**GeneWatch UK response to EFSA's consultation on its Draft guidance on the agronomic and phenotypic characterisation of genetically modified plants**

**November 2014**

The following comments relate to EFSA's consultation document:
http://www.efsa.europa.eu/en/consultations/call/140925.htm

Abstract

Lines 8-9: The scope of the Guidance should be broadened to should be broadened to "*specifications on criteria for field trials and selection of techniques to assess long-term effects of genetically modified plants*" (and the title altered). See more detailed comments under Terms of Reference.

Summary
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**Background as provided by EFSA**

Lines 125-128: It is unclear why EFSA has identified a need to focus field trial requirements solely on the agronomic and phenotypic characterisation of GM plants. Whilst this characterisation is important, other important aspects of the risk assessment include identification of effects on target and non-target organisms, long-term effects, and the effects of the blanket spraying of herbicides on herbicide tolerant crops. Issuing Guidance which is inadequate to cover many of these effects is likely to lead to applicants conducting field trials which are unsufficient for the purposes of risk assessment. EFSA should not lead applicants to believe that providing data on the agronomic and phenotypic characterisation of GM plants is sufficient, when clearly it is not. The terms of reference and scope (and title) of the Guidance should therefore be broadened to "*specifications on criteria for field trials and selection of techniques to assess long-term effects of genetically modified plants*" (consistent with the requirements of the 2008 Council Decision, discussed further below). The criteria for field trials should then be amended to be consistent with the requirements of the environmental and food/feed safety risk assessments and additional experimental and theoretical techniques (e.g. contained trials, computer modelling) should be identified to obtain the necessary data.

**Terms of reference as provided by EFSA**

On 4th December 2008, the Environment Council adopted a number of conclusions on genetically modified organisms (GMOs), arguing that it was necessary to improve implementation of the legal framework: http://register.consilium.europa.eu/doc/srv?l=EN&f=ST%2016882%202008%20INIT This document should have been cited in the Guidance and its recommendations taken into account. For example, the 2008 Council Decision "*EMPHASISES the need to improve harmonisation of the Member States' assessment practices while ensuring that each GMP should be analysed on a case-by-case basis taking account of the characteristics of ecosystems/environments and of the specific geographical areas in which GMPs may be cultivated in accordance with existing legislation*". The decision also "*NOTES with satisfaction that the Commission's mandate to EFSA to further develop and update its guidelines as regards the environmental risk assessments of GMOs includes in particular detailed assessment of the long-term environmental effect of GMPs and covers the following areas: Environmental risk assessment of potential effects of genetically modified plants on non-target organisms, development of criteria for field trials to assess the potential ecological effects of the GMPs in receiving environments, identification of the EU geographic regions where the GMPs*

*may be released, selection of appropriate techniques to assess potential long-term effects of GMPs including experimental and theoretical methodologies, and recommendations for establishing relevant baseline information*" [emphasis added]. The Decision also "*UNDERLINES in particular the need to study the potential consequences for the environment of changes in the use of herbicides caused by herbicide-tolerant GMPs*".

EFSA's Guidance on the environmental risk assessment of genetically modified Plants (http://www.efsa.europa.eu/en/efsajournal/pub/1879.htm ) contains no detailed specifications on criteria for field trials or selection of appropriate techniques to assess potential long-term effects of GMPs (both requirements of the 2008 Council decision). It is therefore puzzling to see EFSA issuing this draft Guidance with a view to specifying criteria for field trials with the much narrower purpose of the agronomic and phenotypic characterisation of genetically modified plants. EFSA itself admits in Section 7 of the draft guidance that its proposals are inadequate for the purposes of assessing effects on target and non-target organisms (a key aspect of the risk assessment). There is therefore a risk that applicants regard the field trials proposed in this guidance as adequate for the purposes of risk assessment, when clearly they are not.

Lines 130-134: The terms of reference and scope (and title) of the Guidance should be broadened to "specifications on criteria for field trials and selection of techniques to assess long-term effects of genetically modified plants" (consistent with the requirements of the 2008 Council Decision). Field trials and other experiments are not restricted to the purpose of assessing agronomic and phenotypic properties and failure to include broader environmental and long-term effects is likely to lead to applicants submitting data that is not fit for purpose. Further, additional experimental and theoretic methodologies (as noted by Council Decision) need to be specified. This is particularly important as the legislation (2001/18/EC) requires a 'step by step' approach in which field trials only take place after sufficient evidence has been provided by contained experiments and other methods (e.g. computer modelling). Finally (again as noted by the Council Decision) baseline information (e.g. regarding target and non-target organisms at a given site) needs to be established prior to any open experiments and EFSA is supposed to provide recommendations for establishing relevant baseline information.

**Assessment**

Lines 137-139: EFSA should have considered the 2008 Council Decision when identifying needs and should have avoided specifying criteria for field trials which are inadequate to meet those needs.

**Introduction**

This should outline, at the start, more detail on the requirements of the relevant legislation, so that the relevance of the guidance to implementing the regulatory requirements is clear.

