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Interaction between SmartStax and the environment

Analyses of Rosenbaum, 2008, Report Number MSL0021061

Christoph Then

Summary:
Genetically engineered plants inherit technically derived features that are not controlled by the plant‘s gene regulation. Technical failures such as genetic instabilities and/or occurrence of undesired components can be triggered by specific environmental conditions. Relevant effects have already been observed in various genetically engineered plants. In general, it is known that genetically engineered plants can show unexpected effects in reaction to environmental conditions such as climate, soil quality and various stressors. Interaction with the environment can impact the plant genome, plant metabolism, cause changes in phenotype and affect different biological properties of the plant (e.g. higher invasiveness and fitness).

In general, the investigation of interaction between the plants and the environment provides insight into the genetic stability of the genetically engineered plants, and is an important starting point in risk assessment. The interaction between the genome and the environment is also relevant to the risk assessment of food and feed, since the composition of the plant's compounds can be affected.

These reactions can and should be measured under controlled conditions, e.g. under laboratory or greenhouse conditions, to identify relevant impact factors before the plants are released in experimental field trials, or used for large scale cultivation.

The investigations were commissioned and paid for by Monsanto. They were conducted in Monsanto Laboratories. Regarding quality control - no independent laboratories were involved, data were not published in peer-reviewed magazines and the wording of the report even indicates manipulation of the data.

The investigations were only performed for one season and on relatively small plots. Rosenbaum only considered the question of whether the plants showed a higher degree of fitness or invasiveness and whether their agronomic properties could be compared to other maize plants.

No food and feed related risks were explored. Neither were there any investigations into metabolic changes within the plants or gene activity, nor were there any detailed analyses of compositional changes throughout the season. Risks related to food and feed cannot be concluded from the data that was presented.

Relevant agronomic criteria were not taken into account (e.g. the date of flowering or viability of pollen). Some significant findings that indicate interactions with the environment or an overall change in gene activity and plant metabolism were dismissed without any further investigations. For

example, a higher incidence of plant disease was found in one site. Further, six criteria were found to be significantly different (e.g. pollen shed, ear height, plant height and grain moisture) in comparing the findings with the control plants at the individual sites. None of these findings were investigated further.

Apparently, several individuals were involved in data collection and data evaluation:

> *"During the process of data summarization and analysis, experienced scientists familiar with each experimental design and evaluation criteria were involved in all steps. This oversight ensured that the data were consistent with expectations based on experience with the crop."*

There is no explanation as to who these experts were or how the data were made consistent with expectations. This wording indicates a possible manipulation of the data. Only one Monsanto member of staff is mentioned under acknowledgements for *"assistance with the statistical analysis of the data."*

In its opinion, EFSA does not discuss Rosenbaum's results in detail. EFSA mentions only very generally that no signals for altered fitness and invasiveness were found, or that they did not consider any other data on unexpected plant reactions caused by genome (x) environment interaction and risks related to food and feed.

According to the statement of experts from Member States, more data and specific data on environmental interactions should be provided. Further, more specific environmental conditions should be taken into account such as abiotic stress through compaction, drought and frost or a higher likelihood of diseases. There was also no comparison made between those plants that were sprayed and those that were not sprayed. Experts from Member States believe that since the relevant raw data is not available, no decisive conclusion can be drawn from the report that was presented. There was also criticism of the small scale of the field trials, as well as the overall design of the study, which was not deemed to be representative for real conditions under commercial cultivation. The experts further criticise the fact that significant findings should not have been dismissed without further examination. More than one season should have been included in the trials as well as other important criteria such as flowering time, pollen size and production or the duration of pollen viability.

Background of the investigation:

As Zeller et al (2010) show, the ecological behaviour of plants can render effects that are highly relevant for the risk assessment of genetically engineered plants. Genetically engineered wheat showed a complex reaction of reduced fitness, higher incidence of fungal disease and higher burden of toxic residues from the fungal disease. These effects only occurred under environmental conditions, but not in the greenhouse. Zeller et al (2010) also point out that so far there has been hardly any investigation of these interactions:

> *"(...) a careful search in the literature for replicated and randomized studies about the ecological behaviour of GM and control plants in glasshouse versus field environments did not return any published references."*

Interactions with the environment can impact genome regulation, plant metabolism, the phenotype as well as different biological plant behaviour (higher fitness and invasiveness). In general, it is known that genetically engineered plants react to environmental conditions such as climate (Chen et al., 2005), soil (Bruns, 2007) and abiotic and biotic stress (Matthews et al., 2005).

Genetically engineered plants inherit technically derived features that are not controlled by the plant‘s gene regulation. Technical failures such as genetic instabilities and the emergence of undesired components can be triggered by specific environmental conditions. Thus, attention must be paid to effects that might occur under certain environmental conditions such as in particular climates (drought, heat, moist conditions). Technical failures and genetic instabilities might give rise to undesirable components in the plants or diminish valuable components.

