Public Comments for Part C Notification C/DE/02/9 (deadline 06/06/2003)
Risk Assessment Report
Insect-protected maize line MON 863 and maize hybrid MON 863 x MON 810

-----Original Message-----
From: [redacted]
Sent: 21 May 2003 16:09
To: SNIF comments
Subject: Comment on notificationC/DE/02/9

The European Commission
DG Joint Research Centre,
Institute for Health and Consumer Protection,
Biotechnology and GMOs Unit

Notification number: C/DE/02/9
Product: Lepidopteran resistant Maize MON 863 x MON 810

[redacted] take issue with the findings of the German Competent Authority (Robert Koch Institute RKI) with regard to Monsanto's notification to place on the market a cross of Bt maize varieties.

RKI have concluded "that no adverse effects to human health or the environment are to be expected from the placing on the market of the genetically modified maize". As such they are happy to rubber stamp the application to market the GMO in Europe. We believe that there is insufficient information available to arrive at this conclusion.

RKI draw attention to the fact that an antibiotic resistant marker (ARM) gene is present. The presence of the ARM is not required in order for the plant to function and, as such, it adds extra risk to the safety of the GMO. RKI have suggested that the registration of the GMO might be subject to the phase out time limit of 31 December 2004 if the Commission's working party to develop a concept for assessing ARMs advise accordingly. As antibiotics can always have a useful function in securing human and animal health, any kanamycin resistance is not to be encouraged. We believe that it is vital that the phase-out time limit must be a condition of any marketing consent that might be given.

Whilst we recognise the fact that the purpose of the release is for the importation of maize as food/feed and not for cultivation, the notification includes the import of maize in grain form and, as such, cultivation of the line in Europe cannot be ruled out regardless of whether this happens by accident or on purpose. This factor has not been adequately addressed by RKI. The potential to contaminate existing maize crops cannot be ruled out. As Europe is essentially a maize grower (about 40 million tonnes was grown in Europe in 2001) rather than a maize importer, it is not worth the risk of allowing this GMO into Europe. We note that Europe only imported 2.5 million tonnes of maize grain in 2001 and the public would prefer the maize to be from a non-GM source.

MON 863 contains a Cry3Bb1 protein variant that attacks grubs of certain insects and the nptII antibiotic marker gene that confers resistance to kanamycin. The gene cassette is under control of the Cauliflower Mosaic Virus promotor.

MON810 contains a Cryl(A)b gene from B. Thuringiensis, the E35S promoter from cauliflower mosaic virus, the intron from the maize hsp70 gene and the 3' untranslated region of the nopaline synthase gene (NOS 3') from the Ti plasmid of Agrobacterium tumefaciens.

There were no studies reported into the safety and potential toxicity of both GM lines, let alone a combination of the two. Toxicity studies mentioned by RKI only relate to oral gavage of mice of the novel proteins themselves not the whole food where the GMO might act completely differently. Subacute feeding studies using rats and chicken were performed on the separate Bt lines but not on the hybrid of the two. We cannot evaluate the results as no data was provided.

The Cryl protein present in the Bt lines is known to be toxic - some [eg, Cryl(A)b] are more so than others. This protein is designed to rupture the gut of certain grubs. The insecticide cannot be removed from the plant by washing or peeling-back. The only information available to support the assertion that the toxicity of this protein is very specific to Lepidoptera and that there is no evidence that it is active against non-target insects, birds, fish or mammals is not from a peer-reviewed scientific paper. On the other hand Hilbeck et al., (1998) in a laboratory feeding experiments showed increased mortality of lacewing larvae fed on a diet of caterpillars which had been grown on Bt maize. On the controversy over the effects of Bt maize on the Monarch and Black Swallowtail butterfly larvae, the final conclusion was that "Bt corn damages butterflies in wild" (http://www.newscientist.com/news/news.jsp?id=ns99991274 New Scientist, 11 September 2001). Other research ("Fine Structural Changes in the Ileum of Mice Fed on -Endotoxin-Treated Potatoes and Transgenic Potatoes"; Nagui H. Fares, Adel K. El-Sayed) has shown that Bt Cryl damages the ileum of mice causing the lining cells of the gut to become enlarged. A damaged ileum would cause distress to digestion and is likely to be diagnosed as mild food poisoning or flu. The study confirms that the toxin is not destroyed in the stomach and has passed on to the last section of small intestine.

As such should similar symptoms arise from the commercial consumption of this GM maize hybrid (ie a digestive disorder of flu symptoms) it is unlikely to be reported to a doctor or to a vet.

We maintain our concern about the cauliflower mosaic virus promoter (CaMV 35S). Stripped of its' protective coating there is potential to do harm. The CaMV promoter is more complicated than just an on-off switch; it sends out such a strong signal that may control not only the new gene that it is inserted with, but also other genes that are already in the plant, with unpredictable results. Even after it is inserted, the gene has the capacity to separate out from the plant's chromosomes and recombine with other genes, such as ones for dormant viruses already in the plant (genetic remnants of an infection), or bacterial genes added as part of the GM process. The CaMV promoter gene can exacerbate any problems associated with horizontal gene transfer. According to Michael Hansen, a research associate at Consumers Union,

organisms normally protect themselves from horizontal invasions of genetic material by chemically "silencing" the new genes. When promoters are added to the mix, however, this defence is much less effective. In fact, one of the main reasons the CaMV promoter is added is to overcome the plant's natural "gene silencing" defences. In short, the CaMV promoter is an unstable piece of genetic material, prone to recombine with other genes found in the plant, including dormant viruses often present in a plant's genetic make-up. As such, scientists suspect that this promoter gene could recombine with genes from bacteria, viruses, and dormant viruses to create new pathogens.

