TECHNICAL REPORT

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Clarifications on EFSA GMO Panel recommendations on the Insect Resistance Management plan for genetically modified maize MON 810

European Food Safety Authority

Abstract

Following a request of the European Commission, the European Food Safety Authority (EFSA) assessed the concerns raised by Monsanto on the previous EFSA GMO Panel's recommendations on its insect resistance management (IRM) plan for maize MON 810. EFSA specifically considered the feasibility of its recommendations for sampling target pests in order to optimise the sampling protocols. EFSA recommends the identification of large geographical areas (e.g. Ebro valley in Spain) where maize MON 810 adoption rate and target pest pressure are high. EFSA suggests focusing the collection of the target pest larvae in three 'sampling zones' instead of sampling extensively over entire 'geographical areas'. In response to Monsanto's concerns, EFSA also re-used the same resistance evolution model as before to assess whether the IRM approach of Monsanto enables the early detection of resistance so that alternative management measures can be adopted to prevent field resistance. Notwithstanding the difficulty to estimate the density-dependent (DD) mortality of target pests, EFSA considered a range of DD values in the new simulations performed with the same resistance evolution model as before. Based on the results of the new model simulations, EFSA concludes that the previous conclusions and recommendations of the EFSA GMO Panel remain valid. EFSA therefore advocates setting the detection limit for resistance allele frequency at 1 % or 3 % depending on the adoption rate of maize MON 810. Moreover, EFSA recommends to annually sample bi-/multi-voltine target pest populations in areas where maize MON 810 adoption rate is at least 60 % of the total cultivated maize.

Key words: annual report, cultivation, insect-resistance management, maize, MON 810, PMEM, sampling

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Summary

Following a request of the European Commission, the European Food Safety Authority (EFSA) discusses the concerns raised by Monsanto on EFSA GMO Panel's recommendations on its insect resistance management (IRM) plan for maize MON 810. EFSA considers the relevance and assesses the implications of these concerns on previous EFSA GMO Panel recommendations.

EFSA considers the feasibility of its recommendations for sampling target pests and acknowledges Monsanto's view to maintain the correct balance between the sampling efforts and the objective to detect resistance in target pests at an early stage.

To optimise the sampling protocol of target pests, EFSA recommends the identification of large geographical areas (e.g. Ebro valley in Spain) where maize MON 810 adoption rate and target pest pressure are high. EFSA also suggests focusing the collection of the target pest larvae in three 'sampling zones' instead of sampling extensively over entire 'geographical areas'.

In response to Monsanto's concerns, EFSA re-uses the same resistance evolution model as before to assess whether the IRM approach followed by Monsanto enables the early detection of resistance so that alternative management measures can be adopted to prevent field resistance. EFSA highlights the difficulty to set a unique value to estimate the density-dependent (DD) mortality of target pests for the model calculations, owing to the lack of information available on the occurrence and density of their natural enemies in maize agro-ecosystems and due to their high variability over time and space. Nevertheless, for the new model simulations, EFSA considers a range of DD mortality values. The newly obtained results presented here only slightly differ from those derived from the previous EFSA GMO Panel model simulations.

EFSA concludes that the previous conclusions and recommendations on Monsanto's IRM plan made by the EFSA GMO Panel remain valid. EFSA therefore advocates setting the detection limit for resistance allele frequency at 1 % for areas with 80 % maize MON 810 adoption rate or 3 % for areas with 60 % maize MON 810 adoption rate, instead of 5 % as proposed by Monsanto. In addition, EFSA recommends to annually sample bi-/multi-voltine target pest populations in areas where maize MON 810 adoption rate is at least 60 % of the total cultivated maize.

Finally, owing to limitations inherent to all models, EFSA recommends that new data are gathered from literature or monitoring to help fine-tuning the model predictions.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Since 1998, genetically modified (GM) maize MON 810 has been authorised[1] to be cultivated in the European Union (EU) subject to a management strategy to delay resistance evolution to the insecticidal Cry1Ab protein in the target pests (i.e. European corn borer (ECB; *Ostrinia nubilalis* Hübner) and Mediterranean corn borer (MCB; *Sesamia nonagrioides* Lefebvre)). Monsanto's insect resistance management (IRM) plan[2] relies on the 'high dose-refuge' strategy[3], and is composed of the following elements: (1) measuring the baseline susceptibility of target pests to the Cry1Ab protein and monitoring changes to that susceptibility over time ; (2) monitoring for potential development of resistance; (3) a communication and education programme aiding farmers to understand the importance of adhering to IRM requirements; and (4) a remedial action plan in the event of any confirmed evolution of pest resistance.

