Shortcomings in the Assessment of Human Health Effects of GM Maize Mon810 by Monsanto (Part I – Technical dossier) Regulation (EC) No. 1829/2003

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EFSA has submitted a positive opinion on the genetically modified maize MON810. It is now up to the member states to comment on the data provided by the applicant.

THIS IS NOT A VALID APPLICATION –THE COMPLETENESS CHECK AND ACCEPTANCE AS A VALID APPLICATION AS ON 29TH JANUARY BY EFSA WAS INCORRECT

LEGAL REQUIREMENTS OF REGULATION (EC) NO. 1829/2003, **ARTICLE 4 (1)**

Food referred to in Article 3(1) must not:

(a) have adverse effects on human health, animal health or the environment;

(c) No GMO for food use or food referred to in Article 3(1) shall be authorised unless the applicant for such authorisation has adequately and sufficiently demonstrated that it satisfies the requirements of paragraph 1 of this Article.

Also Regulation 178/2002 is important as recital (43) of Regulation (EC) No. 1829/2003 shows.

In order to provide a high level of protection of human life and health, animal health and welfare, environment and consumer interests in relation to genetically modified food and feed, requirements arising from this Regulation should apply in a non-discriminatory manner to products originating in the Community and imported from third countries, in accordance with the general principles referred to in Regulation (EC) No 178/2002.

REQUIREMENTS OF REGULATION (EC) NO 178/2002 ARTICLE 14(4):

In determining whether any food is injurious to health, regard shall be had:

(a) not only to the probable immediate and/or short-term and/or long-term effects of that food on the health of a person consuming it, but also on subsequent generations;

(b) to the probable cumulative toxic effects;

(c) to the particular health sensitivities of a specific category of consumers where the food is intended for that category of consumers.

REQUIREMENTS OF DIRECTIVE 2001/18/EC, ANNEX II

"A general principle for environmental risk assessment is also that an analysis of the cumulative long-term effects relevant to the release and the placing on the market is to be carried out. Cumulative long-term effects refers to the accumulated effects of consents on human health and the environment, including inter alia flora and fauna, soil fertility, soil degradation of organic material, the feed/ food chain, biological diversity, animal health and resistance problems in relation to antibiotics"

THE COMMISSION DECISION 2002/623/EC OF 24 JULY 2002 ESTABLISHING GUIDANCE NOTES SUPPLEMENTING ANNEX II TO DIRECTIVE 2001/18/EC

Article 4.2.4 requires that in every risk assessment

"The overall uncertainty for each identified risk has to be described, possibly including documentation relating to:

—assumptions and extrapolations made at various levels in the ERA,

—different scientific assessments and viewpoints,

—uncertainties,

—the known limits of mitigation measures,

—conclusions that can be derived from the data."

SHORTCOMINGS OF LEGAL REQUIREMENTS BY MONSANTO

Table 1: Shortcomings of legal requirements by the Monsanto

Regulation	**Legal Requirements**	**test according to international standards**	**performed test**	**fulfilment by Monsanto**
Reg EC 1829/2003 Art. 4 in conjunction with Article 14 (4) of Reg EC 178/2002	assessment of long-term effects	24 month studies	90 days	no
Reg EC 1829/2003 Art. 4 in conjunction with Article 14 (4) of Reg EC 178/2002	assessment of short term effects	90 days studies	90 days	yes
Annex II of Directive 2001/18/EC	analysis of the cumulative long-term effects	24 month studies single and multiple stressors	-	no
Reg EC 1829/2003 Art. 4 in conjunction with Article 14 (4) of Reg EC 178/2002	effects on subsequent generations	generation study	-	no
Reg EC 1829/2003 Art. 4 in conjunction with Article 14 (4) of Reg EC 178/2002	cumulative toxic effects	multiple exposure study	-	no
Commission decision 2002/623/EC Article 4.2.4	The overall uncertainty for each identified risk has to			no

	be described			

CONCLUSION

The applicant has **not demonstrated adequately and sufficiently** that MON810 has no adverse effects on human health **according to the requirements of Regulation (EC) No. 1829/2003 Article 4 (1 and3)**. It is obvious that not only short term but also long term risks must be assessed to exclude human health impacts seriously. This is also required by article 14 of Regulation EC No 178/2002 and Annex II of Directive 2001/18/EC. Although a long list of legal requirements has not been fulfilled by the applicant the application was checked for completeness and accepted as a valid application on 29th January 2008 by EFSA. Based on the legal requirements this is not a valid application as data on long term effects, subsequent generations etc. and uncertainties are not submitted by the applicant (i.e. Monsanto) and therefore legal requirements are not fulfilled by the applicant see table 1. The Member States and the EC Commission shall return this application back to EFSA until all missing legal requirements are fulfilled.

