A point by point analysis of the arguments put forward by the French government on the 20th February 2012, for a case of emergency concerning MON810 maize

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This analysis refers to the *Note of the French Authorities (here called NAF, in italics and underlined in the following text)* to the European Commission DG-Sanco concerning the planting of genetically modified seeds of maize MON810 [1].

Advice of EFSA on Bt11 (pages 1-3 of NAF)

NAF: « *In contrast to their previous conclusions on Bt11 or on MON810, EFSA underlines, in their new advice based upon the new Directives for evaluation published in 2010, the existence of environmental risks linked to the culture of these GMOs* ».

In fact EFSA in 2011 [4] says:

« *The EFSA GMO Panel concludes that, subject to appropriate risk management measures, maize Bt11 cultivation is unlikely to raise additional safety concerns for the environment compared to conventional maize* ».

NAF: « *The appearance of resistance to CryAb toxin in exposed target populations of Lepidoptera may require the adoption of techniques in the fight against these pests (for example insecticides) that have a greater environmental impact* ».

It should be noted here that this is an agricultural problem (not environmental) since the techniques, which would be used if genetically engineered (GE) plants producing insecticidal

Bt toxin were no longer efficient, would be the same as those used today by farmers using non-GE crops.

In fact EFSA in 2011 [4] does not recommend any ban but rather formulates classical recommendations to control this risk:

« *The EFSA GMO Panel reiterates its earlier recommendation that appropriate insect resistance management (IRM) strategies relying on the 'high dose/refuge' strategy should be employed, in order to delay the potential evolution of resistance to the Cry1Ab protein in lepidopteran target pests* ».

NAF: « *reductions of populations of certain species of sensitive non-target Lepidoptera* ».

In fact EFSA in 2011 [4] says that only 1% of butterfly species are concerned in a hypothetical manner:

« The amounts of maize Bt11 pollen grains found in and around maize fields are unlikely to adversely affect a significant proportion of non-target lepidopteran larvae, except for local populations of lepidopteran species with such hypothetical high sensitivities to the Cry1Ab protein that they comprise just 1% of the total species at risk ».

Here again EFSA in 2011 [4] does not propose any ban but formulates recommendations to control this risk:

« *The EFSA GMO Panel considers that the risks identified during the ERA require management and recommends that appropriate risk mitigation measures be adopted, wherever it is necessary. As an example, if considered proportionate, the planting of border rows of non-*Bt*-maize adjacent to uncultivated margins of maize Bt11 fields, would limit the exposure of those larvae feeding on host-plants present within maize field margins and also would contribute to the required percentage of non-*Bt*-maize necessary to constitute refuge areas for lepidopteran target pests in the framework of IRM. Another example is the establishment of isolation distance to lepidopteran species of conservation concern in protected habitats according to Directive 2004/35/EC* ».

It should be noted that such non-Bt zones exist already if the strategy of the recommended *'high dose/refuge'* zones is correctly implemented. Again this requirement is seen in the new coexistence legislation ordered by the French Government [14] which foresees a border of non-Bt maize around Bt maize plantations.

In addition it should be noted that EFSA [4] underlines that these measures are not necessary in France due to the fact that little Bt-maize is cultivated (21 200 ha in 2007; 0.07% of the French Utilized Agricultural Area):

« *If maize Bt11 (and/or maize MON 810) cultivation remains below 7.5% of the regional Agricultural Unit of Account, the global mortality is predicted to remain below 1%, even for 'extremely sensitive' species, and then risk mitigation measures using non-*Bt*-maize border rows are not required* ».

IN CONCLUSION, the statement by NAF that *"Given the state of urgency and the fact that MON810 is susceptible to pose a grave risk for the environment"* finds no scientific basis in the statements of EFSA.

Answers to arguments presented in the *Annex to NAF* (from page 4)

NAF presents recent scientific publications which will be discussed here.

I.1- Dissemination and persistence of Cry1Ab toxins in soil and water

The impact of of MON810 cultures on non-target aquatic organisms

NAF cites several studies which (according to NAF) show such an impact. The true conclusions of the cited articles are presented alongside the interpretations of NAF.

Tank et al. (2010) [16]

NAF: « *The study conducted by Tank et al. in the United States (2010) shows that fragments of maize may be dispersed by rivers and Cry1Ab toxin may find themselves more frequently than had been previously recognized earlier in the streams draining areas production of maize* ».

