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Evaluation of the environmental risk assessment of application EFSA/GMO/CZ/2008/54 (maize line MON 88017) submitted under Regulation (EC) No. 1829/2003:

Final evaluation report of the Belgian Biosafety Advisory Council

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Introduction (on application & procedure)

The application under consideration EFSA/GMO/CZ/2008/54 concerns the placing on the market of the genetically modified maize line MON 88017 for cultivation purposes by the Monsanto Company. MON 88017 is modified with the *cry3Bb1* and the *cp4 epsps* genes, rendering the maize line resistance to corn rootworm and tolerance to glyphosate. Food and feed uses, import and processing are covered under application EFSA/GMO/CZ/2005/27[1].

The application was submitted to the European Food Safety Authority (EFSA) in accordance with Regulation (EC) No. 1829/2003. EFSA launched a call on May 7, 2008 in accordance with Article 6.3(c) and 18.3(c) to the competent authorities to carry out the initial evaluation of the environmental risk assessment (ERA) of MON 88107 carried out by the applicant. The Belgian competent authority under Directive 2001/18/EC was designated by EFSA to carry out the ERA. The application was declared valid on September 12, 2008 and subsequently assessed by the Belgian Biosafety Advisory Council on request of the Belgian competent authority. On December 3, 2008 (Annex I), March 24, 2009 (Annex II), August 24, 2009 Annex III), December 16, 2009 (Annex IV) and August 19, 2010 (Annex V), the Belgian Biosafety Advisory Council finalised its requests for additional information on the ERA and the post-market environmental monitoring plan and forwarded these to the Belgian competent authority.

The Belgian Biosafety Advisory Council conducted its evaluation of the ERA based on the information in the application, the additional information received by the applicant, the information found in peer-reviewed studies (see References) and the scientific comments raised by the member states within the three month consultation period (May 7, 2008 until August 12, 2008).

[1]Application EFSA/GMO/CZ/2005/27 has been evaluated positively by the Belgian Biosafety Advisory Council (http://www.bio-council.be/bac_advices.html) and EFSA (EFSA, 2009a).

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Environmental risk assessment

1. Background information

1.1. Recipient or parental plant

Maize (*Zea mays L.*) is a highly domesticated annual agricultural crop, originating from Central America. Maize is not considered as having weedy tendencies (Baker, 1974) and is not known as an invasive species in natural ecosystems (CFIA, 1994). Maize is predominantly wind pollinated. There are no other cultivated or wild plant species that are sexually compatible with maize in the EU. Seed survival over-winter is limited under European weather conditions and hence volunteer appearance rare in Europe. Volunteers are generally controlled by farmers, either by the use of herbicides or manual or mechanical removal.

1.2. Genetic modification

MON 88017 has been obtained through *Agrobacterium*-mediated transformation of maize cells with plasmid vector PV-ZMIR39, containing the CP4 EPSPS expression cassette and the Cry3Bb1 expression cassette.

1.3. GM plant

1.3.1. Traits that have been introduced

Properties of the introduced genes conferring herbicide tolerance

Expression of CP4 EPSPS confers tolerance to glyphosate (the active ingredient of Roundup). Glyphosate interferes with normal plant metabolism through inhibiting the enzyme 5-enolpyruvyl-3-phosphoshikimic acid synthase (EPSPS) involved in the biosynthesis of aromatic amino acids. As a consequence of the inhibition of aromatic amino acid biosynthesis, protein synthesis is disrupted, resulting in the plant's death. The application of glyphosate to maize line MON 88017 will be ineffective as CP4 EPSPS will take over the intrinsic plant EPSPS function (OECD, 1999).

Properties of the introduced genes conferring resistance to insects

The introduced gene *cry3Bb1* allows to control certain coleopteran insect pests of the Chrysomelidae family, namely the larvae of the Western corn rootworm (*Diabrotica virgifera virgifera*), but also the Northern (Vaughn *et al*., 2005), Southern (Donovan *et al*., 1992) and Mexican corn rootworm and the Colorado potato beetle. The only corn rootworm species present in the EU is *D. v. virgifera.*

With the insertion of the *cry3Bb1* gene, the plant is able to produce δ-endotoxins (*Bt* toxins). The δ-endotoxin selectively binds to receptors located in the midgut of susceptible species. After binding to receptors, the gut is perforated, enabling enterobacteria from the midgut to enter the body, causing the insect to die from poisoning within 48 to 120 hours.

The activity of the Cry3Bb1 and CP4 EPSPS proteins is not likely to be affected by potential interactions of Cry3Bb1 and CP4 EPSPS with one another, as their mode of action is different. Hence, the Biosafety Advisory Council considered testing the combined effects of the two newly expressed proteins not necessary.

1.3.2. Sequences actually inserted

MON 88017 has been genetically modified to produce the Cry3Bb1 and CP4 EPSPS proteins and contains in a single locus one intact copy of the following cassettes:
(1) an *epsps* gene derived from *Agrobacterium tumefaciens* sp. strain CP4 (CP4 EPSPS), which imparts tolerance to glyphosate, under the regulation of the rice actin 1 gene promoter (P-*Ract1*) and first intron (I-*Ract1*), the nopaline synthase terminator sequences from *A. tumefaciens* and the chloroplast transit peptide 2 sequence from the *epsps* gene of *Arabidopsis thaliana.*
The CP4 EPSPS protein of MON 88107 has an identical amino acid sequence to the CP4 EPSPS protein in soybean GTS 40-3-2 and in one of the two cassettes of maize NK603;
(2) a synthetic version of the *cry3Bb1* gene derived from *Bacillus thuringiensis* (*Bt*) subsp. *kumamotoensis* strain EG4691 conferring resistance to certain coleopteran pests, under the regulation of the enhanced 35S promoter derived from cauliflower mosaic virus, a 5' untranslated leader of the wheat chlorophyll a/b/-binding protein (L-*Cab*), the I-*Ract1* and the transcript termination sequence for wheat heat shock protein 17.3 (T-*Hsp17*).
The amino acid sequences of the Cry3Bb1 protein present in MON 88017 shares a high identity with the Cry3Bb1 of MON 863 and MON 853 (they differ by only one of the 653 amino acid residues). Equivalence of the Cry3Bb1 protein produced by *E. coli* and the GM plant MON 88017 has been demonstrated and positively assessed in the context of application EFSA/GMO/CZ/2005/27.

1.3.3. Expression of the insert

The expression level of Cry3Bb1 and CP4 EPSPS was determined in various tissues of MON 88017 maize relevant for ERA (leaf (over season), root (forage, over season and senescent), grain, silk and pollen). Samples were taken from field trials in the USA (3 sites) during the 2002 growing season (Bhakta *et al.*, 2003), from field trials in Argentina (4 sites) during the 2003-2004 growing season (Dudin & Jennings, 2005) and from European field trials (7 sites located in Germany and Spain) in 2006 and 2007 (Niemeyer & Silvanovich, 2007, 2008). A randomised block design with three replications was used at all sites.

In the evaluation of the data by the Biosafety Advisory Council, main focus was put on the expression values of MON 88017 grown in European fields, as the field trials conducted in the USA and Argentina were considered and positively assessed in the context of application EFSA/GMO/CZ/2005/27. The analysis of the expression levels was considered to be well-performed and the data from the trials conducted over multiple years (2006 and 2007) at different locations (Germany and Spain) were considered sufficient. Cry3Bb1 and EPSPS proteins were found to be expressed in all tissues tested (silk was not tested for *cp4 epsps* expression). The range of mean Cry3Bb1 protein expression levels for tissues of MON 88017 grown in the EU (considering all sites and years) were 100-550 µg/g dry weight (dw) for leaves (depending on growth stage before harvest), 11-480 µg/g dw for root (depending on growth stage), 5.8-28 µg/g dw for grain, 110-330 µg/g dw for silk and 9.7-19 µg/g dw for pollen; CP4 EPSPS protein expression levels were 63-350 µg/g dw for leaves (depending on growth stage before harvest), 5.3-130 µg/g dw for root (depending on growth stage) 1.7-6.6 µg/g dw for grain and 160-440 µg/g dw for pollen (Niemeyer & Silvanovich, 2007, 2008).

In order to verify the conclusion that the expression levels obtained from the EU field trials are in the range of those obtained in the USA, additional historical data from other field trials referred to in the technical dossier were requested by the Belgian Biosafety Advisory Council. This information included expression data obtained from field trials conducted in the EU during the 2007 growing season (Niemeyer & Silvanovich, 2008) and the USA in 2005 and 2006. With this information, the Biosafety Advisory Council could agree with the above-mentioned conclusion and considered non-target studies which have been conducted with toxin doses related to expression levels measured in USA field experiments also valid for the EU.

