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New GMOs, New Threat

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2020
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New Genetic Engineering Techniques

Gene editing:
Is it precise?
Is it safe?

What is genome or gene editing?

Targeted alteration to the DNA of an organism:

- Small base unit changes (deletions/insertions)
- Large deletions
- Small/large insertions

Claim: precise, predictable outcomes, safe

Arguments used for gene editing deregulation in agriculture

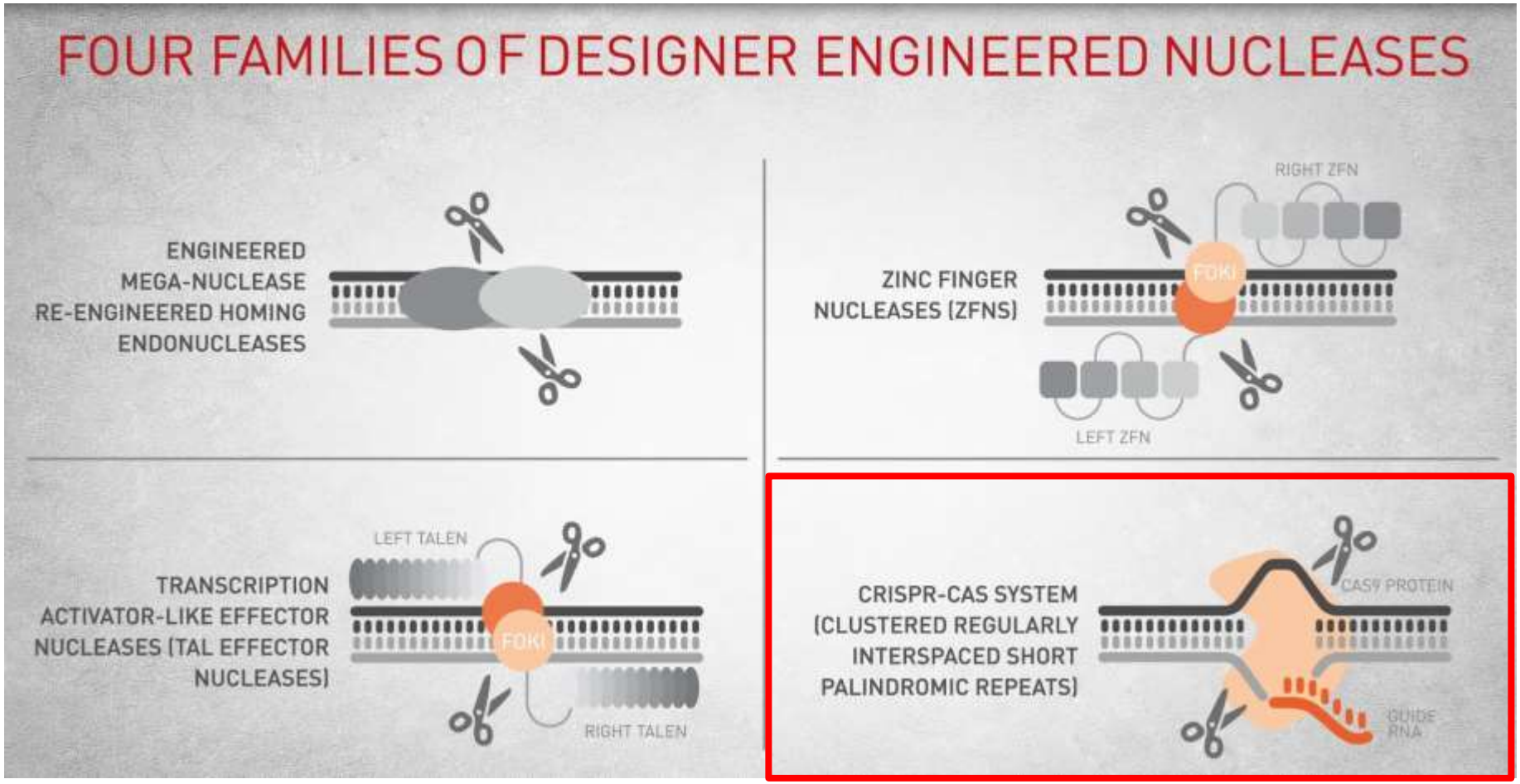
- Only the ***end product*** of the gene editing event(s), whether a microbe, plant or animal, should be considered by regulators, rather than the ***process*** by which the genomic change was obtained.
- The small DNA base unit changes brought about by these methods, which either knock-out (ablate) a gene or modify the function of a gene's protein or RNA product, can ***mimic what may occur naturally through random mutation***.
- The intended changes in a gene(s) are "***precise***" and no other genome alterations occur in the target organism.
- The outcome of the gene editing event(s) is ***totally predictable*** and thus the products derived from this process are ***safe***.

