European Network of Scientists for Social and Environmental Responsibility

Vergleichende Evaluation der Fütterungsstudien von GV Pflanzen für die EU Zulassung

Comparative evaluation of feeding studies for EU approval of genetically modified plants

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BACKGROUND

A 2-year rat feeding study with genetically modified NK603 maize sparked an international debate and policy responses by the European Commission.

The European Food Safety Authority (EFSA) evaluated the study as defective based on conceptual and methodological shortcomings by **retrospective application of a recent guidance.**

OBJECTIVES

EFSA'S **recent guidance**[1] applied to all three relevant NK603 publications, including the feeding study by Monsanto, and evaluated by the same standard.

EFSA's evaluation contextualized with other long-term studies.

[1]*EFSA Scientific Committee. **2011**. EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/ feed. EFSA J 9(12):2438.*

Two application rounds in EU of NK603 HR maize

First application:
Novel Food Regulation (EC) No 258/97 – application was split in two parts: a) the application for import, feed and industrial use was filed in 2001 to the Spanish authority and b) the application for food was filed in 2002 to the Dutch authority.

Second application:
In 2005, Monsanto filed two parallel applications under Regulation (EC) No 1829/2003: one for the renewal of the previous approvals and one that also requested the authorization for cultivation.

Dossier NK603 veröffentlicht http://www.efsa.europa.eu/de/

2001-2002:
As part of their applications, Monsanto submitted data of a 90-day subchronic toxicity test with Sprague-Dawley (SD) rats, including an internal Monsanto study carried out by Dudek (2001).

2005:
When Monsanto resubmitted its files to EFSA, incl. the Dudek-study and a meanwhile published, peer-reviewed publication based on the Dudek-data, i.e. Hammond et al. (2004)

Choice of testing protocol

EFSA (2012a) states: "[g]iven that Séralini et al. (2012) conducted a two-year study, it is unclear why an OECD guideline suitable for a two-year chronic toxicity or carcinogenicity study (i.e. OECD 451, OECD 452 or OECD 453) was not adhered to."

OECD Guideline 452 in its 1981 version (OECD 1981), which would be applicable for the two-year toxicology study started in 2008, **only ten rats out of the 20** per group are necessary for blood and urine sampling and analysis.

OECD 453 – 10 rats for toxicity trials, in parallel 50 rats for carcinogenicty

Tabelle 1: Überblick über relevante OECD-Richtlinien für Fütterungsversuche (Quelle: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788)

Nummer der Richtlinie	Titel	Dauer	Anzahl der Tiere pro Versuchsgruppe und Geschlecht
408	Repeated Dose 90-day Oral Toxicity Study in Rodents (auch sub-chronische Fütterungsstudie genannt)	90 Tage	10
451	Carcinogenicity Studies (Langzeitversuche zur Überprüfung des Risikos für Krebserkrankungen)	24 Monate	50
452	Chronic Toxicity Studies (Langzeitversuche zur Überrprüfung möglicher toxischer Wirkungen)	12 Monate	20
453	Combined Chronic Toxicity\ Carcinogenicity Studies	24 Monate	50

Séralini et al. (2012): OECD 408 - **10 rats per group,** more parameters and extended to two years! OECD 453 – 10 rats up to 12 months if combined with 50 rats study for carcinogenicity trials

Monsanto study: OECD 408 – 20 rats per group but **selected 10 rats for blood and urine analysis**.
No selection criteria for the 10 rats scientifically explained or justified!

Conclusion:
Data on blood and urine analyses in both trials stem from 10 rats only!

RECOMMENDATION:
Data analysis after 90 and 120 days on toxicity parameters!

Choice of rats (Sprague Dawley (SD) Strain)

EFSA notes:
"strain of rats chosen is known to be prone to development of tumours over their life [...] This is neither taken into account nor discussed in the Séralini et al. (2012) publication."

"The biological relevance of the rat strain used should be justified with respect to the design choices."