1.3.4. How the GM plant differs from the recipient plant in reproduction, dissemination and survivability

Both laboratory experiments (Rosenbaum & Horak, 2003) and multi-site field trials conducted in the USA in 2001 (8 sites: Rosenbaum *et al.*, 2003) and 2002 (10 sites: Pester & Woodrum, 2003) and in the EU in 2006 (8 sites located in Germany and Spain: Martin & De Billot, 2008) were conducted to test phenotypic characteristics of maize line MON 88017 compared to conventional maize. A randomised split-plot design with four replications was used in the field study conducted in 2001 and a randomised block design with three replications in the field study of 2002 and 2006. Changes in reproduction (germination, grain moisture, seed dormancy, seedling vigour, silking, pollen shed, and pollen morphology and viability), agronomic characteristics, such as stalk lodging, root lodging, plant and ear height, dropped ears, stay green, yield, disease incidence and insect damage were observed. More information was asked by the Belgian Biosafety Advisory Council on the non-GM control strains used in the field trials in the EU (see Annex I). The breeding tree provided by the applicant and showing that the control lines had a comparable genetic background to MON 88017 was considered appropriate. In addition, the study on pollen morphology and viability (Rosenbaum & Pester, 2004) was asked for as it was missing in the original application.

During the evaluation of the ERA focus was put on the study conducted in the EU, as the set-up of both USA field trials was considered less appropriate to study phenotypic and agronomic characteristics of MON 88017 (negative segregants were used as control and no conventional maize lines were used as comparator). Comparison of MON 88017 maize with the non-transgenic control maize across field sites in the USA did not reveal any phenotypic difference, except for seedling vigour (was greater for MON 88017) in both years of field trial and days to 50% pollen shed (was fewer for MON 88017) in the second year of field trial (2002). In the EU, statistically across-site differences were found for plant height (MON 88017 was shorter) and yield (lower for MON 88017) in field trials conducted in Germany. No across-site differences were found in the Spanish field trials. The relatively small differences detected in the USA and EU field trials were not considered biologically meaningful with respect to plant weed potential. Moreover, in the field trial carried out in the EU, the range of values for agronomic and phenotypic characteristics was shown to fall within the range of values observed for traditional maize hybrids. In addition, it was shown that the susceptibility

MON 88017 to *Ostrinia nubilalis*, *Sesamia nonagrioides* and common smut was comparable to that of non-modified maize plants (Martin & De Billot, 2008).

2. Potential changes in the interactions of the GM plant with the (a)biotic environment

In its evaluation of the ERA, the Biosafety Advisory Council agreed with the assessment provided by the applicant on the issues related to (2.1) persistence and invasiveness, (2.2) selective advantage and disadvantage, (2.3) potential for gene transfer, (2.6) effects on human and animal health and (2.9) issues of interactions with the abiotic environment. On the issues of target (2.4) and non-target (2.5) interactions, (2.7) effects on biochemical processes and (2.8) impacts of specific cultivation, management and harvesting techniques, the Biosafety Advisory Council formulated requests for clarification or additional information (see Annex I to II). For the latter issues, an overview of the evaluation conducted by the Biosafety Advisory Council is given below. To complete the evaluation report, a summary of the assessment provided by the applicant is given for the former issues.

2.1. Persistence and invasiveness

The applicant assessed whether MON 88017 is any more likely to become a weed than the non-transgenic control or other maize currently cultivated in the EU. The assessment took into account the biology of maize, the agronomic and phenotypic characteristics of MON 88017 and the newly introduced traits.

Given (a) the biology of maize (see 1.1), (b) the information that maize line MON 88017 does not exhibit characteristics that would cause it to be more weedy than other maize hybrids (see 1.3.4) and (c) that the traits conferred to MON 88017 are not expected to change the persistence and invasiveness of maize, as maize is incapable of surviving without human assistance under European conditions, it can be concluded that the likelihood of MON 88017 to become more persistent or invasive is negligible.

2.2. Selective advantage and disadvantage

The expression of the insect resistance trait Cry3Bb1 and the herbicide tolerance trait CP4 EPSPS confer specific advantages to **maize in the field**, namely resistance to certain coleopteran pests and tolerance to glyphosate. Comparison of the MON 88017 with the non-transgenic maize comparator and other maize hybrids currently cultivated in the EU did not reveal any changes in reproduction, dissemination and survivability (see 1.3.4). The effect of the transgenes on the biology of maize is therefore negligible. Taking the today's agronomic management practices for maize production into account, it is negligible that the introduced traits in MON 88017 will confer any meaningful selective advantage or disadvantage, except under chrysomelid pest pressure and when glyphosate is used.
As maize does not survive **outside the agricultural environment** and has no wild relatives in the EU, the question of selective advantage and/or disadvantage to plants outside the agricultural environment is not applicable.

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2.3. Potential for gene transfer

As there are no wild relatives of maize in the EU, **vertical gene flow** through cross-pollination from GM maize fields is restricted to plants of the same species. Gene flow might also result from the adventitious presence of GM maize kernels in conventional maize seeds or from seed spillage during transport. Gene transfer might thus result in the occurrence of GM maize volunteers. As the control of these volunteers will be the same as for non-GM maize volunteers, the occurrence of GM maize volunteers will not raise any novel environmental concerns compared to non-GM volunteers.

The possibility of **horizontal gene transfer** between the GM plant and micro-organisms, is considered as a rare event under natural conditions (EFSA, 2006b; Nielsen & Townsend, 2004; Keese, 2008). In the very unlikely case of transfer, maintenance and functional expression of the *cp4 epsps* or *cry3Bb1* gene in micro-organisms of the receiving environment, no impact on the ecology of micro-organism communities and no adverse effect on human/animal health or to the environment are expected.

2.4. Interactions between the GM plant and target organisms

The studies to determine the target specificity of Cry3Bb1 and presented in the application have been conducted in the context of the evaluation of MON 863, using the MON 853 Cry3Bb1 variant which was shown to be biological equivalent to the MON 863 Cry3Bb1 variant (Astwood *et al*., 2001). An issue taken into consideration in the evaluation of the ERA was thus the source of the Cry3Bb1 toxin used in the target (and non-target) toxicity studies (see Annex I) and to see whether studies conducted with other Cry3Bb1 variants are also valid for MON 88017. The sources are different: Cry3Bb1 variants of MON 88017, MON 863 and MON 853 were used*.* Given that the variations in protein sequences between the Cry3Bb1 variants are small (see 1.3.2) and that the biological activity of MON 863 and MON 88017 Cry3Bb1 is equivalent (Duan *et al*., 2003), the Biosafety Advisory Council concluded that studies conducted with MON 853 or MON 863 can be used to assess the safety of MON 88017. However, some uncertainty remains. Experiments describing a side-by-side comparison of the different protein sources, indicating that the amino acid substitution is not expected to have an impact on protein structure and eventually biological activity, are (partly) lacking.

The biological activity of Cry3Bb1 has been tested via dietary laboratory studies. The toxicity of Cry3Bb1 was shown to be restricted to certain coleopteran pests, namely from the Chrysomelidae family (leaf beetles). The larvae of Western corn rootworm (Chrysomelidae: *D. v. virgifera*; Head *et al.*, 2001) are the target organism for maize MON 88107. Also the larvae of the Colorado potato beetle (Chrysomelidae: *Leptinotarsa decemlineata;* Head *et al.*, 2001; Meissle & Romeis, 2009a) were shown to be sensitive for Cry3Bb1. To better document the target spectrum of Cry3Bb1, the applicant was asked to provide a review and references of data on the impact of all Cry3Bb1-containing crops and biopesticides against all *Diabrotica* species (see Annex I).

In conclusion, although it is clear that the specificity of Cry3Bb1 variants is restricted to Chrysomelidae, the biological equivalence between the MON 853 and the MON 88017

Cry3Bb1 variant could have been better demonstrated, as studies with the MON 853 Cry3Bb1 variant were used to demonstrate the target specificity of the MON 88017 Cry3Bb1 variant.

2.5. Interactions of the GM plant with non-target organisms

The potential of maize line MON 88017 to have direct or indirect adverse effects on non-target organisms was evaluated by the Biosafety Advisory Council (for effects on species of soil community, see 2.7). Impacts on non-target organisms due to unintended changes of composition or morphology of the GM maize were not expected to occur, as no compositional and phenotypic differences have been found between the GM maize and its non-GM comparator. The potential of non-target effects due to the expression of the traits (Cry3Bb1 and CP4 EPSPS) is described in this section. In its evaluation the Biosafety Advisory Council concentrated on Cry3Bb1, as non-target effects of CP4 EPSPS have been evaluated in the context of NK603 (EFSA, 2009b). As mentioned in 2.4, the source of the Cry3Bb1 toxin used in the toxicity studies was taken into account in the evaluation. In addition, recently peer-reviewed studies on non-target effects of Cry3Bb1 - some including the MON 88017 Cry3Bb1 variant - published after receipt of the application were considered (see References).

2.5.1. Potential non-target effects due to the expression of Cry3Bb1

Non-target effects on insects

Information on the lack of potential adverse effects on non-target organisms of Cry3Bb1 was obtained from laboratory dietary toxicity studies with insects living above and on-ground and from field studies. Ecological and economical important functional groups, namely beneficial insect predators or natural enemies and pollinators were considered. The laboratory studies provided by the applicant were conducted in the context of the evaluation of MON 863, either using the MON 853 or MON 863 Cry3Bb1 variant; the field studies provided were carried out with MON 863.