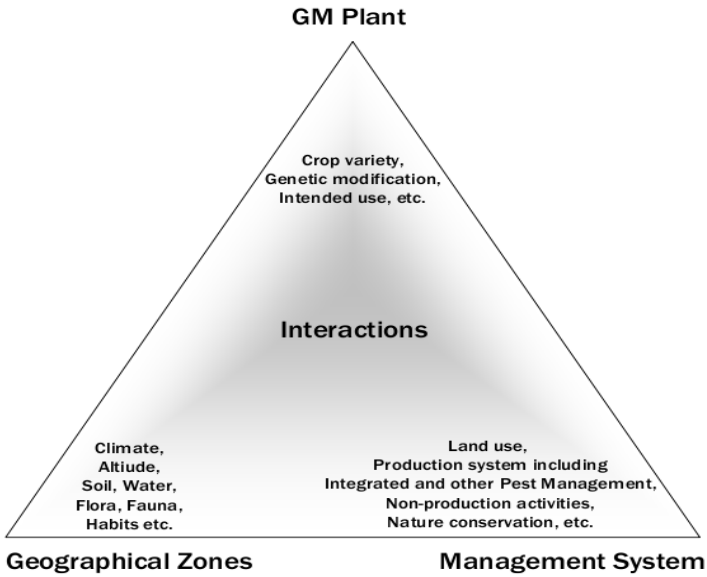
The reactions of genetically engineered plants should be studied under controlled conditions, e.g. in laboratories or greenhouses, to enable identification of relevant impact factors. That is why Then & Potthof (2009) propose a system they call a 'crash test' (or 'stress test'), to systematically investigate the genetic and metabolic reactions of the plants to changing environmental conditions - before release into the environment. Once the plants are released into the environment, they are exposed to a much more complex situation that can make it difficult to determine the impact of particular factors. EFSA (2010c), for example, states:

> *“Laboratory testing provides the best way to control and manipulate experimental conditions (environmental factors, set-up) and to limit complexity and variability. In contrast, field tests allow the evaluation of trait x environment interactions, but they exhibit the highest experimental complexity and provide the lowest ability to control experimental conditions due to large natural variability. ”*

Interactions between the genome and the environment are relevant for environmental risk assessment, as are food and feed related risks, since the composition of the plants might be impacted or plant diseases might trigger toxic residues e.g. from fungal disease. In general, detailed investigation of interactions between the plant and the environment can provide insight into genetic stability and the overall technical quality of the genetically engineered plants, and should be a starting point for risk assessment.

The empirical investigations should take a sufficiently broad range of possible interactions with the receiving environment into account, as for example, indicated by an EFSA diagram (2010c) published in their Guidance for environmental risk assessment.

fig. 1: The receiving environment(s) is characterized by the GM plant, the Geographical Zones, and the Management Systems. Source: EFSA, 2010c



2. Overview of investigations and findings of Rosenbaum

In 2006, SmartStax was grown at four sites in comparison with a conventional hybrid (XE6001). The plots were relatively small (6 rows, 20 feet long), only two of the rows were used for data collection.

Data on the following criteria were collected during one growing season in 2006:

> *"Early stand count, seedling vigor, days to 50% pollen shed, days to 50% silking, ear height, plant height, staygreen, dropped ears, final stand count, stalk lodging, root lodging, grain moisture, test weight, yield, insect stressor, disease stressor, and abiotic stressor."*

Apparently, several scientists were involved in data collection and data evaluation:

> *"During the process of data summarization and analysis, experienced scientists familiar with each experimental design and evaluation criteria were involved in all steps. This oversight ensured that the data were consistent with expectations based on experience with the crop. In addition, the overall dataset was evaluated for evidence of biologically relevant changes, and for possible evidence of an unexpected plant response."*

However, it is not made clear which experts were involved and how "*the data were made consistent with expectations*." Under the acknowledgements only one Monsanto staff member is mentioned for *"his assistance with the statistical analysis of the data"*

No differences were observed except at one site where northern corn leaf blight was found. This was not found in the other fields, thus the report considers it "*unlikely to be of biological concern*".

The protocol and the statistical analyses are said to be "*peer reviewed*", but no external independent scientists can be identified who took part in the review process. Instead, it is stated that "*the Monsanto Quality Assurance Unit audited the statistical analysis results and report*".