There is no monitoring plan proposed in the notification as it is not anticipated that the Bt hybrid will be grown in Europe. As such, monitoring proposed is limited to general surveillance that may not be adequate to identify anything other than major incidents of poor health in humans and livestock. As we have highlighted before, it is unlikely that gastric upsets will be reported.

[redacted] would urge the European Commission to reject this application on the grounds that it is not substantially equivalent to non-GM maize and it is likely to cause harm to both the wider environment where it is grown outside of Europe (or within Europe if grown by accident) and to those species that might consume the product particularly in the unprocessed state as grain.

[redacted]

-----Original Message-----
From: [redacted]
Sent: 02 June 2003 22:09
To: gmoinfo-comments@jrc.it
Subject: Protest against Monsanto application: GM Maize (C/DE/02/9)

Dear Sir,

Provisional comments on the notification by Monsanto for the placing on the EU market of maize grains derived from insect-protected maize lines Mon 863 and Mon 863 x Mon 810 (notification number C/DE/02/9)

I wish to protest strongly against this application, and particularly against the fact that whole appendices have been designated by the applicant as being Confidential Business Information, including:

Appendix I
- PCR analysis and DNA sequence of the insert (Mon 863).
- Confirmation of the genomic DNA sequences flanking the 5' and 3' ends of the insert (mon 863).

Appendix III
• additional information concerning the 3′ junction between the insert and plant DNA (mon 810).
• Bioinformatics evaluation of the DNA sequence from the 3′ junction of Mon 810 insertion event: assessment of predicted polypeptides.
• Additional information concerning the 5′ junction between the insert and the plant DNA (Mon 810)

Appendix IV
• Safety assessment of Cry3Bb1 variants in Mon 863

This is an outrageous abuse of the system, since this is the very information that needs to be in the public domain if meaningful independent assessments are to be made.

I ask you therefore to refuse this application. Please record this protest and keep me informed as to the progress of the application.

Thank you

[redacted]

-----Original Message-----
From: [redacted]
Sent: 03 June 2003 12:57
To: gmoinfo-comments@jrc.it
Subject: Comment on notificationC/DE/02/9

The European Commission
DG Joint Research Centre,
Institute for Health and Consumer Protection,
Biotechnology and GMOs Unit

Notification number: C/DE/02/9
Product: Lepidopteran resistant Maize MON 863 x MON 810

We disagree totally with the findings of the German Competent Authority (Robert Koch Institute RKI) with regard to Monsanto's notification to place on the market a cross of Bt maize varieties.

RKI have concluded "that no adverse effects to human health or the environment are to be expected from the placing on the market of the genetically modified maize". As such they are happy to rubber stamp the application to market the GMO in Europe. We believe that there is insufficient information available to arrive at this conclusion.

RKI draw attention to the presence of the antibiotic resistant marker (ARM) gene. The ARM is not required for the plant to function and it adds extra risk to the safety of the GMO. RKI have suggested that the registration of the GMO might be subject to the phase out time limit of 31 December 2004 if the Commission's working party to develop a concept for assessing ARMs advise accordingly. As antibiotics always have a useful application in securing human and animal health, any

proliferation of antibiotic resistant genes is not to be encouraged. It hardly seems appropriate to market a GMO that has a potential 18 month shelf life.

Whilst we recognise the fact that the purpose of the release is for the importation of maize as food/feed and not for cultivation, the notification includes the import of maize in grain form and, as such, cultivation of the line in Europe cannot be ruled out regardless of whether this happens by accident or on purpose. This factor has not been adequately addressed by RKI. The potential to contaminate existing maize crops cannot be ruled out. As Europe is essentially a maize grower (about 40 million tonnes was grown in Europe in 2001) rather than a maize importer, it is not worth the risk of allowing this GMO into Europe. We note that Europe only imported 2.5 million tonnes of maize grain in 2001 and the public would prefer the maize to be from a non-GM source.

MON 863 contains a Cry3Bb1 protein variant that attacks grubs of certain insects and the nptII antibiotic marker gene that confers resistance to kanamycin. The gene cassette is under control of the Cauliflower Mosaic Virus promotor.

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The Cryl protein present in the Bt lines is known to be toxic - some [eg, Cryl(A)b] are more so than others. This protein is designed to rupture the gut of certain grubs. The insecticide cannot be removed from the plant by washing or peeling-back. The only information available to support the assertion that the toxicity of this protein is very specific to Lepidoptera and that there is no evidence that it is active against non-target insects, birds, fish or mammals is not from a peer-reviewed scientific paper. On the other hand Hilbeck et al., (1998) in a laboratory feeding experiments showed increased mortality of lacewing larvae fed on a diet of caterpillars which had been grown on Bt maize. On the controversy over the effects of Bt maize on the Monarch and Black Swallowtail butterfly larvae, the final conclusion was that "Bt corn damages butterflies in wild" (http://www.newscientist.com/news/news.jsp?id=ns99991274 New Scientist, 11 September 2001).

Other research ("Fine Structural Changes in the Ileum of Mice Fed on - Endotoxin-Treated Potatoes and Transgenic Potatoes"; Nagui H. Fares, Adel K. El-Sayed) has shown that Bt Cryl damages the ileum of mice causing the lining cells of the gut to become enlarged. A damaged ileum would cause distress to digestion and is likely to be diagnosed as mild food poisoning or flu. The study confirms that the toxin is not

destroyed in the stomach and has passed on to the last section of small intestine.

