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KENMERK CGM/110214-02
ONDERWERP Advies m.b.t de documenten 'Guidance on the environmental risk assessment of GM plants' en 'Scientific opinion on the assessment of potential impacts of GM plants on NTOs'

Geachte heer Atsma,

De Europese Commissie is voornemens om een bindend richtsnoer voor de milieurisicobeoordeling van genetisch gemodificeerde (gg-)planten vast te stellen. Als basis voor dit richtsnoer gebruikt zij de 'Guidance on the environmental risk assessment of genetically modified plants' die recent door de EFSA is uitgebracht. Naar aanleiding van uw verzoek om over dit document te adviseren, stuurt de COGEM u de volgende reactie.

Samenvatting
Het richtsnoer geeft een goed overzicht van de vele aspecten die van belang zijn voor een milieurisicoanalyse van gg-planten en beschrijft de verschillende invalshoeken en wetenschappelijke methoden hiervoor. Het gevolg hiervan is echter, dat er te weinig concrete aanbevelingen gedaan worden over hoe een milieurisicoanalyse uitgevoerd moet worden. Het is onduidelijk welke informatie precies benodigd is voor een milieurisicoanalyse. Hierdoor is het niet geschikt als bindend richtsnoer, omdat het niet duidelijk is aan welke concrete eisen een milieurisicoanalyse moet voldoen. Het huidige richtsnoer is in aangepaste vorm wel uitstekend toepasbaar als referentiekader.
De COGEM stelt voor om, in plaats van een algemeen richtsnoer, handleidingen te ontwikkelen die specifiek ingaan op de milieurisicoanalyse van bepaalde categorieën gg-planten, zoals voor insectenresistente planten of planten met een veranderd metabolisme, en deze richtsnoeren bindend te verklaren.
Daarnaast wijst de COGEM erop dat het bepalen van beschermingsdoelen zoals zeldzame flora en fauna een politieke keuze behelst. Dit aspect van de milieurisicoanalyse moet daarom door de relevante competente autoriteiten gedaan worden, en niet aan een aanvrager worden overgelaten.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

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Comments on the 'Guidance on the environmental risk assessment of GM plants' and on the 'Scientific opinion on the assessment of potential impacts of GM plants on NTOs'

COGEM advice CGM/110214-02

1. Introduction

The European Commission intends to develop a legally binding text that can serve as the basis for applicants of GM authorizations, for Member States and for EFSA when assessing the environmental risk of genetically modified (GM) plants. The guidance document on the environmental risk assessment (ERA) of GM plants that was recently published by EFSA, serves as the basis for this document. COGEM has been asked by the Dutch Ministry of Infrastructure and the Environment to advise on the recently published guidance document. Given the time available and the complex nature of the document, COGEM has focused on major concerns.

The guidance document gives a broad overview of the different aspects of the ERA and describes the various approaches that can be used in the ERA. The basic principles of ERA: hazard identification, hazard characterisation, exposure characterisation, risk management strategies and overall risk evaluation, are explained. The document dilates on the risk assessment and uses the basic principles as a guide.

It describes the current ideas with regard to ERA which are based on the present generation of GM-plants, i.e. insect resistant Bt crops and herbicide tolerant crops, and incorporates scientific literature. A disadvantage of this approach is that the document appears to agree less with the ERA of other types of GM plants like plants with a changed metabolism.

The guidance document is very broad as it intends to cover all types of GM plants and to mention all options and methods that might be used in the ERA. However, a consequence of this is that its use as a guidance document is limited. The document does not list exact or precise requirements for data and experiments necessary for the ERA. Moreover, sometimes it appears to be confusing or even inconsistent. Therefore, as a guidance document it will not succeed to provide clarity for applicants, risk assessors and competent authorities. Most likely, the present document will only deepen the discussion between applicants, EFSA and the competent authorities of the different member states concerning the data requirements for specific notifications.

An adapted version of the guidance document is excellently suited as a background document or reference for the ERA of GM plants, but the guidance document does not meet the qualifications of a binding guideline. Therefore, it should not be transformed into a legally binding provision.

Documents that are focused on providing guidance for specific categories of GM plants, e.g. herbicide tolerance, insect resistance, or changed metabolic pathways are more suited as

guidance documents. Therefore, the development of legally binding guidance documents that are more precise and apply to specific categories of GM plants is proposed.

2. General comments

Protection goals and limits of concern should be identified by relevant authorities
In the guidance document it is stated that the applicant has to "identify through relevant assessment endpoints the aspects of the environment that need to be protected from adverse effects". The identification of protection goals cannot be left to an applicant. Protection goals depend on policy goals and should therefore be identified in a political process. The identification of protection goals and the translation of these protection goals in assessment endpoints should be done by the relevant competent authorities. Similar remarks can be made concerning the 'limits of concern'.

Little attention to ERA of new generation of GM plants
The purpose for which GM plants are developed is changing and extending. In the past developments were focused on the introduction of traits resulting in concise and predictable alterations like herbicide tolerance or insect resistance. Currently a new generation of GM plants is under development in which traits are modified resulting in major changes in metabolic pathways. For instance, developments are focused on GM plants with abiotic stress tolerance or otherwise altered metabolic pathways. In the guidance document little attention is paid to the ERA of this new generation of GM plants. Specific guidance is needed with regard to the aspects that are important for the ERA of these GM plants.

Unclear descriptions and data requirements
For several aspects in the ERA, the requirements are formulated vaguely but they appear to involve considerable amounts of data. Words like 'should consider', 'may play a role', 'might be necessary' and 'are recommended' are vague and do not provide guidance with regard to the necessity of the studies mentioned. In addition, it often remains unclear how the large amount of data that is mentioned will be used in the ERA.

For instance, a description of the range of relevant biotic and abiotic interactions likely to occur in the receiving environment has to be given. This request involves vast amounts of data and literature and is formulated vaguely. Since in most cases baseline information on the range of relevant biotic and abiotic interactions will not be present, the requested information will be of little use for the ERA since the information on the GM plant cannot be put in perspective. Only clear and precise requests for information, focusing on data directly applicable in the ERA should be included in the guidance document.