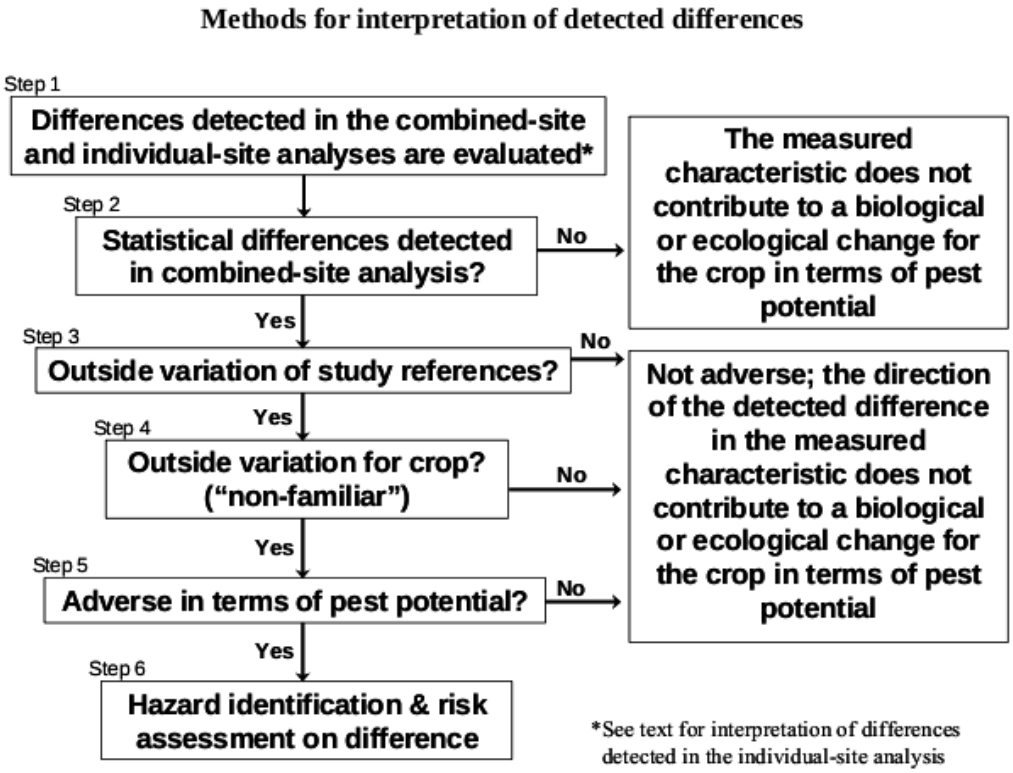
The statistical protocol foresees what is called a "*combined-site analysis*" in which the data from SmartStax "*was compared to the control substance pooled across all sites*". This means that significant findings between the genetically engineered plants and their control plants within a particular site may have easily been diluted in the overall amount of data. The data from particular sites were also analysed, which allows for a much more detailed comparison. This part of the statistical protocol is called "*individual site analyses*".

The data were interpreted in regard to the endpoint of "*biological or ecological change for the crop in terms of pest potential"*:

> *"Ultimately, a weight of evidence approach considering all characteristics and studies is used for the final risk assessment of differences and their significance in terms of increased pest potential."*

Thus, other "*biological or ecological changes"* aside, an increase of pest potential was regarded as not being decisive for the outcome of this study. This is also made evident in what Rosenbaum calls "*methods for interpretation of detected differences*":

fig. 2: Decision making tree for interpretation of significant findings as used by Rosenbaum, 2008. Source: dito



Step 6 of this decision making tree is decisive for the overall outcome of the investigations:

> *"If an adverse effect (hazard) is identified, risk assessment on the difference is conducted. The risk assessment considers contributions to enhanced pest potential of the crop itself, the impact of differences detected in other measured characteristics, and potential for, and effects of trait transfer to feral populations of the crop or a sexually compatible species."*

In effect, this means that criteria relating to risks related to food and feed were not considered.

Phenotypic evaluation:

By using the "*individual site analysis*" six characteristics were found to be significantly different from the test plants. These concern days to 50% pollen shed, days to 50% silking, ear height, plant height, final stand count and grain moisture.

However, by applying the "*combined-site analysis*", that can be used to mask relevant effects by a higher number of secondary data, no differences were detected. Thus, in conclusion, and by referring to the endpoint termed "weed potential" significant findings were dismissed:

> "*Since no differences were detected in the combined-site analysis, the differences detected in the individual site analysis were not indicative of a consistent trend in the data and are*

unlikely to be biologically meaningful in terms of increased weed potential (…)"

Ecological interactions:
A significant difference in the incidence of northern corn leaf blight was found at one site but was not found at the other sites. This is a significant finding, but it was dismissed because it was not found in all sites.

The overall conclusion is:

> *"Thus, the results support a conclusion of no increased pest potential or adverse environmental impact for the combined trait product."*

3. Assessment of the investigations

3.1 Evidence of insufficient testing

The most relevant deficiencies of the Rosenbaum (2008) study are the lack of quality control, inadequate study design and false conclusions.

Regarding quality control, there were no independent scientists involved, the study did not undergo an independent peer review process, and the wording of the report stating that the data were made consistent with expectations even indicates manipulation of the data. Further raw data were not published, so independent examination of the findings is not possible.

