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Testbiotech comment on Assessment of genetically modified maize MON 87411 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-124) of company Monsanto

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Introduction

Companies such as Monsanto & Bayer sell transgenic seeds to grow plants that continually produce insecticides throughout the growing season. Therefore, insects are permanently exposed to the insecticides and can adapt rapidly. This is especially the case with Bt toxins such as Cry3Bb1 produced in maize MON87411. In many maize growing regions of the US, the corn rootworm has already lost its susceptibility and developed resistance. In order to enhance insecticidal toxicity, Cry3Bb1 is now combined with dsRNA. Maize MON87411 also produces an insecticidal miRNA (DvSnf7 dsRNA). In addition, it has been made resistant to glyphosate; and was genetically engineered using *Agrobacterium tumefaciens*.

Regulation (EU) No 503/2013, which foresees 90-day animal feeding studies, an extended literature review, specific monitoring requirements and specific statistical analysis was applied in the risk assessment of maize MON87441.

1. Molecular characterisation

Maize MON87411 is the first genetically engineered plant assessed by EFSA that produces an insecticidal miRNA (double stranded RNA, dsRNA).

The dsRNA produced in the genetically engineered maize is meant to be taken up by so-called pest insects while feeding on the maize. In this case, the target insects are the larvae of the corn rootworm (*Diabrotica* spp.). In the larvae, the dsRNA is taken up from the intestinal gut into the cells of the insects where it interacts with gene regulation.

The dsRNA is meant to kill the larvae by down-regulating the Snf7 gene transcript via RNA interference (RNAi). The Snf7 gene is involved in essential biological processes: its function is part of the ESCRT (Endosomal Sorting Complex Required for Transport) pathway, which plays a crucial role in cellular housekeeping by internalization, transport, sorting and lysosomal degradation of transmembrane proteins. In effect, the functioning of the ESCRT complex is disturbed and the insect will die (Bolognesi et al., 2012; Ramaseshadri et al., 2013).

The dsRNA is produced in addition to the insecticidal Cry3Bb1, which has been used to combat corn rootworm for about 20 years. Due to permanent exposure, the larvae of the rootworm have developed resistance in major maize growing regions of the US. In order to enhance insecticidal toxicity in MON 87411, Cry3Bb1 was combined with the dsRNA. However, its efficacy has to be doubted (Khajuria et al., 2018).

Furthermore, the plants produce a CP4 EPSPS that makes them resistant to spraying with glyphosate.

dsRNA can have many functions and interact with gene regulation in many ways. In most cases, gene activity will be blocked or down regulated (silenced). It belongs to biologically active molecules known under the general term miRNAs, which have cross-kingdom activity. They are known to interact with gene regulation in microorganisms, insects, plants and mammals. Its specificity is dependent on several factors such as its stability, further splicing and regions within DNA where it can interact.

To assess potential off-target effects, the structure of the dsRNA can be compared with genomic regions in organisms that might come into contact with the molecules. Regulation (EU) No 503/2013 says that when silencing approaches with RNAi are used in genetically engineered plants, a bioinformatics analysis is required in order to identify potential 'off-target' genes. An important starting point is the collection of relevant data to make comparisons with the RNA networks of non-target organisms, including mammals and humans that are exposed to the plants via food and feed.

However, in the case of MON87411, the additional dsRNA produced in the plants was compared only with RNA as expressed in plants. EFSA concluded that there was similarity that would raise concerns. However, no comparison was made in regard to mammals and microorganisms.

This gap in risk assessment was also expressed in comments from the experts of Member States (EFSA, 2018b), such as the BVL (Germany):

> "*The applicant has not provided data on potential RNAi-targets of DvSnf7 dsRNA in non-target organisms, including humans. (…) Thus, additional data like bioinformatic evaluations should be considered. As demonstrated by a history of safe consumption of dsRNAs with high homology in conventional food and feed, the identification of sequence similarities between the dsRNA produced by MON 87411 and transcripts of exposed species would not directly indicate an increased risk of adverse effects. Nevertheless, a bioinformatic search for potential targets in transcripts of human and likely exposed non target species (farm animals) would back the weight of evidence approach if no matching targets where identified. The German Competent Authority therefore recommends a bioinformatic evaluation, comparable to study no.: RAR-2015-0373, to identify potential target genes in human and other relevant non target species. Additional information might be recommended according to the outcome of the bioinformatics evaluation.*"