Gene editing: how does is work?

Two approaches:

- Oligonucleotide directed mutagenesis (ODM)
- Site-directed nuclease (SDN)

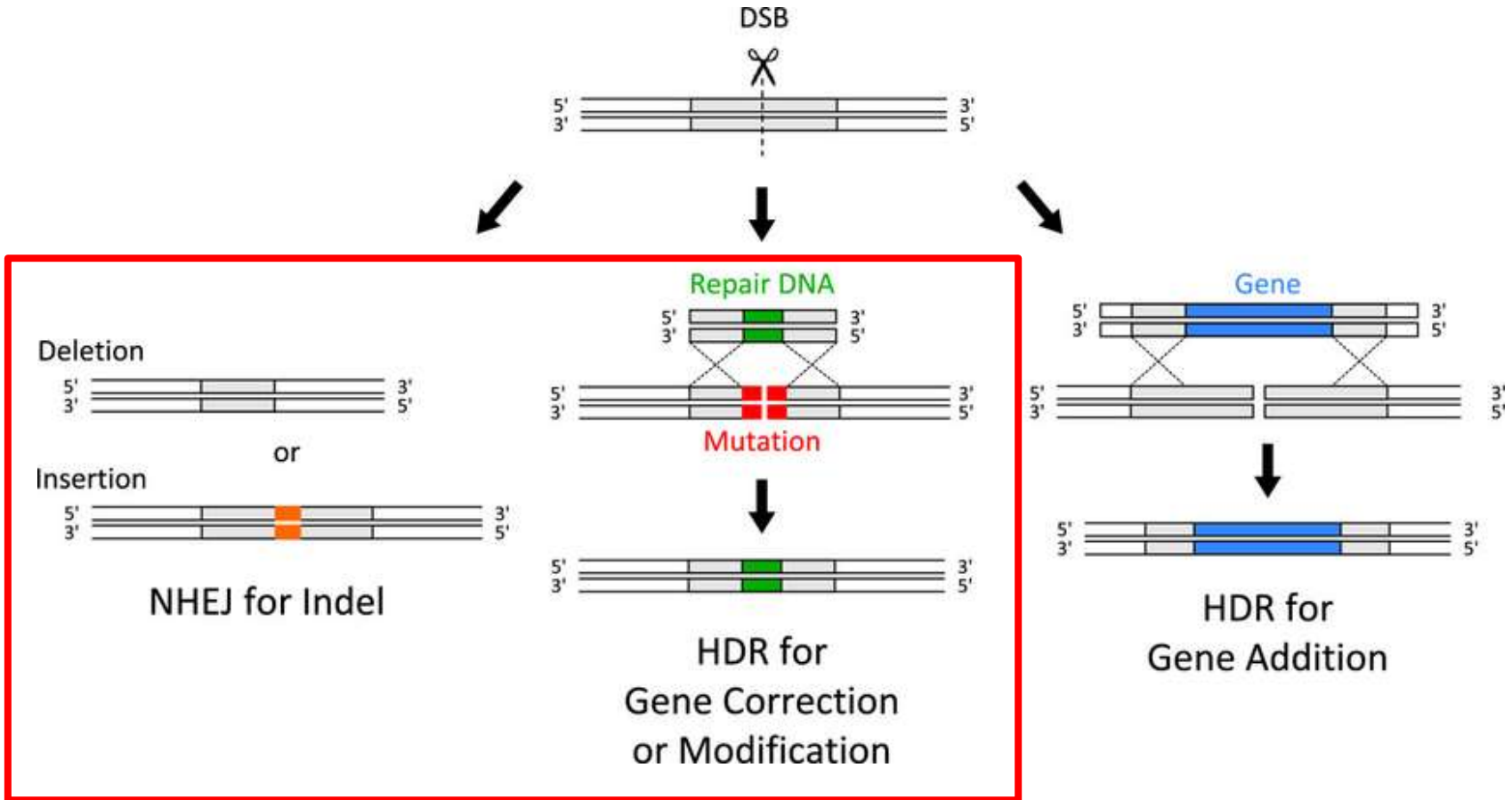
Site-directed nucleases - SDNs



Site-directed nucleases - SDNs

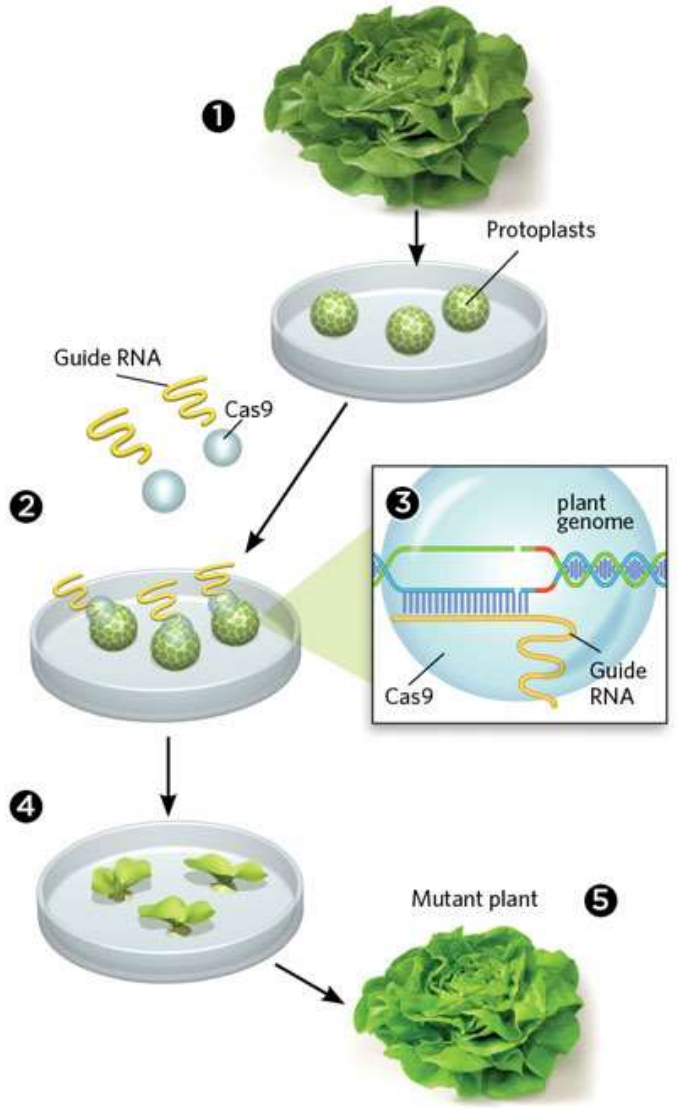
ZFN, TALEN, CRISPR-Cas

Produce double-strand break in DNA at pre-determined site



Procedure of genome editing a plant

Plant tissue culture



Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered with the CRISPR–Cas9 technique can be cultivated and sold without further oversight.

Knock-out of polyphenol oxidase (PPO) gene via NHEJ

The common white button mushroom (*Agaricus bisporus*) has been modified to resist browning

Nature News, 14 April 2016

Huge numbers of gene edited crops and animals await market approval

Calyxt (USA): Edited potato

- TALEN disabled single gene; blocks sucrose conversion to glucose and fructose
- Doesn't accumulate sweet sugars on cold storage; lasts longer
- Won't produce as much acrylamide (suspected carcinogen) when fried

DuPont (USA): low amylose, high amylopectin maize

- CRISPR disabled Waxy gene
- Eliminates amylose
- Kernels with 97% amylopectin

Genome edited farm animals

Hornless cattle

TALEN introgression of POLLED gene via cloning
(Carlson DF et al., Nat Biotechnol. 34: 479, 2016)

Super-muscly pigs created by small genetic tweak

Researchers hope the genetically engineered animals will speed past regulators. *NATURE* | NEWS, 30 June 2015

TALEN knock-out of myostatin gene via cloning

Are claims of precision and predictability of gene editing supported by the evidence?