According to the European legislative framework on GM plants, Monsanto reports the results of its IRM plan to the European Commission and Member States on an annual basis.

Since 2010, the European Commission has asked the scientific panel on genetically modified organisms of the European Safety Authority (hereafter referred to as 'EFSA GMO Panel') to assess the annual post-market environmental monitoring (PMEM) reports submitted by Monsanto on the cultivation of maize MON 810. The EFSA GMO Panel adopted a scientific opinion on the 2009, 2010, 2011, 2012 and 2013 PMEM reports (EFSA GMO Panel, 2011b, 2012, 2013, 2014, 2015). On the basis of the data provided in these reports, the EFSA GMO Panel did not identify adverse effects on the environment, human and animal health due to maize MON 810 cultivation. However, the EFSA GMO Panel noted shortcomings in the methodology, in particular of the IRM plan, and hence made recommendations for improvement of the IRM plan for maize MON 810.

On 20 November 2014, Monsanto sent a letter to the European Commission expressing its concerns about the EFSA GMO Panel recommendations on the IRM strategy of maize MON 810. Specifically, Monsanto claimed that several of the EFSA GMO Panel recommendations are not in line with the current scientific knowledge, experience and practices, and that those recommendations are disproportionate to the level of identified risk.

In response to the mandate of the European Commission received on 24 March 2015, EFSA assesses here the claims brought up by Monsanto in its letter to the European Commission.

2. Data and Methodologies

2.1. Data

In delivering this report, EFSA took into account the claims expressed by Monsanto in the aforementioned letter[4].

2.2. Methodologies

EFSA took into account the appropriate principles described in its guidance document on the PMEM of GM plants (EFSA GMO Panel, 2011a) as well as its assessment of the methodology as reported in

[1] Commission Decision of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. line MON 810), pursuant to Council Directive 90/220/EEC (98/294/EC). OJ L131, 05.05.1998, p. 32–33

[2] See Appendix 1 of 2013 PMEM report published online: http://ec.europa.eu/food/plant/gmo/reports_studies/report_2013_mon_810_en.htm (following the pathway: post-authorisation/reports and studies)

[3] The high-dose/refuge strategy prescribes planting Bt-crops that produce a very high concentration of the Bt-toxin (25 times the amount needed to kill > 99% of susceptible individuals [LC_{99}]), so that nearly all target insect pests that are heterozygous for resistance do not survive on it. In addition, a nearby structured refuge of the non-Bt-crop is required where the target insect pest does not encounter the Bt-toxin. Under these conditions, most of the rare resistant individuals surviving on the Bt-crop will mate with abundant susceptible individuals emerging from nearby refuges to produce heterozygous offspring that are phenotypically susceptible. If inheritance of resistance is recessive, then the hybrid progeny from such matings will die on the Bt-crop

[4] Please consult the EFSA Register of Questions, here (Question number EFSA-Q-2015-00235)

previous scientific opinions on annual PMEM reports on maize MON 810 (EFSA GMO Panel, 2011b, 2012, 2013, 2014, 2015).

3. Assessment

The assessment below is structured into two parts. In Section 3.1, EFSA summarises the main concerns raised by Monsanto on the EFSA GMO Panel recommendations on the IRM of maize MON 810. In Section 3.2, EFSA considers the relevance and assesses the implications of these concerns on previous EFSA GMO Panel recommendations (EFSA GMO Panel, 2011b, 2012, 2013, 2014, 2015).

3.1. Summary of the concerns raised by Monsanto

Monsanto raised both generic and specific comments, listed below. Further details are available in Monsanto's letter[5].

