001) Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. *Theoretical and Applied Genetics* 102: 841-846.

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⁸ Eastham, K. & Sweet, J. (2002) Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. Expert's Corner Series, European Environment Agency, Copenhagen.

⁹ Norris, C.E., Simpson, E.C., Sweet, J.B. & Thomas, J.E. (1999) Monitoring weediness and persistence of genetically modified oilseed rape (*Brassica napus*) in the UK. In: Lutman, P.J.W. (ed.) Gene flow and Agriculture: Relevance for Transgenic Crops. BCPC Symposium Proceedings no. 72, pp. 255-260.

¹⁰ DEFRA (2002) Monitoring large scale releases of genetically modified crops (EPG 1/5/84) incorporating report on project EPG 1/5/30: monitoring releases of genetically modified crop plants. Available at: <http://www.defra.gov.uk/environment/gm/research/epg-1-5-84.htm>

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¹¹ Snow, A.A., Andersen, B. & Jorgensen, R.B. (1999) Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa*. *Molecular Ecology* 8, 605-615.

¹² Ellstrand, N.C. (2001) When transgenes wander, should we worry? *Plant Physiology*, 125, 1543-1545.

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¹⁴ ACRE (2004) *Advice on a notification for marketing of herbicide tolerant GM oilseed rape*
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