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EFSA's Opinion on MON863 hybrids

Some more details on recent findings on the EFSA opinions about the MON863 hybrids MON863xMON810, MON863xNK603 and MON863xMON810xNK603 (EFSA 2005) :

Environmental risk assessment: Member States had explicitly asked for effects of the three hybrids on non-target organisms but EFSA declares such studies unnecessary because the application would be for import and processing only. Experiences with GM crops show repeatedly unintended use, experiences with the feed and food industry show repeatedly unintended or even illegal use. Why not expect similar events for these GMOs?
Member states also asked for the consequence of water and soil exposure to the Bt toxins present in the GM maize via organic waste material and litter or sewage to be addressed. Such expore occur during processing or through spillage. This was not studied because EFSA considers the amount of toxin to be too low to be of interest. No data are given for this assumption even though EFSA acknowledges that Bt toxin from manure can reach susceptible organisms such as soil coleoptera (EFSA 2005a,b,c,d). No consideration is given to the fact that all studied MON863 hybrids have increased Bt Cry3Bb1 toxin levels.

Antibiotic Resistance Gene: MON863 contains an GM antibiotic resistance gene (nptII) against kanamycin and neomycin. EFSA seems to be unaware of political decision to phase out the use of antibiotic resistance genes as markers for GM crops, and instead refers to more than 10 year old studies to claim "a history of safe use" and conclusions predating the EU decision not to allow antibiotic resistance markers: "This conclusion was based on the limited use of kanamycin and neomycin in human and veterinary medicine, the already widespread presence of this gene in bacterial populations [...] NptII is a well-established selection marker with a history of safe use (Nap et al., 1992; Redenbaugh et al., 1994). This conclusion is consistent with earlier safety evaluations of nptII (SCP, 1998)." (MON863xNK603). „The GMO Panel recently concluded that the use of the nptII gene as a selectable marker did not pose a risk to the environment or to human and animal health." (EFSA 2005c: 15)

Mitochondrial DNA: MON863 unintentionally contains mitochondrial DNA in the transgenic insert which was not part of the original intended insert. No risk assessment has been performed for this unintended DNA sequence and the possible effects. Instead, EFSA (2005a: 6) claims that DNA from organelles (mitochondria, chloroplasts) „acquired during the transformation is established as a

normal phenomenon in plant biology and the Panel considered that this would not significantly impact on the present safety assessment..“ This is not the case. The mitochondrial DNA inserted in the MON863 genome is an unintended DNA sequence, and neither part of the nuclear maize genome nor part of the original transgenic insert. As such, it needs to be assessed in a risk assessment. If mitochondria were damaged during the GM transformation to such a degree that their DNA was included in the nucleus, then it is necessary to study possible effects caused by this, as well other possible mitochondrial insert in the rest of the genome.

Bt toxin: In each of the three MON863 hybrids, the Cry3Bb1 toxin levels in the kernels are increased compared with those of MON863 itself (29 µg Cry3Bb1/g dry weight in MON863, 37 µg/g dw in MON863 x MON810 x NK603; see table for details). No further study is done on why there is a consistent increase, and whether this indicates other cumulative effects of the four GM traits. Instead EFSA discards them with one sentence: „This reflects variability in gene expression, which may have been influenced, for example, by environmental factors.“ However, no study is done to confirm that. And if GMO hybrids with stacked GM traits display such a variability in gene expression: can they be considered stable?
Cry1Ab levels in kernels are increased in one hybrid, and decreased in another. Again, no further study is performed.

Composition: several different components are different between the original GM maize varieties MON863, MON810 and NK603 and the hybrids cross-bred from them. For each of the three hybrids, EFSA (2005a: 9) states that „a number of statistically significant differences were observed“ but the Opinions give very little details on which components were different. From those data that are included, a significant decrease in linolenic acid in kernels stands out because it appears in both hybrids involving MON863 and MON810.
The argumentation used by EFSA to discard the observed statistical significance in niacin levels in kernels reduces the whole concept of studying composition differences between control and test group to absurdity:
„Niacin levels decreased from [...] control maize to [...] MON863 x MON810 x NK603 maize, while the latter was slightly outside the background range of commercial varieties [...], but still within the background ranges reported in literature [...] and from previous field trials with maize [...].“ (EFSA 2005d: 10)