The dietary toxicity tests conducted by the applicant on non-target insects and herbivores (putative targets) were overall considered to be well-conducted, except for the *Chrysoperla carnea* larvae (Palmer & Krueger, 1999b) study (see Annex I and II). For this study, the Biosafety Advisory Council was not convinced that the test species was sufficiently exposed to the Cry3Bb1 toxin. Given that, due to the mode of feeding, lacewing larvae will not be exposed much (if at all) to Cry3Bb1 in the field, this study was considered of less relevance.

It was shown (and the Biosafety Advisory Council agrees) that Coleoptera, namely larvae or adults of predatory ladybird beetles (Coccinellidae, *Hippodamia convergens*: Palmer & Krueger, 1999a; Bryan *et al.*, 2001, *Coleomegilla maculata*: Duan *et al.*, 2001a,b, 2002; Lundgren & Wiedenmann, 2002; Ahmad *et al.*, 2006a, *Stethorus punctillum*: Li & Romeis, 2010), larvae of the herbivorous ladybird beetle (*Epilachna vigintioctopunctata*: Shirai, 2006), strawberry leaf beetle larvae (Chrysomelidae, *Galerucella vittaticollis*: Shirai, 2006), larvae or adults of the ground-dwelling carabid beetles (Carabidae, *Poecilus chalcites*: Duan *et al.*, 2006 (see application for reference), *Harpalus caliginosus* and *H. pensylvanicus*: Ahmad *et al.*, 2006a; and other species of Carabidae: Mullin *et al.*, 2005), weevils (Curculionidae and Bruchidae: Head *et al.*, 2001) and red flour beetle (Tenebionidae, *Tribolium castaneum*: Head *et al.*, 2001), Diptera, namely larvae of *Drosophila melanogaster* and *Megaselia scalaris*

(Knecht & Nentwig, 2010), Neuroptera, namely adults of the predatory species green lacewing (*Chrysoperla carnea*: Palmer & Krueger, 1999b; Li *et al*., 2008, 2010), Lepidoptera, namely, the European corn borer (*Ostrinia nubilalis*), the monarch butterfly larvae (*Danaus plexippus*: Mattila *et al.*, 2005) and corn earworm larvae (*Helicoverpa zea*), the parasitic Hymenoptera (*Nasonia vitripennis*: Sindermann *et al*., 2002b) and larvae and adults of honeybees (*Apis mellifera*: Maggi, 1999a,b, 2002; references in Duan *et al*., 2008a), and Hemiptera minute pirate bug (*Orius insidiosus*: Teixeira, 2005; Duan *et al*., 2008b), the rice leaf bug (*Trigonotylus caelestialium*: Rauschen *et al*., 2009) and *Zyginidia scutellaris* (Rauschen *et al*., 2008), would not be affected by Cry3Bb1.

In contrast, a recent paper by Schmidt *et al.* (2009) has reported marginally significant mortality in larvae of the coccinellid *Adalia bipunctata* when exposed to certain concentrations of microbially produced trypsin-activated Cry3Bb protein in its diet. The study of Schmidt *et al.* (2009) was not taken into consideration in the evaluation of the risk assessment as it was considered to have major flaws at the level of methodology used (Meissle & Romeis, 2008; Rauschen, 2010; Ricroch *et al*., 2010). Evidence for the fact that the adverse effects reported by Schmidt *et al*. (2009) were likely artefacts of poor study design were provided by Álvarez-Alfageme *et al*. (2010). Detailed feedings studies with Cry3Bb1 provided either through spider mites that had consumed MON 88017 or as purified protein revealed no adverse effects on a range of life-table parameters of *A. bipunctata* larvae. Another study showed a decrease in survival of the aquatic dipteran species *Chironomus dilutus* exposed to Cry3Bb1, but no effect on growth of the *C. dilutus* larvae (Prihoda & Coats, 2008). However, as stated by the authors themselves, it remains unclear if the observed effects were due to the presence of Cry3Bb1 or other compounds in the root extracts, as no control treatments with increasing concentrations of non-*Bt* maize root extracts were included.

The conclusions of the lower-tier studies were supported by field studies in the USA and Europe. No negative impact of Cry3Bb1-expressing MON 863 was found on field densities of abundant occurring coleopteran species[2], including Carabidae (Ahmad *et al.*, 2005; Bhatti *et al.*, 2005a; Duan *et al.*, 2006), Chrysomelidae, a.o. the corn leaf beetle *Chaetocnema pulicaria* (Bhatti *et al.*, 2005b; Duan *et al.*, 2006), Coccinellidae (Bhatti *et al.*, 2005b; Duan *et al.*, 2006), a.o. *C. maculata (*Al-Deeb & Wilde, 2003, McManus *et al., 2005;* Ahmad *et al.*, 2006a*)*, Lathridiidae (Bhatti *et al.*, 2005a; Duan *et al.*, 2006), Nitidulidae (Bhatti *et al.*, 2005a; Duan *et al.*, 2006) and Staphylinidae (Ahmad *et al.*, 2005; Bhatti *et al.*, 2005a; Duan *et al.*, 2006), the hemipteran species *Orius insidiosus* (Al-Deeb & Wilde, 2003; Bhatti *et al.*, 2005b; Ahmad *et al.*, 2006a; Duan *et al.*, 2006) and *Rhopalosiphum maidis* (Bhatti *et al*., 2005b; Duan *et al.*, 2006) and Nabidae (Bhatti *et al*., 2005b; Duan *et al.*, 2006); the neuropteran species *Chrysoperla carnea* (Bhatti *et al.*, 2005b; Duan *et al.*, 2006), Syrphidae (Diptera; Bhatti *et al*., 2005b; Duan *et al.*, 2006), Formicidae (Hymenoptera; Ahmad *et al.*, 2005; Bhatti *et al.*, 2005a; Duan *et al.*, 2006) and Gryllidae (Orthoptera; Ahmad *et al.*, 2005) in the USA. Also no negative impact of MON 88017 was found on the abundance of ladybirds (Rauschen *et al*., 2010) and the abundant occurring hemipteran species *Trigonotylus caelestialium* (Rauschen *et al*., 2009) and *Zyginidia scutellaris* (Rauschen *et al*., 2008) in Europe.

[2] A recent publication by Meissle & Romeis (2009) indicates that Coleoptera, including Chrysomelids are exposed to Cry3Bb1 in the field.

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As the Cry3Bb1 activity might be broader than to *Diabrotica* and other putative chrysomelid targets, e.g. cereal leaf beetle (*Oulema melanopus*), the applicant was requested to address in its ERA if the cultivation of MON 88017 might impact on non-target Chrysomelids (including threatened and endangered Chrysomelids, if relevant) occurring in and around maize fields in Europe. The provided theoretic quantitative risk assessment indicated that unacceptable adverse effects on non-target Chrysomelids are not expected, and was regarded as sufficient. Recently carried out field experiments substantiate the theoretic assessment. Rauschen *et al*. (2010) showed that Chrysomelidae are one of the most abundant families of Coleoptera in maize fields in Germany, but that there occurrence is mainly restricted to the chrysomelid pests *Phyllotreta* sp. and *Oulema lichenis*. Due to the low occurrence of non-pest chrysomelids in maize fields the risk to these non-target species in field is expected to be minimal, at least in Germany.

In summary, it can be concluded that adverse effects on non-target insects, including non-target Chrysomelids occurring in maize fields, is expected to be negligible.

Non-target effects on other organisms than insects

The Biosafety Advisory Council agreed with the assessment that the non-target effects on other organisms than insects is negligible and has summarised the information in the application, updated with recent information, below.

As the toxicity of Cry3Bb1 is specific (see 2.4), no direct effects on other invertebrates, or on vertebrate organisms are expected. The toxicity and specificity is associated with the binding to specific cell membrane receptors in the brush border membrane vesicles present in the midgut of susceptible insects. In addition, due to the exotic status of *Diabrotica* spp. in the EU, indirect effects of Cry3Bb1 (i.e. reduction of target *Diabrotica* spp.) on biodiversity via shifts in the arthropod food web are considered to be unlikely.

The applicant has examined the potential toxicity of Cry3Bb1 to the crustacean *Daphnia magna* (APHIS, 2005), the springtail *Folsomia candida* (see 2.8), earthworms (see 2.8), birds (Gallagher *et al*., 1999), fish (Li & Robinson, 2004; APHIS, 2005) and mammals (see also EFSA/GMO/CZ/2005/27). The Biosafety Advisory Council considered that no firm conclusions on toxicity could be drawn from the study of Li & Robinson (2004), as the focus of the study was on testing nutritional quality of MON 88017 maize. In addition, Cry proteins of class 3 have not been reported to be toxic for nematodes, mites & protozoa and mammals (Schnepf *et al.*, 1998), and the MON 88017 Cry3Bb1 variant not for the spider *Theridion impressum* (Meissle & Romeis, 2009b), slugs *Arion lusitanicus* and *Deroceras reticulatum* (although only exposed for 3 days, Zürbrugg & Nentwig, 2009). In a subsequent experiment lasting for 16 weeks, Hönemann & Nentwig (2010) detected no significant effects of MON 88017 on the survival, weight change and oviposition of slug *Arion vulgaris*.