Sufficient statistical power not possible in field experiments
The statistical analysis that is proposed in the guidance document stresses the importance of obtaining a high statistical power. Such a requirement is useful and beneficial with regard to laboratory experiments, but for field trials it is almost impossible to obtain sufficient statistical power. Population numbers of organisms in the field have a tendency to fluctuate widely over time and therefore the achievement of a 'sufficient' statistical power involves an unrealistic high number of replicates for each of the NTOs to be examined. The requirement to obtain sufficient statistical power should be eased for field trials.

Too much trust in modelling and meta-analysis
Throughout the guidance document an unwarranted trust is shown in modelling and meta-analysis. Especially when information or data is lacking, modelling is not the answer. Modelling is only possible if data are present as input for the modelling system. Also meta-analysis is only possible if sufficient scientific literature is available.

3. Comments on specific areas

The following comments are listed according to the text in the guidance document. Numbered paragraph headings refer to the exact paragraph as found in the guidance document. A few comments refer to the Opinion on NTOs. This is indicated when this is the case. Otherwise, paragraph headings refer to the guidance document.

2.2.1 Comparative safety assessment as a general principle for the risk assessment of GM plants
In the guidance document a table is presented with examples of environmental protection goals (page 17). This table lists several legal documents that should be considered by the applicant. Often the scope of the legal documents mentioned is the conservation of biodiversity. However, it is not clear how the conservation goals that are mentioned in these documents should be translated into concrete protection goals such as species that should be protected. As mentioned earlier, the identification of protection goals is a political process. Protection goals should therefore be identified by the competent authorities.

2.3.1 Choice of comparators
The ERA strategy is based on the estimation of similarities and differences of a GM plant in relation to a comparator. This section deals with the selection of an appropriate comparator. According to the guidance document, the conventional counterpart should be selected as the comparator. The conventional counterpart is a non-GM line with a genetic background 'as close as possible' to the GM plant.

The guidance document states that for the ERA of single and stacked events, it can be advantageous to include negative segregants as an additional comparator besides the conventional counterpart (page 21 and 23). It has to be noted that in this case differences in the genetic background of the parental lines of the GM plant, and the non-GM plant required for obtaining the negative segregant, have to be taken into account. GM lines to be assessed can be homozygous or heterozygous. In case a negative segregant is obtained from a cross between a heterozygous GM plant and a heterozygous non-GM plant, all kind of combinations of alleles can occur in the progeny, resulting in differences in genetic background of the obtained negative segregant and the GM plant under assessment. The observed differences can be addressed undeservedly to the presence of the introduced trait in the GM plant, as these differences could also have been caused by the different genetic backgrounds. The guidance document should pay attention to this aspect. It should recommend that, in case a negative segregant is selected as comparator, the genetic background of the GM plant and the negative segregant should be as close as possible.

In the guidance document more attention should be paid to the issue of unintended stacking. If a GM plant is able to form feral populations, applicants have to take the potential environmental risk implications of the possible presence of other GM events of the same plant in the same receiving environment into account. These events, possibly produced by other breeding companies, may harbor traits different from the trait introduced in the GM plant under assessment. Unintended stacking of traits can occur by outcrossing of the different GM plants with wild relatives during consecutive years. The guidance document indirectly mentions this aspect in the chapter on 'Receiving environments' (page 24) but no guidance is provided on the risk assessment of this issue.

As stated in the 'General comments', the purpose for which GM plants are developed is changing and extending. Whereas in the past developments were focused on the introduction of traits resulting in concise and predictable alterations like herbicide tolerance or insect resistance, currently a new generation of GM plants is under development in which traits are modified resulting in major changes in metabolic pathways. For instance, developments are focused on the increase of tolerance against abiotic stress like drought or salt. As a result, potential new future cultivation areas arise. According to the guidance document, relevant receiving environments have to be selected for the ERA of a GM plant and potential future areas have to be added (Table 2, page 26). However, for stress tolerant GM plants, it might be the case that the non-GM plant can hardly or cannot at all be cultivated in the potential future area and, consequently, the conventional counterpart (the comparator) can hardly or cannot be cultivated as well. For these cases guidance is needed for the performance of an appropriate ERA. The risk assessment will be based on a theoretical consideration rather than on a comparative risk analysis of field experiments with the GM plant under assessment and an appropriate comparator.

2.3.2 Receiving environments

According to the guidance document, one of the characterizing components of a receiving environment is the 'geographical zone'. Examples are given concerning the elements of a geographical zone. However, the geographical zones themselves are not defined. In appendix A of the guidance document examples are given of zoning concepts for geographical regions. Although the presence of a phytogeographic zoning concept is welcomed, it is not clear which zoning concept should be used in the ERA. A clear choice should be made on the zoning concept to be used in the ERA.

2.3.3 General statistical principles

This chapter in the guidance document deals with the experimental and statistical design of laboratory tests and field experiments. In general COGEM endorses the difference which is made between biological and statistical significance. The rationale behind the statistics is clear. However, there is a major concern relating to field experiments.

Population numbers of organisms in the field have a tendency to fluctuate widely depending on, among other things, the weather. When the statistical analysis as proposed in the guidance document is applied to the field data of different non-target organisms (NTOs) sufficient power is only achievable with unrealistic high numbers of replications.

Furthermore, some criticisms have to be made concerning the proposed statistical methods.

Testing for difference and equivalence are prescribed as statistical methods of choice. However, emphasis is on equivalence testing. Equivalence analysis is relatively new to the field of plant biotechnology. The document fails to explain why equivalence analysis is preferred over testing for difference. The document mentions that the type II error (false positives) is difficult to calculate with difference analysis. However, in case of typical simple statistical testing for difference methods like an ANOVA test or t-test the type II error can be easily computed and there is no need for equivalence testing. This appears to be acknowledged in the opinion on NTOs where it is stated that 'simple statistical methods' should be used (page 47). This appears to be in contradiction to paragraph 2.3.3 of the guidance document.