Toxicity: The toxicity studies are based on the assumption that any relevant effect is dose related

and that they appear to the same degree in male and female animals. This hypothesis has no scientific basis.
Significant differences are discarded because even though there is a decrease in haemoglobin concentration between non-GM and GM feed, the concentration does not get decreased further with more GM feed.
Decreased organ weight were observed in all studies including heart, kidney, thyroid/parathyroid, epididymes and other (not specified) organs. Most of these differences are described as "statistically significant different".
Changes in blood included lower numbers of red blood cells, increased corpuscular hemoglobin for one hybrid and a decrease in another, increased blood urea nitrogen, changes in basophile counts, and an increased neutrophil count (See table).
Other changes included higher feed consumptions of animals: Female rats fed with a small amounts of MON863xMON810 and rats fed high amounts of MON863xNK603 consumed more feed then the control groups (See table).

General remarks on EFSA work: EFSA has a poor understanding of the concept of „statistical significant difference“. In statistics, a difference is significant if it is unlikely to have occurred by chance. In a study to compare a test and control group this means that the difference is caused by the trait or treatment that is different between both groups, and that each test object/animal can be identified as belonging to either of these groups.
EFSA however repeatedly discards such statistically significant differences. It either argues that the difference is not really a difference because the range of data overlaps or because the results are still similar enough. Such interpretation goes against the very basic of the concept of „significant difference“ as explained above, because statistcal signficant differences are calculated to state whether there is a difference or not and to exclude such speculations. This argumentation even gets worse when data from other tests or historical records are used to discard the data from controlled tests.
In other cases EFSA aknowledges the significant differences, but argues that they will have no biological relevance. First of all, the term „biologicall relevant“ there is not based on a scientific concept. Secondly. the unscientific argument of "biological relevance" reduces the whole test to meaninglessness because EFSA basically says that the test as such was not designed to give any relevant information anyway. In this case it would be necessary to repeat the tests with a different values for significance and power, but not to discarded the results as „irrelevant“ or to desing the tests in a different way. Instead EFSA places its assumptions about relevance above the recorded difference between GM crop and the unmodified plants.

Unexpected differences between test and control group, and especially such obvious ones as the increased Cry3Bb1 levels, can be indicators for underlying changes in the plant physiology caused by the transgenic inserts. EFSA, however, seem to consider Bt levels and plant components as some kind of static list of components of a food and feed product and not as indicators for the physiology of a living organisms.

Table: Statistically significant differences betweeen MON863 hybrids and unmodified maize and EFSA's opinion about it

GM line/hybrid	**Difference** *: statistically significant		**Details** All quotes are from the EFSA opinions (EFSA 2005 a,b,c, d)
	Bt toxin levels [µg/g dw] Cry1Ab	Cry3Bb1	
MON863		29	
MON810	0.77		
NK603	--	--	
MON863 x MON810	>0.77 (increase)	>29 (increase)	„Cry3Bb1 and Cry1Ab protein levels in kernels of MON 863 x MON 810 maize were higher than in the individual MON 863 and MON 810 lines." „This reflects variability in gene expression, which may have been influenced, for example, by environmental factors."
MON863 x NK603		34 (increase)	
MON863 x MON810 x NK603	0.65 (decrease)	37 (increase)	
	Compositional analysis		
MON863	* palmitic acid		"Some statistically significant but small differences were observed for palmitic acid (C16:0) between MON 863 and its control."
NK603	* stearic acid		„Compositional data for maize NK603 from two growing seasons revealed a minor, but statistically significant difference for the stearic acid content (C18:0) in kernels compared to non GM maize in one year, but not in the other year."

MON863 x MON810	* a number of significant differences in composition data	„In the comparisons of data from all four combined sites, a number of statistically significant differences were observed." "Comparison of MON 863 x MON 810 maize with controls, both single-trait parental lines and various commercial reference hybrids showed statistically significant differences in several compounds.“
	* decreased linolenic acid	„The average level of linolenic acid (C18:3) in kernels of MON863 x MON810 maize was significantly decreased compared with that of all comparators in each separate location: 1.01 ± 0.02 % of total fatty acids in MON 863 x MON 810 compared with 1.17 ± 0.02 % in MON 863, 1.10 ± 0.02 % in MON 810, 1.19 ± 0.02 % in the control, and 1.19 ± 0.01 % in commercial reference lines.“ „Panel concludes, therefore, that the difference in linolenic acid is not meaningful from a biological point of view.“
MON863 x NK603	• „a number of differences” – no further details given	„A number of statistically significant differences were observed in the comparisons of the composition of kernels from MON 863 x NK603 and its control.“ „The Panel therefore considers the observed consistent differences in arachidic acid content as small in size and not meaningful from a biological point of view.“
	* increased arachidic acid	
MON863 x MON810 x NK603	* „a number of differences” – no further details given	„A number of statistically significant differences were observed in the comparisons of the composition of kernels from MON 863 x MON 810 x NK603 and its control.“
	* increased oleic acid * decreased linoleic acid * decreased niacin	„Niacin levels decreased from 20.81 mg / kg dw in control maize to 18.13 mg / kg dw in MON863 x MON 810 x NK603 maize, while the latter was slightly outside the background range of commercial varieties (18.23 - 31.02 mg / kg dw), but still within the background ranges reported in literature (9.3 - 70 mg / kg dw) and from previous field trials with maize (14.1 - 36.3 mg / kg dw).“
Food/feed safety and toxicological assessment		