Hammond et al. (2004) / Monsanto:
no explanation at all

Dudek (2001)/Monsanto explains:
"[t]he rat [SD] was selected for the study since this species has been traditionally used to assess the safety and wholesomeness of food.
Moreover, there is a historical database for the rat regarding the parameters that were measured."

Conclusion:
Pragmatic explanation underlines the customary use of SD rats in long-term trials - no scientific justification for its use with regard to the design choice as required by EFSA

Confirmed - SD rats used as standard test organism by the two largest toxicity/carcinogenicity research projects worldwide and at least 22 long-term studies:

1. The National Toxicology Program of the U.S. Department of Health and Human Services uses this strain in its 2-year studies, after in-depth studies on the suitability and advantages of the SD rat over previously used strains (King-Herbert et al. 2010).

2. The European Ramazzini Foundation for Oncology and Environmental Sciences (Italy) uses SD rats in its Ramazzini Foundation Cancer Program since more than 40 years (Soffritti et al. 2002b).

Authors	Affiliation	Duration of test [months[	Overall objectives	OECD Guidelines mentioned a	Choice of rat strain discussed a	Measures against bias reported [a]	Storage condition of diet reported [a]	Endpoint reporting a	Power calculation [a]	Number of animals used (and tested) [b]
9) Liang et al. 2010	Peking University & Capital Medical Universiy, China	24	toxicity	no	no	yes	no	yes	no	**20 (10 tested)**
10) Perricone et al. 2010	Michigan State University, University of Houston, Georgetown University, USA	15	toxicity	no	no	no	no	yes	no	**10/20 (5-7 tested)**
13) Lee et al. 2010	Korea Institute of Radiological and Medical Sciences and others, Korea	18	toxicity & carcinogenicity	no	no	no	no	yes	no	**20 (17-18 tested)**

Conclusion:
Sprague Dawley rats are used routinely in long-term toxicology and carcinogenicity studies.

EFSA criteria	Compliance with		
	Séralini et al. (2012)	Hammond et al. (2004)	Dudek (2001)
Study objectives need to be clearly stated a priori in the study protocol	+/-	+/-	+/-
Suitable controls for all treatment groups need to be presented	+	+	+
Biological relevance of the rat strain used should be justified	---	---	---
Measures taken to reduce the risk of bias (e.g. blinding) need to be taken	+	---	---
Critical information about the diet composition need to be reported	---	---	+
Details of the storage conditions of the feed need to be provided	---	---	---
Contamination of the diets, e.g. by mycotoxins, pesticides etc., need to be reported	+/-	---	+
All collected endpoints should be reported openly and transparently	---	+/-	+
The presented data need to ensure the calculation of exposure to the test substance	---	---	---
The sample size (power) calculation must be presented, especially when the study objectives are unclear	---	--- (but reference to OECD GL408)	--- (but reference to OECD GL408)

Dudek 2001/Monsanto reported **77 comparisons** in which **statistically significant** differences between the NK603 treatments and the different controls were measured.

71% (55) assigned to 4 different arbitrary categories of 'meaninglessness': "not biological significant" (19), "not biological relevant" (28), "not biological meaningful" (6), or "not toxicological significant" (2).

Only **29% (22**) of all statistically significant different comparisons were not assigned to one of those categories of ‘meaninglessness’ – BUT:
"[i]n total, some 1050 comparisons were made and approximately 53 of these were anticipated to be significant by ***chance alone*** *at the 5% significance level.”*
Effectively the 5th category of ‘meaninglessness’

Hammond et al. 2004 – No significant differences anymore!?

Two modifications were applied:

i) the only comparator in Hammond et al. (2004) are the mean values of all six reference controls but not of the parental control

ii) double standard deviation is used as upper and lower thresholds to determine statistical significance.

This new statistical approach - obviously unnoticed by EFSA - resulted in eliminating all statistical significant differences as still reported by Dudek (2001).