As such should similar symptoms arise from the commercial consumption of this GM maize hybrid (ie a digestive disorder of flu symptoms) it is unlikely to be reported to a doctor or to a vet.

We maintain our concern about the cauliflower mosaic virus promoter (CaMV 35S). Stripped of its' protective coating there is potential to do harm. The CaMV promoter is more complicated than just an on-off switch; it sends out such a strong signal that may control not only the new gene that it is inserted with, but also other genes that are already in the plant, with unpredictable results. Even after it is inserted, the gene has the capacity to separate out from the plant's chromosomes and recombine with other genes, such as ones for dormant viruses already in the plant (genetic remnants of an infection), or bacterial genes added as part of the GM process. The CaMV promoter gene can exacerbate any problems associated with horizontal gene transfer. According to Michael Hansen, a research associate at Consumers Union, organisms normally protect themselves from horizontal invasions of genetic material by chemically "silencing" the new genes. When promoters are added to the mix, however, this defence is much less effective. In fact, one of the main reasons the CaMV promoter is added is to overcome the plant's natural "gene silencing" defences. In short, the CaMV promoter is an unstable piece of genetic material, prone to recombine with other genes found in the plant, including dormant viruses often present in a plant's genetic make-up. As such, scientists suspect that this promoter gene could recombine with genes from bacteria, viruses, and dormant viruses to create new pathogens.

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We urge the European Commission to reject this application on the grounds that the GM Maize is not substantially equivalent to non-GM maize and it is likely to cause harm to both the wider environment where it is grown outside of Europe (or within Europe if grown by accident) and to those species that might consume the product particularly in the unprocessed state as grain.

[redacted]

-----Original Message-----
From: [redacted]
Sent: 03 June 2003 14:44
To: gmoinfo-comments@jrc.it
Cc: I-SIS press release

Subject: Comment on notificationC/DE/02/9

[redacted]

3rd June 2003

The European Commission
DG Joint Research Centre,
Institute for Health and Consumer Protection,
Biotechnology and GMOs Unit

Notification number: C/DE/02/9
Product: Lepidopteran resistant Maize MON 863 x MON 810

Dear Sir or Madam

We disagree totally with the findings of the German Competent Authority (Robert Koch Institute RKI) with regard to Monsanto's notification to place on the market a cross of Bt maize varieties.

RKI have concluded "that no adverse effects to human health or the environment are to be expected from the placing on the market of the genetically modified maize". As such they are happy to rubber stamp the application to market the GMO in Europe. We believe that there is insufficient information available to arrive at this conclusion.

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can exacerbate any problems associated with horizontal gene transfer. According to [redacted] a research associate at Consumers Union, organisms normally protect themselves from horizontal invasions of genetic material by chemically "silencing" the new genes. When promoters are added to the mix, however, this defence is much less effective. In fact, one of the main reasons the CaMV promoter is added is to overcome the plant's natural "gene silencing" defences. In short, the CaMV promoter is an unstable piece of genetic material, prone to recombine with other genes found in the plant, including dormant viruses often present in a plant's genetic make-up. As such, scientists suspect that this promoter gene could recombine with genes from bacteria, viruses, and dormant viruses to create new pathogens.

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We urge the European Commission to reject this application on the grounds that the GM Maize is not substantially equivalent to non-GM maize and it is likely to cause harm to both the wider environment where it is grown outside of Europe (or within Europe if grown by accident) and to those species that might consume the product particularly in the unprocessed state as grain.

Yours sincerely

[redacted]

-----Original Message-----
From: [redacted]
Sent: 03 June 2003 14:51
To: gmoinfo-comments@jrc.it
Subject: Comment on notificationC/DE/02/9

Please find attached the comments from [redacted] on the
Notification C/DE/02/9

[redacted]

[redacted]

Provisional comments by [redacted] on the notification by Monsanto for the placing on the EU market of maize grains derived from insect-protected maize lines Mon 863 and Mon 863 x Mon 810 (notification number C/DE/02/9)

[redacted] wishes to object very strongly to the lack of transparency with regard to this notification. We believe the public should have full access to all the documents necessary to judge whether adequate risk assessments were conducted. This is also the opinion of the European Parliament, that has –in July 2002- adopted an amendment to the Regulation on genetically modified food and feed that says: " It (the authority) shall also indicate how and where the public can access the files relating to the application and any information submitted subsequently, including the Authority's assessment (...)"

In the light of statements such as these and the general wish of the EU to be transparent we are concerned and surprised that the total dossier of the notification for the placing on the market of grains from genetically modified maize Mon 863 and Mon 863 x Mon 810 was not made accessible to the public. On the Joint Research Centre (JRC) website, the German authority's assessment report was made available but the only other information was the summary notification information format (SNIF) which totals only 26 pages. It is not indicated on the JRC website, nor in any other form by the European Commission or the Competent Authorities in the Member States if and where the full documentation can be accessed.

As a result of the poor level of information provision by the European Commission, it is extremely difficult even for people with detailed scientific and legal knowledge, or the public at large, to deliver meaningful comments on this notification. The way the process is conducted seriously hinders the ability of NGOs and other bodies that have an interest in this notification to ensure that their interests, and the legitimate concerns of the wider public, will be taken into account. The current situation, in which NGOs have to deliver their comments on the basis of only a summary and (in the 'best' cases) some fragments of the underlying documents that were obtained through other channels, is highly undesirable and needs to be addressed urgently.