The claim that gene editing-induced gene changes are similar to what may occur naturally is unproven.
Presently this constitutes at best an untested hypothesis.

These techniques are prone to unpredictable "off-target" and "on-target" mutational effects.

Currently recognized gene editing off-target effects

- **Unintended side-effects from the intended alteration.** For example, alteration in enzyme activity can result in chemical reactions other than those that are intended.
- **Unintended alterations or mutations to other genes in addition to the target gene(s).** Includes mutations from plant tissue culture.

Currently recognized gene editing on-target effects

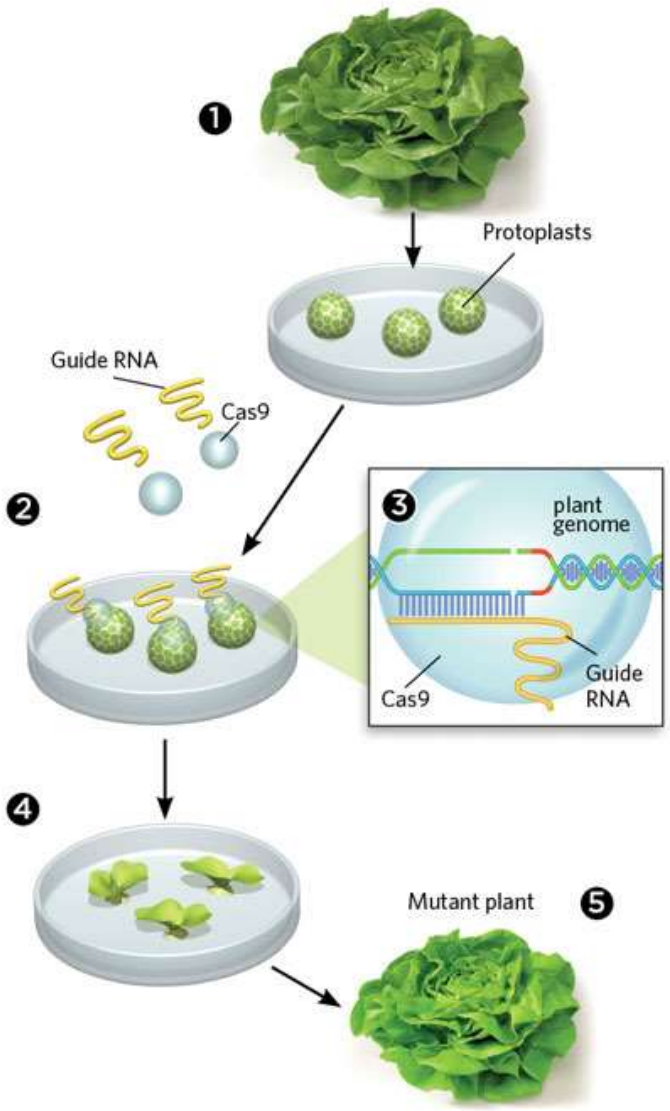
- **Large DNA deletions affecting more than one gene.**
- **Large DNA rearrangements affecting multiple gene functions.**
- **Imperfect knockout of genes resulting in production of mutant proteins**
- **Creation of new gene sequences resulting on new mRNA and proteins.**
- **Insertion of contaminating DNA.**

Consequences of unpredictable off-target and on-target mutations from gene editing

- **Can lead to unintended alterations in the biochemistry of the organism.** In edited plant foods off-target effects could lead to unexpected toxins or allergens, or altered or compromised nutritional value.

- **In order to patent genome edited organisms, industry and academia must argue for novelty and an inventive step.** Contradicts arguments that edited products are no different from organisms that may occur naturally.