For the environmental risk assessment this is 2001/18/EC, specifically Annex II.D:
*In the case of genetically modified higher plants (GMHP)*
*1. Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.*
*2. Any selective advantage or disadvantage conferred to the GMHP.*
*3. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.*
*4. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable).*

*5. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.*
*6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s).*
*7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed.*
*8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).*
*9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.*

The information requirements in Annex IIIA of 2001/18EC are also highly relevant, especially part IV(B):
*B. Interactions with the environment*
*1. predicted habitat of the GMOs,*
*2. studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses,*
*3. genetic transfer capability*
*(a) post release transfer of genetic material from GMOs into organisms in affected ecosystems;*
*(b) post release transfer of genetic material from indigenous organisms to the GMOs;*
*4. likelihood of post release selection leading to the expression of unexpected and/or undesirable traits in the modified organism,*
*5. measures employed to ensure and to verify genetic stability. Description of genetic traits which may prevent or minimise dispersal of genetic material. Methods to verify genetic stability,*
*6. routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc.,*
*7. description of ecosystems to which the GMOs could be disseminated,*
*8. potential for excessive population increase in the environment,*
*9. competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s),*
*10. identification and description of the target organisms if applicable,*
*11. anticipated mechanism and result of interaction between the released GMOs and the target organism(s) if applicable,*
*12. identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction,*
*13. likelihood of post release shifts in biological interactions or in host range,*
*14. known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens,*
*15. known or predicted involvement in biogeochemical processes,*
*16. other potential interactions with the environment.*

In general, the draft Guidance is poor on the aspects of the risk assessment related to altered management and to environmental interactions, which are requirements of 2001/18/EC which should have received greater attention. More detailed comments are given in relevant sections below.

In the case of food and feed and impacts on human and animal health, the relevant legislation is 1829/2003/EC, which contains the requirement to ensure a high level of protection of human life and health, animal health and welfare, environment and consumer interests (Article 1). Establishing the composition and nutritional value and effects of the food/feed (including on subpopulations) are important requirements in 1829/2003/EC, which should be better reflected in the Guidance, especially in relation to tests on the edible parts of GM plants.

Examples of effects not properly considered in the draft include: impacts on gene expression and plant composition due to altered environmental conditions (i.e. different environments can alter the phenotype of the crop) and the development of resistant weeds or pests or the establishment of secondary pests which reduce crop yields (i.e. environmental responses can alter the agronomic characteristics of the crop). These changes can have significant impacts on the risk assessment.

For example, the current generation of GM crops consist primarily of herbicide-tolerant (HT) and insect-resistant traits (Bt crops), including stacked traits. Known adverse environmental impacts of these crops include:

- Negative impacts on biodiversity due to harm to wildlife habitats associated with blanket spraying of HT crops, including a 90% reduction in the Monarch butterfly populations in the United States (e.g. Flockhart, D. T. T., Pichancourt, J.-B., Norris, D. R., & Martin, T. G. (2014). Unravelling the annual cycle in a migratory animal: breeding-season habitat loss drives population declines of monarch butterflies. Journal of Animal Ecology. doi:10.1111/1365-2656.12253; Pleasants, J. M., & Oberhauser, K. S. (2013). Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. Insect Conservation and Diversity, 6(2), 135–144; Hartzler RG (2010) Reduction in common milkweed (Asclepias syriaca) occurrence in Iowa cropland from 1999 to 2009. Crop Protection 29(12):1542–1544; Zalucki MP, Lammers JH (2010) Dispersal and egg shortfall in Monarch butterflies: what happens when the matrix is cleaned up? Ecological Entomology 35(1):84–91) , and loss of habitat for other species elsewhere including farmland birds (Squire GR, Brooks DR, Bohan DA, et al. (2003) On the rationale and interpretation of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Philos Trans R Soc Lond B Biol Sci. 358(1439):1779–1799.);
- The spread of herbicide-tolerant weeds, which impact on agronomic properties such as yield (e.g. Sandermann H (2006) Plant biotechnology: ecological case studies on herbicide resistance. Trends in Plant Science, 11(7), 324-328; Binimelis, R, Pengue, W, Monterroso, I (2009) ‘‘Transgenic treadmill”: Responses to the emergence and spread of glyphosate-resistant johnsongrass in Argentina. Geoforum, doi:10.1016/j.geoforum.2009.03.009.; Bonny S (2011) Herbicide-tolerant Transgenic Soybean over 15 Years of Cultivation: Pesticide Use, Weed Resistance, and Some Economic Issues. The Case of the USA. Sustainability, 3(12):1302–1322.);
- Impacts of these herbicide-tolerant weeds on crop management, i.e. increased spraying with more herbicides, in an attempt to regain weed control (e.g. Benbrook CM (2012) Impacts of genetically engineered crops on pesticide use in the U.S. - the first sixteen years. Environmental Sciences Europe 24(1):24;  ) and consequences for wildlife (e.g. more habitat loss) and food safety (higher herbicide residues, see: Bøhn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R (2013) Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. Food Chemistry. doi:10.1016/j.foodchem.2013.12.054.);
- The spread of Bt-resistant pests , which also impact on agronomic properties such as yield (e.g. Dhurua S, Gujar GT (2011) Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), from India. Pest

Management Science 67(8):898–903; Gassmann AJ, Petzold-Maxwell JL, Keweshan RS, Dunbar MW (2011) Field-Evolved Resistance to Bt Maize by Western Corn Rootworm. PLoS ONE. 6(7):e22629; Gunning RV, Dang HT, Kemp FC, Nicholson IC, Moores GD (2005) New resistance mechanism in Helicoverpa armigera threatens transgenic crops expressing Bacillus thuringiensis Cry1Ac toxin. Appl. Environ. Microbiol. 71(5):2558–2563.; Tabashnik BE, Gassman AJ, Crowder DW, Carriére Y (2008) Insect resistance to Bt crops: evidence versus theory. Nature Biotechnology, 26, 199 – 202.; Van Rensburg JBJ (2007) First report of field resistance by the stem borer, Busseola fusca (Fuller) to Bt-transgenic maize. South African Journal of Plant and Soil, 24(3):147–151; Van den Berg J, Hilbeck A, Bøhn T (2013) Pest resistance to Cry1Ab Bt maize: Field resistance, contributing factors and lessons from South Africa. Crop Protection 54:154–160; Wan P, Huang Y, Wu H, et al. (2012) Increased Frequency of Pink Bollworm Resistance to Bt Toxin Cry1Ac in China. PLoS ONE. 7(1):e29975.; Jin L, Wei Y, Zhang L, Yang Y, Tabashnik BE, Wu Y (2013) Dominant resistance to Bt cotton and minor cross-resistance to Bt toxin Cry2Ab in cotton bollworm from China. Evolutionary Applications. 6(8):1222–1235; Tabashnik BE, Brévault T, Carrière Y (2013) Insect resistance to Bt crops: lessons from the first billion acres. Nat Biotech. 31(6):510–521) and impacts of altered pest management as a result (e.g. more spraying);