The study design does not allow any judgment upon the relevant issues. There is a general lack of sufficiently defined environmental conditions that could be used to identify relevant impact factors. There was no investigation of metabolic changes within the plants or of gene activity. There were also no detailed analyses of compositional changes throughout the season. Relevant agronomic criteria were not taken into account (such as date of flowering or viability of pollen). The investigations were only performed for one season and on relatively small plots.

The conclusions drawn from the data are adversely affected by an incorrect decision making process for risk assessment, which only takes fitness and invasiveness as relevant endpoints. Risks connected to food and feed cannot be concluded from the presented data. Even fitness and invasiveness were not fully investigated, since relevant criteria and the behaviour of the segregating progeny were not included. Significant findings that might indicate specific interaction with the environment, or an overall change in gene activity and plant metabolism were dismissed without any further investigation.

3.2. Assessment by EFSA and by experts from EU Member States

In its opinion, EFSA (EFSA 2010a) does not discuss the results of the Rosenbaum study in detail. EFSA mentions only very generally that no signals for altered fitness and invasiveness were found, thereby agreeing on the endpoints concluded by Rosenbaum. EFSA did not consider any other data on ecological plant behaviour such as genome x environment interaction:

> *" (…) a series of field trials with maize MON 89034 x 1507 x MON 88017 x 59122 was conducted at four locations within major maize-growing regions of the USA in 200631. Information on phenotypic and agronomic characteristics was provided to assess the*

agronomic performance of maize MON 89034 x 1507 x MON 88017 x 59122 in comparison with its conventional counterpart. These field trial data did not show changes in plant characteristics that indicate altered fitness and invasiveness of maize MON 89034 x 1507 x MON 88017 x 59122 plants (...)" (page 25)

Experts from three Member States (including Norway) came up with comments on the Rosenbaum paper (EFSA 2010b). Two of them, Austria and Germany, are raising a detailed debate on the topic.

According to the Member States' statement, more and specific data on environmental interactions should be provided. Many more specific environmental conditions should be created during field trials, such as abiotic stress through compaction, drought and frost or higher risk of diseases. There was no comparison between sprayed and non-sprayed plants. Since relevant raw data material is missing, no decisive conclusions can be drawn from the existing report. The small scale of the field trials was criticized, and the overall design of the study was not representative of real conditions under commercial cultivation. The significant findings should not have been dismissed without further investigation. More than one season should have been included and other important criteria such as flowering time, pollen size and production and the duration of pollen viability.

The Austrian experts believe that the differences that were observed would be even more significant under the standard criteria used to investigate new plant varieties. Because of the high range of variation within certain criteria, it is stated that it would be likely that these plants would be rejected under variety registration law.

In their mostly formalistic answers, EFSA refers to their Guidance that only requests field trials for stacked events over period of one year, and confirms that the relevant endpoints of the investigations are fitness and invasiveness only. EFSA also refers to field trials with the parental lines that did, according to EFSA, not show unexpected findings. They agree with Rosenbaum that findings not consistent in all locations can be dismissed without further investigation.

Table 1: Relevant comments from the experts of Member States and the answer from EFSA 2010b

Member State	Statement	answer from EFSA
Austria	However, the phenotypic characteristics evaluated in the field trial merely comprise standard agronomic parameters as used by plant breeders and agronomists. Ecologically important characteristics as for instance the flowering time, pollen size and production or the duration of pollen viability have not been assessed. For the ecological evaluation, the small size of the field trial plots may limit the significance of the results. The assessment of ecological behavior should furthermore be addressed with a specific design of the trial to establish the occurrence of certain environmental conditions during the field trial, e.g. the demonstration of the occurrence of the respective pest or disease in the growing area (testing under pest/disease pressure) in order to assess the susceptibility to pests and diseases.	The EFSA GMO Panel thanks Austria for drawing its attention to this detail in the comparative analysis. In section 4.1.2 describing the field trial design for the comparative analysis, the EFSA GMO Panel's opinion addresses the issue of herbicide treatment with the target herbicides as follows: "Given the fact that previous assessments of the herbicide-tolerant single events MON 88017, 1507 and 59122 considered both plants treated with the target and conventional herbicides and plants treated with only conventional herbicides, the EFSA GMO Panel does not consider it necessary to ask for compositional data on maize MON 89034 x 1507 x MON 88017 x 59122 that was treated with conventional herbicides (i.e. not with the target herbicides)." The herbicide treatment is described in more detail in the appendix production plan 06-01-52-04. In addition, in response to a query by the EFSA GMO Panel, the applicants have explained that the doses of glufosinate-ammonium and glyphosate-based herbicides are representative of those used in commercial practice.