The conclusions of the lower-tier studies were supported by field studies in the USA. No negative impact of Cry3Bb1-expressing MON 863 was found on field densities of spiders (Ahmad *et al.*, 2005) and Chilopoda (Bhatti, 2005a).

2.5.2. Potential non-target effects due to the expression of CP4 EPSPS.

The expression of CP4 EPSPS is not expected to have adverse effects on non-target organisms, as the CP4 EPSPS protein shares no significant homology with toxic proteins, is homologous with the wild type CP4 EPSPS protein ubiquitous in plants and has been proven by laboratory studies to be safe for arthropods (Levine & Uffman, 2007; Uffman & Levine, 2007). The conclusions of the laboratory studies were supported by field studies conducted with maize NK603 in the EU (Rosca, 2004; Rodriguez *et al.*, 2006; Schier, 2006) and the Phillipines (Reyes, 2005). In addition, the lack of toxicity of CP4 EPSPS to mammals and avian species was confirmed in toxicity studies (Harrison *et al.*, 1996) and nutritional equivalence studies. Moreover, the potential non-target effects of CP4 EPSPS have been assessed by EFSA as being negligible (EFSA, 2009b).

2.6. Effects on human and animal health

It was demonstrated that there is a lack of structurally relevant similarity between the Cry3Bb1 protein and any known toxic or pharmacologically active proteins relevant to human and animal health (TOXIN5 database, 2001). The Belgian Biosafety Advisory Council requested to demonstrate if this conclusion is still up-to-date by using the TOXIN6 database (2008). The applicant demonstrated that this was the case.

For further evaluation of effects on animal and human health due to accidental consumption, we refer to the food/feed evaluation of maize line MON 88017 (EFSA, 2009a).

2.7. Effects on biogeochemical processes

The Biosafety Advisory Council took the same approach as described in the introduction of 2.5 in its evaluation of the assessment of effects on biogeochemical processes.

2.7.1. Effects due to the expression of Cry3Bb1

As other *Bt* toxins, Cry3Bb1 can be introduced into the soil via leaching from root exudates (Icoz & Stotzky, 2007; Icoz *et al*., 2008) and incorporation of plant residues after harvest. Laboratory and field studies showed that Cry3Bb1 does not persist in the soil environment and is degraded rapidly (Ahmad *et al.*, 2005; Icoz & Stotzky, 2007; Icoz *et al.*, 2008; Miethling-Graff *et al.*, 2010; Zurbrügg *et al.*, 2010). According to Prihoda & Coats (2008) the half-life of Cry3Bb1 in decomposing MON 863 maize tissues in microcosms is less than 5 days. In a litterbag study carried out during the winter period, the decomposition rate of Cry3Bb1 in MON 88017 senescent leaves was determined at 48% after 3 weeks and 95% after 6 weeks (Zürbrugg *et al*., 2010). Similarly, Miethling-Graff *et al.* (2010) reported a 99.99% decline in the Cry3Bb1 concentration in the roots of field-collected stubbles from MON 88017 maize within a 7 months period.

The tests conducted by the applicant on soil (micro-)organisms were overall considered to be well-conducted. However, the Biosafety Advisory Council was of the opinion that the study by Sindermann *et al.* (2002a) did not provide prove for the absence of adverse effects on *Eisenia fetida*, but rather gave indications of absence of adverse effects (see Annex I and II).

Exposure to Cry3Bb1 has not shown to cause any immediate or delayed effects on soil organisms. No adverse effects have been detected in laboratory studies on earthworms (*Lumbricus terrestris*: Ahmad *et al.*, 2006b and *Enchytraeus albidus:* Hönemann & Nentwig, 2009) and Collembola (*Folsomia candida*: Teixeira, 1999) due to toxicity of Cry3Bb1. A study on *Eisenia fetida* (Sindermann *et al.*, 2002a) gave indications that also no adverse effects occur on this earthworm species. Field trials conducted in the USA showed that there were no significant differences in numbers of Collembola (on-ground or below-ground: Al-Deeb *et al.*, 2003; Ahmad, 2005; Bitzer, 2005), Acarina (Al-Deeb *et al.*, 2003; Ahmad, 2005), nematodes (Al-Deeb *et al.*, 2003) and earthworms (Zeilinger *et al.*, 2010). A recent 9 months leaf litter-bag field study conducted in Switzerland with MON 88017 revealed no difference in the decomposer community when compared to the near isoline and other conventional maize varieties (Hönemann *et al.*, 2008).

No deleterious effects on soil microbial communities due to the presence of Cry3Bb1 toxins were found (Devare *et al.*, 2004; Carson *et al.*, 2005). Field studies with MON 863 did not reveal any negative impact on soil micro-organisms diversity or decomposition function of the microbial community (Icoz *et al.*, 2008; Lawhorn *et al.*, 2009). Similarly, Miethling-Graff *et al.* (2010) did not detect any significant differences between the rhizosphere bacterial community structure of MON 88017 maize compared to the non-transformed near-isogenic counterpart and two conventional cultivars in a three year field study.

The results of Poerschmann *et al.* (2008) indicate that the roots of MON 88017 have a slightly increased total lignin content (by 7 %) compared to the near-isogenic line. No difference in lignin content was found for the leaves. The latter was confirmed by Zurbrügg *et al.* (2010). Their litter bag study revealed that MON 88017 leaf litter is readily degraded and that degradation does not differ from the near isoline maize or other conventional maize varieties. It is not expected that the small increase in lignin content in the roots will cause differences in carbon sequestration over the longer term. It has been shown that even distinct increases in decomposition resistant compounds such as lignin result in only modest increases in organic carbon in the topsoil. Changes in soil management have a much more pronounced effect (Sessitsch *et al.*, 2004).

In summary, it can be concluded that adverse effects on non-target insects and microbes of the soil community is expected to be negligible.

2.7.2. Effects due to the expression of CP4 EPSPS.

CP4 EPSPS can be introduced into the soil via incorporation of plant residues after harvest. No adverse effects have been detected in laboratory studies on earthworms (*Eisenia fetida:* Sindermann *et al.*, 2004) due to toxicity of CP4 EPSPS. Field trials showed that there were no significant differences in numbers of Collembola (Bitzer *et al.*, 2002)[3].
The CP4 EPSPS of MON 88017 is homologous to the EPSPS proteins found in plants and micro-organisms. It is therefore considered unlikely that it will affect the microbial community and hence biogeochemical processes adversely. CP4 EPSPS was shown not to alter key soil microbial processes, such as carbon and nitrogen transformation via laboratory studies

[3] This field trial was conducted with soybean GTS-40-3-2

(Carson *et al.*, 2004) and field studies (Liphadzi *et al.*, 2005[4]; Philippot *et al.*, 2006). Moreover, the potential non-target effects of CP4 EPSPS have been assessed by EFSA as being negligible (EFSA, 2009b).

2.8. Impacts of the specific cultivation, management and harvesting techniques

In the application is stated that the specific cultivation, management and harvesting techniques used for MON 88017 are comparable to those used for other commercially available maize, with the exception of the environmental monitoring plan. Additionally, the possibility of using glyphosate 'over the top of the crop' in the cultivation of MON 88017 is added to the farmer's weeding options.

It is acknowledged by the applicant that the use of glyphosate 'over the top of the crop' in the cultivation of MON 88017 could result in potential biological relevant indirect adverse effects[5] for maize agro-ecosystems (i.e. effects on biodiversity due to reduction of weeds), but notices that this also might occur with current agronomic practices. The applicant believes that any indirect effects associated with the use of MON 88017 maize in the EU, will be within the range of currently acceptable indirect effects. The Biosafety Advisory Council is of the opinion that the applicant has not provided sufficient information to substantiate this statement. As herbicide tolerant maize will allow the use of non-selective herbicides as glyphosate 'over the top of the crop', less weeds might result in biologically relevant adverse effects for maize agro-ecosystems. The Biosafety Advisory Council is of the opinion that the use of glyphosate 'over the top of the crop' must not interfere with biological functions of non-target organisms (such as biological control and decomposition). First field studies in Spain do not indicate an impact of NK603 and its associated weed management on biological control (Albajes *et al.,* 2009).

In conclusion, given the fact that management and utilisation of a GM crop may vary from region to region, farm to farm and over time, the Biosafety Advisory Council acknowledges the difficulty to predict the range of farming practices that will be deployed with the GM crop and the consequences for biological functions. The risk assessment should have taken this unpredictability of farm management and its consequences for biological functions better into account, e.g. by relating this to monitoring. The Biosafety Advisory Council is of the opinion that the farmer questionnaires are a good tool to detect changes in biological functions, but that the questionnaire should be adapted to cover this issue (for requests see general surveillance).