Besides that we recommend a Council hearing on EFSA, as EFSA failed to fulfil legal requirements in any application of GMOs so far. When EFSA members are not willing or unable to fulfil legal requirements then the panel members must be replaced.

MOLECULAR CHARACTERIZATION IS INSUFFICIENT

NO UPDATED MOLECULAR CHARACTERIZATION AND FLANKS SEQUENCING BY MONSANTO

Quote by Monsanto:

„... evidence from a body of independent peer-reviewed literature on MON 810 that does not raise any safety issues (see Annex 3.1 of the "Specific Information" in this renewal application**), do not indicate the need to update the information on molecular characterization and flanks sequencing**. The additional information generated in the context of MON 810 containing stacks and therefore reviewed by EFSA has been consolidated into a report cited in Part I - Technical dossier (Scanlon et al., 2007). The data in this report confirm the original characterization data previously reported".

COMMENT

Monsanto referred to a non peer-reviewed report (Scanlon et al., 2007) concluding that there **might be no need to update the information on molecular characterization and flanks sequencing**. Monsanto claims that "The DNA sequence of the insert and of the flanks in MON 810 is commercially sensitive information". But Monsanto fails to argue why this information is "commercially sensitive". SCANLON et al 2007 is not cited in Annex 3.1 the list of peer reviewed articles on MON810, so the public is not able to analyse the data.

In contrast to Monsanto's refusal to analyse in detail the flanking regions of the insert with state of the art technology (which definitely has evolved significant since the initial application) new scientific findings on the insert were provided by independent scientists. Rosati et al (2008)[1] demonstrate that there are

[1] Rosati A, Bogani P, Santarlasci A, Buiatti M (2008): Characterisation of 3′ transgene insertion site and derived mRNAs in MON810 YieldGard® maize. Plant Molecular Biology. Accepted: 16 February 2008 Published online: 28 February 2008. DOI: 10.1007/s11103-008-9315-7.

- Several new fusion RNA´s transcripted from the construct and the flanking regions
- The two MON810 flanking regions do not belong to the same DNA locus
- Data suggest that that the transformation event may have involved the truncation of the 3´ end of putative HECT endogenous gene leading to the partial loss
- The flanking regions are so far not identified i.e. DNA from unknown origin

Rosati et al (2008)[1] clearly demonstrate that there is a need to fully analyse the construct with state of the art methods to get a complete picture of what is happening in the genome.

Without a complete updated molecular characterization and flanks sequencing, it is not possible to demonstrate adequately and sufficiently that MON810 is safe as required by the Regulation (EC) No. 1829/2003.

MONSANTO'S INSUFFICIENT ANALYSES OF EXPRESSION OF POTENTIAL FUSION PROTEINS

Monsanto's analyses show that there might be transcription and there might be translation of the open reading frames of the insert and the flanking regions, but concluded that.*" No biologically relevant structural similarities to allergens, toxins, or pharmacologically active proteins were observed for any of the putative polypeptides"*. Monsanto failed to acknowledge that also RNAs can act as biological active structures (see details below).

FUSION RNAS NOT IDENTIFIED AND IMPACT NOT ANALYSED BY MONSANTO

As Monsanto failed to fulfil its legal obligations (assisted by EFSA which did not demand a new molecular characterization), Monsanto did not explore RNAs transcribed from the open reading frames in the construct. Rosati et al (2008)[1] report several RNA transcripts from the insert and the flanking regions. It is obvious that these RNAs themselves might be biologically relevant RNA structures. Monsanto failed to address this question.

SYNTHETIC RNAS AND ITS BIOLOGICAL CONSEQUENCES

RNAs are known to trigger allergenic or other autoimmune effects. Researchers identified a food RNA of shrimp to be a mayor allergen for humans (NAGPAL et al. 1987)[2]. This shows that not only food-proteins are able of triggering allergenic reactions. There is also evidence that immunostimulatory nucleic acid sequences may temporary attenuate allergenic diseases like asthma (SILVERMAN and DRAZEN 2003)[3].