The facts: The presence of insecticidal Cry protein in aquatic media has been known for a long time.

Tank et al. furnish quantitative data on the Cry1Ab protein present in aquatic environments in the USA, a country in which the Bt cultivation is widespread. However, they draw no conclusions as whether this represents a menace for the environment.

Chambers et al. (2010) [16]

NAF: « *Another study conducted by Chambers et al. (2010) demonstrated negative growth effects in certain aquatic organisms of the trichopterans family* ».

In fact, these effects are only observed during forced feeding in the laboratory and are not seen in natural media. The conclusion of Chambers *et al*. was « *Our in situ findings did not support our laboratory results* ».

Jensen et al. (2010) [17]

NAF: « *Jensen et al. (2010) have also demonstrated negative effects if Bt on the growth and survival of certain non -target invertebrates such as isopods and tipulid crane fly which feed on vegetable debris in watercourses…* ».

The conclusion of the article is quite different from that stated by NAF:

« Overall, our results provide evidence that adverse effects to aquatic non-target shredders involve complex interactions arising from plant genetics and environment that cannot be ascribed to the presence of Cry1Ab proteins ».

In fact the entire question of impact on aquatic organisms has been the subject of a recent review article by **Carstens *et al.* (***in press***)** [18]. This review is not cited by NAF.

« *Based on exposure estimates, shredders were identified as the functional group most likely to be exposed to insecticidal proteins. However, even using worst-case assumptions, the exposure of shredders to Bt maize was low and studies supporting the current risk assessments were deemed adequate* ».

IN CONCLUSION, the allegations of NAF regarding the effects of Bt-cultivation on aquatic organisms is without any scientific foundation.

Persistence of the Cry1Ab protein in soils

It has been known for a long time that Cry1Ab protein may fix on soil particles while conserving its insecticidal activity. The document of NAF only cites a single publication by ***Sanders et al. (2010)*** [19]. This publication describes only the physico-chemical mechanisms of soil adsorption, but gives no characterization of an in situ risk. The same group has recently published other articles of this type: **Madliger *et al.* (2010)** [20]; **Madliger *et al.* (2011)** [21] (not cited in the NAF document). No environmental risk is characterized in these articles.

A recent article by **Gruber *et al.* (2012)** [22] (not cited in NAF) describes a 9-year study of consecutive Bt-cultures in 4 sites. The Cry1Ab protein was never found in the spring following the year of culture.

The article demonstrates that there is no experimental proof of the accumulation of Cry1Ab after long-term cultivation in various different soils.

I.2- Appearance of resistance in target pests

Certain elements of the opinion (December 22, 2009) of the French High Council of Biotechnologies are cited by NAF using words like ' *this makes any prediction random*' and *'impossible to know'* regarding this agronomic risk, inferring that its management is not feasible.

In particular two publications by Prof. Fangneng Huang (***Huang et al. 2007*** [23]*;* ***Wu et al. 2009*** [24]) are cited in support of these claims.

We have questioned Prof. Huang on this subject and his replies are given below:

I have noted that in a recent French government document two of our publications (Huang et al. 2007 [23] *and Wu et al. 2009* [24]*) related to Bt resistance in sugarcane borer, Diatraea saccharalis, were cited to justify a ban of MON810 Bt corn in France. Since 2004 I have worked on how to effectively use transgenic Bt corn for managing D. saccharalis in the U.S. mid-southern region. Our research results suggested that effective resistance management is important for the sustainable use of transgenic Bt corn, but the results did not provide any data to justify a ban of MON810. In fact, the overall research results in the United States including ours showed that Bt corn including MON810 is an effective tool for managing corn borers including D. saccharalis in the United States. Field resistance to Bt corn in any corn borer species has not been observed after 16 years of use of Bt corn in the USA. It is also improper to cite our ppublications together with the Busseola fusca -MON810 case. Our*

resistant strain of D. saccharalis was developed by using an F2 screening method in the laboratory and thus it should be considered as a laboratory selected resistance. In contrast, the resistance in Busseola fusca should be considered as a case of field resistance. As pointed out in our paper (Huang et al. 2007 [23]), the discovery of a major Bt resistance allele in a field population of D. saccharalis in northeastern Louisiana does not necessarily indicate an immediate threat of resistance to Bt corn in the field. Actually Bt corn is still effective against D. saccharalis and no field resistance has been observed in this area. In addition, as stated in the French document, D. saccharalis even is not a corn pest in Europe. Therefore, I believe it is inappropriate to cite our research results to justify a ban of MON810 or any other currently commercialized Bt corn in Europe. Let me know if you have any more questions.