However, no such data were requested by EFSA. Instead, EFSA seems to be of the opinion that such data would not allow reliable prediction of the potential effects of such molecules. The protocol of the EFSA panel meeting (EFSA, 2017) states:

> *"In plants a set of parameters allows for a reasonable prediction of RNAi off-target genes while in human and animals the extent of complementarity between the small RNA and the target is more limited and therefore these prediction tools do not allow for sufficiently reliable predictions (Pinzón et al., 2017). Therefore the GMO Panel considers that only the search for small RNA off-targets in the GM plant could have value for the risk assessment of GM plants."*

This is an interesting statement since it exposes some limitations in current knowledge. Pinzón et al. (2017) show that further research would be needed to make reliable predictions in regard to miRNA

effects in mammals. It can not be used as justification not to assess health risk in the case of MON87411. But EFSA neither tries to overcome these limitations of current knowledge, nor does it consider that risk assessment cannot be concluded without sufficient data and meaningful analysis.

Instead, EFSA (2018a) simply accepts these limitations by restricting its considerations and risk assessment to potential off-target effects in the plants, leaving aside effects in humans and livestock and their gut microbiomes that are exposed to the maize via the food and feed chain. This is akin to someone who has lost something in the dark and then only searches where street lamps shed light because that is where the light is available.

A similar approach was taken by EFSA in assessing the concentration of dsRNA and its downstream metabolic products in the plants. EFSA (2018a) states:

> *"The applicant provided a measure of the levels of DvSnf7 dsRNA in different tissues including grain and forage. However, the dsRNA is an intermediate molecule which is processed by dicer to siRNA molecules and the levels of dsRNA are not a good proxy for the levels of the active siRNAs in the plant (Paces et al., 2017). Therefore, the levels of the DvSnf7 dsRNA were not considered relevant for the risk assessment of maize MON 87411."*

As a result, the data on the concentration of the biologically active molecules in the plants were not assessed. However, such data are necessary to assess the risks for the food chain and the fate of these molecules in the environment (see below).

Instead of performing detailed risk assessment, EFSA, in contradiction to scientific publications (see below) simply assumes that:

> *"the amount of RNAs taken up and absorbed after oral ingestion is considered negligible in humans and animals (mammals, birds and fish)."*

EFSA´s risk assessment of the dsRNA expressed in the plants on a molecular level might be described as the perfect example of a 'don´t look – don´t find' strategy incompatible with existing regulation.

There are further gaps in risk assessment:

- EFSA did not assess additional unintended gene products, such as other unintended miRNA, that can emerge from the insertion of the transgenes.

- Further, no detailed consideration was undertaken regarding the extent to which the modification of the Bt protein Cry3Bb1 will change biological characteristics. In order to enable further independent risk assessment, the full DNA sequence inserted into the plants should be made available, including all open reading frames.

- EFSA also did not request any detailed analysis based on so-called -omics (transcriptomics, metabolomics, proteomics) to investigate changes in the overall metabolism in the plants. EFSA assumed that the data from phenotypic characteristics and compositional analysis would not indicate any need for further investigations. However, these data did show many significant changes (see below). In general, data on phenotypic characteristics and compositional analysis can be used as complementary data, but these are not as sensitive as -omics data and cannot replace them.

- Expression data were provided on the new intended proteins. It is known that the Bt content in the plants depends on environmental impact. For example, environmental stress can cause

unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). Therefore, the plants should have been subjected to a much broader range of defined environmental conditions and stressors in order to gather reliable data on gene expression and functional genetic stability. The same investigations should be performed in regard to dsRNA produced in the maize.

- Further, the method used to determine the amount of Bt toxins (ELISA) is known to be dependent on the specific protocols used. The data are not sufficiently reliable without further evaluation by independent labs. For example, Shu et al. (2018) highlight difficulties in measuring the correct concentration of Bt toxins produced by the genetically engineered plants (see also Székács et al., 2011). Without fully evaluated test methods to measure the expression and the concentration of the Bt toxins and the dsRNA (and its metabolites), risk assessment will suffer from substantial methodological gaps. Based on such poor and inconclusive data, the dietary exposure to Bt toxins within the food chain cannot be determined as required by Regulation (EU) No 503/2013. A similar problem emerges from the dsRNA produced in the plants.

Consequently, the risk assessment of molecular characteristics is not conclusive and is not sufficient to show food and feed safety.

2. Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)
Field trials were only performed in Argentina during one growing season (2011–2012). This is not acceptable, since maize produced for the EU is also grown in other regions such the US and Brazil.

Of the 9 constituents assessed in forage and the 53 constituents assessed in grain, around one third were significantly different from those of the comparator plants. If the plants were treated with glyphosate the number of significant differences was strongly increased in grain (from 16 to 28 endpoints). One of the constituents measured (palmitic acid) fell outside the range of comparable data. Despite the huge number of significant differences, EFSA (2018a) decided that no more investigations would be needed.

Taken as isolated data these differences might not directly raise safety concerns, nevertheless, the large overall number of effects should have led to further investigations. Therefore, EFSA should have requested further studies e.g.

- data from omics (proteomics, transcriptomics, metabolomics),
- data representing more extreme environmental conditions such as those caused by climate change,
- data representing more areas of commercial maize cultivation,
- more data on stress reactions under controlled conditions
- and impact of the dosage of the complementary herbicide sprayed onto the plants.

Instead, EFSA has relied solely on the newly introduced statistical method known as the “test of equivalence”. This method can be helpful to make some assumptions on the relevance of the significant findings. However, it cannot replace a detailed assessment of the high number of significant differences.

Based on the available data, no final conclusions can be drawn on the safety of the plants.

Toxicology
The company conducted a 90-day feeding trial with maize MON87411 in rats. In this feeding trial only one dosage of maize (33 %) was included as part of the diet, instead of several dosages as requested by existing guidance. Nevertheless, EFSA still accepted the data.

The stability of the test and control materials was not tested; therefore it remains unclear if the diet is comparable to diets fed under practical conditions if, for example, the maize is fed to animals closer to the date of harvest.

The most relevant finding was weight depression in the rats fed with the maize. As EFSA (2018a) summarises :

> *"Statistically significant lower mean feed consumption (as g/cage per day only) were observed in males fed test diet (~ 9% in study week intervals 5–6, 9–10, 10–11, 11–12). This was associated with a statistically significant decrease in mean body weights, compared to the concurrent control (~ 7% in weeks 11 and 12) and in mean cumulative body weight (~ 12% in study week intervals 0–10, 0–11 and 0–12). Moreover, statistically significant lower mean weekly body weight change was also observed in males (study week intervals 0–1, 3–4, and 6–7) and in females (study week interval 7–8) fed the test diet, compared to the concurrent controls."*

However, in the absence of test diet-related clinical signs and histopathological changes in the digestive tract, the GMO panel considered the changes to be non-adverse. Further, EFSA, without citing specific references, very generally questions whether the uptake of the dsRNA can be expected at all:

> *"Dietary ncRNAs [non coding RNAs] are generally rapidly denaturated, depurinated and degraded shortly after ingestion due to enzymes and conditions (e.g. pH) in the gastrointestinal tract lumen; in addition, the presence of barriers (e.g. mucus, cellular membranes) limits the cellular uptake of ncRNAs by gastrointestinal cells, and a rapid intracellular degradation of possible uptaken ncRNA occurs. Due to the above, the amount of RNAs taken up and absorbed after oral ingestion is considered negligible in humans and animals (mammals, birds and fish)."*

This assessment of toxicology has to be rejected for several reasons:

- In 2012, it was reported for the first time that miRNA produced by plants can enter the bloodstream of mammals (including humans) at the stage of consumption (Zhang et al, 2012). These findings were called into question by several experts (see, for example, US-EPA 2014; EFSA, 2014). However, looking at more recent publications, one has to assume that plant miRNA can indeed enter the bloodstream, organs, milk and urine of mammals after ingestion (Yang et al., 2015; Liang et al., 2015; Hirschi et al, 2015, Lukaski & Zielenkiewicz, 2014).
- There is evidence that small RNAs taken up from the intestine do indeed interfere with gene regulation in humans and animals. For example, it was found that miRNA transferred via milk shows biological activity (Baier et al., 2014). Small RNAs produced by plants are able to interfere with the immune system in humans and animals (Zhou et al., 2015; Cavalieri et al., 2015).
- It is also known from several studies that uptake of miRNA from the mammalian gut and its detection is dependent on specific factors. For example, Liang et al. (2015) describe mechanisms for uptake and measurement that need to be taken into account to successfully quantify the uptake, Yang et al. (2015) as well as Wang et al. (2012) show that the health

status of the recipient can be decisive; Baier et al. (2014) show that packaging in liposomes enhances uptake; Yang et al. (2015) show that dosage and also prolonged duration of exposure is important.