While the added value of equivalence testing in certain cases is acknowledged, in several cases such an added value does not exist and it is questionable how useful it is to prescribe equivalence testing as the only method to be used.

2.3.3.3 Power analysis

The guidance document states that each study should have sufficient power to provide reasonable evidence of safety or no effect. This is a generally accepted notion. In case of laboratory experiments sufficient statistical power can be achieved since it involves standardised tests performed under highly controlled conditions and the number of necessary replicates can easily be achieved. However, field experiments are notoriously difficult in this respect. The effects found in the field test will strongly depend on the scale of the set-up, the time that the experiment lasts and the surrounding landscape. Field tests are almost always carried out on a small scale and it is not clear whether scaling-up to crops will produce similar results. A small plot of maize in a landscape dominated by potato fields is likely to attract different NTOs (mainly those that move a few meters from a potato to a maize plant) than a large maize field. Also many NTOs will take years to find a crop and build up significant populations. It is well known that plants can escape from herbivores by being non-apparent. This has the consequence that results of field tests are much more variable than those of laboratory tests and will be strongly context dependent.

Moreover, for computing power the standard deviation should be known before the experiment starts.[1] In field testing, it is simply not possible to estimate standard error in a variable (like e.g. the number of ladybirds) beforehand. This will depend on the surrounding landscape, previous weather conditions and unknown factors (like the fraction attacked by parasites). Because of build-up of populations it would even be very difficult to estimate standard deviation in year 2 from that in year 1. Many insect populations, bumblebees included, show large variation in population numbers over time.

The guidance document appears to acknowledge these problems. It states that "for ecological field trials the restriction on the land available for experimentation combined with unavoidable environmental heterogeneity usually necessitates some compromise…." (page 29). It continues with the remark that "optimal experimentation design shall be directed to attain power as high as possible" (page 29). However, further on in the text it is stated that "For each study, applicants shall provide an analysis that estimates the power for each difference test on each measurement endpoint, based on the stated effect size and assuming a

[1] Perry JN *et al.*, (2009). Statistical aspects of environmental risk assessment of GM plants for effects on non-target organisms. Environ. Biosafety Res. 8(2): 65-78.

5% type I error rate" (page 29). Requiring a high statistical power for each functional group in a field experiments presents a difficult if not impossible task.

To illustrate this problem, a rough calculation can be made for aphids (as an example of a NTO) on potato. If interpreted the guidance document correctly, it is estimated - based upon data in the literature, a publicly available computer program[2], 2-sided P-values and a limit of concern of 50% - that according to the guidance requirements approximately 120 replicates are needed (or higher if the variation in numbers of aphids present is highly divergent between plots). It should be noted that even then, this only leads to a false sense of security, since the results are based upon arbitrary chosen values, confusing statistical significance with biological significance.

2.3.3.5 Experimental design

In this paragraph of the guidance document it is stated that earlier explicit requirements for replication to achieve representativeness do not apply to confirmatory field data for the assessment of unintended effects on for example NTOs. Consequently, there is no requirement for a minimum number of sites and/or years (page 31). However, while the requirements for representativeness are relaxed the requirement for statistical power remains unaltered. This seems to be contradictory because to achieve a 'sufficient' statistical power with field experiments an unreasonable high number of replicates have to be made, as shown above.

In conclusion: paragraph 2.3.3 in the guidance document provides an insight in statistical methods and set-up of experiments. The main concern with this paragraph is the required statistical power for field experiments. It is doubtful whether what is considered 'sufficient' in the text, can be achieved for all instances in field experiments. In case of potential effects on NTOs numerous replicates are necessary to achieve sufficient statistical power, but this will not significantly contribute to the ERA. Therefore, the requirements for statistical power should be relaxed for field experiments.

2.3.4 Long term effects

According to the guidance document, an analysis of the long-term effects of the release of a GM plant has to be carried out by the applicant. Due to the complexity of the analysis of long-term effects, it is understandable that the guidance document does not give clear criteria for the analysis. Still, the applicant is required to make an estimate whether long-term effects are expected to occur and how the monitoring plan for cultivation should be adjusted accordingly. In the guidance document it is stated that applicants should propose indicators and reference databases for EU-wide surveillance of long-term effects. Each member state has its own regulations for monitoring environmental effects outside the field. It is questionable that the applicant is supposed to have knowledge on the subject of indicators and reference databases and is asked for specific information, while the guidance document does not clearly specify criteria for an analysis of long-term effects. Since the analysis of long-term effects is closely connected to general surveillance, it is strongly advised that the analysis of long-term effects is incorporated into the chapter on post-market environmental monitoring.

[2] http://www.quantitativeskills.com/sisa/calculations/power.htm

2.3.5 Risk assessment of GM plants containing stacked transformation events

In the guidance document is stated that for the risk assessment of stacked events, the risk assessment of the single events included in a stack is always a pre-requisite (page 38). An argumentation for this pre-requisite is lacking. It has to be noted that in case of the ERA, it is not relevant to assess the single events themselves if they will never be used for import, processing or cultivation. Furthermore, if stacked GM crops are to be cultivated, the ERA of a single event is not always relevant because wild relatives are often lacking and as a consequence segregation by outcrossing with wild relatives will not take place. Feral populations do not occur and volunteers are rare. This is for instance the case for maize and soybean.

According to the guidance document, the ERA of a GM plant containing three or more events shall cover all sub-combinations of these events as independent stacks because after cultivation of a stacked event, sub-combinations of events may arise by segregation (pages 38-39). However, the ERA on sub-combinations of a stacked event is not always relevant because:

- as mentioned above for maize and soybean, when wild relatives are lacking, volunteers are rare and establishment of feral populations has never occurred. Consequently, segregation by outcrossing with wild relatives will not take place,
- farmers do not propagate seeds for future use since F1 hybrids are involved and, due to patent restrictions, farmers are not allowed to. Consequently, sub-combinations will not occur in the field,

In addition, it is not necessary to perform an ERA on each possible sub-combination of events present in the stack if there is no evidence that their gene products synergistically or antagonistically interact, *e.g*. when a Bt gene is combined with a herbicide tolerance gene.