Sincerely yours,

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NAF cites a document by ***Kruger et al. in 2011*** [25], which NAF interprets as proof of the inefficiency of risk management for the appearance of resistant insects (the method of refuge zones). Firstly, it should be noted that the target insects are not those of France but of a South African species. Secondly, these same authors [25] (not cited by NAF) show that the strategy of refuge zones was not applied in the correct conditions and they speak of irresponsible management.

IN CONCLUSION, the extrapolations of the NAF document regarding the appearance of resistant insects result in an inexact interpretation of the cited publications. It should also be noted that the same risks are present for all insecticides on the market; though these are not banned. This fact is not considered by NAF.

I.3- Impact of MON810 maize on not target invertebrates **(studies in the laboratory and in the field)**

Here we will not return upon certain publications already cited (***Chambers et al. 2010***, [16]**;** ***Jensen et al. 2010***, [18]*)* concerning aquatic species.

Elements arising from the opinion of the French High Council of Biotechnologies (December 22, 2009)

This section concerns meta-analyses on which the text of NAF is very confusing. These meta-analyses show clearly two things:

1. A positive impact of Bt cultivation on non-target invertebrates, relative to treatment by chemical insecticides

2. When a comparison is made with conventional cultures without treatment it is demonstrated that, in MON810 fields, there is a notable reduction in a parasitoid specializing on the target organism the European Corn borer (since this latter has largely disappeared from the field)

For the more recent meta-analyses from the laboratory of Steve Naranjo (***Wolfenbarger et al. 2008,*** [27]**;** ***Naranjo 2009*** [6]**)** we have sought the opinion of the author. His complete reply on the NAF interpretations (acceptable or erroneous) is given below in Annex 1.

Recent scientific publications

The publication of ***Lang and Otto in 2010*** [28] is cited twice. It consists of a compilation of already published studies, none of which are more recent than those already examined by EFSA. None of these studies were interpreted by EFSA as indicating a serious menace for non-target insects and thus justifying a ban on the culture. The authors express a certain number of opinions, and regret particularly a predominance of studies in the USA relative to Europe and call for long-term large-scale studies under realistic conditions.

The authors do not underline an important fact: namely such studies are difficult in Europe due to field destructions by anti-GMO activists and more specifically in France due to a ban on GMO cultivation by the French government.

Bohn et al. (2010) [30]

This publication claims to have observed negative effects due to feeding Bt maize to Daphnia, as compared to conventional maize. Survival, fertility and demographic growth were affected. However the studies were exclusively laboratory experiments where the Daphnia were force fed.

Ricroch *et al.* [3] have already published in 2010 a critical article on the Bohn *et al*. publication. Briefly, difference may exist between the two varieties of maize which are not due to the MON810 character. The non-characterization of the composition of the two aliments did not allow exclusion of the hypothesis that the differences observed were due to these composition variations. In any case the artificial situation used bears no relationship to the field situation where the Daphnia have a more varied food supply.

It should be noted that the German agency ZKBS [29] confirmed the analysis of Ricroch *et al*. [3].

Kramarz et al. (2009) [31]

NAF: *« A study by Kramarz et al. (2009) on the snail Cantareus aspersus equally showed a negative long term effect of MON810 on growth ».*

Yet if one reads the article:

« The hazard analysis of Bt-maize which we performed, based on a worst-case scenario, i.e. snails having no food choice, should now be complemented by other simple measurements, e.g. food intake, to understand the underlying mechanisms involved ».

Thus it is a laboratory study where the authors used an extreme scenario. Nothing indicates an effect in the natural environment. We also note that this is an organism considered a nuisance by farmers. The publication by Kramartz *et al.* [31] derives from the EC program ECOGEN [32], which did not find negative effects of Bt-maize on non-target organisms.

Virla et al. (2010) [33]

This publication is considered as preliminary by its authors. It does not concern MON810 maize but a maize producing another insecticide protein (Cry1F) which has no authorization for cultivation in Europe.

Elements arising from the EFSA Opinion of 8 October 2011 [4]

This question concerns the effect of Bt-maize on ultra-sensitive insects. We have posed the question to a member of the EFSA GMO Panel (who wishes to remain anonymous). Here is his answer:

I think the two Perry et al. papers are misused.