[redacted] has based the following comments on a copy of the dossier supplied by Monsanto, which was provided by the UK authorities. However, whole appendices have been designated by the applicant as being Confidential Business Information, including:

Appendix I

- PCR analysis and DNA sequence of the insert (Mon 863).
- Confirmation of the genomic DNA sequences flanking the 5' and 3' ends of the insert (Mon 863)

Appendix III

- additional information concerning the 3' junction between the insert and plant DNA (mon 810).
- Bioinformatics evaluation of the DNA sequence from the 3' junction of Mon 810 insertion event: assessment of predicted polypeptides.
- Additional information concerning the 5' junction between the insert and the plant DNA (Mon 810)

Appendix IV

- Safety assessment of Cry3Bb1 variants in Mon 863

As a consequence, the following comments cannot be considered as complete. They are general and very provisory. [redacted] is only sending you these provisional and incomplete comments, because we have no alternative, as we are faced by an official deadline. [redacted] also wants to state clearly that it will send additional comments or changes to previous comments made by us, in case additional documentation becomes available, even if formal deadlines have expired.

1. Mon 863 and Mon 863 x Mon 810 should not be approved because of the presence of the antibiotic resistance gene nptII.

Article 4.2 of Directive 2001/18 states that

Member States and the Commission shall ensure that GMOs which contain genes expressing resistance to antibiotics in use for medical or veterinary treatment are taken into particular consideration when carrying out an environmental risk assessment, with a view to identifying and phasing out antibiotic resistance markers in GMOs which may have adverse effects on human health and the environment. This phasing out shall take place by the 31 December 2004 in the case of GMOs placed on the market.

A working group has been set up the Commission to examine the phasing out of antibiotic resistance marker genes in GM crops, but this will not be meeting until 2004. It is premature to make a decision on this crop in advance of the deliberations of the expert committee, and illogical to allow approval of a product which is likely to be removed from the market within two years. The German authority suggests that it would limit the registration to 31 December 2004, however [redacted] strongly argues that the crop should not be approved at all until its safety has been established. It does not seem at all reflective of the precautionary underpinnings of the Directive to allow approval of a product containing a genetic construct which the Commission and member states have committed to phasing out.

Mon 863 contains an antibiotic resistance gene, *nptII*, which according to the German authorities confers resistance to a number of antibiotics, including neomycin, kanamycin, geneticin, butirosin, gentamicin A and B and paramomycin. The German authorities suggest that because these antibiotics are not widely used, transfer of the nptII gene is of little consequence. However, this position ignores the fact that these drugs are still important for specific purposes, such as bowel sterilisation prior to surgery and treatment of neo-natal infections, and that therefore transfer of the gene could still cause adverse effects on human and animal health in those cases where these medicines are being used.

Consideration of horizontal gene transfer

The German authorities state that "*dissemination of the Bt toxin gene and the nptII gene integrated into the Mon 863 and Mon 863x Mon 810 lines from the genetically modified maize to micro-organisms of the digestive tract is not to be expected, due to the absence of a selective advantage*". However, [redacted] believes that this assessment is based on an inaccurate consideration of the evidence.

In their application, Monsanto state that "*transfer of DNA from plants to gut bacteria has not been reported*". However, research published by the UK government in 2002 showed that bacteria in human intestines had in fact taken up a novel gene from processed food containing GM soya[1]. This transfer occurred at a seemingly high rate, as it was recorded in seven out of twelve of the volunteers in the study. Selective advantage is not necessary for horizontal gene transfer to occur and, in fact, this research indicates that such gene transfer readily occurs in human intestines.

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[1] Netherwood T, Matin-Orue SM, O'Donnell AG, Gockling S, Gilbert HJ and JC Mathers 2002. *Transgenes in genetically modified soya survive passage through the human small bowel but are completely degraded in the colon* UK Food Standards Agency, Research Report G01008: Evaluating the risks associated with using GMO in human foods

Monsanto argues that gene transfer is unlikely because the plant DNA will be rapidly degraded by human and animal digestion. However, research examining GM maize fed to pigs has found that novel genetic material can last in the animals' intestines for up to 48 hours[2], while research examining GM maize fed to sheep found that an intact copy of the Bt gene Cry1A(b) could be extracted from grains after 24 hours in the rumen of sheep[3].

Monsanto also argue that for the *nptII* gene to be incorporated by competent bacteria, its "*eukaryotic regulatory signal would have to be exchanged against prokaryotic ones*" (notification C/DE/02/9, p 102). However, this would not actually be necessary because the *nptII* gene is controlled by the 35S CaMV promoter, which is known to be active in bacteria, including *E coli*[4] [5]. This means that there is no reason that nptII should not be transferred to bacteria in the intestines of humans and livestock.

The German authority's assessment of risk from the nptII gene is not sufficient because:

- It ignores important therapeutic uses of the antibiotics to which resistance is provided by the *nptII* gene
- There is recent evidence that gene transfer from plant DNA to bacteria in the human intestine has occurred and can do so at high frequency.
- The nptII gene is under control of a promoter that can be utilised by intestinal bacteria
- Assessment of the likely breakdown of DNA in the intestine is contradicted by published research

2. The Assessment of the safety of Mon 863 and Mon 863 x Mon 810 for use in food and feed is inadequate.

Assessment of Cry3Bb1 as an allergen

The applicant argues that there is no evidence that Cry3Bb1 is likely to cause an allergic reaction because it is claimed that there is a history of safe use and lack of resistance to digestion. However, this is not demonstrated in the dossier.