- Explosions in <u>secondary pests</u> in some Bt crops, again impacting on agronomic properties such as yield (e.g. Catangui MA, Berg RK (2006) Western Bean Cutworm, Striacosta albicosta (Smith) (Lepidoptera: Noctuidae), as a Potential Pest of Transgenic Cry1Ab Bacillus thuringiensis Corn Hybrids in South Dakota. Environmental Entomology 35:1439–1452.; Lu Y, Wu K, Jiang Y, et al. (2010) Mirid Bug Outbreaks in Multiple Crops Correlated with Wide-Scale Adoption of Bt Cotton in China. Science 328(5982):1151–1154.; Wang S, Just DR, Andersen PP (2008) Bt-cotton and secondary pests. International Journal of Biotechnology 10(2/3):113; Zhao JH, Ho P, Azadi, H (2011) Benefits of Bt cotton counterbalanced by secondary pests? Perceptions of ecological change in China. Environ Monit Assess, 173:985–994; Tay WT, Soria MF, Walsh T, et al. (2013) A Brave New World for an Old World Pest: Helicoverpa armigera (Lepidoptera: Noctuidae) in Brazil. PLoS One 8(11)) and impacts of altered pest management as a result (e.g. more spraying).

Other direct negative impacts may also exist (e.g. toxicity of Bt toxins to some beneficial species: Hilbeck A, Schmidt J (2006) Another view on Bt-proteins - how specific are they and what else might they do? Biopesticides Int. 2:1–50.; Lang A, Otto M (2010). A synthesis of laboratory and field studies on the effects of transgenic Bacillus thuringiensis (Bt) maize on non-target Lepidoptera. Entomologia Experimentalis et Applicata 135(2):121–134.), as well as the risks of persistence and spread of traits (e.g. Schafer MG, Ross AA, Londo JP, et al. (2011) The Establishment of Genetically Engineered Canola Populations in the U.S. PLoS ONE 6(10):e25736.) and direct negative impacts of the herbicide management regime (e.g. on amphibians, see for example: Wagner N, Reichenbecher W, Teichmann H, Tappeser B, Lötters S. (2013) Questions concerning the potential impact of glyphosate-based herbicides on amphibians. Environmental Toxicology and Chemistry. 32(8):1688–1700). However, the major known negative impacts listed above occur as a result of <u>interactions between the crop, the management regime and the environment, over the longer term</u> i.e. it is necessary to measure the crop in different environments, under relevant management regimes, and to take account of how ecosystems evolve in response over time (including human responses such as increased spraying to tackle herbicide-tolerant weeds). It is clear that these impacts are required to be covered by the risk assessment according to 2001/18/EC (cited above), but it is less clear that the draft Guidance is adequate to capture such effects. The 2$^{nd}$ generation of GM crops will be resistant to more toxic herbicides such as 2,4-D and dicamba, exacerbating many of these problems and adding to the importance of capturing data on these risks within the risk assessment process conducted by EFSA.

Other categories of future crops may raise new challenges. In the case of disease-resistant plants (an active area of GM crop research), the evolution of pathogens to overcome resistance will occur, particularly in the case of monogenic traits (polygenic resistance – more likely to be introduced through conventional breeding or MAS – is more durable): Palloix, A., Ayme, V., & Moury, B. (2009). Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. The New Phytologist, 183(1), 190–199. This again highlights the importance of considering changes in the agronomic and phenotypic characteristics of the GM crop over the longer term (in response to evolving environmental conditions and ecosystem responses such as evolution of the target pathogen).

Environmental interactions will also be important for other types of GM crops that may be developed in the future, such as nutrient-altered crops (some of which have already been submitted for consideration for import as food and feed, but others of which are still under development). For example, GM crops which concentrate zinc from the soil might potentially be used to reduce zinc deficiency in human diets but the efficacy and safety of this approach depends on the composition of the plants and the dose received by people eating them (depending also on the rest of their diets and whether they suffer from any condition where zinc overload is a problem, including people with the common iron-overload disease haemochromatosis). Since such plants also concentrate cadmium, they might also be used to clean up soils contaminated with cadmium: but these plants may then become toxic to humans if grown on contaminated soils even if they benefit humans if grown on uncontaminated soils. Thus, it is critical to the risk assessment required by 1829/2003/EC to know the dose of zinc and other heavy metals in the edible plants in different environments (with or without contaminated and zinc-deficient soils). See: Zhao, F.-J., & McGrath, S. P. (2009). Biofortification and phytoremediation. Current Opinion in Plant Biology, 12(3), 373–380; Gómez-Galera, S., Rojas, E., Sudhakar, D., Zhu, C., Pelacho, A. M., Capell, T., & Christou, P. (2010). Critical evaluation of strategies for mineral fortification of staple food crops. Transgenic Research, 19(2), 165–180; Palmgren, M. G., Clemens, S., Williams, L. E., Krämer, U., Borg, S., Schjørring, J. K., & Sanders, D. (2008). Zinc biofortification of cereals: problems and solutions. Trends in Plant Science, 13(9), 464–473. For many traits, the dose of the particular protein or proteins expressed in the GM plant will be important and will vary according to the environment, but this example shows that when the phenotype of the plant is significantly altered, understanding the GM plant's interactions with the environment as a whole may be critical to the food safety assessment (in the example above, the plant's uptake of metals from soils and the concentration of them in edible parts of the plant). The draft Guidance is wrongly focused on the subset of cases where a single protein of interest is synthesised within the plant, rather than on more complex changes to metabolism. Environmental interactions may also lead to unintended environmental (rather than food safety) impacts. For example, GM plants engineered to enhance bioavailability of calcium (in an attempt to improve human diets) can remove defence compounds such as oxalate, rendering the plant more attractive to pests, alter the ripening process, or reduce the plant's tolerance to stress (Dayod, M., Tyerman, S. D., Leigh, R. A., & Gilliham, M. (2010). Calcium storage in plants and the implications for calcium biofortification. Protoplasma, 247(3-4), 215–231). Some of these adverse effects on agronomic properties may not be detected if test environments do not include the relevant abiotic and biotic stresses (e.g. water shortage or presence of a particular pest).