3.1.1. Generic comments

The generic comments relate to the business interest and sustainability of a GM product on the market, the economic nature of the risk of resistance evolution of target pests, the requirements for resistance monitoring for GMOs being disproportionate to those for insecticides, the familiarity and experience with maize MON 810 as well as the success of the IRM plan for maize MON 810 in the EU so far.

3.1.2. Specific comments

Monsanto supports its assertion that the likelihood for resistance evolution in target pests in the EU is extremely low due to scarcity of the Cry1Ab resistance alleles, high mobility of target pests and good levels of farmer compliance with non-Bt-maize *refugia*. Monsanto therefore questions the need to revise its current IRM approach[6] and in particular:

- the sampling protocol of ECB and MCB for monitoring changes in baseline susceptibility of target pests to the Cry1Ab protein,
- the monitoring protocol designed for the early detection of resistance evolution.

Over several years, susceptibility of target pests to the Cry1Ab protein was assessed by Monsanto for laboratory colonies and for larvae collected in maize fields in e.g. Czech Republic, France, Germany, Hungary, Slovakia, Poland, Portugal, Romania, and Spain. Concerning the latter, Monsanto reports that ECB are sampled in three maize growing areas of the Iberian Peninsula alternating every two years (Northeast Iberia (i.e. Ebro Valley), and jointly Central Iberia and Southwest Iberia).

The standard operating procedures to sample ECB and MCB are described in Appendix 2 of EuropaBio's harmonised IRM plan for cultivation of Bt-maize (single insecticidal trait) in the EU[7]. According to the standard operating procedure specific to the sampling of ECB, larvae are collected from "*three different fields/collection sites within a geographic homogeneous area. To cover the genetic variability, it is considered to collect 300 larvae per field".* For MCB, a minimum of 400 larvae should be sampled from at least three different fields. For both target pests, the sampled fields should be at least 50 km apart, but not more than 250-300 km.

Monsanto points out that the sampling approach as recommended by the EFSA GMO Panel is disproportionate and cannot be put into practice. Monsanto also seeks clear and harmonised definitions of the terminology (e.g. hotspot, geographical area, zone, province/county) used by the EFSA GMO Panel in its recommendations for sampling locations.

In accordance with the harmonised IRM plan, the monitoring approach is designed to detect resistance when the frequency of the resistant allele reaches about 1-5 %. In its letter, Monsanto emphasizes that decreasing the allele frequency detection limit from 5 % to 1 % - as recommended

[5] Please consult the EFSA Register of Questions, here (Question number EFSA-Q-2015-00235)

[6] See Appendix 1 of 2013 PMEM report published online: http://ec.europa.eu/food/plant/gmo/reports_studies/report_2013_mon_810_en.htm (following the pathway: post-authorisation/reports and studies)

[7] See Appendix 1 of 2013 PMEM report published online: http://ec.europa.eu/food/plant/gmo/reports_studies/report_2013_mon_810_en.htm (following the pathway: post-authorisation/reports and studies)

by the EFSA GMO Panel - implies additional sampling efforts without the guarantee of early detection of resistance evolution. In the unlikely event that a 5 % level of resistance allele frequency is detected, Monsanto, in contrast to the EFSA GMO Panel, considers that there is sufficient time to adapt the IRM strategy.

3.2. Evaluation of the implications of Monsanto's concerns on previous EFSA GMO Panel conclusions and recommendations

The aforementioned generic comments (see Section 3.1.1) are more of normative nature and therefore are not addressed by EFSA in the present report as falling outside its remit.

In this report, EFSA focuses its assessment on the following main and recurrent concerns:

(1) the sampling frequency of target pests;

(2) the sampling sites of target pests.

Overall, EFSA considers here to what extent the IRM protocol of maize MON 810 as followed by Monsanto is designed for the early detection of resistance in target pests.

3.2.1. Sampling frequency of target pests

Previous discussions and conclusions

Monsanto adheres to the following sampling approach:

- ECB and MCB are not collected in areas where Bt-maize[8] occupies less than 20 % of the total maize acreage within these areas (see also Section 3.2.2),
- Univoltine[9] and multivoltine[10] target pest populations are sampled every two years in areas where Bt-maize adoption rate varies between 20 and 80 % of the total maize cultivated area,
- Annual sampling of multivoltine target pest populations is foreseen only in exceptional circumstances in areas of high adoption rate (i.e. > 80 % of the total maize cultivated area and therefore where non-Bt-maize *refugia* have not been fully implemented).