In order to adopt emergency measures relating to genetically modified organisms pursuant to Article 34 of Regulation No 1829/2003, it is necessary for the existence of a risk to human health, animal health or the environment to be established, which is not merely hypothetical, and for the probability of such harm occurring to be significant, even though it has not necessarily been determined precisely (See EU Court Case decision on French Safeguard Clause).

In EFSA's Bt11/MON810 opinion - which is largely based on the Perry et al. papers - we have indicated a hypothetical risk dependant on highly sensitive species being exposed to certain quantities of pollen. Situations where this risk is a reality have not been reported in Europe (yet) and current scientific literature so far has indicated no harmful effects to lepid

populations. Thus the situation is that current risk levels are either hypothetical as no at-risk species have been identified as being exposed, or are low, based on current research results. Therefore the same expressions as the EU Court judgement on the French Safeguard Clause are valid regarding MON810: the probability of harm actually occurring is currently low and thus not significant. So EFSA/Perry's opinions are still in line with the judgement. The situation might only change if a butterfly species was identified as being at risk due to its high sensitivity and/or exposure in a particular environment in France. It would interesting to get data from Arvalis on current insecticide use on maize in France and the effects this is likely to be having on NT Lepids, so that a fair comparison could be made of the relative risks of changing to Bt maize.

IN SUMMARY, these are theoretical risks and no insect in this category has been identified in France.

I.3 c- Development of secondary pests

This theme appears in several parts of the NAF document (page 7):

« The maize MON810 favorizes the survival of a new pest of maize »,

*« The culture of resistant cotton favorizes the development of other non-target insects that become pests for cotton » (**Lu et al. 2010** [37]; see comments below).*

Part 1.3.c is dedicated to this theme. Three publications are cited, ***Virla et al. (2010)*** [33]**;** ***Dorhout and Rice (2010)*** [34], as already mentioned above, it does not concern MON810 maize) and ***Meissle et al. (2011)*** [35]. We have questioned one of the authors of the latter article, Dr Joerg Romeis, who replied:

Reference to our paper (Meissle et al. 2011) as evidence for the outbreaks of secondary pests in Bt maize is incorrect. We do not provide any data on this. We simply review the existing

literature and point to publictions that have reported an increased abundance of the Western Bean Cutworm in parts of the US. This fact is also addressed in the other papers cited in this paragraph.

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In summary, these publications mention the case of the expansion of the pest *Striacosta albicosta* (the western bean cutworm) in the USA. The idea that this is exclusively due to the cultivation of Bt maize is criticized by **Hutchinson *et al*. in 2011** [36]. This article is not cited by NAF.

As to the last paper cited by NAF (***Lu et al. 2010)*** [37] concerning minor pest insects that develop in Bt cotton cultures (and NOT maize) in China, this has been the subject of a scientific publication (**Bergé and Ricroch 2010)** [38] not cited by NAF. This publication places the phenomenon in its true framework (any selective use of an insecticide, while respecting the non-target fauna, may also preserve non-target pests. The publication also proposes management solutions. It should be noted that no country in the world ever proposed a ban because of this phenomenon (a decision which would favour the use of methods that do not respect auxiliary insects) which has to deal with by integrated pest management approaches.

IN CONCLUSION, the NAF document is of mediocre scientific quality: selection of specific publications of which the context or the conclusions are not given in a faithful manner, omission of other publications that testify against the thesis defended by NAF, confusion between risks that can be managed and serious risks that cannot be managed.

ANNEX 1. Reply of Dr Steve Naranjo to those parts of the NAF document that cite his publications

The underlined italic parts have been translated from the French NAF document relating to Naranjo in 2009 [6] on all non-target invertebrates.

NAF: Laboratory studies concern only a limited number of phyla (n = 3), class (n =8), orders (n = 16), families (n = 43) of type (n = 79) or of species (n = 99) invertebrates. This is related to difficulties or inability to raise or to reproduce some insect species in captivity.

Naranjo Response: This is merely a summary of the data I present in Table 2 where I catalog all the non-target studies completed up to late 2008 that meet the criteria laid out on p. 5 of the review. I cannot find reference to the final statement about rearing animals in the lab. I merely state (p. 10) that field studies have been more inclusive and primarily focused on arthropods.