In addition to the above shortcomings, the principle outlined in 2001/18/EC Preamble (24) should have been stated and taken into account throughout the guidance. This states:
*The introduction of GMOs into the environment should be carried out according to the 'step by step' principle. This means that the containment of GMOs is reduced and the scale of release increased gradually, step by step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken.*

In line with this principle, the Guidance should not have focused solely on field trials but also on experiments necessary prior to release, including, for example; "*studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses*" (2001/18/EC, Annex IIIA part IV.B(2), as cited above).

Lines 148-149: should say "*… by assessing whether intentionally and unintentionally modified properties of the GM plant, its interactions with the environment, or its cultivation, management or harvesting techniques, alter the level of risk or give rise to additional risks*". This is a requirement of 2011/18/EC (see Annex II.D (9) and Annex IIIA part IV.B), as cited above. This scope is very important in order to meet the legal requirements and protect human health and the environment, as outlined above.

Line 155. Should add: "*Both these tests (of difference and equivalence) may vary with environmental conditions (including the management conditions of the crop and local conditions such as soil type) and over time (particularly as ecosystems evolve in response to the GM crop or its management)*". Some of the evidence for this is cited above. The Guidance should also note explicitly that long-term effects must be taken into account in the risk assessment. 2001/18/EC preamble (19) states:
*A case-by-case environmental risk assessment should always be carried out prior to a release. It should also take due account of potential cumulative long-term effects associated with the interaction with other GMOs and the environment.*

Lines 156-160: Should note that compositional characterisation may depend strongly on the environment where the crops are planted and on management of the crop. For example, environmental conditions and gene-environment interactions (GxE) are known to have important effects on nutrient composition of soybeans (Whent M, Hao J, Slavin M, et al. Effect of Genotype, Environment, and Their Interaction on Chemical Composition and Antioxidant Properties of Low-Linolenic Soybeans Grown in Maryland. J Agric Food Chem. 2009;57(21):10163–10174) and such effects can vary at different developmental stages (Han Y, Xie D, Teng W, Zhang S, Chang W, Li W. Dynamic QTL analysis of linolenic acid content in different developmental stages of soybean seed. Theor Appl Genet. 2011;122(8):1481–1488). Management conditions may also alter impacts on human or animal health. For example, in the case of glyphosate-resistant GM crops, application of glyphosate alters the nutrient profile as well as leaving pesticide residues on the soybeans (Bellaloui N, Abbas HK, Gillen AM, Abel CA. Effect of glyphosate-boron application on seed composition and nitrogen metabolism in glyphosate-resistant soybean. J Agric Food Chem. 2009;57(19):9050–9056.; Bøhn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R. Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. Food Chemistry. 2014;153:207–215). It is therefore essential to include studies of the product as it is expected to be produced, with the intended herbicide. The composition of the final product to be consumed may also be important, even if this is not the GM plant itself. For example, the addition of GM soybean oil or seeds to animal feed is an active topic of research, with the aim of altering milk fat composition (Bernal-Santos G, O'Donnell AM, Vicini JL, Hartnell GF, Bauman DE. Hot topic: Enhancing omega-3 fatty acids in milk fat of dairy cows by using stearidonic acid-enriched soybean oil from genetically modified soybeans. J Dairy Sci. 2010;93(1):32–37.) Further, it is likely that a similar approach could be applied to meat and eggs where diet is known to affect fat composition (e.g. Berthelot V, Bas P, Schmidely P. Utilization of extruded linseed to modify fatty composition of intensively-reared lamb meat: effect of associated cereals (wheat vs. corn) and linoleic acid content of the diet. Meat Sci. 2010;84(1):114–124.; Oliveira DM, Ladeira MM, Chizzotti ML, et al. Fatty acid profile and qualitative characteristics of meat from zebu steers fed with different oilseeds. J Anim Sci. 2011;89(8):2546–2555).

Lines 161-166: It should be noted that there are a wide variety of complex environments within the EU and environmental impacts may vary with location and their existence or severity may depend on complex interactions between the GM crop and the local environment. For example, the consumption of Bt-containing pollen from GM maize by herbivorous larvae of butterflies (*Lepidoptera*) may vary in effect according to whether the butterflies are univoltine i.e. having a single generation a year (as is the case for the protected butterfly the protected butterfly *Inachis io* in Northern Europe), when Bt-maize pollen may not be present on the food plant at the same time as the larvae, or bivoltine (as is the case for the same butterfly in Southern Europe), when Bt-maize pollen and the second generation *I. io* larvae would coincide, and an increased mortality of the larvae is predicted (Holst, N., Lang, A., Lövei, G., & Otto, M. (2013). Increased mortality is predicted of *Inachis io* larvae caused by Bt-maize pollen in European farmland. Ecological Modelling, 250, 126–133). In this example, EFSA should clearly require data that is relevant to both these regions (North and South Europe) for input to the risk assessment in both regions. However, in practice, due to the existence of many different species and associated lifecycles throughout the EU, it is likely that far more than two different environments must be considered. In all cases, worst-case-scenarios, i.e. including all vulnerable species and environments within the EU, must be considered.