For further details, please consult Table 4 of EuropaBio's harmonised IRM plan[11] for cultivation of Bt-maize (single insecticidal trait) in the EU.

The harmonised IRM plan then states that the monitoring approach is designed "*to detect resistance when the frequency of the resistant allele reaches about 1-5 %".*

The EFSA GMO Panel previously assessed whether the IRM approach followed by Monsanto enables the early detection of resistance evolution, in order to undertake management measures that would prevent field resistance (EFSA GMO Panel, 2011b, 2013). For this assessment, the EFSA GMO Panel used the model Populus[12] of Alstad and Andow (1995) which, starting from a given resistance allele frequency estimates the number of generations required for the resistance allele frequency of 50 % to be reached[13]. The Alstad and Andow (1995) model constitutes a well-established tool to predict resistance allele frequency based on well recognised concepts of population dynamics, and hence the onset of resistance in target pests. The EFSA GMO Panel ran simulations for maize MON 810 adoption rates ranging from 20 to 90 % and considering various parameters with fixed values such as the initial

[8] Maize MON 810 is the only Cry1-expressing maize cultivated in the EU so far. However, the EFSA GMO Panel already recommended that in future the applicant takes into consideration the overall adoption rate of Cry1-expressing maize when fine tuning its protocol for sampling target pests.

[9] Uni-/mono-voltine population is a population producing one brood/generation in a season.

[10] Bi-/multi-voltine population is a population having several broods/generations in a season.

[11] See Appendix 1 of 2013 PMEM report published online: http://ec.europa.eu/food/plant/gmo/reports_studies/report_2013_mon_810_en.htm (following the pathway: post-authorisation/reports and studies)

[12] Using the shareware software Populus, Version 5.4. Copyright © 2007 DN Alstad, University of Minnesota, available at http://wwumnw.cbs.edu/populus

[13] A target pest population is considered resistant when the resistance allele frequency has reached 50 %.

resistance allele frequency estimated at 0.006 for ECB, and a density-dependent (DD) mortality parameter[14] estimated at 0.7.

The EFSA GMO Panel concluded that, in areas of high maize MON 810 adoption rate (e.g. 60-80 %), the detection level of 5 % resistance allele frequency, as suggested by the applicant, is not appropriate as it would not leave enough time to undertake management measures sufficient to prevent field resistance. According to previous EFSA GMO Panel outputs (EFSA GMO Panel, 2011b, 2013), an efficient monitoring programme should be aimed at detecting an allele frequency below 3 % for maize MON 810 at adoption rates lower than 50 %. In the case of higher adoption rates, a detection limit of 1 % was deemed necessary. For further details, please consult Appendix 2 of EFSA GMO Panel (2011b) and Appendix A of EFSA GMO Panel (2013). Moreover, based on the previous EFSA GMO Panel simulations with the Alstad and Andow (1995) model, annual sampling of target pests was also recommended in areas where maize MON 810 adoption rate is high and bi-/multi-voltine target pests occur (see Table 1 in EFSA GMO Panel, 2013).

In response to the arguments provided by Monsanto, the assumptions made by the EFSA GMO Panel during its previous model simulations are discussed here. Results of new model simulations are also discussed.

Preliminary discussion on the choice of the model

In its letter, Monsanto claims that "*models such as the Populus model that keep track of population size typically assume that the 'natural' mortality rate increases as population size increases,* i.e. *that there is density-dependent mortality".*

In order to assess whether different hypotheses on DD mortality would affect its previous recommendations, EFSA uses the Populus model[15] to run additional simulations to estimate the number of generations required to reach a resistance allele frequency of 50 % from the detection of a 1, 3 or 5 % frequency under different maize MON 810 adoption rates. For these new calculations, EFSA estimates different DD mortality with a range of '*b*' values from 0.1 (strong inversely DD mortality) to 0.9 (strong DD mortality), while in previous simulations a fixed value of 0.7 was adopted (EFSA GMO Panel, 2011b, 2013). Results obtained for the extreme *b* values (i.e. 0.1 and 0.9) are not reported here since they are considered quite unlikely. Results obtained for DD mortality of 0.3, 0.5 and 0.7 are given below in Tables 1 and 2.