No history of safe use

Mon 863 was only deregulated in the United States in 2002, so there is no history of consumption of the GM product anywhere in the world. Instead, the claim for the GM maize is actually upon the use of microbial pesticides containing Cry3Bb1. The applicant states that "Cry3Bb1 has a history of safe consumption. Microbial pesticides including Raven® which contains Cry3Bb1, are commercially available". This statement is flawed for the following reasons:

1. The two proteins are not the same; as outlined in the notification, the version of Cry3Bb1 produced in Mon863 is a truncated version of the microbial pesticide, which is produced by bacterial fermentation, and thus the two proteins may exhibit different properties.
2. There is unlikely to have been any real exposure of the human population to Cry3 proteins because no foliar *Bt* insecticide containing Cry3 proteins has achieved significant market share in any crop[6]

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[2] Reuter T & Aulrich K. 2003. Investigations on genetically modified maize (Bt-maize) in pig nutrition: fate of feed-ingested foreign DNA in pig bodies *European Food Research and Technology* Vol 216(3) 185-192

[3] Duggan PS, Chambers PA, Heritage J, Michael Forbes J. 2003. Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. *British Journal of Nutrition* 89(2) pp 159-66

[4] Assaad FF and Signer ER (1990). Cauliflower mosaic-virus p35S promoter activity in *Escherichia-coli*. *Molecular and General Genetics* 223(3): 517-520:

[5] Lewin A, Jacob D, Freytag B, Appel B (1998). Gene expression in bacteria directed by plant-specific regulatory sequences. *Transgenic Research* 7(6): 403-411.

[6] Environmental Defense, Institute for Agriculture and Trade Policy, Science and Environmental Health Network, Centre for Food Safety, Consumer Policy Institute Consumers Union, 2000. *COMMENTS*

and because foliar applied Bt sprays rapidly break down in sunlight, so there is unlikely to have been any consumer exposure.

3. Even considering possible environmental exposure to Cry3Bb1, it should be noted that a Danish study examining naturally occurring *Bacillus thuringiensis* serovars did not find the subspecies *kumamotoensis*[7], indicating that this micro-organism may not be found commonly in Europe. In fact, a search of relevant academic databases (eg pubMed) does not find any published material examining exposure to Cry3 proteins.

Bt proteins have been shown to cause allergies

Information on human exposure to other Bt sprays indicates that there is potential for allergic responses as a result that exposure. A study published in 1999 examined whether conventional Bt pesticide sprays might lead to allergic sensitivities in farm workers[8]. The study found 2 out of 123 workers who picked and packed vegetables on a farm where Bt sprays were used exhibited reactivity to the Bt pro delta-endotoxin. The cry protein in the conventional sprays was present at much lower concentrations than in Mon 863 and Mon 863 x Mon 810 maize grains. Similarly, recent research from Denmark[9], which examined the health effects of a number of microbial pesticides, found that agricultural workers exposed to products of Bacillus thuringiensis were more likely to exhibit immune responses than workers exposed to other bacteria used in microbial pesticides. A study examining health effects on local residents from aerial spraying of a Bt pesticide found an increase in respiratory health effects associated with allergic responses, such as hay fever reactions[10].

These studies indicate that exposure to Cry3Bb1 could cause an immune response and hence allergic reactions. According to guidance produced by the EU Scientific Steering Committee, in such a case "*specific serum screening of the expressed protein should then be undertaken with appropriate sera from patients allergic to the source material using relevant validated immunochemical tests.*"[11] . This has not been done.

Allergen screening inadequate

The direct assessment of the allergenicity of Cry3Bb1 consists of sequence comparisons using allergen databases and in vitro digestion analyses. The applicant used sequence analysis to determine homology to look for a match of eight or more linearly contiguous amino acids in Mon863 to sequences within the allergen database. However, best practice would suggest that a search should examine sequences down to 6 amino acids[12], and indeed the applicant notes later in the dossier that "*the hypothetical minimum requirement for a peptide to elicit an allergic response would be six (to 15) amino acids*" (Notification by Monsanto, ref C/DE/02/9, p.83). It is therefore unclear why Monsanto has failed to undertake a suitable

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[7] Damgaard, PH, Hansen BM, Pedersen JC, Eilenberg J. 1997. Natural occurrence of Bacillus thuringiensis on cabbage foliage and insects associated with cabbage crops. *J Applied Microbiology* 82(2) pp 253-8

[8] Bernstein IL, Bernstein JA, Miller M, Tierzieva S, Bernstein DI, Lummus Z, Selgrade MK, Doerfler DL, Seligy VL. 1999 Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides *Environ Health Perspect* Jul;107(7):575-82

[9] "Sundhedsmæssige problemer ved brug af mikrobiologiske bekæmpelsesmidler i væksthuse" (Health problems associated with the use of microbial pesticides in greenhouses) Bekæmpelsesmiddelforskning nr. 61, Miljøstyrelsen 2002

[10] Petrie K, Thomas M, Broadbent E. 2003. Symptom complaints following aerial spraying with biological insecticide Foray 48B *New Zealand Medical Journal* 116(1170): U354

[11] *Guidance document for the risk assessment of genetically modified plants and derived food and feed 6-7 march 2003* Prepared for the Scientific Steering Committee by The Joint Working Group on Novel Foods and GMOs European Commission Health & Consumer Protection Directorate-General

[12] Moneret-Vautrin DA 2003 The allergic risk of transgenic foods strategy for prevention *Ann Pharm Fr* 61(2):96-102

sequence search, but it effectively invalidates its conclusion that there is no match to "immunologically relevant" amino acid sequences.