Furthermore, it has to be noted that, if sub-combinations occur by segregation, segregants can markedly differ in their genetic background depending on the genetics of the parental lines. This hampers the comparison for the potential unintended effects of the inserted genes.

In conclusion, it should be noted that there are circumstances where assessing single events and sub-combinations of events included in a stack is not relevant, and as a consequence, without contributing significantly to the ERA raises the costs for notifications considerably as the assessment is time consuming and cost demanding. Therefore, it is recommended to only require an evaluation of single and different combinations of events, if these events will segregate in the field or in the environment, and if there is a scientific reason that there can be synergistic or antagonistic effects between the different events. In this way, irrelevant notifications are prevented and subsequently, needless administrative workload and eventual delay in authorization processes is significantly precluded.

3.1 Persistence and invasiveness including plant-to-plant gene flow

Fitness of the GM plant plays a key role in the assessment of the potential adverse effects due to persistence or invasiveness of the GM plant. Therefore, a clear and unequivocal definition of ‘fitness’ is of paramount importance. The guidance document uses the word ‘fitness’ more or less casually throughout the text. A clear definition of ‘fitness’ will lead to a clear description of requirements for the ERA.

The scientifically based definition of fitness is ‘the average contribution to the gene pool of future generations that is made by an average individual of the specified genotype or phenotype’. However, this definition is of limited value for measurements on fitness in practice. The definition of ‘fitness’ in the guidance document (footnote 18, page 40) by Crawley *et al*. (1993) is more workable. This definition states that fitness is ‘the number of seeds (or propagules) produced per seed sown, including the whole life cycle of the plant’. However, this definition only applies to a selfing annual species without a seed bank. Especially in the case of a polycarpic perennial and/or plants with a long-lived seed bank it will be extremely difficult to evaluate fitness differences within a reasonable time. For those plant species that Crawley’s fitness definition does not apply to, information and/or experiments will be needed on several of the characteristics mentioned in section 3.1.2.2. This important limitation of the definition of fitness by Crawley *et al.* should be included in the guidance.

In the guidance document a staged approach for information requirements relating to persistence and invasiveness is described (page 41 and further, including figure 4). The approach seems to be a guide to perform the hazard characterisation step and test formulated hypotheses on adverse effects and hazards. Because the staged approach is described in the section on problem formulation it seems that experiments are necessary in this first step of the risk analysis. Therefore, the description of the scheme in figure 4 and the figure itself should be placed under the section on hazard characterization (3.1.2).

The guidance document remarks that outbreeding depression could cause wild relatives to decline locally or become extinct (page 40). It should be noted that in the case of e.g. problematic weeds, this should be seen as an advantage.

The guidance document states that the questions in figure 4 (page 43) are meant to lead to an estimate of the likelihood of occurrence of adverse effects in ruderal, semi-natural and natural environments. Agricultural environments should be included in this sentence, since a transgene might spread to relatives on an agricultural field, possibly increasing a potential adverse effect.

The second most important purpose of the staged approach is to ensure that information requirements remain proportionate to the potential risk. The information requirements for the different stages in the staged approach are given (pages 44-45). The data requirements as outlined here are very large and it remains unclear if data on all proposed characteristics should be made available. The required data should not be necessary for all crops. For instance, if a crop has characteristics that will overrule any other weedy or invasiveness characteristics, like the maize characteristic that its kernels are hardly scattered during ripening of the cob and harvest, newly generated data on weedy or invasiveness characteristics are of no additional value. Also, studies on effects on reproduction, germination, seed persistence, invasiveness and hybridization (page 21 and pages 44-45) might not be relevant in all cases. The plant species under assessment might not occur outside production systems in Europe, due to its specific properties. In the case of for instance maize, cotton and soybean, wild relatives are fully lacking in Europe and volunteers are almost absent.

One of the characteristics for which information should be given to determine whether the GM plant and its progeny can reproduce and hybridise under EU conditions, is attractiveness to pollinators. Field experiments to assess if a plant is more or less attractive to pollinators than its appropriate comparator are difficult, time-consuming and costly. The guidance document does not specify which approach should be used for the experiments. Also, these experiments will be of little value for an ERA. Moreover, most plants have some degree of cross-pollination, even if they are self-pollinated. When suitable pollinators are not present in the receiving environment, wind pollination might take place, although inefficiently. Therefore, a worst case approach should be used: no data on attractiveness to pollinators should be required as all GM plants should be considered to be cross-pollinated to some extent.

Another characteristic necessary to determine reproduction and hybridization potential of a GM plant is seed persistence leading to volunteer occurrence (page 45). It should be noted that post-harvest field inspection data will not provide indications on the overwintering potential of GM plant seeds, unless a suitable comparator is used and data on these plants is collected after winter. In addition, it is stated here that seed burial experiments might be used. However, these experiments take years to conduct. It is questionable if the merit of these experiments for the ERA outweighs their cost.

The guidance document states that for stage 3, experiments may be required to measure relative fitness in semi-natural and natural environments (page 47). It is important to note that these experiments are difficult, take years to execute and are expensive. Moreover, the experiments often do not lead to a clear conclusion on a change in fitness. Especially in the case of perennials fitness experiments in (semi-) natural environments are not realistic and should not be obligatory. However, this aspect can be taken into account in the post-market monitoring phase.

For cultivated plant species that have a low potential for becoming feral or invasive in the EU, such as maize or soybean, experiments measuring relative fitness should not have to be performed. This should be included in the staged approach as described in figure 4.

On the other hand, it should be noted that if potentially invasive GM-plants are tested on field plots in semi-natural or natural environments, the risk of these tests could be high. Therefore, field testing of potentially invasive GM plants should be carefully considered and in some cases a conclusion might be drawn that the development of the GM plant should be ceased.

In conclusion, for GM plant species that have a general low potential for ferality, invasiveness and/or hybridisation, the guidance document should state that the ERA should start with considering whether the introduced trait leads to a significant change in these characteristics. If a significant change is not anticipated, extensive risk analysis on these aspects is not necessary.