None of these issues were discussed or assessed by EFSA (2018a). Further, an external study commissioned by EFSA (Paces et al., 2017) overlooked several relevant studies. Moreover, in its conclusions it does not support the position of EFSA that uptake cannot generally be expected. Paces et al. (2017) summarise the discussion as follows:

> *"Thus, it is apparent that four years after the original report (Zhang et al., 2012(...)), the field remains split. The essential questions concerning the existence of the proposed mechanism emerged already in 2012. Further research is necessary to clarify the basis of the aforementioned contradictory observations."*

Paces et al. (2017) also mention that the findings (Zhang et al., 2012), which although disputed are not in contradiction to the general findings in this field:

> *"In 2012, the article by Zhang et al. proposed that miRNAs from ingested plants could traverse into the bloodstream and suppress genes in the liver (Zhang et al., 2012 (...)). The report sparked an ongoing debate because of potential implications these data could have. It should be pointed out that, while the article reported unexpected and surprising results, it was not breaking any conceptual dogma. The idea that information could be transmitted from food in a form of a large organic molecule that would traverse into the human organism has been an integral part of the prion hypothesis, which brought a concept of food-borne infectious particles made only of proteins (...). The prion hypothesis, for which Stanley Prusiner received a Nobel Prize in 1997, is nowadays a biology textbook knowledge. Furthermore, cross-kingdom regulation by small RNAs was discovered in RNA silencing field already in its early years – long dsRNA expressed in bacteria could induce repression of worm genes with complementary sequences when worms were fed with such bacteria (...). Furthermore, in 2012 it was already well known that feeding on a plant carrying an RNAi-inducing transgene can induce RNAi in nematodes, insects, or fungi (...). Thus, the article by Zhang et al. was not bringing any major shift in existing paradigms. The article essentially extended knowledge of RNA silencing spreading by reporting an example of a miRNA activity transferred from plants to mammals through feeding."*

There are at least two ways in which the additional dsRNA expressed in the plants can impact mammalian health:

(1) Uptake from the gut into the bloodstream in the same way as other plant miRNAs as described (see, for example, Yang et al., 2015; Liang et al., 2015; Hirschi et al, 2015; Beatty et al., 2014). If the bioactive molecules produced in the plants start to interfere with mammalian gene regulation, the effects might be drastic: in humans dysfunction of the ESCRT complex is associated with numerous pathologies, including cancer and various neurodegenerative diseases (Henne et al., 2012).

Based on current knowledge, this scenario cannot be excluded. This is especially true in the light of the specific circumstances described by Liang et al. (2014), Zhang et al. (2012) and Yang (2015) that are relevant for the uptake of miRNA from the gut. The need for further investigation is supported by the outcome of a FIFRA scientific panel workshop held in the US in 2014, maintaining that in particular the risks for immune-compromised individuals should be tested (USEPA 2014):

> *"The stability of dsRNA should be tested in individuals that manifest specific diseases (e.g.,*

Crohn's, colitis, irritable bowel syndrome, etc.), the immune compromised, elderly, as well as children. These individuals may have compromised digestion or increased sensitivity to dsRNA exposure."

(2) It is well known that miRNA plays a key role in gene regulation in the gut microbiome, as well as in the communication between the mammalian host and its gut microbiome (see, for example, Williams et al., 2017). It is plausible that the dsRNA produced in maize MON87411 can interact with the gut microbiome directly without direct uptake from the gut. At least for yeast, the essential role of the Snf7 as part of the ESCRT pathway is well described (see www.yeastgenome.org/locus/S000004015). Thus, there is a plausible hypothesis on how the additional dsRNA might affect the gut microbiome community.

Interaction with the microbiome also might explain the findings from animal feeding studies showing weight differences without pathological effects.

These aspects were mostly overlooked by EFSA (2018a) in its risk assessment even though a 2014 EFSA workshop (ESFA 2014) identified the following issues as relevant for risk assessment of health effects:

"Throughout the different discussion topics, the following issues were identified as knowledge gaps, where more research could be warranted:

- The RNAi and metabolic profiling in RNAi-based plants could be further explored and corroborated to support risk assessment. In this context, 'omics' techniques should be further investigated as supporting tools.
- The use of bioinformatics to predict potential off target effects in consumers should be further explored.
- Possible changes in microbiota, residing in human or animal guts, following consumption of food and feed products derived from RNAi-based plants could be a research topic."