In addition, EFSA recognises the difficulty to consider this specific parameter in the simulation exercise since data on possible uniform effects of natural enemies on target pest populations across all EU receiving environments are scarce. In practice, it is difficult to predict such effects as the occurrence and density of natural enemies vary over space and time. Finally, the impact of natural enemies on target pest mortality rate is generally considered to be too low to have relevance in maize agro-ecosystems (Orr and Landis, 1997; Naranjo and Ellsworth, 2002).

The new values to feed the model are not in accordance with those provided by Monsanto, and EFSA points out some ambiguities in the choice of model parameters by Monsanto. For instance, in the context of DD mortality the fecundity parameter, '*F*', was recalibrated without supporting explanations, whereas the '*b*' value remained unchanged (see Monsanto's letter for further details).

Finally, EFSA recognises that the model assumptions might be too conservative since resistance to Cry1Ab protein in target pests has not yet been observed in the field. For its model simulations (EFSA GMO Panel, 2011b, 2013), the EFSA GMO Panel used an initial resistance allele frequency set at the conservative value of 0.006. However, the actual value of the initial resistance allele frequency might be lower in those regions where maize MON810 has been cultivated and this could explain why resistance has not yet been detected in the field (Engels et al., 2010).

[14] The density-dependent mortality is here defined by the impact of predation on population dynamics (e.g. mortality rate caused by natural enemies under different pest densities) of target pests. The density-dependent mortality value of 0.7 was previously adopted by the EFSA GMO Panel and considered conservative in absence of specific literature data.

[15] Populus, Version 5.5. https://www.cbs.umn.edu/research/resources/populus

New simulations with resistance evolution model

Results newly obtained for DD mortality of 0.3, 0.5 and 0.7 are given below in Tables 1 and 2. Results are provided for maize MON 810 adoption rates of 60 % (i.e. adoption rate in some Spanish provinces such as Lleida) and 80 % of the total maize cultivated area.

Both Tables 1 and 2 show that DD mortality assumptions affect the model's predictions in terms of resistance evolution. However, they hardly affect the number of generations required to reach a resistance allele frequency of 50 % from the detection of a 1, 3 or 5 % initial frequency.

Table 1: Number of generations of European corn borer (ECB) populations required, from the detection of a resistance allele frequency of 1 % (or **0.01** in bold, below), 3 % (or ***0.03*** in bold italic, below) and 5 % (or <u>0.05</u> underlined, below), to reach the in-field resistance of the target pest populations corresponding to a resistance allele frequency of 50 % (0.50 highlighted in grey, below). Calculations are reported for three density-dependent (DD) mortality estimates (i.e. *b* = 0.3, 0.5 and 0.7) in areas where maize MON 810 adoption rate is at least of 80 %.

Number of ECB generations	**DD mortality estimates for ECB (value '*b*' hereafter)**		
	***b* =0.3**	***b* =0.5**	***b* =0.7**
0	0.0060	0.0060	0.0060
1	0.0065	0.0067	0.0071
2	0.0076	0.0091	**0.0115**
3	0.0082	0.0099	0.0125
4	0.0098	**0.0127**	0.0170
5	**0.0106**	0.0138	0.0187
6	0.0127	0.0179	0.0257
7	0.0139	0.0197	0.0289
8	0.0172	0.0265	***0.0428***
9	0.0191	***0.0301***	<u>0.0506</u>
10	0.0244	0.0437	0.0873
11	0.0280	<u>0.0526</u>	0.1165
12	***0.0381***	0.0884	0.2624
13	0.0459	0.1206	0.4332
14	0.0696	0.2595	0.7840
15	0.0932	0.4369	0.9333
16	0.1702	0.7636	0.9837
17	0.2798	0.9248	0.9958
18	0.5429	0.9816	0.9990
19	0.7957	0.9953	0.9998
20	0.9424	0.9989	0.9999
21	0.9846	0.9997	1.0000
22	0.9965	0.9999	1.0000
23	0.9991	1.0000	1.0000
24	0.9998	1.0000	1.0000

Table 1 shows that, regardless of the DD mortality value, ECB populations with a detected resistance allele frequency of 5 % would be considered in-field resistant (i.e. reaching the 50 % frequency) in approximately 4-5 generations. In case of bi-/multi-voltine ECB populations, the approximately 4-5 generations could be achieved in two years. EFSA concludes that this does not allow sufficient time for farmers to respond (e.g. by increase planting of non-Bt-maize *refugia*, implement alternative IRM measures).