Multiple types and large number of unpredictable mutations from gene editing



Mutations from plant tissue culture and transformation process

Off-target and on-target mutations

Process-based and product-based regulation must be applied

Given that gene editing:

- Uses laboratory-based, artificial DNA modification procedures
- Does not in itself involve natural cross-breeding
- Results in functional alterations of one or more DNA sequences
- Cause unintended and/or unpredictable off-target effects at DNA, RNA and protein levels

Gene editing is a GM procedure and regulations applied to their products should be process-based as well as product-based, as with the current EU GMO regulations.

Advantages of process-based regulation

- Process-based regulation can highlight mechanisms of unintended and off-target/on-target gene function disruption effects

- Process-based regulation is true to the state of this science and technology.

- **Attempts to argue that such regulation is superfluous or excessive are disingenuous and place public health and the environment at risk.**

EURACTIV

The EU must not de-regulate gene-edited crops and foods

https://www.euractiv.com/section/agriculture-food/opinion/the-eu-must-not-de-regulate-gene-edited-crops-and-foods/

Scientific and technical facts about genome editing show that organisms produced by these procedures are GMOs and give rise to novel health risks.

This demands that all products of genome editing should be regulated:

- In accord with strictest GMO regulations (e.g. EU regulations)
- As permitted by the Cartagena Protocol on Biosafety and Codex Alimentarius

Evidence of harm from gene editing?

No studies conducted to date

Claims of safety are hypothetical

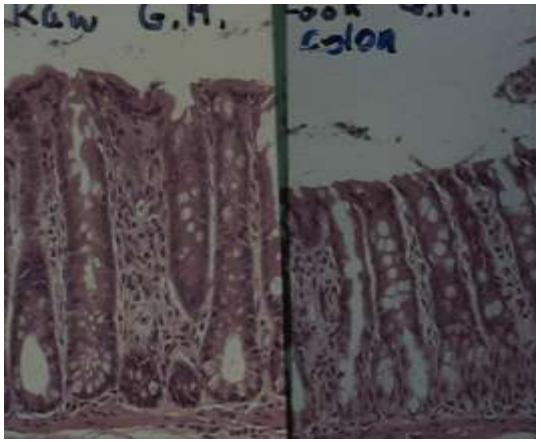
Numerous studies show evidence of harm from consumption of old-style transgenic GM crops

Controlled animal feeding studies show clear signs of toxicity linked with GM crops

Revealed by GM vs isogenic non-GM comparison

Feeding studies conducted by academics: non-commercialised crops

Cell proliferation similar to a pre-cancerous condition in gut of rats fed GM potatoes containing snowdrop GNA insecticide protein (Ewen SWB and Pusztai A, *Lancet*, **354**, 1353-1354, 1999):



GM Non-GM
Rat Colon

Rats fed GM Bt rice: significant differences in gut bacterial populations and organ weights (adrenals, testis, uterus) (Schrøder *et al*., 2007).

GM peas cause surprise allergic reaction: bean α-amylase inhibitor in peas caused marked immune response and allergic type reactions in mice (Prescott VE et al. *J Agri Food Chem.*, **53:** 9023-9030, 2005).