3.2 Plant to micro-organism gene transfer

This section in the guidance document appears to be based on concerns regarding the spread of antibiotic resistance genes in the microbial environment and the consequences this could have for the use of antibiotics in human and veterinary medicine. However, several antibiotic

resistance and herbicide tolerance genes are present abundantly in natural microbial communities. No ERA should be required for those genes that are already present abundantly in the environment.

The applicant is required to address several aspects concerning horizontal gene transfer, e.g. provide a molecular characterisation of the inserted DNA sequences and consider the possible presence of recipient micro-organisms in the receiving environment(s) (page 50). In addition, an analysis of the overall risk is required (page 52). It is unclear what kind of information has to be provided for an ERA of plant to micro-organism gene transfer. It seems to range from a full description or characterization of the entire soil community to the statement that horizontal gene transfer is unlikely. The latter is useless and the first hardly possible. Also, the guidance document recognizes the limitations to experiments on microbial communities. However, it does not clearly state if hazard, exposure and risk characterizations require a theoretical argumentation or if and when laboratory tests and field experiments are necessary. In addition, it is not clear how the information provided will lead to conclusions on the environmental risk and the envisaged methodology is obscure.

Most importantly, in this section it is stated that horizontal gene transfer is a rare event under natural conditions. Although horizontal gene transfer from plant to bacteria can be provoked under artificial laboratory and greenhouse conditions, it has never been described under field conditions. The guidance document should be changed on this point.

Also, the guidance for the ERA of plant to micro-organism gene transfer should be based on the fact that horizontal gene transfer has never been described under field conditions. It should therefore be limited to those cases where traits have been introduced in the GM plant that might enhance the occurrence of horizontal gene transfer. If this is the case, it should be considered whether the transgene could provide a certain selective advantage which could lead to the subsequent establishment of the transgene in the microbial community.

In conclusion, the ERA should be limited to those aspects that might enhance horizontal gene transfer. In addition, genes that exist abundantly in natural microbial communities should not be included in the ERA.

3.3 Interactions with target organisms

This section of the guidance document discusses the development of resistance of target organisms. However, it is questionable whether development of resistance is an environmental problem. Resistance of target organisms renders the GM plant useless and can lead to crop losses and damage for farmers. This is a well-known problem in agriculture which can occur in any pest resistant conventional crop and is not GM specific. Therefore, it is an agronomical or economical problem and not an environmental issue.

Besides, the considerations in this section appear to be based mainly on the ERA of insect resistant Bt crops. Although it is mentioned that various strategies can be deployed to obtain resistance to plant pathogens and pests, it deals only with plants expressing toxic substances against herbivorous arthropods. The data, which apparently have to be collected (or considered?) for the hazard, exposure and risk characterization, apply to arthropods and not to fungi or other plant pathogens. Moreover, it is assumed that the GM plant produces a toxin.

However, there are other strategies which can be employed to obtain resistant GM plants, like inducing hypersensitive responses or expression of repellents. Most likely, pathogens will also develop resistance against these approaches in time. The proposed data collection such as determination of expression levels or intake of transgenic products will give no insight or prediction in the occurrence of resistance development.

The applicant is requested to submit a vast amount of information or data on the target organism. For example on the biology, life cycle, population genetics, behaviour and distribution in environments in Europe. It is questionable whether this information is available and it is unclear what this information contributes to the ERA or to the prediction of resistance development.

The paragraph on plant pathogen interaction (page 53) is one of the rare occasions that the text deals with other pathogens than insects. Unfortunately, the remarks on potential mechanisms for evolving resistance involving complementation and heterologous encapsidation are not correct. Firstly, the previous perceived risks of complementation and heterologous encapsidation have little to do with the development of resistance and concern the development of new viruses. Secondly, it is based upon outdated views on the risks involved with GM plants expressing viral coat proteins.[3]

In view of the above, this paragraph should be deleted from the guidance document and should be replaced by the recommendation to plant refugee areas with susceptible non-GM host plants in case of arthropod resistant GM crops. The refuge strategy has been shown to effectively delay resistance.[4]

3.4 Interactions of the GM plant with non-target organisms

In addition to the section on NTOs in the guidance document a scientific opinion on the assessment of potential impacts of GM plants on NTOs was published. The guidance provided in this document is presented in a condensed format in the overall guidance document. Unless mentioned otherwise, the comments listed refer to both documents.

General statement

The guidance document states that in all cases the possible impact of a GM plant on NTOs should be tested. This is a new requirement in the ERA. It has to be noted that the required laboratory tests in many instances cannot be carried out because no transgenic proteins are expressed in the GM plants. Moreover, laboratory tests carried out on a limited set of arthropods will not be informative or have a predicative value. Also, because the nature of the potential effect is unknown. Therefore, an assessment of the possible impact should only be required when there is an indication that expression of the transgene could adversely affect NTOs. If there is no reason to expect a negative impact on NTOs, field or laboratory tests should not be required

[3] Prins M, Laimler M, Noris E, Schubert J, Wassenegger M, Tepfer M (2008) Strategies for antiviral resistance in transgenic plants. Molecular Plant Pathology 9: 73-83.

[4] Tabashnik B Gassmann AJ, Crowder DW, Carriére Y (2008). Insect resistance to Bt crops: evidence versus theory. Nature Biotechnology 26: 199-202

In case an adverse effect can be expected than testing is required. However, if laboratory and semi-field tests show no significant adverse effects, specific field testing should not be required. The presence of NTOs could be monitored during field trials that are carried out for other purposes. In addition, during General Surveillance possible effects on NTOs should be monitored.

Specific comments
The guidance document defines potential NTOs as all those species directly and/or indirectly exposed to the GM plant, and which are not targets of the newly expressed metabolite(s) in these plants (page 55). An adverse effect on pest species that are not the target of a newly expressed metabolite could be considered a beneficial effect. In addition, when the effect of a GM plant on natural enemies like parasitoids and predators is investigated one should be aware that a negative effect is not always a direct effect of the GM plant, but can also be the result of a reduced quantity or quality of prey or host organisms. In the latter cases the observed effect should not be attributed to the GM plant. These aspects should be taken into account in the ERA.