RNAs of unknown origin transcribed in the flanking regions of the insert may also trigger other immune responses as the human immune systems has several receptors for foreign (= not self) nucleic acids. One of the major pathways to detect foreign DNA/RNA in mammals is via Toll-like-receptors (TLR). TLRs are evolutionary conserved from the worm *C. elegans* to mammals. The members of the Toll-like receptor (TLR) family recognize conserved molecular patterns, including peptidoglycans, lipopolysaccharides (LPS), and, most

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[2] Nagpal S, Metcalfe DD, Rao PV (1987) *Identification of a shrimp-derived allergen as tRNA*. The Journal of Immunology **138**(12): 4169-4174

[3] Silverman ES, Drazen JM (2003) *Immunostimulatory DNA for Asthma: Better than Eating Dirt*. American Journal of Respiratory Cell and Molecular Biology **28**(6): 645-647

interestingly, nucleic acids. Four (TLR3, TLR 7, TLR 8, TLR 9) of twelve to date known Toll-like receptor (TLR) are able to recognize foreign RNA or DNAs (PAWAR et al. 2006)[4].

But RNAs and DNA can also trigger immune responses via Toll-like receptor independent pathways like the retinoic acid-inducible protein1 (RIG-1) The retinoic acid-inducible protein1 (RIG-1) seems to be responsible for TLR independent response when cells are challenged with viral DNA or RNA (WAGNER and BAUER 2006)[5].

MONSANTO FAILED TO ANALYSE THE STRUCTURE OF FUSION RNAS AND TO ANALYSES POTENTIAL INTERACTIONS WITH THE IMMUNE SYSTEM

Monsanto failed to analyse in MON810 the structure of fusion RNAs and to analyse potential interactions with the immune system via the Toll-like receptor (TLR) pathway or via Toll-like receptor independent pathways such as the retinoic acid-inducible protein1 (RIG-1). Similar to MON810, irregularities were also found in genetic insert of Roundup Ready soybeans. Windels et al (2001)[6] showed that the initial molecular characterization provided by Monsanto was incomplete. Windels et al (2001) first identified the occurrence of sequence of unknown origin which does not belong either belong to the insert, nor to the soybean genome. 10 years after the approval of Roundup Ready Soybean, Rang et al (2004)[7] detected that this "sequences of unknown origin" are transcripted into RNAs.

Also in its renewal application of MON810 Monsanto refused to analyse the structure of the insert with state of the art scientific methods and 10 years later, independent scientists Rosati et al (2008)[1] identify synthetic RNAs from the synthetic transgene and the flanking regions.

It seems to be a common phenomenon that transgenic plants produce synthetic RNA of unknown origin. It is therefore highly important to assess the abundant synthetic RNAs to the human immune system before placing GMOs on the market.

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[4] Pawar RD, Patole PS, Wornle M, Anders HJ (2006) *Microbial nucleic acids pay a Toll in kidney disease*. Renal Physiology **291**(3): F509-F516

[5] Wagner H, Bauer S (2006) *All is not Toll: new pathways in DNA recognition*. Journal of Experimental Medicine **203**(2): 265-268

[6] Windels P, Taverniers I, Depicker A, Bockstaele Ev, Loose Md (2001) *Characterisation of the Roundup Ready soybean insert*. Eur Food Res Technol **213**: 107-112

[7] Rang A, Linke B, Jansen B (2005) *Detection of RNA variants transcribed from the transgene in Roundup Ready soybean*. European Food Research and Technology **220**(3 - 4): 438-443

ACUTE TOXICITY STUDY WITH CRY1Ab PROTEIN INVALID- VIOLATION OF THE CASE BY CASE PRINCIPLE

Quote page 92:

"An acute mouse gavage study with the Cry1Ab was performed to directly assess potential toxicity associated with the Cry1Ab " "The black box is the original censored black mark in the public available document"

a. Why Monsanto claimed this report as "confidential" is unknown. Did Monsanto want to hide important data on food safety before the public? Monsanto obviously fears that the consumers might in detail challenge Monsanto's so-called proof of the food safety.

b. But also from Monsanto's sparse information on the report it is clear that Monsanto has violated the obligatory rule to assess all risks according to the "Case by Case Principle" .