In vitro digestion analysis inadequate

Monsanto's analysis of potential allergenicity rests heavily on *in vitro* digestion studies using Cry3Bb1. These studies do not accord with guidance provided by the EU Scientific Steering Committee on this matter.

Monsanto state that Cry3Bb1 is rapidly degraded in simulated gastric fluid and states that this indicates that it is not an allergen. However, the EU Scientific Steering Committee note that "*no absolute correlation exists*" between pepsin degradation and allergenicity and the Scientific Steering Committee states that "*further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic*" such as serum testing, IgE binding, analysis of cross-reactivity and/or sensitising potential. Other than the sequence analysis previously mentioned, no further tests of allergic potential were conducted. In addition, in simulated intestinal fluid the Cry3Bb1 protein degraded to a stable fragment of approximately 57kDA. The Scientific Steering Committee recommend that, when examining protein digestion, "*Stable breakdown products should be characterized and evaluated with regard to the hazards linked to their biological activity.*"

Expression level of Bt proteins

Mon 863 maize contains the novel gene *cry3Bb1*, a synthetic variant of the wild type gene *cry3Bb* from *Bacillus thuringiensis* ssp. *kumamotoensis*. The expressed protein Cry3Bb1 differs from the wild type by 7 additional amino acids, increasing its potency as an insecticide. The novel protein is expressed in Mon 863 and the hybrid Mon 863 x Mon 810 at much greater levels than in any previously approved GM maize varieties containing proteins derived from Bacillus thuringiensis and greater even than the levels of Cry9C found in Starlink maize (see Table). For example, Cry3Bb1 is expressed in Mon 863 maize at a level 70,000 times greater than the expression of Cry1Ab protein in Bt 176 maize.

Cry3Bb1 protein and event	**Protein Expression Level (µg/g) in grains**	**Level compared to Mon 810 (0.46 µg/g Cry1Ab)**	**Level compared to Bt 11 (1.4 µg/g Cry1Ab)**	**Level compared to Bt176 (0.001 µg/g Cry1Ab)**	**Level compared to Starlink (18.6 µg/g Cry 9C)**
Cry3Bb1 in Mon863 (1999)	70 (average) 49-86 (range)	x 152	x 50	x 70,000	x 3.8
Cry3Bb1 in Mon863 (2000)	42.7 (average) <0.096 – 84.1 (range)	x 93	x 30.5	x 42,700	x 2.3
Cry3Bb1 in Mon863 x Mon 810 (2000)	61.1 (average) 38.5 – 83.1 (range)	x 132	x 43.6	x 61,100	x 3.3

The data provided by the applicant indicate that all the Bt endotoxin proteins are expressed at higher levels in the hybrid than in either parent. In the case of Cry3Bb1, the applicant notes that "*Average levels of the Cry3Bb1 protein in tissues of Mon 863xMon 810 were estimated to be 1 -2 fold higher than in Mon 863*" (Notification by Monsanto, ref C/DE/02/9, p.62). In the grain the average level was 43 per cent higher in the hybrid grains (61.1 µg/g fw) than Mon 863 parent grains (42.7 µg/g fw). Similarly the average level of Cry1Ab was 83% higher in the hybrid (0.84 µg/g fw) than in Mon 810 parent grains (0.46 µg/g fw).

These findings are not investigated or interpreted further by the applicant on the basis of the "safety characteristics" of the protein. However, [redacted] considers the safety assessment of Cry3Bb1 to be insufficient, as discussed above.

Details of feeding studies not provided

Monsanto claims that its conclusions on the safety of Cry3Bb1 are further supported by animal feeding studies. However, while summary findings of these studies are presented in the notification, the reports and detailed results of the studies have not been provided. The reports themselves have not been peer reviewed or published but are internal Monsanto documents. In the absence of peer review it is essential that Monsanto make the entire reports and detailed results available for consideration, as the validity of the conclusions can only be ascertained by an examination of the methods used. Until such time as this is done it is not acceptable to simply take the word of the applicant that there were no adverse findings.

In conclusion, Monsanto has not demonstrated that there is low risk of Cry3Bb1 being an allergen because:

- Evidence exists that Bt proteins can induce allergic reactions in humans
- The suggestion that there is a safe history of use of Cry3Bb1 is not supported by any evidence
- Amino acid sequence comparison was limited to matches of eight or more amino acids, which is not in accordance with good practice.
- Specific tests for allergic potential were not conducted.
- The stable breakdown product of Cry3Bb1 was not investigated further.
- Reports on animal feeding studies have not been made available.

3 The Environmental Risk Assessment and monitoring plan is not adequate

The applicant has undertaken a very poor assessment of the potential for dissemination and accidental release of this GMO. Both the applicant and the German authorities conclude that, because the application is for import of food and feed only, there is negligible risk to the environment and that a monitoring plan is unnecessary. [redacted] strongly disagrees with this, as the position is directly contradicted by the experiences in Mexico; despite the fact that only food and feed imports of GM maize were allowed, local landraces of maize were still found to be contaminated with GM constructs. It has been suggested that GM maize grains sold as food or feed were inadvertently planted and that this was the cause of the widespread contamination of the Mexican varieties[13].

Despite the widespread industrialisation of European agriculture, maize seed saving is still practiced and regional landraces of maize are still abundant in some areas, and have been documented in Spain, Portugal, Italy and France and Greece. They represent an important resource of agricultural biodiversity for Europe and so should be given sufficient protection. A monitoring plan is essential to ensure that if these GM maize grains are sold for food and feed, they really are restricted to this purpose and that the situation in Mexico is not repeated in the EU, with the contamination of European landraces.