New generation of GM plants
The considerations in this section of the guidance document appear to be based on insect resistant Bt crops. According to the guidance document, in general the first step of the assessment of the potential impact of the GM plant on NTOs are laboratory experiments where the NTOs are exposed to purified metabolites (tier 1a) and GM plant material (tier1b). It is mentioned that metabolic pathways can be altered (page 61), but no specific guidance is given for the assessment of GM plants in which no new metabolites are expressed (silencing), complete metabolic pathways are altered, or plant architecture is changed. On basis of the general guidance in this section, it could be assumed that for GM plants that have altered metabolic pathways known to affect NTO-plant relationships, laboratory experiments with GM plant material should be conducted and that for GM plants with an altered plant architecture semi-field experiments are required. These examples could be mentioned in the guidance document in order to give a general idea of the type of experiments that are required for the assessment of new types of GM plants. It should be determined case by case which experiments are needed precisely.

Non-arthropod NTOs
In the guidance document it is stated that the ERA should address the potential environmental impact on population levels of herbivores, natural enemies, symbionts, parasites and pathogens (page 55). The section on the interaction of the GM plant with NTOs is however focused on non-target arthropods. Although other NTOs e.g. gastropods, nematods, rhizobacteria and mycorrhiza, are mentioned, terms like ‘eggs’ and ‘instar’ (page 66) reflect the focus of this section on non-target arthropods. In the guidance on laboratory experiments with natural enemies only those aspects that are important for the assessment of possible effects on predators and parasitoids are mentioned. No attention is paid to natural enemies like pathogens or parasites, or to symbionts. Although the current generation of GM plants is not expected to have impacts on NTOs other than non-target arthropods, it is possible that in the future GM plants are developed to affect organisms like fungi, bacteria or nematods. For

these GM plants guidance on the assessment of potential impacts on NTOs should be developed in due time.

3.4.1.2 Definition of assessment endpoints

Selection of focal species

The guidance document states that species selection shall be performed according to four steps (page 56). Species selection is focused on those species that are important for ecosystem functions and services. In addition, according to the guidance document estimation of ecosystem functions and services could replace data on focal species (page 58). Although the guidance document states that additional species of economic or aesthetic or cultural value, or species of conservational importance considered as threatened or endangered may need to be included, the focus on ecosystem functions in the species selection suggests that biodiversity is not considered important.

Except for a table with examples of functional groups (table 3, page 57), no concrete guidance is given on the actual species that should be included in the ERA. No recommendations are given with regard to the (combinations of) NTO species that should be selected. The report issued by Scholte & Dicke (2005) gives a more concrete indication of specific NTO groups per crop and event.[5] The approach of Scholte & Dicke (2005) should be adopted in the guidance document.

3.4.1.3 Considering the exposure patterns to NTOs

The guidance document states that when the GM plant is not cultivated, the ERA has to focus on indirect exposure to products of the GM plant (e.g. through manure and faeces from the gastrointestinal tracts of animals fed the GM plant, and by-products of industrial processes) (page 60). Further concrete guidance on how specific testing on this aspect should be executed remains absent. In addition, this exposure route is only relevant in very specific cases like pharmaceutical crops (which will most likely not be used as feed) and the necessity to include indirect exposure in the ERA should therefore be determined on a case by case basis.

If gene flow to cross-compatible wild/weedy relatives and feral plants inside or outside the areas of cultivation is likely to occur, exposure of NTOs over life cycles and seasons should, according to the guidance document, be assessed (page 60). It remains, however, unclear whether data is required for the assessment. In most cases, NTOs that are relevant to cross-compatible wild/weedy relatives are also relevant to the crop itself. In these cases NTO tests with the GM plant will provide sufficient information on the possible effects on NTOs in case gene flow occurs. Only when there is a reason to assume a deviation in the NTOs present on cross-compatible wild/weedy relatives additional data on the NTOs on these relatives should be requested.

[5] Scholte E. & Dicke M. (2005) Effects of insect-resistant transgenic crops on non-target arthropods: first step in pre-market risk assessment studies. COGEM Research Report 2005-06

3.4.1.4 Definition of measurement endpoints

In the guidance document, behavioural characteristics (e.g. searching efficiency, predation rates, food choice) are mentioned as a sub-lethal effect that may be considered when appropriate. It is not stated when the assessment of behavioural characteristics is considered necessary. In addition, no guidance is given on the assessment of behaviour. Laboratory experiments are not suited for the assessment of behavioural characteristics as they fail to encompasss realistic conditions. The unsuitability of laboratory experiments to study effects on behaviour is recognized for some organisms (page 30). It is stated that for these organisms field testing is of special importance. However, the assessment of behaviour in field experiments is almost impossible. The effect of a GM plant on NTOs is reflected much better in the relative fitness of a NTO. The assessment of sub-lethal effects through growth pattern, development rate or reproduction parameters is already part of the guidance document. The assessment of behavioural characteristics should therefore be removed from the guidance document.

3.4.1.6 Specific hypothesis-driven investigation

A decision tree is presented that depicts the questions that need to be answered in order to determine the requirements for NTO studies (figure 6, page 64). The decision tree starts with questions about laboratory studies with purified proteins (tier 1a) and the GM plant (tier 1b). This indicates that laboratory experiments are always necessary, even if there is no reason to assume an adverse effect on NTOs based on the characteristics of the transgene. This would mean that for instance events with a herbicide tolerance gene or potatoes with an altered amylose content have to be tested for NTO effects in the laboratory. However, such effects have never been demonstrated. NTO tests should only be required when the transgene gives reason to assume an adverse effect on NTOs. As a consequence an additional question box should be added on top of the decision tree. In this question box it should be asked whether there is an indication that the GM plant could affect NTOs adversely. If the answer to this question is “no”, no further NTO studies are required. If the answer to this question is “yes” one should proceed to the first question of the current decision tree.