In case of a biennial sampling programme[16] as proposed by Monsanto, the effectiveness of the monitoring plan in preventing the onset of resistance is even more affected by the fact that information on allele frequency may be available at the earliest the year after the limit has been reached.

Moreover, note that when allele frequency is above 10 %, resistance will be exhibited in a number of individuals and some damage is expected in field crops (Roush and Miller, 1986; Roush and Osmond, 1997).

Table 1 also shows that, in areas where maize MON 810 adoption rate is at least 80 %, even a detection limit of the resistance allele in ECB populations set at 3 % might not provide sufficient time (i.e. a minimum of five to six generations in the case of a bi-/multi-voltine ECB populations) for farmers to put in place mitigation measures in order to prevent field damages and delay the onset of resistance in bi-/multi-voltine target pest populations. Therefore, in areas of high to very high maize MON 810 adoption rates, a detection threshold of resistance allele frequency should preferably be set at 1 %.

Table 2: Number of generations of European corn borer (ECB) populations required, from the detection of a resistance allele frequency of 1 % (or 0.01 in bold, below), 3 % (or 0.03 in bold italic, below) and 5 % (or 0.05 underlined, below), to reach the in-field resistance of the target pest populations corresponding to a resistance allele frequency of 50 % (0.50 highlighted in grey, below). Calculations are reported for three density-dependent (DD) mortality estimates (i.e. *b* = 0.3, 0.5 and 0.7) in areas where the maize MON 810 adoption rate is 60 %.

Number of ECB generations	DD mortality estimates for ECB (value '*b*' hereafter)		
	***b*=0.3**	***b*=0.5**	***b*=0.7**
0	0.0060	0.0060	0.0060
1	0.0062	0.0063	0.0064
2	0.0068	0.0075	0.0087
3	0.0070	0.0078	0.0091
4	0.0078	0.0094	**0.0117**
5	0.0082	0.0098	0.0122
6	0.0093	**0.0119**	0.0159
7	0.0098	0.0126	0.0167
8	**0.0112**	0.0156	0.0227
9	0.0119	0.0166	0.0242
10	0.0138	0.0212	***0.0349***
11	0.0149	0.0229	0.0383
12	0.0175	***0.0307***	<u>0.0613</u>
13	0.0191	0.0339	0.0705

[16] See Table 4 of the EuropaBio's harmonised IRM plan for cultivation of Bt-maize (single insecticidal trait) in the EU, September 2012

Number of ECB generations	DD mortality estimates for ECB (value '*b*' hereafter)		
	***b* =0.3**	***b* =0.5**	***b* =0.7**
14	0.0230	0.0486	0.1330
15	0.0254	0.0560	0.1707
16	***0.0316***	0.0895	0.3586
17	0.0359	0.1117	0.5097
18	0.0465	0.2015	0.7401
19	0.0550	0.2867	0.8670
20	0.0755	0.4864	0.9413
21	0.0951	0.6656	0.9736
22	0.1399	0.8292	0.9888
23	0.1933	0.9172	0.9951
24	0.2976	0.9642	0.9979
25	0.4287	1.0000	1.0000
26	0.6090	1.0000	1.0000
27	0.7638	1.0000	1.0000
28	0.8834	1.0000	1.0000
29	0.9443	1.0000	1.0000
30	0.9765	1.0000	1.0000
31	0.9896	1.0000	1.0000
32	0.9957	1.0000	1.0000
33	0.9981	1.0000	1.0000
34	0.9992	1.0000	1.0000

Table 2 shows that, regardless of the DD mortality value, ECB populations with a detected resistance allele frequency of 5 % would be considered in-field resistant (i.e. reaching the frequency of 50 %) in approximately 5-7 generations. A successful contingency plan can therefore be efficiently applied only for annual samplings in areas of adoption rate of 60 %. A detection limit of the resistance allele in ECB populations set at 3 % would provide more time for farmers to put in place mitigation measures in order to delay the onset of resistance, even in bi-/multi-voltine target pest populations.