As the BSE crisis showed, the risk of bioactive compounds being transmitted via the food and feed chain poses a high risk for farm animals and humans (see Paces et al., 2017). Therefore, uncertainties and knowledge gaps identified in the current risk assessment cannot be accepted.

In addition, the need for more detailed assessment is underlined by publications showing that the Bt toxins also raise further questions in regard to feed and food safety:

(1) There are several partially diverging theories about the exact mode of action of the Bt toxins at the molecular level (see Then, 2010; Hilbeck & Otto, 2015). Thus, it cannot be excluded a priori that the toxins are inert in regard to human and animal health as maintained under risk assessment for food and feed.

(2) There are further uncertainties regarding the specificity of Bt toxins (Venter and Bøhn, 2016). Changes in specificity may emerge from structural modifications performed to render higher efficacy. For example, the proteins are truncated to become activated (see Hilbeck and Schmidt, 2006).

(3) In addition, there are findings in mammalian species showing that Bt toxicity is a relevant topic for detailed health risk assessment: some Cry toxins are known to bind to epithelial cells in the intestines of mice (Vázquez-Padrón et al., 1999).

(4) As far as potential effects on health are concerned, several publications (Thomas and Ellar 1983; Shimada et al., 2003; Mesnage et al., 2013; Huffman et al., 2004; Bondzio et al., 2013) show that Cry proteins may indeed have an impact on the health of mammals. For example, de Souza Freire et al., (2014) confirm haematological toxicity of several Cry toxins. Some of these effects seem to occur where there are high concentrations and tend to become stronger over longer periods of time.

(5) Further, the toxicity of Bt toxins can be enhanced through interaction with other compounds, such as plant enzymes (Zhang et al., 2000, Zhu et al., 2007; Pardo-López et al., 2009); other Bt toxins (Sharma et al., 2004; Tabashnik et al., 2013; Bøhn et al. 2016, Bøhn 2018); gut bacteria (Broderick et al., 2009); residues from spraying with herbicides (Bøhn et al. 2016, Bøhn 2018) and other (Kramarz et al., 2007; Kramarz et al., 2009; Khalique and Ahmed, 2005; Singh et al., 2007; Zhu et al., 2005; Mason et al., 2011; Reardon et al., 2004).

In this context, it is relevant that Bt toxins can survive digestion to a much higher degree than has been assumed by EFSA. Chowdhury et al., (2003) and Walsh et al. (2011) showed that when pigs were fed with Bt maize, Cry1A proteins could frequently and successfully still be found in the colon of pigs at the end of the digestion process. This means that Bt toxins are not degraded quickly in the gut and can persist in larger amounts until digestion is completed; and that there is enough time for interaction between various food compounds.

Further, as far as the exposure of the food chain with Bt toxins is concerned, EFSA should have requested data on the overall combined exposure to Bt toxins resulting from the introduction of Bt plants in the EU. Currently, there are already 30 events that produce Bt toxins authorised for import. The accumulated exposure stemming from these imports should have been taken into account. For example a new study testing corn with a combination of Bt toxins (Cry1Ab and Cry34Ab1) indicates health impacts in rats (Zdziarski et al., 2018).

We conclude the need for more detailed investigation. Further, more detailed (e.g. using several dosages) and long-term feeding studies, taking into account the functioning of the microbiome, would be necessary to assess potential health impacts. These studies should include -omics data from animals, as well as detailed assessment of the impact of higher dosages of glyphosate sprayed on the plants (as can be expected under practical conditions).

In any case, the toxicological assessment carried out by EFSA (2018a) is not sufficient to show food and feed safety.

Allergenicity

Bt toxins are known to be immunogenic. They appear to act as allergens and adjuvant effects are likely to occur. In regard to immunogenicity (non-IgE-mediated immune adverse reactions), it is generally acknowledged that Bt toxins are immunogenic (Rubio-Infante & Moreno-Fierros, 2016; Adel-Patient et.al., 2011; Andreassen et.al., 2015a,b; Andreassen et.al., 2016; see also Then & Bauer-Panskus, 2017). Thus, there are some substantial reasons for concern that reactions to allergens can be enhanced. This is relevant since in food/feed the Bt toxins can be mixed with allergens from soybeans, amongst others. Mixing with soybeans can also substantially prolong the degradation of the Bt toxins in the gastric system (Pardo-López et al., 2009).