3.4.1.7 Data requirement for the evaluation of possible unintended effects investigation

According to the guidance document all GM plants should be assessed for possible unintended effects on NTOs. Firstly, in our opinion ‘unexpected effects’ would be a better description of the type of effects that are meant in this paragraph, because one could argue that all effects on non-target organisms are unintended effects.

In the guidance on the evaluation of possible unintended effects it remains unclear which NTOs should be tested for unintended effects of the GM plant. In addition, it is stated that applicants should ensure that some field generated data are included. However, in the Opinion on NTOs it is stated that field generated data are a fundamental source of information in the *majority of cases*. Therefore, it is not clear whether and when field generated data are considered indispensable for the assessment of unintended effects.

As mentioned before, assessing the possible impact of a GM plant on NTOs should only be required when there is an indication that expression of the transgene could adversely affect NTOs. If there is no reason to expect a negative impact on NTOs, field or laboratory tests should not be required. The purpose of post-market environmental monitoring (General

Surveillance) is the detection of possible unanticipated effects. Therefore, detection of these effects on NTOs should be assessed in the context of General Surveillance.

3.4.2.1. Laboratory studies

In the section on laboratory studies it is stated that when the aim is to demonstrate the equivalence of the GM plant to the appropriate comparator, the standard tests should include this comparator as a negative control […] as well as a positive chemical control to prove the functionality of the experimental setup, as advised in the pesticide test guideline. However, in the section on the choice of comparators (page 20) it is recommended to include the conventional counterpart with and without pest control management. The presence of data of relevant baselines is important in NTO testing, therefore this recommendation should be repeated in the section on laboratory studies.

Furthermore, the type of positive chemical control that should be used to prove the functionality of the experimental setup is not identified. The guidance document refers to the pesticide test guideline, but this guideline is not listed in the references. A relevant pesticide should be used as a positive control.

3.4.2.2 Field trials

The information in the guidance document on the necessity of field trials is confusing. From figure 6 (page 58) it can be concluded that field testing is not required when laboratory and semi-field tests show no significant adverse effects. However, the importance of field trials is underlined in paragraph 3.4.2.2 (page 67). Therefore, it is unclear when field trials should be conducted and which organisms should be studied. Specific field testing should only be required to investigate trait versus environment interactions when laboratory tests give reason to assume an adverse effect in the field. If no adverse effects are detected during laboratory tests, field tests are not required, but the presence of NTOs could be monitored during field trials that are carried out for other purposes. In addition, during General Surveillance possible effects on NTOs should be monitored.

Field testing should be done in an environmental setting relevant for the specific application. In the guidance document it is stated that field generated data from outside the EU may be informative, but the relevance of this data to the receiving environments in the EU should be justified reflecting relevant meteorological, ecological, soil and agronomic conditions. In addition, according to the guidance document applicants must provide explicit reasons if data from field trials in EU Member States are not available. Field testing data from outside the EU can never be sufficient to conclude that possible adverse effects on NTOs are absent within the EU, since NTOs in the EU differ from those outside the EU. Therefore, field generated data from relevant agricultural areas in the EU should be obligatory when laboratory tests give reason to assume an adverse effect in the field.

Opinion on NTOs

Protection goals

The guidance document refers to the opinion on NTOs for examples on how to consider protection goals in general (page 15). In the road map on the ERA of NTOs (appendix I) it is mentioned that when analyzing relevant protection goals protected and endangered species in protected areas should be considered by the applicant. The road map does not demonstrate

how protection goals that are linked to protected and endangered species should be selected. In addition, in the main text of the opinion the selection of protected or endangered species does not play a major role. The selection of protection goals that are linked to protected or endangered species should be described in more detail.

Selection of relevant areas for field tests
In the Opinion on NTOs the table that presents the schematic steps for the selection process of relevant areas for field tests is unclear (table 2). It is unclear to what extent focal NTO guilds from relevant functional groups should be identified. Should all focal NTO guilds in all possible cultivation areas be identified or should only those cultivation areas be considered were the GM plant is most likely to be cultivated? In addition, why should focal NTO guilds be identified before the region/zone in which the GM plant will be cultivated is established (step 4)? The table should be adapted in order to reflect that field tests should be conducted in areas that are representative for the cultivation areas, the management practices, and the NTOs that are present in the production systems and its adjacent habitat.

In the Opinion on NTOs a road map is included to illustrate the process underlying the proposed ERA for NTOs (appendix I). The process of species selection is part of this road map. The road map does not provide insight into the process of species selection. The table that illustrates the species selection process includes a rating for each of the taxa in the table. However, it is unclear how the rating follows from the prioritisation criteria that are listed for each of the different taxa in the table. In addition, the conclusion from the prioritisation process does not follow logically from the ranking in the table. In conclusion, it is unclear how the final focal species are identified and therefore the added value of the road map is questionable.

3.5 Impacts of the specific cultivation, management and harvesting techniques
In the guidance document it is pointed out that it is a requirement in Directive 2001/18/EC to assess the environmental impact of specific management and production systems associated with the GM plant. Indeed, it is stated in Annex II of the Directive, that information on possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the genetically modified higher plant (GMHP), where these are different from those used for non-GMHPs, should be included, as appropriate, in notifications with a view to assist in drawing conclusions on the potential environmental impact from the release or the placing on the market of GMOs.

The guidance document states that applicants are asked to carry out a scenario analysis which shall consider scenarios representative of the diversity of situations that may occur and assess their potential applications. Apparently this includes changes in pest management systems, irrigation regimes, changes in tilling, crop rotation and cropping systems, rate of adoption of the GM plant, substitution of other crops, pest pressure evolution etc.

It is debatable whether the present guidance document is within the spirit of the Directive. No doubt the introduction of a GM plant can lead to changes in production systems and these changes can affect the environment. But the guidance document appears to go beyond the question of the effects of specific cultivation or management techniques directly linked to the GM plant.