[4] This field trial was conducted with soybean GTS-40-3-2

[5] The potential (direct) effects of the herbicide use on the environment were considered under Directive 91/414/EEC (a.o. direct effects on non-target organisms (insects, soil organisms, birds, fish and mammals) and development of weed resistance) and not reconsidered in this evaluation. Indirect effects (i.e. effects on biodiversity due to reduction of weeds), however, were not addressed under Directive 91/414/EEC and are taken into consideration in the evaluation of the impacts of the specific cultivation, management and harvesting techniques (point 2.8).

2.9. Potential interactions with the abiotic environment

Expression of the introduced traits, of which the wild type variants are naturally present in the soil environment, will not alter the natural interactions of maize plants with the abiotic environment.

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Monitoring

As no potential adverse effects were identified for the environment and human health, the Biosafety Advisory Council agrees with the applicant that case-specific monitoring is not considered necessary during the cultivation of MON 88017. However, to avoid the development of insect resistance, the applicant provided an insect resistance management (IRM) plan for case-specific monitoring of resistance development in corn rootworms. Also a glyphosate resistance management plan was set up to address the potential development of resistant weeds. The latter plan was set up in the framework of Directive 91/414/EEC and therefore not reconsidered in this evaluation. The evaluation of the Biosafety Advisory Council was restricted to the scientific quality of the monitoring plans proposed, including the IRM plan and the general surveillance plan (see Annex III – V).

1. Case-specific Monitoring: IRM

When assessing the IRM plan, the receiving environment was taken into account. In the EU, MON 810 expressing Cry1Ab, is already cultivated. Potential implications on IRM of MON 88017 due to the combined presence of MON 810 and MON 88017 should be no issue given the specificity of Cry1Ab and Cry3Bb1 proteins to Lepidoptera and Coleoptera (Chrysomelidae), respectively.

The IRM plan proposed to delay insect resistance to Cry3Bb1 recommends a 20% refuge for areas larger than 5 hectares as requested for *Bt* maize events expressing Cry1Ab and Cry1F in high dose (i.e. 99,9% efficacy[6]). As the expression of Cry3Bb1 is considered low-to-moderate (Meihls *et al*., 2008 and references therein), the applicant was requested to provide clearer argumentation to substantiate their claim that the Cry3Bb1 dose in MON 88017 is high enough to lead to > 99% larval mortality and the appropriateness of the proposed measures.

The Biosafety Advisory Council is of the opinion that no convincing evidence is given by the applicant to claim 99,9% efficacy of MON 88017. Moreover, recently Hibbard *et al.* (2010) reported that the dose (density-independent mortality) of the Cry3Bb1-expressing event DAS-59122 is rather 96,71% than 99,88% as previously calculated by the equation of Storer *et al.* (2006). Also Binning *et al.* (2010) showed that the efficacy of DAS-59122 was lower than the earlier predictions (Storer *et al.*, 2006): in their plant study neonate mortality was determined to be 99,5%. The findings of Hibbard *et al*. (2010) and Binning *et al.* (2010) are suggestive that Cry3Bb1-expressing events fail to meet the high dose criteria. Given this information, the Biosafety Advisory Council is reluctant to rely on the outcomes of the stochastic modelling approach to support the appropriateness of the 20% refuge strategy.

[6] High dose is here defined as 10-25 amount needed to kill 99,9% of susceptible individuals (ILSI, 1999).

Preliminary information on the feeding behaviour of the Western corn rootworm larvae (they avoid feeding on root parts expressing Cry3Bb1, Clark *et al*., 2006) on MON 863 suggest that the emerging adults may not have been exposed to Cry3Bb1 (or only to a limited extent). The accomplishment of the larvae to survive may be due to subtle differences in Cry3Bb1 expression in the roots. As pointed out by Clark *et al*. (2006) this information raises questions on the proposed refuge requirements. If the *Bt* crop yields susceptible adults, the crop itself can act as refuge. Uncertainty remains if the larval feeding behaviour observed for MON 863 is the same for MON 88017, as the applicant notes that the latter has a more even distribution of the Cry3Bb1 protein in the roots and provides more consistent root protection compared to MON 863. However, neither the less heterogeneous Cry3Bb1 expression of MON 88017 compared to MON 863, nor the feeding behaviour of larvae on MON 88017 has been clearly demonstrated.

Given the remaining uncertainties (gaps in knowledge) on the feeding behaviour of Western corn rootworm on MON 88017, the better performance of MON 88017 compared to MON 863 and the potential of Western corn rootworm to become resistant (Lefko *et al*., 2008; Meihls *et al*., 2008), the Biosafety Advisory Council supports the adoption of the proposed 20% refuge strategy as described in the IRM plan. However, the Biosafety Advisory Council is of the opinion that the importance of the refuge in delaying resistance against MON 88017 should be further investigated. If the above assumptions on larval feeding behaviour and Cry3Bb1 expression in MON 88017 are true, it follows that the 20% refuge strategy may be a too highly conservative measure to delay the occurrence of insect resistance. In addition, the Biosafety Advisory Council wishes to note that care should be taken to continuously evaluate and, if needed, adjust the recommended measures in the plan, particularly if large scale adoption of the *Bt* maize would change existing eradication/containment cropping measures (e.g. crop rotation) possibly affecting abundances of the target population.

In addition, the IRM plan describes the methodology of resistance monitoring for MON 88017 when cultivated in the EU. This methodology to monitor resistance is considered appropriate. The Biosafety Advisory Council points out that a sub-lethal seedling assay described by Nowatzki *et al*. (2008) might be an additional useful method to establish baseline susceptibility and monitor changes in that susceptibility.

2. General Surveillance

The applicants will conduct general surveillance for MON 88017 maize throughout the period of validity of the authorisation. In particular, the general surveillance will take into consideration and be proportionate to the extent of cultivation of MON 88017 maize seed products and use thereof in the Member States. It will focus on growers cultivating MON 88017 maize seed products. The Biosafety Advisory Council is of the opinion that the current general surveillance plan needs to be adapted to allow identification of unanticipated adverse effects on non-target organisms (see 2.8 and Annex III), and of management regimes that do not have an environmental performance at least as good as current regimes. The following is requested:

- The GS questionnaire should clarify what is meant with insects under 3.8, i.e. whether the term refers to pests or insects other than pests.
- Insects other than pests, i.e. used in biological control of maize, should be considered separately in the general surveillance plan.
- In the farmer questionnaire, insect control treatments applied on the MON 88017 refuges should be requested in order to check the efficacy of the IRM measures.

The Biosafety Advisory Council wants to point out to the member states authorities the applicant's commitment to develop a "Technology Use Guide for the European MON 88017 markets", describing recommendations for the use of their product, including more detailed weed control recommendations specific to each region and local needs. Such a Use Guide has already been set up for Spain (Monsanto, 2007).

Overall conclusions

Based on the information in the application, the additional information received by the applicant, the information found in peer-reviewed studies and the scientific comments raised by the member states, the Belgian Biosafety Advisory Council considers that no risks concerning the environment and human and animal health were identified as a result of cultivation of MON 88017, except for potential indirect adverse effects related to the use of glyphosate over the top of the crop. The Biosafety Advisory Council is of the opinion that the applicant should have linked the latter ERA issue better to monitoring. Therefore, the Biosafety Advisory Council requests that the potential consequences for biological functions of non-target organisms due to the use of glyphosate are better considered in the post-market monitoring plan and that the proposals made in its report are implemented.

The Biosafety Advisory Council wants to note that some recent research results on IRM of Cry3-expressing maize question the importance of the 20% refuge strategy. Given the current knowledge gaps, the Biosafety Advisory Council supports the proposed refuge strategy as described in the IRM plan, but is of the opinion that the IRM plan needs further development and continuous updating taken into account the results of ongoing scientific research.

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

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Déclaration minoritaire annexée au document BAC_2010_0928

Déclaration minoritaire de Damien Winandy relatif au rapport final du Conseil Consultatif de Biosécurité (ref. BAC_2010_0928) sur l'évaluation du risque environnemental du maïs MON88017 (Dossier EFSA/GMO/CZ/2008/54 introduit dans le cadre du Règlement (EC) 1829/2003)

The submitted ERA does not sufficiently take into account the potential consequences on environment of an increased utilization of glyphosate, including the development of weed resistance. Scientific data from region where glyphosate resistant GM crops are grown for many years could have been presented and analyzed.

Bioveiligheidsraad
Conseil de Biosécurité

Secretariaat
Secrétariat

03 December 2008

O./ref.: WIV-ISP/BAC/2008_853

Environmental Risk/Safety Assessment (ERA) of maize line MON88017 (EFSA-GMO-CZ-2008-54):

Requests of the Belgian Biosafety Advisory Council for clarification on the ERA

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 26 September 2008

Coordinator: Prof. dr. ir. Dirk Reheul

Experts: Patrick De Clercq (UGent), Adinda De Schrijver (SBB), Patrick du Jardin (FUSAGx), Jean-Luc Hofs (FUSAGx), Joerg Romeis (Reckenholz-Tänikon Research Station ART, Switzerland)

Domains of expertise of experts involved: genetics, molecular characterisation, genetic engineering, transgene expression, agronomy, ecology, plant-insect relations, effect on non-target species, impact on bio-diversity, nature conservation, biosafety research risk analysis

Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

Request 1. Side-by-side comparison with relevant Cry3Bb1 variants, including the MON88017 variant, in order to conclude on their equivalence in terms of biological activity would be helpful. Any available data complementing the data by Astwood et al. (2001b) and Duan et al. (2003) should be provided.