**1.1. Objectives**

Lines 186-191: food safety and nutritional assessment implications should be added here. This is because the composition of the crop may vary significantly in different environments (as noted above) and this may make a significant difference to food safety (see examples provided under Introduction). This is likely to be major issue for nutritionally-altered crops.

Lines 194-195. The 2nd aim to "ensure the best use of the agronomic and phenotypic data to inform the food/feed and environmental risk assessments" is undermined by the shortcomings noted above (i.e. failure to consider sufficient environments and evolutionary adaption of these environments to the cultivation of the GM crop over the longer term). This is partially acknowledged by EFSA in Section 7, where it is noted that the proposed field trials will have limited value to assess interactions with target and non-target organisms. This is a major shortcoming as consideration of these effects is a requirement of 2001/18/EC for the environmental risk assessment. In fact, EFSA should have considered the need for a much wider range of experiments including, for example; "*studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses*" (2001/18/EC, Annex IIIA part IV.B(2), as cited above).

Lines 196-203: There is a tension between the need to provide some harmonisation (especially ensuring applicants meet minimum standards and there is a level-playing field) and the need to include a mixture of hypothesis-generating and hypothesis-testing experiments, covering the major expected areas of risk and hoping also to identify unexpected effects and thus prevent harm to humans or the environment. The draft Guidance fails to consider the extent to which numbers and types of tests and field sites may be (at least in part) hypothesis-driven in the context of particular types of crops. This could have been done by considering the main known areas of risk for different crops and traits (some examples are provided here in comments on the introduction) e.g. herbicide-tolerant, insect-resistant, nutrient-altered, disease-resistant, stacked traits etc. For example, nutrient-altered crops require testing in multiple environments to establish likely doses to humans and animals in all edible parts of the crop, of both the target nutrient and other major components. This is likely to be even more important for GM crops designed to alter nutrient uptake from the soil, rather than simply expressing a particular protein (see the example of zinc-enhanced crops and the possibility of risks from planting in cadmium-contaminated soils above). Feeding trials will be needed using relevant products (e.g. soya cooking oil, soya milk, lecithin) from a wide variety of

environments for most-nutrient-altered crops as doses may vary considerably and dose may be important in efficacy and safety. In the case of herbicide-tolerant GM crops, it may be more important to consider the long-term effects of habitat loss due to blanket spraying with herbicide and the development of herbicide-tolerant weeds. Although this will again require many different habitats to be considered (because vulnerable species and their lifecycles will vary), testing a variety of soil conditions may be less important than considering the impact of the herbicide management regime on soils. Further (as noted above), it is a mistake to consider field trials as the only or main mechanism to obtain much of this data. A wider variety of e.g. soil types or non-target pests can be studied under contained conditions, and this would be more consistent with the 'step by step' approach required by 2001/18/EC.

**1.2. Scope**

Lines 209-215: The scope of the Guidance should be broadened to "*specifications on criteria for field trials and selection of techniques to assess long-term effects of genetically modified plants*" (and the title altered), with reference to the 2008 Council Decision. See more detailed comments under Terms of Reference.

The draft Guidance fails to consider the extent to which numbers and types of tests and field sites may be hypothesis-driven in the context of particular types of crops, and in order to meet the requirements of the 2008 Council Decision (for example, in order to assess the impacts of the blanket spraying of herbicide on HT crops). This could have been done by considering the main known areas of risk for different crops and traits (some examples are provided here on comments in the introduction) e.g. herbicide-tolerant, insect-resistant, nutrient-altered, disease-resistant, stacked traits etc. As well as different traits, different crops have different habitats and properties which influence the different risks (e.g. oil seed rape vs. soya). EFSA should draw on the prior knowledge of risks reported in the literature from both experimental development and commercial uses of all these types of GM crops. This would help devise experiments which are able to characterise known risks (such as habitat loss associated with the use of HT crops). A broader range of data will be needed to consider uncertainties and potential unexpected effects. This approach would lead to a more realistic assessment of the number and location of trials, and prior contained experiments, needed to identify potential adverse effects.

As noted by the 2008 Council Decision baseline information (e.g. regarding target and non-target organisms at a given site) also needs to be established prior to any open experiments and EFSA is supposed to provide recommendations for establishing relevant baseline information. These recommendations should be included in the Guidance.

**2. Selection of sites and test materials**

Account must be taken of the need to identify gene-environment interactions (see comments on Section 6.3) and long-term effects. This section of the draft Guidance is inconsistent with the 2008 Council Recommendation, including its recognised need to take account "*of the characteristics of ecosystems/environments and of the specific geographical areas in which GMPs may be cultivated*…" (as cited above). The proposals also ignore the 2008 Council Decision which requires that the environmental risk assessments of GMOs includes in particular detailed assessment of the long-term environmental effect of GMPs and covers the following areas: Environmental risk assessment of potential effects of genetically modified plants on non-target organisms, development of criteria for field trials to assess the potential ecological effects of the GMPs in receiving environments, identification of the EU geographic regions where the GMPs may be released, selection of appropriate techniques to assess potential long-term effects of GMPs including experimental and

theoretical methodologies, and recommendations for establishing relevant baseline information. The Decision also "UNDERLINES in particular the need to study the potential consequences for the environment of changes in the use of herbicides caused by herbicide-tolerant GMPs".