Overall, EFSA recommends:

- to set the detection limit for resistance allele frequency at 1 % or 3 % (rather than 5 %) depending on the adoption rate of maize MON 810 (i.e. 80 % or 60 % maize MON 810 adoption rate, respectively);
- to annually sample bi-/multi-voltine target pest populations in areas where maize MON 810 adoption rate is at least 60 % of the total cultivated maize (see Table 3, below).

Table 3: Recommended sampling frequency of target pests

Maize MON 810 adoption rate [17] (% maize MON 810 on total maize acreage in a geographical area)	Sampling frequency	
	Uni-/Mono-voltine target pest population	Bi-/multi-voltine target pest population
< 20 %	None	None
20 % - ≤ 60 % (R allele frequency of 3 %)	Biennial	Biennial
›60-80 % (R allele frequency of 1 %)	Biennial	Annual
> 80 %[18]	Annual	

3.2.2. Sampling sites of target pests

EFSA shares Monsanto's view that a good trade-off exists between the sampling efforts deployed and the targeted objective of the early detection of resistance evolution in target pests.

Nevertheless, EFSA also emphasizes that the IRM plan should be designed to prevent field resistance to evolve. The new model simulations reported in Section 3.2.1 confirm that resistance evolution is slow at the beginning and may remain undetected for a long period of time. However they also illustrate that, once a certain resistance allele frequency has been reached, resistance evolution speeds up drastically. This is why one should aim at maintaining resistance allele frequency at very low levels. For that purpose, a strict compliance with the *refugia* implementation is crucial. In addition, the IRM plan should aim at detecting increased levels of resistance wherever and whenever they occur. EFSA recognises that this is a challenge as resistance may occur randomly and it is impossible to monitor every maize field. Nevertheless, EFSA considers that optimal sampling strategies can be put in place without additional effort. The rationale of the EFSA recommendations is that, rather than monitoring the average resistance level of target pest population in a large geographical area, an optimal sampling scheme should focus on those zones where resistance, should it occur, is likely to evolve more quickly, i.e. those zones with the highest selection pressure.

EFSA clarifies here the previously recommended sampling approach and provides the requested definitions of the terminology used by its GMO Panel (EFSA GMO Panel, 2011b, 2012, 2013, 2014, 2015). For the sake of harmonisation across all EU Member States, EFSA no longer refers to 'politically defined' areas such as province/county/lander owing to the variability in scale across Member States.

For the selection of appropriate sampling sites, EFSA therefore suggests applicants to follow a two-step approach, narrowing down the geographical scale:

(1) Identification of geographical areas

EFSA agrees with Monsanto's definition of '*geographical area*'; i.e. a "*geographical zone where maize is typically grown following similar agronomic practices isolated from other maize areas by barriers that might impair an easy exchange of target pests between those areas*".

As in the harmonised IRM plan, EFSA supports the procedure suggested by EuropaBio that "*the sampling will be intensified in areas where high levels of Bt-maize adoption occur and where target pest pressure is higher*". The Bt-maize adoption rate is expressed as a fraction of total maize cultivation in the same geographical area.

For Spain, given the past and current maize MON 810 adoption rates, EFSA therefore recommends to focus the sampling effort in North-East Iberia (i.e. Ebro valley), where maize MON 810 adoption rates and target pest pressure are high.

[17] At the time of adoption of this opinion, maize MON 810 is the sole Cry1-expressing maize cultivated in the EU. However, the EFSA GMO Panel recommends that in future the applicant takes into consideration the overall uptake of Cry1-expressing maize when identifying zones of high adoption for sampling target pests.