Though the technical dossier (page 25) claims that "*the Cry3Bb1 proteins in MON 863 and in MON88017 have been extensively characterized*", the applicant should carefully address the issue of the possible specificities of the MON88017 variant and better summarise all the available information.

Request 2. The tests provided on non-targets organisms (insects and soil organisms) and herbivores (putative targets) are overall well-conducted. However, the applicant needs to argue the results of some non-target studies in order to be able to take these studies up as confirmatory evidence that no risks are expected on non-target organisms from the exposure to Cry3Bb1.

General Comment:

We want to note that a rationale as to why the insect species are chosen for testing should be part of each application. This information should include (a) proof that the species tested are representative for a specific crop and (b) information on how these species come into contact with the Cry protein. This information is lacking in the current application.

Comments on non-target insect and soil organism studies:

* In Bryan *et al.* (2001) no clear arguments are given as to why a certain Cry3Bb1 test concentration was selected and how it relates to potential field exposure levels. This information should be provided.

* We have some reservations regarding the toxicity test done by Palmer & Krueger (1999b) to assess susceptibility of *Chrysoperla carnea* to Cry3Bb1. Although this study has provided for a toxic reference (using a "stomach" poison, potassium arsenate) which is used to show the effectiveness of the exposure method, we do not fully understand how the test was done and how exactly the insect was exposed. Uncertainties with respect to the outcome of the *C. carnea* test are related to:

- the way in which lepidopteran prey eggs were mixed with the toxic solutions
- the amount of solution used per weight or volume unit of prey eggs
- the mode of action of the compound used in the toxic reference
- the degree of absorption of the test compounds in the prey eggs
- the actual degree of exposure of the predator to the test compounds

* One weakness of the earthworm study *Eisenia fetidae* (Sindermann *et al*., 2002a) is the fact that bioactivity of the Cry3Bb1 protein could not be confirmed in soil samples after 14 days, probably due to degradation. Since the bioactivity was only tested on day 0 (start of the experiment) and day 14 (termination of the experiment) it is unclear for which period of time the earthworms were exposed to biologically active Cry3Bb1. In addition, it is difficult to figure out how the experiment was replicated.

* Sindermann *et al.* (2002b) base their calculation of the test concentration on pollen expression levels even though there is no published record available reporting that the test insect in this study, *Nasonia vitripennis*, consumes pollen. The applicant should clarify this.

* In the springtail bioassay (Teixera, 1999) the Cry protein was provided in form of MON88017 leaf power mixed with brewer's yeast. Stability and bioactivity of the test protein

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was confirmed for the study period. However, no proof is given that the test insects actually ingest the Bt maize powder when mixed with brewer's yeast.

Additional requests:

The applicant should address in its ERA if the cultivation of MON88017 might impact on non-target Chrysomelids (including threatened and endangered Chrysomelids where relevant) and other herbivores (putative targets) occurring in and around maize fields.

Notes:

* The hymenopteran parasitoid *Nasonia vitripennis* is not an ecologically relevant indicator species for maize.

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Request 3. Given that maize MON88017 confers combined protection against insect pests and the use of broad-spectrum herbicides, the applicant should assess the combined effects of the insecticide present, the associated herbicide treatments and agricultural practices on populations of non-target organisms (soil organisms, insects and species higher in the food chain, e.g. birds) and more broadly on biodiversity.

There is no discussion on the biodiversity within the crop and adjacent non-crop habitats likely to be affected by the GM crop and its cultivation (farming practices) in the short and long term. This should be included in the application.

Taking into account that the management and utilisation of a GM crop may vary from region to region and farm to farm, it may be difficult to predict the range of farming practices that will be deployed with the GM crop and their potential impacts. The risk assessment should take the unpredictability of farm management on biodiversity into account and relate this to monitoring.

More specifically we request the applicant to discuss if invertebrates/vertebrates might be adversely affected by shifts in host species (due to the presence of insecticide traits) and the potential reduction in host plants (due to weed management practices, including the use of herbicides) and relate these issues to monitoring, if considered relevant.

Request 4. We request the applicant to argue his conclusions on the following points:

* In the technical dossier p. 31:
The dossier states "*Expression of this Cry3Bb1 protein provides protection against certain coleopteran insect species pests, including members of the corn rootworm complex (Diabrotica spp.), which includes Western corn rootworm, Northern corn rootworm and Southern corn rootworm*". The applicant should provide a review and references of data on the impact of all Cry3Bb1-containing crops and biopesticides against all above-mentioned *Diabrotica* species. As this application is the first for the cultivation of Cry3Bb1-containing maize in the EU, the dossier could be more detailed on this issue. The data would also add certainty to the conclusions that the Cry3Bb1 protein specifically acts against Chrysomelids.

* In the technical dossier p. 65:
When comparing the expression of the Cry3Bb1 toxin in roots in US trials (table 11 to 13) *vs.* EU trials (table 5 to 7), significant differences are noticed (higher levels in US than in EU). At some stages, the ranges do not overlap. The applicant acknowledges that the expression levels from the EU data are generally lower than those of the US, but the conclusion (page 65 of the technical dossier) is that the observed levels are "*similar to the corresponding range of expression values obtained from historical data collected from other field trials where Cry3Bb1 and CP4 EPSPS protein expression levels in MON 88107 were assessed".* We are of the opinion that the only valid conclusions are those based on the data included in the dossier, hence "historical data from other field trials" should thus be either included or ignored. When clarifying this, the applicant should discuss the possible role of different ecological conditions, genotypic backgrounds, *etc.* in the observed discrepancies. The relevance of the expression level of Cry3Bb1 for its efficacy and for insect resistance management should also be discussed by the applicant.
In addition we want to note that in tables 5 to 7 showing expression data from US and tables 11 to 13 showing the corresponding data from EU, the growth stages are indicated by symbols "OS L/R/WP – 1, 2, 3 or 4", but their meaning is different from one location to the other (US *vs*. EU). For example, OSR-3 corresponds to growth stage V8 in table 7 (US), whilst the same OSR-3 corresponds to growth stage V10-V12 in table 13 (EU). The applicant is requested to avoid such misleading presentation of the data.

* In the technical dossier p. 92:
The conventional maize DKC3945 (Germany trials) and DKC5 143 (Spain trials), claimed to have genetic backgrounds similar to MON88017, are not shown in the breeding tree of MON 88017 (Figure 20 on page 92 of the technical dossier), hence their genetic relationships can not be understood. In Martin 2007 (CBI Appendix, page 13), MON 88017 is said to have DKC3945 and DKC5143 as genetic backgrounds, but no more information is given. The applicant should clarify this.

* In the technical dossier p. 139:
Potential structural similarities shared between the Cry3Bb1 protein and proteins in the TOXIN5 database (2001) were evaluated. Results demonstrated the lack of structurally relevant similarity between the Cry3Bb1 protein and any known toxic or pharmacologically

active proteins relevant for human and animal health. The applicant should demonstrate if this conclusion is still up-to-date by using the TOXIN6 database (2008).

p.o.

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

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Bioveiligheidsraad
Conseil de Biosécurité

24 March 2009

Secretariaat
Secrétariat

O./ref.: WIV-ISP/BAC/2009_912

Environmental Risk/Safety Assessment (ERA) of maize line MON88017 (EFSA-GMO-CZ-2008-54):

Further requests of the Belgian Biosafety Advisory Council for clarification on the ERA

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 26 September 2008

Coordinator: Dirk Reheul (UGent)
Experts: Patrick De Clercq (UGent), Adinda De Schrijver (SBB), Patrick du Jardin (FUSAGx), Jean-Luc Hofs (FUSAGx), Joerg Romeis (Reckenholz-Tänikon Research Station ART, Switzerland)
Domains of expertise of experts involved: genetics, molecular characterisation, genetic engineering, transgene expression, agronomy, ecology, plant-insect relations, effect on non-target species, impact on biodiversity, nature conservation, biosafety research and risk analysis
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

Request 1. Side-by-side comparison with relevant Cry3Bb1 variants, including the MON88017 variant, in order to conclude on their equivalence in terms of biological activity would be helpful. Any available data complementing the data by Astwood et al. (2001b) and Duan et al. (2003) should be provided.

We have no further requests.

Request 2. The tests provided on non-targets organisms (insects and soil organisms) and herbivores (putative targets) are overall well-conducted. However, the applicant needs to argue the results of some non-target studies in order to be able to take these studies up as confirmatory evidence that no risks are expected on non-target organisms from the exposure to Cry3Bb1.

Overall we're satisfied with the information provided by the applicant on the rational as to why non-target insect species are chosen for testing (p. 4-8 of PB/YD/shv (2008) 3520180) and information provided on the non-target studies (p.9-14). However, we do not fully agree with the reply on some specific non-target studies. We ask the applicant to take note of our comments. In addition, we ask the applicant to reconsider its assessment of impacts on non-target Chrysomelids.