The starting point of the Guidance should be that specifications on criteria for field trials and selection of techniques to assess long-term effects of genetically modified plants (including, but not limited to, the agronomic and phenotypic characterisation of GM plants) must be sufficient to protect human and animal health and the environment. Because "worst-case scenarios" may occur in some environments but not others (e.g. where particular species occur, or where growing conditions increase the dose of a toxin in the plant or express it at the wrong point in the lifecycle of a beneficial organism) this requires a very wide variety of environments to be considered, including e.g. a range of soil types, different management regimes, different target and non-target organisms and the presence or absence of secondary pests. Realistically, the first step to generating the necessary data should be more contained trials where a wide range of experimental conditions and responses to abiotic and biotic stressors can be considered, including the evolution of these environments over time (particularly the development of resistant weeds or pests, or increases in secondary pests). EFSA must also select appropriate techniques to assess potential long-term effects of GMPs, including experimental and theoretical methodologies, as specified by the 2008 Council Decision. The number of contained experiments and subsequent field sites must be adequate for this purpose. The short-term nature of the trials proposed here is also likely to be inadequate to study long-term effects e.g. the development of herbicide-resistant weeds and their impact on agronomic characteristics and the wider environment.

Recommendations for establishing relevant baseline information should also have been included.

2.1. Suitability and representativeness
2.1.1. Receiving environments

Lines 290-291: Areas where the plant or seeds may be spilled should be included as there are reported instances where GM plants have established in areas where they are not cultivated (Price, B., & Cotter, J. (2014). The GM Contamination Register: a review of recorded contamination incidents associated with genetically modified organisms (GMOs), 1997–2013. International Journal of Food Contamination, 1(1), 5).

2.1.2. The extent of receiving environments
2.1.3. The assessment of site suitability
2.1.4. The assessment of site representativeness

Representativeness will also depend on whether the sites and conditions tested are sufficient to identify the composition of the plant and provide adequate materials for feeding trials. As noted above (comments on the Introduction) the dose of a target nutrient in a nutrient-altered GM plant can vary considerably in different environments, and in the case of complex pathways may even differ completely in its chemistry (e.g. in the example of cadmium, rather than zinc, being concentrated in the plant from contaminated soils). Altered nutrients can also increase susceptibility to pests (e.g. in the case of calcium, see references above), but identifying this requires the presence of the relevant pests at the trial site. The number of sites chosen (and earlier contained experiments performed) must be sufficient to identify "worst case scenarios" for environmental and food safety assessment.

Line 347: It is unclear why EFSA regards the MidWest USA as potentially representative of EU environments. Although US data may indeed be relevant (especially long-term data from commercial

cultivation) the 2008 Environment Council Decision requires guidance from EFSA to also take into account "*characteristics of ecosystems/environments and of the specific geographical areas in which GMPs may be cultivated in accordance with existing legislation*". This means areas within the EU.

Line 355: It is unclear why EFSA regards it as adequate for the applicant to define the set of factors which define suitability. The Guidance should be more explicit on what is required.

2.1.5. The assessment of non-GM reference varieties suitability
2.2. Spatial and temporal representativeness

3. Quality of starting materials used as test materials
3.1. Seed production conditions
3.2. Seed purity
3.3. Seed health and germination capacity

**4. Design of field trials**

It is unclear why EFSA is advising applicants to design field trials that it accepts will not capture interactions with target and non-target organisms (Section 7) and will therefore be of limited value in the Environmental Risk Assessment (ERA). Alternative designs are possible which can identify relevant environmental risks e.g. the impacts of HT crops on habitat loss, such as the Farm Scale Evaluations conducted in the UK (Squire GR, Brooks DR, Bohan DA, et al. (2003) On the rationale and interpretation of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Philos Trans R Soc Lond B Biol Sci. 358(1439):1779–1799). These trials led to plans to commercialise HT GM crops in the UK being abandoned, whereas in the USA the lack of any such trials or adequate regulation has led to a 90% reduction in Monarch butterfly populations due to loss of the milkweed habitat where they lay their eggs (e.g. Flockhart, D. T. T., Pichancourt, J.-B., Norris, D. R., & Martin, T. G. (2014). Unravelling the annual cycle in a migratory animal: breeding-season habitat loss drives population declines of monarch butterflies. Journal of Animal Ecology. doi:10.1111/1365-2656.12253; Pleasants, J. M., & Oberhauser, K. S. (2013). Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. Insect Conservation and Diversity, 6(2), 135–144; Hartzler RG (2010) Reduction in common milkweed (Asclepias syriaca) occurrence in Iowa cropland from 1999 to 2009. Crop Protection 29(12):1542–1544; Zalucki MP, Lammers JH (2010) Dispersal and egg shortfall in Monarch butterflies: what happens when the matrix is cleaned up? Ecological Entomology 35(1):84–91). Thus trials such as the FSEs had to be conducted prior to commercialisation in order to identify serious adverse effects. Further, the requirement for a 'step by step' approach (2001/18/EC) must be implemented, and this means that today (where ample evidence of harm is available from commercial growing of GM HT crops in the USA) such trials themselves may be regarded as posing unnecessary risks because the scale of release should be increased gradually, step by step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken (2001/18/EC, Preamble (24)).