Feeding studies conducted by academics: commercialised crops: Bt maize/corn

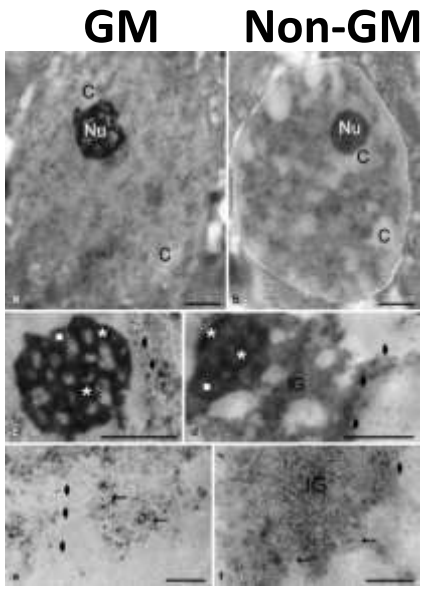
- **Rats fed GM Bt corn over three generations:** areas of necrosis to **liver and kidneys** and alterations in blood biochemistry (Kilic & Akay, 2008).
- **Old and young mice fed GM Bt corn MON810:** marked disturbance in immune system cells and in biochemical (cytokine) activity (Finamore *et al*., 2008).
- **Pigs fed GM Bt corn variety MON810 for 31 days:** differences in immune cell type numbers (e.g. CD4+ T cells, B cells, macrophages) and biochemistry (cytokine levels; e.g. IL-12, IFNg, IL-6, IL-4, IL-8) (Walsh *et al*., 2011).
- **Ewes and their lambs fed GM Bt corn variety Bt176 over three generations:** hyperplasia of ruminal epithelial basal cells in ewes and a disturbed gene functioning of **liver** and pancreas in lambs (Trabalza-Marinucci *et al*., 2008).

- **Rats fed MON810 GM Bt corn for 91 days: multiple organ changes in weight, biochemistry; severe damage in structure and function including to *liver, kidney, testes, intestines* (Gab-Alla *et al*., 2012; El-Shamei *et al*., 2012).**

Feeding studies conducted by academics: commercialised crops: HT RR soya

- **Mice fed GM soy:** disturbed **liver**, pancreas and testes function; abnormally formed cell nuclei and nucleoli in **liver** cells, indicating increased metabolism and potentially altered patterns of gene expression (Malatesta *et al*., 2002; Malatesta *et al*., 2003; Vecchio *et al*., 2004).

Mice fed GM soy over their lifetime (24 months): more acute signs of ageing in the **liver**; significant changes in the expression of 49 proteins. Significant decrease in senescence markers (e.g. regucalcin, HSPs); lower metabolism. Structure of liver cell nuclei suggest marked lowering of gene function (Malatesta *et al*., 2008):



A long-term toxicity study on pigs fed a combined genetically modified (GM) soy and GM maize diet

Carman JA et al. (2013) *J Organic Systems* **8**: 38-54

Gastric and uterine differences in GM ration fed pigs:

- **Marked increase in severe stomach inflammation (4-fold males; 2.2-fold females)**
- **Uteri 25% heavier**