[18] In some regions where farmers do not comply with non-Bt-maize *refugia* implementation

(2) Selection of sampling zones within a geographical area

In the selected geographical area (i.e. Ebro valley), EFSA suggests not to sample extensively over the entire 'geographical area' but to collect larvae from three 'sampling zones' of smaller scale within that given geographical area. A 'sampling zone' is defined by an area of approximately 10 km x 10 km within a geographical area where the Bt-maize adoption rate and the target pest pressure are high to very high. A 'sampling zone' of high Bt-maize adoption rate might be described as a zone where Bt-maize MON 810 occupies more than 50 % of the total maize cultivation for at least three consecutive years. The three 'sampling zones' should be separated from each other by a minimum of 50 km.

For each individual sampling zone, the objective is to collect the targeted[19] number of larvae (i.e. 300 ECB larvae) regardless of the number of Bt-maize fields sampled within that given sampling zone.

In order to detect potential inter-population variation in the susceptibility of target pest populations (EFSA GMO Panel, 2012), EFSA recommends to separately analyse the data from samples taken in different sampling zones.

In the view of EFSA, Monsanto's opinion that the previous recommendations of the EFSA GMO Panel imply an extra burden in terms of sampling target pests and cannot be implemented (EFSA GMO Panel, 2011b, 2012, 2013, 2014, 2015) is based on a misinterpretation of EFSA's recommendations. In this respect, EFSA confirms that sampling is not required for a total of 24 'zones' (i.e. three samples for each of the eight provinces/zones of the Ebro valley) as referred to by Monsanto in its letter.

Moreover, in situations where the Bt-maize adoption rates and/or the target pest pressure are very high or increasing over time, or where farmers have indications of possible resistance evolution, EFSA is of the opinion that repeated sampling of the same sampling zones over time would be more suitable for resistance monitoring.

4. Conclusions

In the present report, EFSA considers the feasibility of its recommendations for sampling target pests and acknowledges Monsanto's view to maintain the correct balance between the sampling efforts and the objective to detect resistance in target pests at an early stage.

To optimise the sampling protocol of target pests, EFSA recommends the identification of large geographical areas (e.g. Ebro valley in Spain) where maize MON 810 adoption rates and target pest pressure are high. EFSA also suggests focusing the collection of target pest larvae in three 'sampling zones' instead of sampling extensively over the entire 'geographical areas'.

In response to Monsanto's concerns, EFSA re-uses the same resistance evolution model as before to assess whether the IRM approach followed by Monsanto enables the early detection of resistance so that alternative management measures can be adopted to prevent field resistance. EFSA highlights the difficulty to set a unique value to estimate the DD mortality of target pests for the model calculations owing to the lack of information available on the occurrence and density of their natural enemies in maize agro-ecosystems and due to their high variability over time and space. Nevertheless, for the new model simulations, EFSA considers a range of DD mortality values. The newly obtained results presented here only slightly differ from those derived from the previous EFSA GMO Panel model simulations (EFSA GMO Panel, 2011b, 2013).

EFSA concludes that the previous conclusions and recommendations on Monsanto's IRM plan made by the EFSA GMO Panel remain valid. EFSA therefore advocates setting the detection limit for resistance allele frequency at 1 % for areas with 80 % maize MON 810 adoption rate or 3 % for areas with 60 % maize MON 810 adoption rate, instead of 5 % as proposed by Monsanto. In addition, EFSA recommends to annually sample bi-/multi-voltine target pest populations in areas where maize MON 810 adoption rate is at least 60 % of the total cultivated maize.

Finally, owing to limitations inherent to all models, EFSA recommends that new data (e.g. on initial resistance allele frequency, DD mortality estimates) are gathered from literature or monitoring to help fine-tuning the model predictions.

[19] See Appendix 2 of EuropaBio's harmonised IRM plan for cultivation of Bt-maize (single insecticidal trait) in the EU

Documentation provided to EFSA

1. Letter, dated 24 March 2015, from the European Commission to the EFSA Executive Director requesting scientific assistance on the arguments provided by Monsanto with regard to EFSA's opinion on Monsanto's Insect Resistance Management Strategy (see Monsanto letter, dated 20 November 2014, to the European Commission).

2. Acknowledgement letter, dated 22 April 2015, from the EFSA Executive Director to the European Commission.

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