**4.1. Data generation for the characterisation of GM plants**

Lines 496-498: The recommendation that applicants generate protein expression and compositional data in the same field trials as those performed for the agronomic and phenotypic characterisation of the GM plant is welcome. However, this means that sufficient environments must be studied to characterise variability in composition which may have important impacts on food safety. This should be made much clearer in the Guidance. As highlighted repeatedly above, it also makes little sense to make recommendations for field trials that do not encompass all the aspects necessary for

environmental risk assessment (i.e. not just agronomic and phenotypic properties of the plant itself) as well as food safety and nutritional aspects. The scope of the Guidance should be broadened to include these aspects.

Lines 514-517: Baseline information is also required if the data is to be relevant to environmental risk assessment (e.g. presence/absence of target and non-target species). Guidance on establishing the baseline is required by the 2008 Council Decision. Numerous additional categories of information relevant to assessing ecosystem impacts therefore need to be added to the list provided.

4.2. Description of the receiving environment of field trials
4.2.1. Location of field trials and their size
4.2.2. Agrometereological data
4.2.3. Soil type and mean soil characteristics
4.2.4. Cropping history
4.2.5. Post-harvest conditions

**4.3. Crop management**

**4.3.1. Herbicide regimes in the case of GM herbicide tolerant plants**

The requested information is important, but the Guidance is inadequate to ensure the provision of information needed for the environmental risk assessment. This should include "*the potential consequences for the environment of changes in the use of herbicides caused by herbicide-tolerant GMPs*" (2008 Council Decision).

4.4. Experimental design
4.4.1. Plot size and shape
4.4.2. Inter-plot distances and buffer/guard rows

**5. Agronomic and phenotypic endpoints**

The scope of the Guidance should be broadened to include relevant environmental endpoints, as required by the environmental risk assessment e.g. effects on non-target species (including increases in secondary pests) and effects of herbicide applications on HT crops on habitats and biodiversity.

5.1. Mandatory agronomic and phenotypic endpoints
5.1.1. Stand count
5.1.2. Crop cover
5.1.3. Herbicide injury
5.1.4. Flowering
5.1.5. Lodging
5.1.6. Plant height
5.1.7. Maturity
5.1.8. Seed loss
5.1.9. Fruit count
5.1.10. Seeds per fruit
5.1.11. Seed moisture at harvest
5.1.12. Seed weight
5.1.13. Yield

**5.1.14. Plant responses to biotic stressors**

It is unclear why plant responses to biotic stressors such as pests are included, but pest responses to the plant are not (e.g. development of resistant pests, or increase in secondary pests) – even though these feedback to affect agronomic properties such as yield in the longer term - and nor are impacts on e.g. pollinators or other species e.g. birds. The scope of the Guidance should be expanded, as stated above, so that field trials encompass all the necessary endpoints for the environmental risk assessment. Otherwise there will either be unnecessary risks and costs associated with conducting additional trials, or important and necessary information will be missing from applications. It is also necessary to include guidance on contained trials and other methods (including computer modelling) which can provide controlled conditions to examine the interactions of the GMP with multiple pests and other species under a variety of conditions.

**5.1.15. Plant responses to abiotic stress**

The guidance should also cover contained trials which can be used to examine response to abiotic stressors.

**5.2. Additional endpoints to be considered on a case-by-case basis**

In line with the proposed increased scope of the Guidance, additional endpoints must be considerably expanded to include e.g. impacts on target and non-target organisms. Problem formulation must include the issues specifically identified by the 2008 Council Decision, such as long-term effects, effects on non-target species and effects on the environment of herbicide use with HT crops.

Reference should be made to the need to provide particularly detailed information on composition for nutritionally-altered crops in a variety of environments, and to enable feeding trials to be conducted using all edible parts and products (e.g. for soya, cooking oil, soya milk, lecithin etc.).

5.2.1. Pollen characteristics
5.2.2. Seed characteristics

The investigation of seed characteristics should not be optional but should be performed for all applications as it is likely to be central to environmental and food safety risk assessments.

Reference is made to the possibility of altered oil characteristics in seed but not to other strategies for nutrient-altered crops which may e.g. seek to enhance iodine in the leaves of lettuces (Blasco, B., Ríos, J. J., Leyva, R., Cervilla, L. M., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M. M., … Romero, L. (2011). Does iodine biofortification affect oxidative metabolism in lettuce plants? Biological Trace Element Research, 142(3), 831–842. doi:10.1007/s12011-010-8816-9) or seek to move minerals to edible tissues in plants (Karley, A. J., & White, P. J. (2009). Moving cationic minerals to edible tissues: potassium, magnesium, calcium. Current Opinion in Plant Biology, 12(3), 291–298. doi:10.1016/j.pbi.2009.04.013). The proposed additional endpoints in seeds are therefore inadequate to deal with the wide range of potential applications in the field of nutritionally altered plants: all edible parts of the plant must be considered (any part consumed by humans or animals, including pests).