Monsanto states that

c. The Cry1Ab gene encoding the natural identical full length Cry1Ab Protein

was introduced into *E.coli*

On this statement it is clear that

- Monsanto has not used the original protein structure i.e. the truncated protein
- Monsanto has not used the protein from the plant.

This practice by Monsanto is a clear violation of the "Case by Case Principle". As small changes in the protein structure (see BSE-proteins) can trigger substantial effects, it is clear that a acute toxicity test of a Cry1Ab Protein which is not derived from Mon810 **is scientifically invalid and does not provide any safety information of MON810.**

TESTING OF THE WHOLE GM FOOD/FEED TECH REPORT NO 7.8.4. PAGES 98-100 –

VIOLATION OF THE "CASE BY CASE PRINCIPLE" AND VIOLATION OF REGULATION (EC) NO. 1829/2003 ARTICLE 30 (3)

13 weeks feeding study 90 days subchronic study

Reference cited by Monsanto with authors censored (=black mark):

13 week feeding study in rats with grain from Yield Gard (MON810) corn grain (DK 551 Bt) preceded by a 1-week baseline food consumption determination with PMI certified rodent diet #5002 Monsanto Technical Report MSL 17596

a) Why Monsanto claimed this report was confidential is unknown. But perhaps Monsanto wants to hide important data on food safety before the public. Monsanto obviously fears that the consumers might in detail challenge Monsanto's so called proof of the food safety. This is a clear violation of Regulation (EC) No. 1829/2003 Article 30 (3): *"Information relating to the following* ***shall not be*** *considered confidential:*
d) effects of the GMO, food or feed referred to in Articles 3(1) and 15(1) on human and animal health and on the environment"

b) As all applications have to be evaluated case by case, this study cannot be used by a competitor who has also to comply with the case by case principle. To claim this study as confidential business information is not comprehensible and not in line with legal obligations by Regulation (EC) No. 1829/2003. So why the author of the study is confidential is not comprehensible.

c) In contrast to the BROILER CHICKEN PERFORMANCE test. Monsanto gave no information in detail on the results of the study and very little general information (two pages) on the whole food study.

Monsanto failed even to give information on the full test design. Monsanto only states that:
"For quantitative measures, MON 810 test group was compared with

a) its control counterpart and
b) the population of rats fed the non-transgenic commercial (Monsanto failed to inform how many reference control groups has been used
c) maize historical controls for the testing laboratory"

COMMENT ON THE STATISTICAL VIOLATIONS IN THE STUDY

The test performed is scientifically invalid for several reasons:

a) To compare significant changes to "maize historical controls for the testing laboratory is a violation of the "ceteris paribus" clause (i.e: other things being equal) of any statistical analyses of data. The data is derived from organisms which have a different genomic background, different age etc., and were fed on different feed or at least different patch of control feed, were exposed to different daylight, room temperature conditions etc., and cannot be used as a control group to deny statistical significant differences.

b) To include a various number (Monsanto failed to give detailed information on that in the publicly available document) of reference lines and to compare results to reference control group is not scientifically valid. It is a comparison 1 against 7-10[8] controls. It is clear that Monsanto has chosen this experimental design to dilute any statistically significant effect in the greater variation of the 7-10 control lines (reference control group and control group). To get real information if there is a statistically significant difference 7 GMO lines have to be tested against 7 reference control lines. Only from such experimental design can deviations of statistical significant difference be made.

c) To exclude effects which are not dose related is not in line with the scientific literature: Especially chronic and subchronic effects or the trigger of autoimmune diseases are commonly known to act not dose or sex-related. There are several examples as Lahita (1996) points out: "The autoimmune diseases are more common in women than men. The actual prevalence ranges from the high of 10 to 15 females for each male for systemic lupus erythematosus to four females for every male with rheumatoid arthritis"[9]. Also Sobel et al (2005) report non dose related effects show that chemicals are able to trigger auto immune effects at low concentrations i.e. 4-fold lower than the NOEL. He suggests that "an effect on autoimmunity might be a sensitive toxic end point (an effect that occurs at doses lower than other adverse effects) for the pesticide, methoxychlor, and therefore of **particular interest for risk assessment". (See also** Rao et 1999 just to name a few).[10]

COMMENT ON INSUFFICIENT LENGTH OF THE 90 DAYS SUBCHRONIC TOXICITY STUDY

Significant differences identified in a subchronic study must be addressed and not downplayed. The minimum is to perform a chronic 24 months study and to use sensitive endpoints like the trigger of auto immune diseases to rule out any potential health effects.