[redacted] submits that Mon 863 and Mon 810 x Mon 863 maize should not be approved because it contains an unacceptable risk to human health through the presence of the antibiotic resistance marker gene *nptII*. [redacted] considers that, in line with Article 4.2, this gene may have adverse effects on human health and the environment.

In addition, [redacted] believes that the food and feed safety assessment, the environmental risk assessment and the monitoring plan are all far from adequate and that these do not constitute sufficient evidence of the safety of the GM maize lines.

[redacted]

[13] CIMMYT Press Release. Director General Iwanaga Gives CIMMYT's Position on Issue of Transgenes in Mexican Landraces and Implications for Diversity Worldwide 5 December 2002

-----Original Message-----
From: [redacted]
Sent: 03 June 2003 16:49
To: gmoinfo-comments@jrc.it
Subject: comment on notification C/DE/02/9

The European Commission
DG Joint Research Centre,
Institute for Health and Consumer Protection,
Biotechnology and GMOs Unit

Notification number: C/DE/02/9
Product: Lepidopteran resistant Maize MON 863 x MON 810

We disagree totally with the findings of the German Competent Authority (Robert Koch Institute RKI) with regard to Monsanto's notification to place on the market a cross of Bt maize varieties.

RKI have concluded "that no adverse effects to human health or the environment are to be expected from the placing on the market of the genetically modified maize". As such they are happy to rubber stamp the application to market the GMO in Europe. We believe that there is insufficient information available to arrive at this conclusion.

RKI draw attention to the presence of the antibiotic resistant marker (ARM) gene. The ARM is not required for the plant to function and it adds extra risk to the safety of the GMO. RKI have suggested that the registration of the GMO might be subject to the phase out time limit of 31 December 2004 if the Commission's working party to develop a concept for assessing ARMs advise accordingly. As antibiotics always have a useful application in securing human and animal health, any proliferation of antibiotic resistant genes is not to be encouraged. It hardly seems appropriate to market a GMO that has a potential 18 month shelf life.

Whilst we recognise the fact that the purpose of the release is for the importation of maize as food/feed and not for cultivation, the notification includes the import of maize in grain form and, as such, cultivation of the line in Europe cannot be ruled out regardless of whether this happens by accident or on purpose. This factor has not been adequately addressed by RKI. The potential to contaminate existing maize crops cannot be ruled out. As Europe is essentially a maize grower (about 40 million tonnes was grown in Europe in 2001) rather than a maize importer, it is not worth the risk of allowing this GMO into Europe. We note that Europe only imported 2.5 million tonnes of maize grain in 2001 and the public would prefer the maize to be from a non-GM source.

MON 863 contains a Cry3Bb1 protein variant that attacks grubs of certain insects and the nptII antibiotic marker gene that confers resistance to kanamycin. The gene cassette is under control of the Cauliflower Mosaic Virus promotor.

MON810 contains a Cryl(A)b gene from B. Thuringiensis, the E35S promoter from cauliflower mosaic virus, the intron from the maize hsp70 gene and the 3' untranslated region of the nopaline synthase gene (NOS 3') from the Ti plasmid of Agrobacterium tumefaciens.

There were no studies reported into the safety and potential toxicity of both GM lines, let alone a combination of the two. Toxicity studies mentioned by RKI only relate to oral gavage of mice of the novel proteins themselves not the whole food where the GMO might act completely differently. Subacute feeding studies using rats and chicken were

performed on the separate Bt lines but not on the hybrid of the two. We cannot evaluate the results as no data was provided.

The Cryl protein present in the Bt lines is known to be toxic - some [eg. Cryl(A)b] are more so than others. This protein is designed to rupture the gut of certain grubs. The insecticide cannot be removed from the plant by washing or peeling-back. The only information available to support the assertion that the toxicity of this protein is very specific to Lepidoptera and that there is no evidence that it is active against non-target insects, birds, fish or mammals is not from a peer-reviewed scientific paper. On the other hand Hilbeck et al., (1998) in a laboratory feeding experiments showed increased mortality of lacewing larvae fed on a diet of caterpillars which had been grown on Bt maize. On the controversy over the effects of Bt maize on the Monarch and Black Swallowtail butterfly larvae, the final conclusion was that "Bt corn damages butterflies in wild"
(http://www.newscientist.com/news/news.jsp?id=ns99991274 New Scientist, 11 September 2001).

Other research ("Fine Structural Changes in the Ileum of Mice Fed on -Endotoxin-Treated Potatoes and Transgenic Potatoes"; Nagui H. Fares, Adel K. El-Sayed) has shown that Bt Cry1 damages the ileum of mice causing the lining cells of the gut to become enlarged. A damaged ileum would cause distress to digestion and is likely to be diagnosed as mild food poisoning or flu. The study confirms that the toxin is not destroyed in the stomach and has passed on to the last section of small intestine.

As such should similar symptoms arise from the commercial consumption of this GM maize hybrid (ie a digestive disorder of flu symptoms) it is unlikely to be reported to a doctor or to a vet.