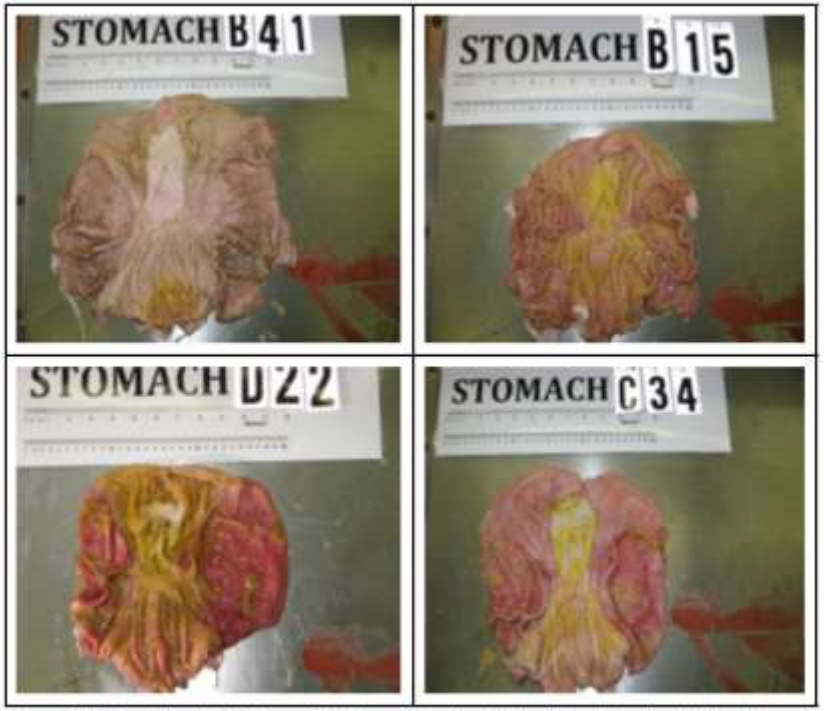
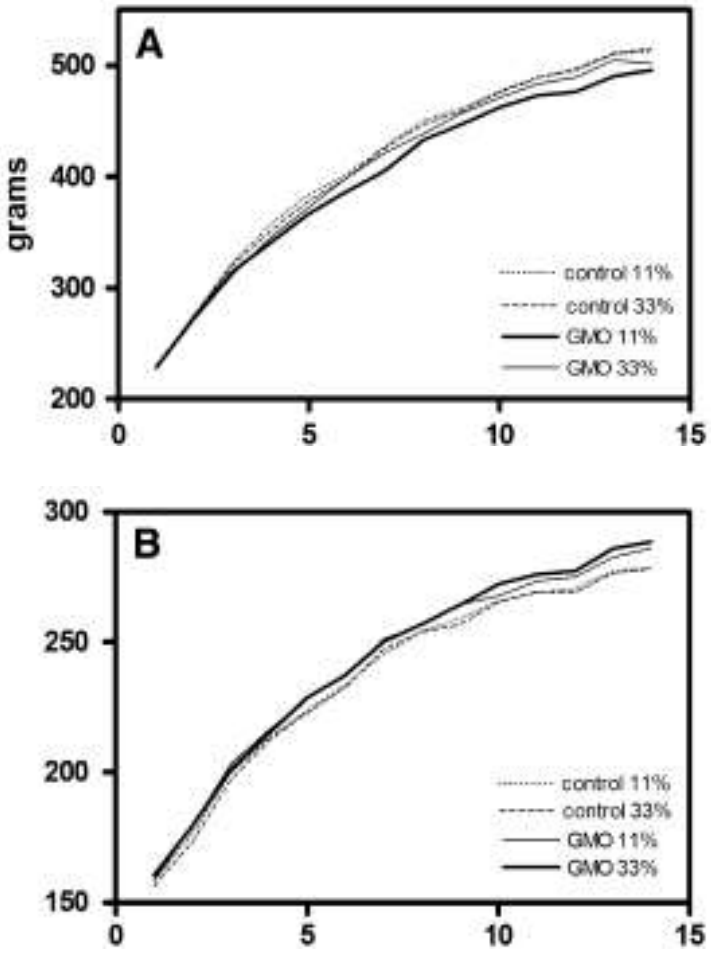


Figure 1. Different levels of stomach inflammation found (clockwise from top left): nil (from a non-GM-fed pig, number B41), mild (from a non-GM-fed pig, number B15), moderate (from a GM-fed pig, number C34) and severe (from a GM-fed pig, number D22).

Feeding studies conducted by industry

Rats fed commercialised insecticide-producing MON863 Bt corn:

- Grew more slowly
- Sex differences
- Showed higher levels of certain fats (triglycerides) in their blood
- Problems with liver and kidney function (Séralini *et al*., 2007).



	Week	m 11%	m 3 %	f 11%	f 33%
Liver parameters					
Albumin/globulin ratio	5	11*	-3	-9	4
Albumin/globulin ratio	14	6	-2	-18**	7
Albumin	5	-3	-2	-2	5*
Albumin	14	-2	3	-6*	5
Globulin	5	-12*	2	9*	1
Globulin	14	-8	7	15*	-2
Alanine aminotransferase	14	-30*	-8	37	4
Total protein	14	-5*	5*	1	3
Triglycerides	5	22	-2	-11	40**
Triglycerides	14	15	-1	24*	6
Liver weight	14	-1	-2	7**	6
Liver/brain ratio	14	-1	-3	6*	4
Kidney parameters					
Creatinin	14	-7	13*	13*	-2
Urine sodium	14	-23	-25*	11	-26
Urine sodium excretion	14	3	-35*	35	-24
Urine chloride excretion	5	35	3	50*	67*
Urine potassium	5	35*	-20	-3	-13
Urine phosphorus	5	3	-35*	24	-15
Urine phosphorus	14	-34	-31*	12	-8
Urea nitrogen	14	-8	4	17*	-1
Kidney weight	14	-3	-7*	3	2
Kidney/brain ratio	14	-3	-7*	1	1
Kidney % body weight	14	-1	-5*	-1	-1
Pancreas					
Glucose	14	-4	9	9*	10**
Bone marrow					
Neutrophils	5	5	22*	-14	3
Eosniophils	14	32	54*	20	0
Reticulocytes	14	15	-17	-35	-52*
Reticulocytes % RBC	14	16	-16	-36	-55*