Member State	Statement	answer from EFSA
	The experimental design did not include plots which were not treated with the non-selective herbicides (glyphosate and glufosinate-ammonium), and thus, did not allow for a comparison between treated and untreated plots. To address the abovementioned concerns the notifier should submit further data on ecologically relevant parameters such as duration of pollen viability, flowering time or susceptibility towards pest and disease (investigated under pest or disease pressure). (page 6)	The EFSA GMO Panel considers that the information provided in relation to the agronomic performance assessment is sufficient considering the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122, which excludes cultivation. In an environmental risk assessment, agronomic/phenotypic data give an indication on whether the fitness, persistence and invasiveness of the GM plant differ from that of its non-GM counterpart. Information on phenotypic and agronomic characteristics usually is obtained from agronomic field trials conducted at a range of locations representative of different environments where the GM crop may be grown. To assess the agronomic performance of the GM plant, different plant characteristics are recorded to establish differences between the GM plant and its non-GM counterpart. Fitness differences between the GM plant and its non-GM counterpart are usually inferred from a composite measure of relative plant germination, emergence, growth, survivorship, biomass and fecundity. Previous field trials have shown that there are no indications of altered fitness of the single maize events MON 89034, 1507, MON 88017 and 59122 and the two double stacks 1507 x 59122 and MON 89034 x MON 88017, as compared to their conventional counterparts. In addition to the field trials carried out with the single events and maize stacks (EFSA, 2004, 2005a, b, 2007b, 2008, 2009a, b, c, 2010), a series of field trials with maize MON 89034 x 1507 x MON 88017 x 59122 was conducted at four locations within major maize-growing regions of the USA in 2006. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of maize MON 89034 x 1507 x MON 88017 x 59122 in comparison with its conventional counterpart. These field trial data did not show changes in plant characteristics that indicate altered fitness and invasiveness of maize MON89034 x 1507 x MON88017 x 59122 plants, though there is a potential for enhanced biomass production when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests. On the basis of the available data on the single events and maize stacks (1507 x 59122, MON 89034 x MON88017 and MON 89034 x 1507 x MON 88017 x 59122), the EFSA GMO Panel considers it very unlikely that the segregating progeny of MON 89034 x 1507 x MON 88017 x 59122 would have any increased persistence and invasiveness in EU receiving environments. In addition, the EFSA GMO Panel is not aware of any scientific report of increased establishment, spread or any change in survival capacity including overwintering of maize MON89034 x 1507 x MON88017 x 59122, or maize with comparable properties such as single events and sub-combinations of maize MON 89034 x 1507 x MON

Member State	Statement	answer from EFSA
		88017 x 59122. Since maize MON 89034 x 1507 x MON 88017 x 59122 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include all sub-combinations of the individual events) will not differ from that of the single maize events (MON 89034, 1507, MON 88017 and 59122), the two double stacks (1507 x 59122 and MON 89034 x MON 88017), or from that of conventional maize varieties. (page 6-8)
Austria	a) General comments: Because of the lack of raw (individual) data on all phenotypic parameters it cannot be concluded that there are no relevant differences between test substance (MON88017 x 1507 x MON88017 x 59122) and control substance (XE6001). Providing of the raw data in electronic form would be appreciated in order to reproduce the reasoning of the study. Due to little disease pressure at the sites no real evaluation of the different substances (control, test, reference) is possible, especially with regard to the efficacy of the insect resistance traits. Although the authors of the study noted once "The purpose of this study was to assess the phenotypic characteristics and ecological interactions of the combined trait corn products MON 89034 x TC1507 × NK603 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 compared to a conventional corn control", this respective triple stack is not again mentioned in the whole paper (…). b) Study design: Use of randomised complete block (RCB) design with three replications and the use of SAS® analysis software, as well as the length and width of the plots, are in accordance with modern standards as used by plant breeders and agronomists. However, with respect to the ecological evaluation, the small size of the field trial plots may limit the significance of the results. Comparing the study design with legal standards for authorisation of new plant varieties in Austria (Republik Österreich 1997; AGES 2002), it should be mentioned that at	It is noted by the EFSA GMO Panel that the field trials performed for the comparative analysis need not necessarily comply with the requirements for variety testing given that the purpose of the latter (e.g. demonstration of a certain difference with the comparators) is different from that of the comparative safety assessment. The EFSA GMO Panel's guidance on the assessment of stacked events thus mentions in section 3.2.2: "Possible differences in phenotypic characteristics and agronomic properties of stacks must be assessed in field trials over at least one season" In section 4.1.4 of its opinion, the EFSA GMO Panel comments on the outcomes of the study on agronomic/ phenotypic characteristics as follows: "In the present application, the analyses of agronomic and phenotypic characteristics of maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart and twelve commercial maize varieties included a range of parameters related to plant morphology, physiology, appearance and performance, including stressors and plant health. A number of parameters showed statistically significant differences in the per-location statistical analysis of the comparison between maize containing stack MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart but this was not consistently observed in each location." In section 4.1.2 describing the field trial design for the comparative analysis, the EFSA GMO Panel's opinion addresses the issue of herbicide treatment with the target herbicides as follows: "Given the fact that previous assessments of the herbicide-tolerant single events MON 88017, 1507 and 59122 considered both plants treated with the target and conventional herbicides and plants treated with only conventional herbicides, the EFSA GMO Panel does not consider it necessary to ask for compositional data on maize MON 89034 x 1507 x MON 88017 x 59122 that was treated with conventional herbicides (i.e. not with the target herbicides)." The herbicide treatment is described in