Causes for controversy over rat feeding study:

No accepted standard testing protocols for GMOs.
All discussed protocols were developed for testing of CHEMICALS

Why?
Because adverse effects of GM HR plants were postulated to be unlikely. Hence, no testing necessary

How?
By applying a narrow scope risk assessment and excluding the effects of corresponding herbicides from the beginning (but of course including them in the benefit analysis – asymmetrical risk assessment) – **concept of substantial equivalence/comparative safety approach**

Principles for the application of substantial equivalence to the assessment of foods from organisms developed by the application of biotechnology:

-If the new or modified **food or food component** is determined to be substantially equivalent to an existing food, then further safety or nutritional concerns are expected to be insignificant;

OECD 1993, p. 11-12

[illegible]

- Where a product is determined not to be substantially equivalent, the identified differences should be the focus of further evaluations;
- Where there is no basis for comparison of a new **food or food component**, ... then the new food or food component should be evaluated on the basis of its own composition and properties.

OECD 1993, p. 11-12

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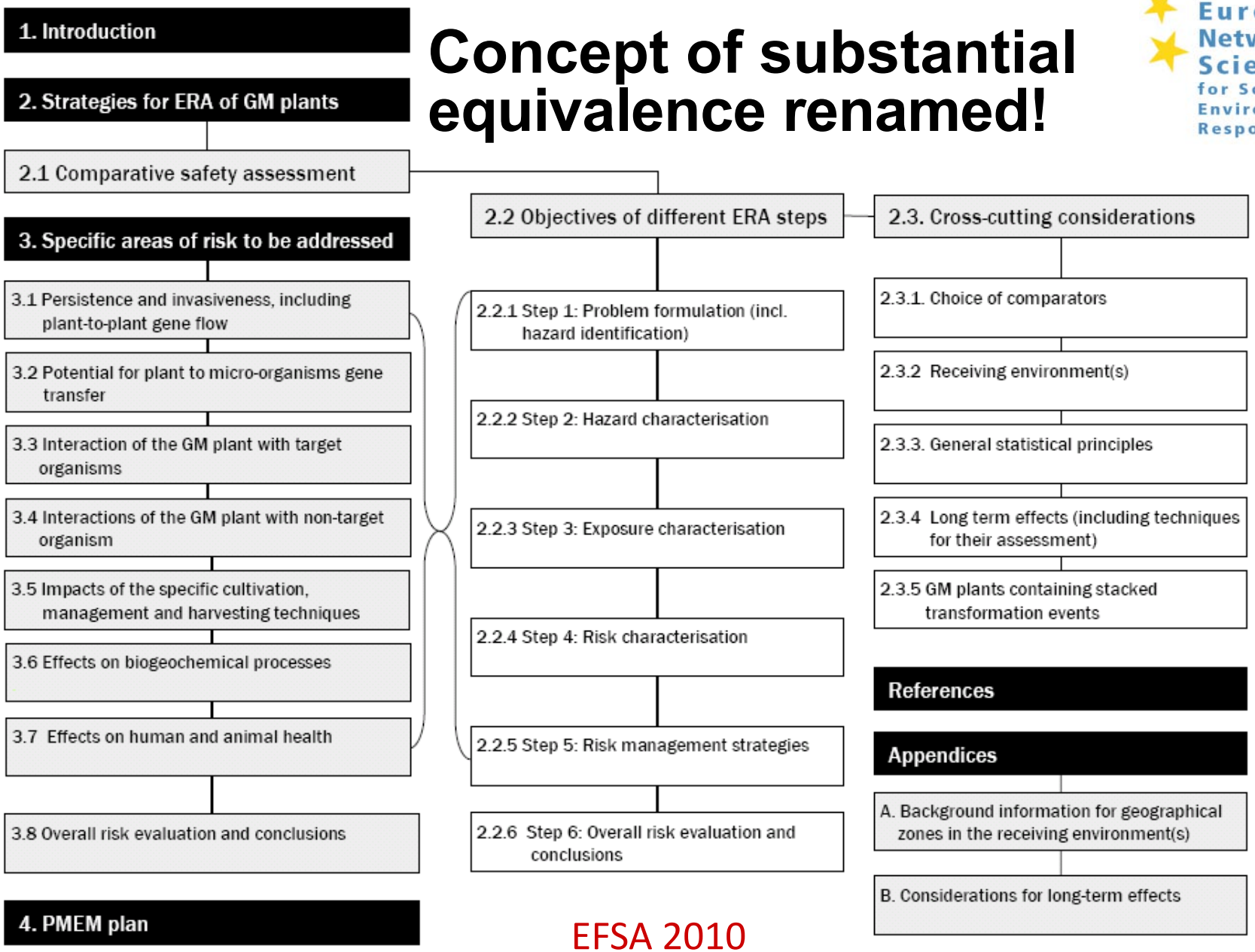
Concept of substantial equivalence renamed!