We maintain our concern about the cauliflower mosaic virus promoter (CaMV 35S). Stripped of its' protective coating there is potential to do harm. The CaMV promoter is more complicated than just an on-off switch; it sends out such a strong signal that may control not only the new gene that it is inserted with, but also other genes that are already in the plant, with unpredictable results. Even after it is inserted, the gene has the capacity to separate out from the plant's chromosomes and recombine with other genes, such as ones for dormant viruses already in the plant (genetic remnants of an infection), or bacterial genes added as part of the GM process. The CaMV promoter gene can exacerbate any problems associated with horizontal gene transfer. According to [redacted] a research associate at [redacted] organisms normally protect themselves from horizontal invasions of genetic material by chemically "silencing" the new genes. When promoters are added to the mix, however, this defence is much less effective. In fact, one of the main reasons the CaMV promoter is added is to overcome the plant's natural "gene silencing" defences. In short, the CaMV promoter is an unstable piece of genetic material, prone to recombine with other genes found in the plant, including dormant viruses often present in a plant's genetic make-up. As such, scientists suspect that this promoter gene could recombine with genes from bacteria, viruses, and dormant viruses to create new pathogens.

There is no monitoring plan proposed in the notification as it is not anticipated that the Bt hybrid will be grown in Europe. As such, monitoring proposed is limited to general surveillance that may not be adequate to identify anything other than major incidents of poor health in humans and livestock. As we have highlighted before, it is unlikely that gastric upsets will be reported.

We urge the European Commission to reject this application on the grounds that the GM Maize is not substantially equivalent to non-GM maize and it is likely to cause harm to both the wider environment where it is grown outside of Europe (or within Europe if

grown by accident) and to those species that might consume the product particularly in the unprocessed state as grain.

yours faithfully,

[redacted]

-----Original Message-----
From: [redacted]
Sent: 04 June 2003 10:04
To: gmoinfo-comments@jrc.it
Subject: Comments, Joint Res C, insect protected maize Notification no. C/DE/02/9

Comments to Joint Research Centre re Monsanto's application for GM insect protected maize 4.6.03
To gmoinfo-comments@jrc.it

Comments re. Notification number C/DE/02/9:
Monsanto applying to place on the EU market their maize grains derived from insect protected maize, Mon 863 and Mon 863 x Mon 810

1. Gene transfer risk
Monsanto asserts that gene transfer is unlikely because the plant DNA will be rapidly degraded by human and animal digestion. However, scientific research which examined pigs fed GM maize revealed that novel genetic material can last in the animals' intestines for up to 48 hours
Similarly, research on sheep fed GM maize revealed an intact copy of the Bt gene Cry1A(b) in grains after 24 hours in the sheep rumen. And has there even been any such research on cows?

2. Inadequate safety assessment of: Mon 863; Mon 863 x Mon 810 for food and feed use.
e.g. the assessment of Cry3Bb1 as an allergen
Monsanto say here is no evidence that Cry3Bb1 is likely to cause an allergic reaction and that there is a history of safe use and lack of resistance to digestion.
However, this is not backed up by any evidence in their dossier.

3. No "safe use" history
Mon 863 was deregulated in the USA very recently- i.e. 2002. There is no history of consumption of this GM product anywhere in the world.
Instead, the claim for the GM maize is actually based solely on the use of microbial pesticides containing Cry3Bb1.
Also, Monsanto write: "Cry3Bb1 has a history of safe consumption. Microbial pesticides including Raven® which contains Cry3Bb1, are commercially available".
This statement is wrong in that:
a. The two proteins are not the same. As explained in the notification, the version of Cry3Bb1produced in Mon863 is a truncated version of the microbial pesticide, produced by bacterialfermentation. So the two proteins may well exhibit different properties.

b. No foliar Bt insecticide containing Cry3 proteins has achieved significant market share in any crop, thus making it unlikely that many people have been exposed to Cry3 proteins.

4. Antibiotic resistance markers - no thank you
Mon 863 and Mon 810 x Mon 863 maize should not be approved because they contain an unacceptable risk to human health through the presence of the antibiotic resistance marker gene nptII.
The EU is supposed to be phasing out the use of antibiotic markers!

Please _reject_ this application - the maize is not substantially equivalent to non-GM maize and it may well cause harm to the wider environment and to any creatures that might consume it, especially as unprocessed grain.

[redacted]

-----Original Message-----
From: [redacted]
Sent: 06 June 2003 00:30
To: gmoinfo-comments@jrc.it
Subject: consultation GM maize C/DE/02/9

[redacted]

European Commission,-DG Joint Research Centre,
Institute for Consumer and Health Protection
Biotechnology and GMOs Unit.

Dear Sir/Madam,
Re Consultation on application by Monsanto Ltd. to market maize lines Mon 863 and Mon 863 ¥ Mon 810, notification no. C/DE/02/9.

I wish to object to the marketing of this GM maize.
1. Mon 863 contains the antibiotic resistance gene npt 11 which confers resistance to a number of antibiotics. This is against the intention of EU Directive 2001/19/EC and will soon become illegal. It is not correct to suggest that because these antibiotics are not often used then any transferred resistance is irrelevant.

2. The inclusion of the insecticide in the maize itself is a retrograde step which is in principle quite wrong. All insecticides, however mild, whether present in natural bacteria or not, are by their nature, toxic.

To incorporate them into food that will be eaten by animals or humans is undesirable and unnecessary. The actual dose received in food cannot be calculated exactly neither can the dose that is absorbed from the intestine. Even if the dose received is not toxic there is always the possibility of irritation to the intestine as has been shown in the paper by Nagui H. Fares and A.K. El-Sayad of the Department of Zoology and Entomology at Ains Sams University, Cairo. Natural Toxins, Vol 6 issue 6 1998.

3. A very likely consequence will be the development of insect resistance to the toxin which is a valuable aid when used in limited circumstances in organic farming..

4. No feeding trials have been carried out on cattle whose digestive processes are quite different to mice and rats.

Yours sincerely

[redacted]