Member State	Statement	answer from EFSA
	least two years of testing at eight different sites is considered necessary to enable sound ecological assessment. From this point of view, a number of four sites seems to be rather low. Additionally, ecologically important characteristics as for instance the flowering time, pollen size and production or the duration of pollen viability have not been assessed. The assessment of ecological behavior should, furthermore, be addressed with a specific design of the trial to establish the occurrence of certain environmental conditions during the field trial, e.g. the demonstration of the occurrence of the respective pest or disease in the growing area (testing under pest/disease pressure) in order to assess the susceptibility to pests and diseases. We are, therefore, of the opinion that the notifier should submit further data on ecologically relevant parameters (e.g. duration of pollen viability, flowering time or susceptibility towards pest and disease (investigated under pest or disease pressure). Moreover, the experimental design did not include plots which were not treated with the non-selective herbicides (glyphosate and glufosinate-ammonium), and thus, did not allow for a comparison between treated and untreated plots. (page 8-10)	more detail in the appendix production plan 06-01-52-04. In addition, in response to a query by the EFSA GMO Panel, the applicants have explained that the doses of glufosinate-ammonium and glyphosate-based herbicides are representative of those used in commercial practice. The EFSA GMO Panel considers that the information provided in relation to the agronomic performance assessment is sufficient considering the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122, which excludes cultivation. In an environmental risk assessment, agronomic/ phenotypic data give an indication on whether the fitness, persistence and invasiveness of the GM plant differ from that of its non-GM counterpart. Information on phenotypic and agronomic characteristics usually is obtained from agronomic field trials conducted at a range of locations representative of different environments where the GM crop may be grown. To assess the agronomic performance of the GM plant, different plant characteristics are recorded to establish differences between the GM plant and its non-GMM counterpart. Fitness differences between the GM plant and its non-GM counterpart are usually inferred from a composite measure of relative plant germination, emergence, growth, survivorship, biomass and fecundity. Previous field trials have shown that there are no indications of altered fitness of the single maize events MON 89034, 1507, MON 88017 and 59122 and the two double stacks 1507 x 59122 and MON 89034 x MON 88017, as compared to their conventional counterparts. In addition to the field trials carried out with the single events and maize stacks (EFSA, 2004, 2005a, b, 2007b, 2008, 2009a, b, c, 2010), a series of field trials with maize MON 89034 x 1507 x MON 88017 x 59122 was conducted at four locations within major maize-growing regions of the USA in 2006. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of maize MON 89034 x 1507 x MON 88017 x 59122 in comparison with its conventional counterpart. These field trial data did not show changes in plant characteristics that indicate altered fitness and invasiveness of maize MON89034 x 1507 x MON88017 x 59122 plants, though there is a potential for enhanced biomass production when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests. On the basis of the available data on the single events and maize stacks (1507 x 59122, MON89034 x MON88017 and MON89034 x 1507 x MON88017 x 59122), the EFSA GMO Panel considers it very unlikely that the segregating progeny of MON 89034 x