EFSA 2010

Conventional breeding of up to (4) GM plants, 8 GM traits – Smartstax – NO TESTING

D. INFORMATION RELATING TO THE GM PLANT

1. **Description of the trait(s) and characteristics which have been introduced or modified**

MON 89034 × 1507 × MON 88017 × 59122 is produced by crossing plants containing MON 89034, 1507, MON 88017 and 59122 using conventional breeding methods and expresses :

- two distinct *Bacillus thuringiensis* proteins, Cry1A.105 and Cry2Ab2 which provide a dual effective dose against feeding damage caused by the key

lepidopteran pest complex in maize

- the *Bacillus thuringiensis* var *aizawai* Cry1F insecticidal protein which provides a third activity against the lepidopteran pest complex
- the modified Cry3Bb1 protein, derived from *Bacillus thuringiensis* subsp. *kumamotoensis* that provides protection against corn rootworm (*Diabrotica* spp.) larval feeding and the CP4 EPSPS protein, derived from *Agrobacterium* sp. strain CP4 which provides tolerance to glyphosate.
- the *Bacillus thuringiensis* Cry34/35Ab1 binary insecticidal protein that provides a second mode of activity against corn rootworm larval feeding (*Diabrotica* spp.). 59122 also produces the PAT protein which provides tolerance to glufosinate-ammonium.

Commercialisation of MON 89034 × 1507 × MON 88017 × 59122 will therefore provide substantial benefits to growers by reducing the risk from insecticide and herbicide use to humans and the environment and by limiting yield losses from insects feeding damage while at the same time limiting weed pressure.

5-6 Bt Toxine!
-Cry1A.105
-Cry2Ab2
-Cry1F
-Cry3Bb1
-Cry34/35Ab1 (binary)

Resistent gegen 2 Totalherbizide:
- Roundup (Glyphosat)
- BASTA (Glufosinat)

ZEIT ONLINE | UMWELT

GRÜNE GENTECHNIK

Die Risikoabschätzung gentechnisch veränderter Pflanzen ist unzureichend

In der Grünen Gentechnik beginnen Fragen zur Sicherheit, wo Entwickler-Interessen aufhören. Es reicht nicht, gentechnisch veränderte Pflanzen wie Chemikalien zu testen.

von Angelika Hilbeck;Hartmut Meyer I 07. März 2012 - 10:17 Uhr

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CONCLUSION

If Seralini et al. 2012 study is insufficient to arrive at a conclusion, **all studies are insufficient!**
Hence, there is no basis for a safety conclusion!

EU risk assessment and decision making on GM crops is still largely based on regulatory science and methodologies developed under and for the contrasting U.S. regulatory approach.

Critical double standards revealed in the evaluation of feeding studies submitted as proof of safety for regulatory approval to EFSA.

CONCLUSION

Only recently, the EU authorities began to build up an implementing system based on its own legislation and supportive of its approach.

The debate about the results still to come!

Until these double standards are resolved, we do not expect that neither the public acceptance will increase nor the scientific debate subside.