MON863 x MON810	Feed consumption	„Small deviations in food consumption by females on test diets containing MON 863 x MON 810 were observed as compared with those on the control diet.“
	* **decreased haemoglobin concentration**	„Analysis of the clinical chemistry and pathology data showed statistically significant decreases in mean corpuscular haemoglobin concentration in male animals in the 11% and 33% test diet groups. Values of 32,7 g/dl for the control group, 31,6 g/dl for the 11% group and 31,6 g/dl for the 33% group and therefore these values were not dose-related.“
	* **different basophile counts**	„Another statistically significant, but slight difference in basophil counts was observed but only in males that received the 11% test diet. The Panel considers the changes observed to be of no toxicological relevance.“
	* different organ weights	„Concerning organ weights, some statistically significant differences were observed.“
	* lower hyroid/ parathyroid weight	„For example, lower mean absolute and relative thyroid/parathyroid weights compared with the control group (0,257 g) were observed in female animals of groups fed the 11% (0,200 g) and 33% (0,219 g) test diets.“
	* lower kidney weight	„The mean kidney weight relative to body weight was statistically significantly lower in females in the 33% test diet group.“

MON863 x NK603	* higher feed consumption	"With regard to feed consumption, the average daily intake per animal was statistically significantly higher during various weeks in the male and female 33% test groups. However, the ranges of individual values overlapped with each other."
	lower heart weight	„Lower mean heart weight and heart/brain ratio in male rats fed 11% MON 863 x NK603 compared with the controls."
	*** less red blood cells**	„With regard to haematology parameters, the only observed statistically significant differences were lower values for mean red blood cell count and higher values for mean corpuscular hemoglobin in male rats fed 11% MON 863 x NK603 compared with the control group."
	*** increased corpuscular hemoglobin conc.**	
	* increased blood urea nitrogen	„The value for mean blood urea nitrogen was statistically significantly higher in female rats in the 11% test group than in the control group"
MON863 x MON810 x NK603	increased neutrophil count	„The results of blood cell analysis showed that the average absolute counts of neutrophils in females fed 11% transgenic maize were higher than those in animals fed control maize."
	less urine	„The volume of urine was lower in males in both test groups as compared with that in the control group."
	increased epididymes weight	„Higher absolute weights of the epididymes were observed in males of the test group fed a diet containing 11% transgenic maize as compared with the corresponding weights in the control group."
other		
MON863	mitochondrial DNA in the transgenic insert	„Nucleotide sequences at the junctions between the insert and parental DNA were determined and bioinformatic analysis revealed the presence of mitochondrial DNA at both the 5' and 3' flanks. The integration of organellar DNA within the nuclear plant genome – being already present or acquired during the transformation- is established as a normal phenomenon in plant biology and the Panel considered that this would not significantly impact on the present safety assessment."

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Sources:

EFSA 2005a: Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/DE/02/9) for the placing on the market of insect-protected genetically modified maize MON 863 x MON 810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto, The EFSA Journal (2005) 251, 1-22.

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EFSA 2005c: Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-UK-2004-06) for the placing on the market of insect-protected glyphosate- tolerant genetically modified maize MON 863 x NK603, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto, The EFSA Journal (2005)255, 1-21.

EFSA 2005d: Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-BE-2004-07) for the placing on the market of insect-protected glyphosate- tolerant genetically modified maize MON 863 x MON 810 x NK603, for food and feed use, import and processing under Regulation (EC) No 1829/2003 from Monsanto, The EFSA Journal (2005) 256, 1-25.

This text and table have been prepared by Antje Lorch on behalf of Greenpeace.
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