NAF: The meta-analysis of Naranjo (2009, 5) includes 84 studies of Cry1Ab toxin produced pure or by Bt corn

Naranjo Response: Not sure where they got this number. I never broke out the number of studies in each crop as the review was meant to look at general patterns overall. In truth there were a total of 64 Cry1Ab laboratory studies in the analysis with 50 coming from maize, 3 from rice and 11 others using pure protein.

NAF: The effects are highlighted include: (1) slight reduction of predators development does not, however, results in a reduction in their survival or their reproductive rate and (2) a decrease in development time and survival many lepidopteran pests they are non-target species or symbolic (eg. Nymphalidae, Papilionidae, Saturniidae, and Bombyxidae Lycaenidae).

Naranjo Response: 1) a paraphrase of what I said. The exact words were "Within the natural enemy group predators showed a slight but significant reduction in developmental rate when exposed to Bt proteins directly compared with non-Bt control. Conversely, Bt proteins had no affect on survival or reproduction of either predators or parasitoids". 2) Again, a paraphrase but relatively accurate. It is well-known and accepted that non-target lepidoptera are susceptible to some Bt proteins. What they fail to mention is that the vast majority of studies for "symbolic" species were done on Monarch butterfly and exhaustive field studies in the USA (and published in a series of PNAS papers) showed that there is negligible risk to field populations of this insect. To my knowledge, such field studies have not been done for the other families noted and in some cases it is hard to imagine how they would even be exposed (e.g., silkworm moths)

NAF: Furthermore, analysis of Naranjo (2009) [6] clearly shows an effect on the quality of host / prey on development, reproduction and survival of parasitoids and predators. These three parameters are assigned only if the host / prey are first weakened through exposure to Bt toxins to which they are sensitive. In contrast, parasitoids and predators grow, reproduce and survive normally on hosts / prey naturally insensitive or become resistant to Bt toxins These results conclude that the effects on parasitoids and predators are generally - if not exclusively - indirect.

Naranjo Response: They have represented this quite fairly. However, one could question the use of the term "indirect". In reality, without the proper controls, there is no way to know if tri-trophic studies with susceptible hosts is even testing Bt protein toxicity. It is testing prey quality affects for certain.

NAF: The latest meta-analysis to date (Naranjo, 2009) [5] includes 14 additional studies. The results of meta-analyzes Marvier et al. (2007 [39]), Wolfenbarger et al. (2008) [27] and Naranjo (2009) [6] can be summarized as follows:

a. The abundance of non-target invertebrates is generally higher in non conventional maize plots than in insecticide-treated plots MON810.

Naranjo Response: Not true. Figure 4 on my CAB review shows that only parasitoids in maize are significantly reduced in Bt corn compared with unsprayed non-Bt corn. The use of insecticides in non-Bt corn negatively affects most groups compared with unsprayed Bt corn.

b. Specifically, the parasitic wasps - mainly species wasps belonging to the Braconidae and Ichneumonidae - are less frequent in plots of corn producing the Cry1Ab toxin than in those containing corn untreated conventional. This reduction is almost exclusively a diminished abundance of Macrocentrus grandii, a specialist parasitoid of European corn borer corn, Ostrinia nubilalis. The effect on parasitoids is certainly an effect secondary related to the reduction of the densities of their host and thus the efficiency of corn MON810 on its main target: O. nubilalis.

Naranjo Response: Basically a true statement. The exception would be that it is entirely Macrocentrus that drives this relationship. In Wolfenbarger et al. we removed that one species from the meta-analysis and the effect on the remaining parasitoids in corn was non-significant. This is an ecological effect that would be observed with any effective technology for reducing the pest population.

c. Collembola are on average less frequent in corn toxin-producing Cry1Ab than in conventional corn. Marvier et al. (2007)[39] believe, however, that this difference is based on an insufficient number of studies (n = 3) to be considered as significant.

Naranjo Response: They did not get this result from my CAB review or the Wolfenbarger paper. In the CAB review the effect of Bt corn compared with unsprayed Bt corn on detritivores overall was completely neutral (Fig. 4). There was a significant decline in Collembola in unsprayed Bt corn compared with sprayed non-Bt corn in an analysis by Wolfenbarger et al.

Basically we showed that this was due to the insecticides reducing populations of carabid beetles that use Collembola as prey (see P. 7 of the PloS One paper). It is basically a

resurgence of Collembola when biological control of carabids is removed.

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