Member State	Statement	answer from EFSA
		1507 x MON 88017 x 59122 would have any increased persistence and invasiveness in EU receiving environments. In addition, the EFSA GMO Panel is not aware of any scientific report of increased establishment, spread or any change in survival capacity including overwintering of maize MON89034 x 1507 x MON88017 x 59122, or maize with comparable properties such as single events and sub-combinations of maize MON 89034 x 1507 x MON 88017 x 59122. Since maize MON 89034 x 1507 x MON 88017 x 59122 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include all sub-combinations of the individual events) will not differ from that of the single maize events (MON 89034, 1507, MON 88017 and 59122), the two double stacks (1507 x 59122 and MON 89034 x MON 88017), or from that of conventional maize varieties. (pages 8-12)
Austria	Phenotypic Evaluation and Ecological Interactions (Rosenbaum 2008) [cont.]: c) Definitions of characteristics: Definitions of some plant characteristics seem to be incomprehensible and misleading: - "Grain moisture: Percent moisture of shelled grain at harvest or after drying." In fact, provided data can only represent percent moisture at harvest. It would really make no sense to collect data on moisture contents after drying procedures. - "Test weight: Weight of shelled grain per unit volume at harvest or after drying." Again this is not understandable. - "Yield: Calculated from grain weight moisture, and test weight". It is not clear how the yield was calculated, and it is also not clear how much percent moisture was used as standard for this calculation. And, why and in what manner was the test weight included in the calculation, as the yield is totally independent of the test weight? d) Conducting the study: Obviously, singling and gapping after germination and emerging of the plants were not performed. This is a standard procedure in Austria field testing in order to guarantee	Table 6 of the appendix “production plan 06-01-52-04” mentions that grains harvested at the R6 stage were dried down to 12-15% moisture. With regard to drying practices in more general terms, it is noted by the EFSA GMO Panel that drying grains after harvest down to a lower moisture level (i.e. not completely dry) appears to be common practice in commercial agriculture, particularly if the harvest takes place during a wet/moist period leading to a high moisture content of the grain, in order to prolong the storability of the harvested grain (e.g., grain with a high moisture content can only be stored during a very short period). Data on herbicide applications are provided in Table 5 of the appendix production plan 06-01-52-04, which also contains data on environmental factors and agronomic treatments. Table 6 of this appendix mentions that grains harvested at the R6 stage were dried down to 12-15% moisture. The report by Rosenbaum indicates which differences are statistically significant. In section 4.1.4 of its opinion, the EFSA GMO Panel comments on the outcomes of the study on agronomic/phenotypic characteristics, including the statistically significant differences observed in the across-location (no differences) and per-location statistical analyses, as follows: “In the present application, the analyses of agronomic and phenotypic characteristics of maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart and twelve

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	homogeneity of the population. However, due to lack of raw data no conclusions can be drawn on the homogeneity of the populations (control, test, reference). Additionally, data on weed infestation and herbicide application are missing. e) Results: Few differences regarding the phenotypic characteristics were found between test and control substances for mean values across all sites. However, those statistically significant differences found for mean values at the different sites could have been caused by minimal environmental effects or minimal measuring errors. For instance, at site IL-1 the average plant height of the test substance was 11 centimetres higher than the control (105.5 / 101.2 inches). Pest pressure and infestation in general seemed to be negligible, as no or only little stressor symptoms were found only. Similarly, no abiotic stress through compaction, drought or frost was observed. As a consequence, statistical evaluation did not provide any differences between the test, the control and the reference substances. However, the reverse argumentation that actually there are no differences is not allowed. Furthermore, it is conspicuous that grain moisture is significantly higher (on average 1.2%) in the test substance MON88017 x 1507 x MON88017 x 59122. In Austria the test substance would thus be rated as dent maize, i.e. 30 FAO units higher, which means later ripening. At one single site the difference for grain moisture was 3.1% (25.8 vs. 22.7), which would mean ca. 80 FAO units! In addition, higher values of staygreen (6.4), days to pollen shed (56.6) and days to silking (55.7) were found. The yields though were considerably lower in the test substance (152.9 vs. 161.0)! According to the current standards for authorisation of new plant varieties in Austria (Republik Österreich 1997; AGES 2002), both a difference of 30 FAO units and lower yields of about 5% would be enough to refuse authorisation. However, this report does not give indication whether differences for grain moisture and yields arr statistically significant, and due to missing raw data this cannot be verified. f) Other comments:	commercial maize varieties included a range of parameters related to plant morphology, physiology, appearance and performance, including stressors and plant health. A number of parameters showed statistically significant differences in the per-location statistical analysis of the comparison between maize containing stack MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart but this was not consistently observed in each location." Concerning the apparent decrease in some leaf diseases at one location at a later stage that Austria points to, the EFSA GMO Panel notes that certain diseases proceed through various stages of plant infection (e.g. primary infection followed by secondary infection), which may also account for certain temporal variations in observed disease severity and progression, depending on environmental conditions (e.g. humid versus dry conditions). The EFSA GMO Panel considers that the information provided in relation to the agronomic performance assessment is sufficient considering the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122, which excludes cultivation. In an environmental risk assessment, agronomic/ phenotypic data give an indication on whether the fitness, persistence and invasiveness of the GM plant differ from that of its non-GM counterpart. Information on phenotypic and agronomic characteristics usually is obtained from agronomic field trials conducted at a range of locations representative of different environments where the GM crop may be grown. To assess the agronomic performance of the GM plant, different plant characteristics are recorded to establish differences between the GM plant and its non-GM counterpart. Fitness differences between the GM plant and its non-GM counterpart are usually inferred from a composite measure of relative plant germination, emergence, growth, survivorship, biomass and fecundity. Previous field trials have shown that there are no indications of altered fitness of the single maize events MON 89034, 1507, MON 88017 and 59122 and the two double stacks 1507 x 59122 and MON 89034 x MON 88017, as compared to their conventional counterparts. In addition to the field trials carried out with the single events and maize stacks (EFSA, 2004, 2005a, b, 2007b, 2008, 2009a, b, c, 2010), a series of field trials with maize MON 89034 x 1507 x MON 88017 x 59122 was conducted at four locations within major maize-growing regions of the USA in 2006. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of maize MON 89034 x 1507 x MON 88017 x 59122 in comparison with its conventional counterpart. These field trial data did not show changes in plant characteristics that indicate altered fitness and