General remark:

In several places, it has been mentioned that some non-target tests supersede others:

- The *Poecilus chalcites* test supersedes the *Nasonia vitripennis* test
- The *Orius insidiosus* test supersedes the *Chrysoperla carnea* test

We do not understand the rationale behind this. The respective species are not taxonomically related and have very different feeding habits.

Further, on p. 5 the applicant states that the *Coleomegilla maculata* test was developed to replace the *Hippodamia convergens* system, but on p. 9 the exact inverse is stated: the *H. convergens* system was developed to replace the *C. maculata* system. This is highly inconsistent.

p.10 toxicity test by Palmer & Kreuger

We're not convinced that the toxic reference proves that the *Chrysoperla carnea* larvae have ingested the Cry3Bb1 toxin from feeding on the treated lepidopteran eggs. A dose response may also be the consequence of contact exposure (i.e. contact with the pesticide residue on the eggs and contaminated surfaces). Despite the fact that potassium arsenate is called a "stomach poison" there are strong indications that the compound also has contact toxicity. Potassium arsenate has been used widely as a toxic compound in flypaper, to speed up killing of trapped flies.
We acknowledge that the peer-reviewed study by Li *et al*. (2008) adds further certainty to the negligible risk conclusion for this test species.

p. 11-12: Cry3Bb1 activity in the earthworm study

The explanation why there was no measurable toxin activity in soil samples after 2 weeks does not circumvent the fact that the earthworms were exposed to bioactive toxin for a very short period of time. We believe that the decline in toxin activity was almost exclusively due to protein degradation and not due to toxin adsorption to surface-active particles in soil. In the latter case the material should still cause a response in sensitive insect bioassays with larvae of the Colorado potato beetle. We agree that safety is added to the study by testing high toxin doses, but nevertheless exposure was likely to be very short in duration.

p. 12-13: *Nasonia vitripennis* testing

We would like to note that "… pollen-feeding seems to be rather uncommon among hymenopteran parasitoids" (Wäckers, 2005)[1] and that we believe that Rohrig *et al*. (2008) do not provide evidence for direct pollen feeding in this group.

p. 14-16: Risk for non-target Chrysomelidae

As a response to our question "*The applicant should address in its ERA if the cultivation of MON88017 might impact on non-target Chrysomelids (including threatened and endangered Chrysomelids where relevant) and other herbivores (putative targets) occurring in and around maize fields*", the applicant provided an assessment of potential effects on non-target Chrysomelids outside the maize fields.

From literature (Web of Science) and the EU Directive 92/43/EEC indeed no information can be retrieved on the presence of (endangered) Chrysomelids in the EU and more specifically in and around maize fields. However, the absence of this information does not necessarily mean that no (endangered) Chrysomelids occur in and around maize fields. Possibly, the absence of studies on the occurrence of Chrysomelids in and around maize fields may be the reason why no information can be retrieved (we were informed by IUCN that no Chrysomelids are present in the 2008 Red List as no assessments on Chrysomelids have been submitted yet). We do not ask the applicant to identify chrysomelid species in and around maize fields, but request the applicant to take the uncertainty on the presence of non-pest Chrysomelids in and around maize fields.

The applicant should be careful to refer to the Colorado potato beetle as the "most sensitive species". It is much more sensitive to Cry3Bb1 when compared to corn rootworms, however, few other chrysomelid species have been tested for their sensitivity.

The assessment provided is focussed on non-target Chrysomelids occurring outside the maize field and does not include non-target Chrysomelids potentially occurring in the maize field. As non-target and putative target Chrysomelids, e.g. cereal leaf beetle, may potentially come into contact with maize plant materials in the field, exposure in the field to maize plant materials should be considered in the risk analysis.

To conclude, we cannot support the reasoning that has been followed to assess the potential adverse effects on non-target Chrysomelids in and around maize fields.

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[1] Wäckers FL (2005) Suitability of (extra-)floral nectar, pollen, and honeydew as insect food sources. In: Plant-Provided Food for Carnivorous Insects (Wäckers FL, van Rijn PCJ, Bruin J, es.), Cambridge University Press, Cambridge, UK, pp. 17-74.

Request 3. Given that maize MON88017 confers combined protection against insect pests and the use of broad-spectrum herbicides, the applicant should assess the combined effects of the insecticide present, the associated herbicide treatments and agricultural practices on populations of non-target organisms (soil organisms, insects and species higher in the food chain, e.g. birds) and more broadly on biodiversity.

We're satisfied with the argumentation provided by the applicant that it is unlikely that the presence of the insecticide trait (Cry3Bb1) will cause any unacceptable shifts in the maize-arthropod food web and hence affect biodiversity adversely.

Concerning the indirect effects on biodiversity related to the application of the herbicide, we are of the opinion that in its assessment the applicant should focus on the potential changes in management practices of the GM maize that might differ from commonly used agricultural practices (as has been done in the technical dossier). In case of MON88017, focus should be put on the consequences of the herbicide use, namely the use of glyphosate 'in crop' (on maize) *versus* the use of glyphosate 'of crop' (between two maize crops), and not on agronomic practices that will remain applicable for maize cultivation in general (e.g. conservation tillage methods). For the latter it is expected that their indirect effects will be similar for GM and non-GM maize (as demonstrated e.g. by Schier, 2006 and Rodriguez *et al*., 2006).

Glyphosate is currently used in the EU as an 'of crop' herbicide to control weeds pre-emergence. Although we recognise that post-emergence weed control is a common agricultural practice in the EU, we note that HT maize offers new possibilities for post-emergence weed control in European cropping systems, as it allows non-selective herbicides as glyphosate to be used 'in crop'. We therefore do not agree entirely with the statement "*in crop use of glyphosate should not be considered as a novel agronomic or management technique*". The use of non-selective herbicides in crop could be considered as a novel practice, potentially causing changes in the agro-ecosystem.

Although we recognise that weed reduction, weed community shifts and consequent effects on biodiversity occur in any weed control system, an assessment of the potential indirect effects of the herbicide use should be included in the ERA, as indirect effects of the herbicide have not been addressed under Directive 91/414/EEC and are required to be addressed under Directive 2001/18/EC. We are happy to see that the applicant recognises that "*any weed control operation, mechanical or herbicidal (including glyphosate), can indirectly affect arthropod populations and species compositions in a cropped field by altering - as expected - the density and composition of vegetation*" and request the applicant to take this statement up in the ERA. Moreover, it should be discussed in the ERA if the use of glyphosate 'in crop' might result in any biological relevant adverse effects for maize agro-ecosystems. We are of the opinion that the use of glyphosate 'in crop' must not interfere with biological functions of non-target organisms (such as biological control and decomposition).

In conclusion, we ask the applicant to put the focus of the risk assessment on the indirect effects of the herbicide use on the biological functions of non-target organisms, as a change in glyphosate use ('in crop' *versus* 'of crop') may change and impact agro-ecosystems adversely. If considered relevant, this issue should be related to management measures and/or monitoring.

Request 4. We request the applicant to argue his conclusions on the following points:

We're satisfied with the information given under request 4 and have no further comments.

We want to note that the study on pollen morphology and viability (reference is made to this study on p. 88-89 of technical dossier) is lacking in AP54 and should be provided by the applicant.

p.o

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

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Secretariaat
Secrétariat

24 August 2009

O./ref.: WIV-ISP/15/BAC/2009_01190

Environmental Risk/Safety Assessment (ERA) of maize line MON88017 (EFSA-GMO-CZ-2008-54):

Requests of the Belgian Biosafety Advisory Council for clarification on the post-market monitoring

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 26 September 2008

Coordinator: Dirk Reheul (UGent)

Experts: Patrick De Clercq (UGent), Adinda De Schrijver (SBB), Patrick du Jardin (FUSAGx), Jean-Luc Hofs (FUSAGx), Joerg Romeis (Reckenholz-Tänikon Research Station ART, Switzerland)

Domains of expertise of experts involved: genetics, molecular characterisation, genetic engineering, transgene expression, agronomy, ecology, plant-insect relations, effect on non-target species, impact on biodiversity, nature conservation, biosafety research and risk analysis

Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

Request 1. As the expression of Cry3Bb1 is considered low-to-moderate, the applicant is requested to provide clearer argumentation to substantiate the appropriateness of the proposed refuge strategy.

The IRM plan proposed by the applicant (Appendix 1 of the Technical dossier), suggesting a 20% refuge for farmers growing more than 5 ha of MON88017 is based on the IRM plan for GM maize expressing Cry1Ab and Cry1F proteins targeting lepidopteran pests. The detailed reasoning behind the 5 ha threshold is explained in Appendix 2 of the IRM plan. In short, the reasoning is based on: (1) the high fragmentation of the European agricultural landscape, (2) the lack of economic feasibility for providing refugia on farms with less than 5 ha of Bt-maize, (3) the negligible risk of resistance development in Bt-maize areas smaller than 5 ha.