New findings (Santos-Vigil et al., 2018) now indicate the allergenic potential of Cry toxins after intra-gastric administration in a murine model. Thus, the EFSA assumption that a detailed assessment of the allergenic potential of Cry toxins is not necessary is simply wrong.

Consequently, the assessment on allergenicity cannot be regarded as conclusive.

Others

According to Regulation (EU) No 503/2013, the applicant has to ensure that post-market monitoring is developed to collect reliable information on the detection of indications of whether any (adverse) effects on health may be related to genetically modified food or feed consumption. Thus, the monitoring report should at least contain detailed information on

i) actual volumes of maize MON87411 imported into the EU,
ii) the ports and silos where shipments of maize MON87411 were unloaded,
iii) the processing plants where maize MON87411 was transferred to,
iv) the amount of maize MON87411 used on farms for feed, and
v) transport routes of maize MON87411.

The applicant is further requested to explain how the PMM of maize MON87411 in mixed GMO commodities imported, processed or used for food/feed is put into practice. Since traders may co-mingle maize MON87411 with other imported commercial GM maize that is processed or used for food/feed, the applicant is requested to explain how the monitoring will be designed to distinguish between potential adverse effects caused by MON87411 and those caused by other GM maize.

The monitoring should be run in regions where viable MON87411 is be transported, stored, packaged, processed or used for food/feed. In case of substantial losses and spread of MON87411, all receiving environments need to be monitored.

Environmental risk assessment

EFSA acknowledges that potential gene transfer between maize and weedy Zea species, such as teosintes and/or maize-teosinte hybrids, can occur (Trtikova et al., 2017).

Much more detailed investigation would be needed to assess the potential introgression of wild teosinte populations with gene constructs inserted in maize MON87411 and its effects on fitness of any progenies. For example, in the light of Fang et al (2018) it has to be assumed that the transgenic plants will render their offspring higher fitness compared to conventional plants. Therefore, EFSA (2018 a) is wrong in its statement saying:

> *"Even if cross-pollination would occur, the GMO Panel is of thE opinion that environmental effects as a consequence of the spread of genes from occasional feral Gm maize plants in Europe will not differ from that of conventional maize varieties."*

Further, as shown by Pascher (2016), EFSA also underestimates the risks posed by the occurrence of volunteers from maize plants.

In addition, the fate in the environment of the Bt proteins and the dsRNA molecules need much more attention. As experts from Member States (BfN, Germany) state:

> *"For Bt proteins an exposure route via manure from cattle fed with Bt maize has been demonstrated (Gruber et al. 2011; Gürtler et al. 2010). Paul et al. (2010) observed that 44% of the immunoreactive Cry1Ab from MON810 present in feed was transferred to the feces (Paul et al. 2010) while 34% of the Cry1Ab protein levels in feed could be detected in liquid manure (Gruber et al. 2011). As Gruber et al. (2011) demonstrated Cry1Ab is relatively stable in liquid manure (decrease of 49% in 24 weeks). The bioactivity of Cry proteins in wastewater or manure is unknown as no bioassays have been carried out so far.*

Based on the above finding it is likely that Cry3Bb1 protein event MON87411 will also be transferred from processing or feed directly or indirectly into the environment. Thus, the applicant should provide a detailed analysis on the fate of the Cry3Bb1 protein in the environment and subsequent exposure of non-target organisms."

"Analogue to the Bt protein the exposure of DvSnf7 should be analysed and data for the concentration of DvSnf7 in feed, wastewater, and feces including urine should be provided. A study performed by Monsanto (Dubelman et al. 2014) examined the fate of the DvSnf7 in soil. The results show a considerable variability for different soil types. The study used lyophilized, and presumable grinded, plant material and found a DT90 of <35 h under experimental conditions. The data show indeed that persistence of freely available Snf7 in soil is low. However, it is not possible to conclude on the environmental fate of DvSnf7 in the media described above. Plant material, feed-material or feces may retain dsRNA for long periods of time which is relevant for the environmental fate of the dsRNA (see also US-EPA 2014). It is also critical to understand the fate of waste material or wastewater containing DvSnf7 in aquatic environments. In addition no information is available on whether DvSnf7 is transferred within the food web."

Consequently, environmental risk assessment carried out by EFSA is not acceptable.

Conclusions and recommendations
The EFSA risk assessment has to be rejected.

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