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	Symptoms of the plant disease 'grey leaf spot' assumedly diminished between the 3rd and the 4th observation for all plant populations at the York site (NE). This is the same for the disease 'stressor rust'. The notifier is therefore requested to explain this behaviour. (page 12-14)	invasiveness of maize MON 89034 x 1507 x MON88017 x 59122 plants, though there is a potential for enhanced biomass production when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests. On the basis of the available data on the single events and maize stacks (1507 x 59122, MON 89034 x MON 88017 and MON 89034 x 1507 x MON 88017 x 59122), the EFSA GMO Panel considers it very unlikely that the segregating progeny of MON 89034 x 1507 x MON 88017 x 59122 would have any increased persistence and invasiveness in EU receiving environments. In addition, the EFSA GMO Panel is not aware of any scientific report of increased establishment, spread or any change in survival capacity including overwintering of maize MON 89034 x 1507 x MON 88017 x 59122, or maize with comparable properties such as single events and sub-combinations of maize MON 89034 x 1507 x MON 88017 x 59122. Since maize MON 89034 x 1507 x MON 88017 x 59122 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include all sub-combinations of the individual events) will not differ from that of the single maize events (MON 89034, 1507, MON 88017 and 59122), the two double stacks (1507 x 59122 and MON 89034 x MON88017), or from that of conventional maize varieties. (page 12-16)
Germany (BfN)	In particular the information to assess expression, composition and phenotypic characteristics (both agricultural and ecological) of MON89034 x 1507 x MON88017 x 59122 maize need to be expanded. The characterization of the GMO should be based on experiments in more than one planting season and the number of field sites should allow to test for possible gene-environment interaction including biotic and abiotic (climatic) factors in a statistically sound design. (page 50)	The EFSA GMO Panel's guidance on the assessment of stacked events, section 3.2.2, notes that “For the stacked events at least one year of field trial data is required, with trials performed together with appropriate controls in geographical localities representative of the climatic conditions under which such crops will be cultivated.”
Germany (BfN)	The submitted study of Rosenbaum (2008; MSL-0021061) does not allow to conclude on possible ecological interactions of MON89034 x 1507 x MON88017 x 59122 maize. For an assessment of ecological interactions with non-target organisms or in terms of pest and disease incidence additional information and further field tests would be required. In this respect information on pest pressure, disease	Taking into account the scope of the application, both the rare occurrence of feral maize plants and low levels of Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 protein exposure in maize MON89034 x 1507 x MON88017 x 59122 grains or through other routes indicate that the risk to target and non-target organisms is extremely low. (page 56)

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	pressure or abiotic stressors have not been provided. Therefore, the baseline to compare stressor symptoms is missing. In fact data from Table 6 of MSL-0021061 indicate that pest pressure of the target organisms was week or absent. Moreover the application of different kinds of pesticides (see production plan) is counterproductive when assessing parameters listed in Tables 6 to 8. To conclude on the risk assessment the notifier is requested to i) to give the criteria on which the representativeness of locations has been established, ii) present further field data (several years, including treatments with and without HR) and to iii) statistically analyse the data giving the achieved statistical power. To allow conclusions on ecological characteristics of the GMO we strongly recommend including data from additional field seasons. We also recommend including data on the occurrence of volunteers during cultivation of MON89034 x 1507 x MON88017 x 59122 maize to facilitate a better assessment of effects of loss and spillage. (page 56/57)	
Norway	Stacked events: The applicant is asked to test the maize for genetic stability of the inserts for more than one generation, e.g. three growing season and multiple locations representing different environmental conditions. (page 74)	The molecular data supplied by the applicants do not suggest a structural modification due to the conventional crossing of the single events in the stacked lines. The stability of the single events was determined over several generations, stability of the stacked event over one generation. This is considered to be sufficient from a safety point of view. (page 74)

4. Conclusion

The Rosenbaum (2008) data should be rejected as a basis for risk assessment because it is inadequate and of dubious quality. The data as provided by Monsanto show the need for clear standards, problem formulation and endpoints for the risk assessment of ecological interactions of genetically engineered plants. They further show the need for rigid quality control and independent investigations.

Several significant differences were found within particular sites indicating major biological differences between the comparator plants and the genetically engineered maize. It is unacceptable that the EFSA opinion more or less just ignores these findings and simply follows Monsanto’s interpretation.

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