However, this paradigm has been developed within the "high dose/refuge" concept. Whereas Bt-maize events expressing Cry1Ab and Cry1F proteins can be considered “high dose”, those expressing Cry3Bb1 (including MON88017) are considered "low to moderate dose" (see Meihls *et al*. 2008[1], and references therein). As pointed out in Meihls *et al*. (2008), WCR may develop resistance to Cry3Bb1 expressed by transgenic maize under high selection pressure at a relatively fast rate. These findings emphasise the importance of maintaining adequate refuges to avoid development of resistance in WCR to Bt-maize expressing Cry3Bb1. Therefore, the IRM plan developed for high dose Bt-maize events may not be appropriate for MON88017 and the applicant should provide clearer argumentation to substantiate the appropriateness of the proposed 20% refuge with a 5 ha threshold for the MON88017 event expressing low to moderate doses of Cry3Bb1.

[1] Meihls *et al*., 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. PNAS 105: 19177-19182.

Request 2. The GS questionnaire can be improved by adjusting the possible replies to the questions on diseases, pests and weeds, and by clarifying what is meant with insects under 3.8.

The category of rating currently used in the GS plan for questions on diseases, pests and weeds is "low", "as usual" and "high". We wonder if this is the best option, as "as usual" is another category of rating than "low" and "high", and will often mean the same as "'low". We propose to use the following rating scale: "none or less (than usual)", "as usual" and "higher (than usual)" (cf. wording in 3.5).

Under 3.3 and 3.4 of the questionnaire, questions are asked on the occurrence of pest insects in the fields. Under 3.8 of the questionnaire information is asked on insects. Most likely with the term "insects" in 3.8, insects other than pests are considered, as pests have already been considered in 3.3 and 3.4. We ask the applicant to clarify this point.
In addition, given that the GM maize considered in the application is insect-resistant, it is advisable to let the farmer answer to the occurrence of mammals, birds and insects separately. In contrast to the current question format, this will allow determining if the farmer has, or has not, looked at insects (other than pests) in the field.

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

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Conseil de Biosécurité

16 December 2009

Secretariaat
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O./ref.: WIV-ISP/15/BAC/2009_01574

Environmental Risk/Safety Assessment (ERA) of maize line MON88017 (EFSA-GMO-CZ-2008-54):

Additional requests of the Belgian Biosafety Advisory Council for clarification on the post-market monitoring

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 26 September 2008

Coordinator: Dirk Reheul (UGent)

Experts: Patrick De Clercq (UGent), Adinda De Schrijver (SBB), Patrick du Jardin (FUSAGx), Jean-Luc Hofs (FUSAGx), Joerg Romeis (Reckenholz-Tänikon Research Station ART, Switzerland)

Domains of expertise of experts involved: genetics, molecular characterisation, genetic engineering, transgene expression, agronomy, ecology, plant-insect relations, effect on non-target species, impact on biodiversity, nature conservation, biosafety research and risk analysis

Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

Request 1. As the expression of Cry3Bb1 is considered low-to-moderate, the applicant is requested to provide clearer argumentation to substantiate the appropriateness of the proposed refuge strategy.

In response to the comment that the expression of Cry3Bb1 in MON88017 is low-to-moderate, the applicant argues that "*the doses are high enough to produce a 96-99% reduction in adult corn rootworm (CRW) emergence, which corresponds to >99% mortality of CRW larvae once the widely recognized impact of density-dependent mortality factors are taken into consideration*" and refers to Storer (2003) and Meihls *et al*. (2008) for substantiation.

A review of the relevant literature indicates that the density dependence of mortality in the larval stages of CRW following establishment of the first instar on the roots has as yet not been demonstrated experimentally. Dose calculation of the toxin of this event and related transgenic corn events with protection against CRW (Storer *et al*., 2006) should arguably be based on knowledge of the biology of the pest, more in particular on density-dependent and density-independent mortality factors during larval life. Density-dependent mortality in later larval stages (i.e. from the 2^{nd} instar on) was assumed by Onstad *et al*. (2001) and later this assumption was taken over in the modelling exercise by Storer (2003). To date, however, no field study has directly tested this assumption by determining adult emergence over a sufficiently wide range of initial densities of eggs and by comparing low and high egg densities in the same environment (unlike Onstad *et al*., 2006). Hence, there is no direct evidence to show that mortality between the time of establishment of the first instar and adult emergence is density-dependent, or rather density-independent. The outcome of such a study may have a critical impact on the correct calculation of the effective dose of a toxin against corn rootworms[1].

The study by Meihls *et al*. (2008) does not provide substantiation for the hypothesis of density dependence and is thus misquoted. Also, the figure of >99% actual survival on transgenic plants cited from this study is based on personal communication of "unpublished data" from other workers. Further, the applicant's criticism on the greenhouse study by Meihls *et al.* (2008) is not always justified. For instance, the line with continuous exposure to Bt corn was not increased on isoline corn "essentially after every generation": there were 6 generations of selection and the colony was increased on isoline corn only after generations 2, 4 and 5. The criticism that artificial selection as in Meihls *et al.* (2008) "is not the same as would be observed in the field" is evidently true, but the same can be said for the modelling work of Storer (2003) upon which the argumentation of the applicant is built. Bottom line is that Meihls *et al.* (2008) found that resistance quickly evolved without effective refuges.

In conclusion, no convincing evidence is given that the Cry3Bb1 dose in MON 88017 is high enough to lead to > 99% mortality of CRW larvae. Therefore, we retain our comment that the applicant has not sufficiently demonstrated that a 20% refuge for farmers growing more than 5 ha of Bt corn is appropriate for the non-high dose event MON 88017.

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[1] We want to draw the attention of the applicant to an upcoming publication: see http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=244076.

An IRM plan for MON 863 has been set up by the EPA in cooperation with Monsanto (EPA, 2007)[2]. We would like to receive feedback from the applicant if the information available (e.g. on dose, refuge size, refuge treatment and monitoring approaches) in this IRM plan has been considered when setting up an IRM plan for the EU. For example, the IRM plan of EPA states (page IID15) "*Soil applied insecticides to control CRW larvae are acceptable on refuge acres*." Whether such treatment is advised or not in the refuge implementation plan for EU is not mentioned in the application. In case this option would be left open based on the actual infestation level of the refuge zone, the actual practices should be recorded in the monitoring plan, more specifically in the farm questionnaire.

[2] EPA (Environmental Protection Agency, US), 2007. Bt Cry3Bb1 corn biopesticide registration action document. http://www.epa.gov/pesticides/biopesticides/ingredients/tech_docs/cry3bb1/2_d_c

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Request 2. The GS questionnaire can be improved by adjusting the possible replies to the questions on diseases, pests and weeds, and by clarifying what is meant with insects under 3.8.

We have no further comments and hope our comments on the GS plan will be useful in the process of harmonising the monitoring plans.

p.o.

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

Bioveiligheidsraad
Conseil de Biosécurité

19 August 2010

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2010_0798

Environmental Risk/Safety Assessment (ERA) of maize line MON88017 (EFSA-GMO-CZ-2008-54):

Final notes of the Belgian Biosafety Advisory Council for clarification on post-market monitoring

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 26 September 2008

Coordinator: Dirk Reheul (UGent)

Experts: Patrick De Clercq (UGent), Adinda De Schrijver (SBB), Patrick du Jardin (FUSAGx), Jean-Luc Hofs (FUSAGx), Joerg Romeis (Reckenholz-Tänikon Research Station ART, Switzerland)

Domains of expertise of experts involved: genetics, molecular characterisation, genetic engineering, transgene expression, agronomy, ecology, plant-insect relations, effect on non-target species, impact on biodiversity, nature conservation, biosafety research and risk analysis

Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

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Request 1. As the expression of Cry3Bb1 is considered low-to-moderate, the applicant is requested to provide clearer argumentation to substantiate the appropriateness of the proposed refuge strategy.

We have no further requests for information on the IRM plan. However, we ask the applicant to clarify the following:

In its reply to our request on the use of insecticides in the refuges, the applicant stated "*soil-applied insecticides for larval CRW control is not expected to adversely affect adult CRW emergence*". This sentence is confusing as insecticides are expected to adversely affect adult CRW emergence.

In addition, we are of the opinion that (1) clearer text should be provided in the IRM plan on the issue of insecticide use in the refuge and (2) information on insecticide treatments in the refuge should be taken up in the farmer questionnaire.

Dr. D. BREYER

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

Bioveiligheidsraad
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Déclaration minoritaire annexée au document BAC_2010_0928

Déclaration minoritaire de Damien Winandy relatif au rapport final du Conseil Consultatif de Biosécurité (ref. BAC_2010_0928) sur l'évaluation du risque environnemental du maïs MON88017 (Dossier EFSA/GMO/CZ/2008/54 introduit dans le cadre du Règlement (EC) 1829/2003)

The submitted ERA does not sufficiently take into account the potential consequences on environment of an increased utilization of glyphosate, including the development of weed resistance. Scientific data from region where glyphosate resistant GM crops are grown for many years could have been presented and analyzed.