BROILER CHICKEN PERFORMANCE WHEN FEED DIETS CONTAINING MON810 - PAGES 100-106

The broiler chicken study is a study used to measure food conversion and the performance of MON810 maize as feed in broilers. There are no toxicological relevant endpoints, to conclude any safety from this study. Subchronic or chronic effects cannot be derived from this study. Monsanto's method to derive safety conclusions from such kind of feed conversion study are not consistent with any scientific standard.

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[8] In recent 90 days study Monsanto used at least 6 reference control lines, so we suppose that also in this case, Monsanto has used at minimum 6 reference control lines

[9] Lahita RG. The connective tissue diseases and the overall influence of gender. Int J Fertil Menopausal Stud. 1996;41:156–165.

[10] Sobel et al (2005) ,Environ Health Perspect. 2005 March; 113(3): 323–328.
Rao T, Richardson B. Environmentally induced auto-immune diseases: potential mechanisms. Environ Health Perspect. 1999;107(suppl 5):737–742

THE IMPORTANCE OF THE "CASE BY CASE PRINCIPLE" - REMARKS BY THE EC IN THE WTO CASE[11]

734: The Codex Alimentarius Commission adopted the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology, and the Draft Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants and Microorganisms (Codex). The principles for the safety assessment **dictate a case by case pre-market assessment** on the basis of a comparative safety assessment (CSA) ... The **safety of the gene product must be assessed on a case-by-case basis.** ... In addition to investigating health risks directly associated with food production, the broadening of the Codex risk assessment to include indirect effects now encompass effects of novel food on the environment that may have an indirect impact on human health.

735**. A case by case** assessment considering any organisms derived from a transformation event as well as different receiving environments is broadly recognised as the best framework for assessing environmental risks of GMOs.

MONSANTO MISSED TO APPLY IN DUE TIME FOR THE RENEWAL OF THE APPLICATION – THE AUTHORISATION AS FOOD IS NO LONGER VALID.

The authorisation by the EC commission was 22 April 1998 (consent by French authorities was 03 August 1998) the application for renewal was 04 May 2007. Critical is the term "were first placed on the market " from Regulation 1829/2003. According to the EC register http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

Date of placing on the market:

- 01/01/1997 (as food additives, feed additives and feed materials) deadline 01/01/2007 because application was submitted on 04 May 2007.
- 05/05/1998 (as feed) i.e. deadline 05/05/2007 OK 04 May 2007.
- 05/02/1998 (as food) i.e. deadline 05/02/2007 missed because application was submitted on 04 May 2007.

According to the register the applicant failed to apply in due time for use as food, use as food additives, feed additives and feed materials. Only application as feed was in due time. This would mean that the use as food is illegal because the authorization has expired.

Only for feed authorisation is valid because only when the applicant has in due time applied for renewal, than is the authorization extended as long as the renewal authorization process is finished i.e. a positive or negative decision has been made.

Article 11 of Regulation (EC) No. 1829/2003:
Authorisations under this Regulation shall be renewable for 10-year periods, on application to the Commission by the authorisation-holder at the latest one year before the expiry date of the authorisation.
Article 8 Status of existing products of Regulation (EC) No. 1829/2003:

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[11] COMMENTS BY THE EUROPEAN COMMUNITIES ON THE REPLIES BY THE SCIENTIFIC EXPERTS TO THE QUESTIONS POSED BY THE PANEL, 28 JANUARY 2005 - ANNEX 14, WT/DS291/R/Add.7 , WT/DS292/R/Add.7, WT/DS293/R/Add.7, Page I-197

Within nine years from the date on which the products referred to in paragraph 1(a) were first placed on the market, but in no case earlier than three years after the date of application of this Regulation, operators responsible for placing them on the market shall submit an application in accordance with Article 11, which shall apply mutatis mutandis.