Because, no or unclear limitations have been specified in the guidance document for which effects have to be assessed, the demanded scenario studies result in an assessment of agriculture in general, beyond the ERA of a GM plant. It is a legitimate question for policy makers and politicians whether for example an increase in the acreage of maize (due to the success of a GM trait or because of other reasons) is desirable, as it can result in a decrease of biodiversity. However, this is not an assessment that should be made by applicants and has little to do with the ERA of a GM variety.

As mentioned before, according to the guidance document the applicant has to "identify through relevant assessment endpoints the aspects of the environment that need to be protected from adverse effects". In this case it is especially important that the authorities have set the endpoints that need to be protected and that the identification of protection goals is not left to an applicant. For example, drought tolerant GM plants need less irrigation which can lead to lower water levels and a shift to more drought tolerant weeds, changes in insect populations, or the occurrence of other animals. Whether this should be regarded as a positive or negative matter depends on policy goals and these should be translated in assessment endpoints by the competent authorities.

As mentioned before, the applicant is asked to carry out a scenario study. It is unclear what is meant by this. Scenario studies can be characterised as a systemic way to organize our thinking about the future, resulting in descriptions of possible futures. They can be predictive, analytic or explorative. Most likely a predictive scenario study is envisaged in the guidance document. This asks for a clear description of the objectives and boundaries of the systems and explicit and clear definitions of the various variables, which are lacking in this case. In the demanded scenario study one variable is the introduction of the GM plant. The other variables however are a multitude of different factors since agriculture is dynamic and crops and management and cultivation practices change over time. Consequently, a prediction can only be made when regarding the current practices as invariable. However, according to the guidance document the applicant has to take anticipated future changes into account (page 73) and the identified potential impacts have to be seen in the context of the already existing and evolving range of current management and production systems. Three different scenarios have to be considered a field level, landscape level and a worst-case scenario.

Basically, the demand for a scenario study asks of the applicant to perform a practically impossible task, while the results of such a scenario study will be debatable and will not lead to clear answers. Only in those cases where there can be relied on previous experiences with cultivation in non-EU countries an educated guess can be made on the effects in the EU. But even this will be challenging and difficult due to differences in receiving environments.

Monitoring can be an effective tool to detect adverse effects of cultivation or management techniques. Instead of asking the applicant to devise an ill-founded scenario with unfounded mitigating measures, the emphasis in this section should be on monitoring. If adverse effects are detected, appropriate measures can be taken, like adjustment of the management techniques, limiting the acreage or even withdrawal of the GM crop.

A part of this section (boxed text) deals with specific considerations for GM herbicide tolerant (HT) plants. Presently, the effects of herbicide applications are the only known 'indirect

effects' of a management or cultivation technique directly linked to a GM plant. Rightfully the guidance document states that these effects should be assessed. However, a political decision has to be made whether this assessment should take part under Directive 2001/18 or under Regulation 1107/2009. In the guidance document it is stated that the applicant is requested to assess the potential environmental effects of the use of herbicide treatment with GM HT crops. It has to be noted that in the new revised Regulation 1107/2009 considerations on the impact of plant protection products on biodiversity are mandatory. Including the assessment of potential environmental effects of the use of herbicide treatment with GM HT crops in the guidance document will lead to duplication and possible conflicts with the pesticide regulation process. Taking into account that the competent authorities and other involved organisations under Regulation 1107/2009 have more expertise on the risk assessment of herbicides, it is recommended that the environmental impact of herbicide treatments linked to GM HT plants is dealt with under the Plant Protection Product Regulation and not under Directive 2001/18. Consequently, this section should be stricken from this guidance document and could be transferred to a guideline under Regulation 1107/2009.

In conclusion: a political decision should be taken concerning the limits of an assessment of indirect effects cq effects of changes in management, cultivation or harvesting techniques. The assessment of potential effects should be limited to effects directly linked to the incorporated traits in the GM plant. More emphasis should be on monitoring of effects caused by changes in management or cultivation techniques and less on the prediction of effects. The assessment of the environmental effects of herbicide treatments linked to cultivation of GM herbicide tolerant crops should take place under Plant Protection Product Regulation 1107/2009.

3.6 Effects on biogeochemical processes

The applicant is requested for information on 'losses of production sites or systems' to air or water like greenhouse gas emissions. This also includes the loss due to operations that occur outside the GM plant production site, like manufacture and transport of fertilizer. These processes indeed affect the environment, but these should not be included in an ERA of GM plants as they are not specific additional risks of GM plants. Agriculture in general is associated with the use of e.g. fertilizer, insecticides and biocides and resulting environmental effects. Therefore the subject of 'losses of production sites or systems' goes beyond the scope of the ERA of GM plants

Glossary

According to the glossary of the guidance document the definition of 'receiving environment' is the environment into which the GM plant will be released and into which the transgenes may spread. However, in the guidance document it is mentioned that the receiving environment is characterized by the GM plant, the geographical zones and the management systems (page 23). This implies that management systems are also part of a 'receiving environment', but this aspect is not mentioned in the definition. The definition should be changed and the management system should be included.

4. Conclusion

The guidance document gives a broad overview of all aspects of the ERA and describes different angles that can be used in the ERA. It offers insight in the considerations on the different aspects of the ERA and lists various scientific views on these aspects. However, a consequence of the broadness of the guidance document is that its use as a guidance document is limited. It does not offer protocols or specification of the objectives which have to be fulfilled at each stage of the risk assessment. Therefore, the present document does not meet the qualifications needed for a binding guideline. An adapted version of the document is excellently suited as a background document or reference for the ERA of GM plants, but should not be transformed into a legally binding provision.

Documents that are focused on providing guidance for specific categories of GM plants, e.g. herbicide tolerance, insect resistance or changed metabolic pathways are more suited as guidance documents. Therefore, COGEM proposes the development of legally binding guidance documents that are more precise and apply to specific categories of GM plants.

In addition, COGEM would like to emphasize that the task of identifying protection goals and setting ‘limits of concern’ should not be left to the applicant. Protection goals depend on policy goals and should therefore be identified in a political process. The identification of protection goals and the translation of these protection goals in assessment endpoints and setting the ‘limits of concern’ should be done by the competent authorities.