**6. Data analysis**

6.1. Data submission
6.2. Statistical analysis

**6.3. Analysis of Genotype*Environment interactions**

Lines 1301-1305. EFSA is correct to state that special attention should be paid to genotype*environment (G*E) interactions. However, the Guidance fails to consider issues of the statistical power needed to identify such interactions. The detection of G:E interactions requires experimentation in multiple conditions of interest and large population sizes are often needed to obtain sufficient statistical power. There is scope for hypothesis-generating research in multiple environments by subjecting the GM crop to environmental perturbation and studying the emerging phenotypes, but this requires carefully designed experiments (e.g. Joosen, R. V., Arends, D., Li, Y., Willems, L. A., Keurentjes, J. J., Ligterink, W., … Hilhorst, H. W. (2013). Identifying genotype-by-environment interactions in the metabolism of germinating Arabidopsis seeds using Generalized Genetical Genomics. Plant Physiology, pp.113.216176.). The Guidance as drafted is clearly inadequate to identify such interactions and must be expanded to properly include them. For example, environmental conditions and gene-environment interactions (GxE) are known to have important effects on nutrient composition of soybeans (Whent M, Hao J, Slavin M, et al. Effect of Genotype, Environment, and Their Interaction on Chemical Composition and Antioxidant Properties of Low-Linolenic Soybeans Grown in Maryland. J Agric Food Chem. 2009;57(21):10163–10174), which may have important implications for food safety assessment. To deal with this issue the phenotypic and agronomic properties of the plant will need to be studied systematically under contained conditions which represent a broad range of environmental conditions (e.g. soil types) and abiotic and biotic stressors (drought, pests etc.). "Worst case scenarios" must be included. Further experiments will need to be devised to study the potential for the ecosystem to adapt over time (e.g. to identify the development of resistant weeds or pests, or the rise of secondary pests). Computer models may also assist in developing and exploring relevant scenarios.

6.4. Correlated endpoints

**7. Relevance of agronomic and phenotypic data for environmental risk assessment**

Lines 1336-1355: It is noted that the proposed field trials will have limited value to assess interactions with target and non-target organisms. This is a major shortcoming as consideration of these effects is a requirement of 2001/18/EC for the environmental risk assessment. In fact, EFSA should have considered the need for a much wider range of experiments to address these issues including, for example; "*studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses*" (2001/18/EC, Annex IIIA part IV.B(2), as cited above).

Proposing field trials which are inadequate to assess environmental risks is inconsistent with the 2008 Council Decision which: "*NOTES with satisfaction that the Commission's mandate to EFSA to further develop and update its guidelines as regards the environmental risk assessments of GMOs includes in particular detailed assessment of the long-term environmental effect of GMPs and covers the following areas: Environmental risk assessment of potential effects of genetically modified plants on non-target organisms, development of criteria for field trials to assess the potential ecological effects of the GMPs in receiving environments, identification of the EU geographic regions where the GMPs may be released, selection of appropriate techniques to assess potential long-term effects of GMPs including experimental and theoretical methodologies, and recommendations for establishing relevant baseline information*" [emphasis added]. The Decision also "*UNDERLINES in particular the need to study the potential consequences for the environment of changes in the use of herbicides*

*caused by herbicide-tolerant GMPs*". For example: where are the required criteria for field trials to assess the potential ecological effects of the GMPs in receiving environments?

Meeting these requirements requires a fundamental revision of the scope and content of the draft Guidance in order to (1) include other experimental and theoretical methodologies (including contained experiments); and (2) propose field trials that capture appropriate data regarding environmental harms (including long-term risks) and establish the relevant baseline information. The UK Farm Scale Evaluations (cited above) are examples of studies that can capture adverse ecological effects.

As highlighted above, it makes little sense to make recommendations for field trials that do not encompass all the aspects necessary for environmental risk assessment (i.e. not just agronomic and phenotypic properties of the plant itself). It also makes little sense to progress to field trials unless adequate contained use experiments and analysis have been conducted (which may lead to a decision not to progress to field trials, under the 'step by step' approach, or which may feed in to the experimental design of any field trials). The scope of the Guidance should be broadened to include these aspects.

**7.1. Persistence and invasiveness of the GM plant**

Lines 1386-1388: It is unclear why the Guidance does not consider the persistence and invasiveness of cross-compatible wild/weedy relatives that may acquire transgenes through vertical gene flow. This is a key aspect of the risk assessment and should be included within a new draft Guidance document of broader scope.

Areas where the plant or seeds may be spilled should be included as there are reported instances where GM plants have established in areas where they are not cultivated (Price, B., & Cotter, J. (2014). The GM Contamination Register: a review of recorded contamination incidents associated with genetically modified organisms (GMOs), 1997–2013. International Journal of Food Contamination, 1(1), 5).

**7.1.1. Decision tree**

Lines 1395-1396: Where is the Guidance for the "studies performed under controlled conditions" mentioned here, and why are these restricted to only the issues of persistence and invasiveness? The scope of the Guidance should include studies under controlled conditions for all aspects of relevance to the risk assessment. It is also unclear why levels 2 and 3 (additional endpoints) are considered necessary only for the issue of persistence and invasiveness and not for other aspects of the assessment (e.g. effects on non-target organisms, or effects of herbicide spraying on habitats).

7.1.2. Data requirements

**Conclusions**

It is inappropriate to focus the experimental design of field trials on the characterisation of phenotypic and agronomic characteristics of the GM plant, when other aspects (impacts on the wider environment) must also be included in field trial design. Restricting the scope of the guidance in this way makes no sense because it may lead to more field trials being conducted than is necessary (in order to obtain other endpoints in future), or to the lack of important data (if applicants think that only the data required by this guidance is needed). In addition, the lack of a 'step-by-step' approach, which should first specify data that can be obtained from contained

experiments and other approaches (e.g. computer modelling), is incompatible with the legislation (2001/18/EC).

**Documentation provided to EFSA**

It is notable that the draft Guidance arises from a self-task mandate, instead of being driven by the mandate laid out in the 2008 Environment Council Decision, which would have led to a more comprehensive approach.

**References**

EFSA should draw on the prior knowledge of risks reported in the literature from both experimental development and commercial uses of all these types of crops, in order to aid the design of experimental and theoretical methodologies and field trials (as required by the 2008 Council Decision). Impacts of herbicide use on HT crops and effects on target and non-target organisms (including increases in resistant and secondary pests) have been identified as of particular importance. Some relevant additional references have been cited in the comments above.

**Appendix A. Decision tree to inform the problem formulation for the persistence and invasiveness assessment of GM plants**

It is unclear why oil seed rape is rated only medium risk: this should be high risk.