Note: * & ** indicate statistical significance

Feeding studies conducted by industry

Rats fed commercialised GM Bt corn varieties MON863 and MON810 and Roundup tolerant NK603: signs of toxic effects on liver and kidneys. (de Vendomois *et al*., 2009).

Parameters	Week	Males 11%	Males 33%	Females 11%	Females 33%
BONE MARROW					
Absolute Lymphocytes	14	-12	29	-1	-23 *
Neutrophils	14	13	-34 **	4	16
Lymphocytes	14	-3	8 **	0	-2
Eosinophils (p)	5	38 *	-19	43	-13
Lar Uni Cell	5	4	-6	33 **	6
HEART					
Heart Wt	14	6	11 **	0	4
Heart % Body Wt	14	5	9 **	2	1
Heart % Brain Wt	14	6	9 *	-2	4
KIDNEY					
Urine Phosphorus	5	-15	67 **	-1	40 *
Urine Phosphorus	14	-10	97 **	12	28
Urine Sodium (p)	14	23	44 *	-7	37
Urine Potassium	14	-6	34 *	4	-13
Urine Creatinine Clearance	5	20	42 **	0	29
Blood Urea Nitrogen	5	-14 *	-13 *	13	-14
Creatinine	5	-25 *	-23 **	-6	-17
Phosphorus	5	2	-7 *	2	-8
Potassium	14	4	-2	5	13 **
LIVER					
Liver Wt	14	2	10 *	-4	1
Liver % Body Wt	14	1	5 *	-2	-2
Alkaline Phosphatase	14	2	3	29 *	16

Differences in NK603 fed rats and control animals fed isogenic non-GM maize.

Note: * & ** indicate statistical significance

Séralini et al. Environmental Sciences Europe 2014, 26:14
http://www.enveurope.com/content/26/1/14

Environmental Sciences Europe
a SpringerOpen Journal

RESEARCH Open Access

Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize

Gilles-Eric Séralini[1*], Emilie Clair[1], Robin Mesnage[1], Steeve Gress[1], Nicolas Defarge[1], Manuela Malatesta[2], Didier Hennequin[3] and Joël Spiroux de Vendômois[1]

Conceptual flaws of agricultural genetic engineering

Bound to fail: The flawed scientific foundations of agricultural genetic engineering (part 2)

Published: 21 November 2018

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The new understanding of "omnigenics" tells us GM food and crop technology is conceptually flawed - and genome editing won't change that, writes Dr Michael Antoniou

https://www.gmwatch.org/en/news/latest-news/18593

No gene works in isolation

Function of ALL genes required to impart complex traits: "OMNIGENICS"

Genes work as a highly co-ordinated NETWORK

Adding a new gene of altering the function of just one gene will have far reaching consequences in the network

The whole is greater than the sum of its parts; study of parts cannot predict the function of the whole

European Network of Scientists for Social and Environmental Responsibility (ENSSER)

Statement on new GM techniques

[https://ensser.org/publications/ngmt-statement/]

ENSSER Statement
27 September 2017

Products of new genetic modification techniques should be strictly regulated as GMOs

We encourage all scientists to sign on to this statement

What you can do

- When the UK is politically stable, write to your MPs (and MEPs if Brexit doesn't happen), asking them to ensure that all GMOs remain strictly regulated and labelled: https://gmwatch.org/en/news/latest-news/18984. Access same page by going to gmwatch.org; in the right-hand menu, click "GENE-EDITED CROPS AND FOODS: Help stop the new threat".
- Subscribe to GMWatch's free newsletters to stay up to date at gmwatch.org: click "Subscribe to news".
- Buy our book, **GMO Myths & Truths: A Citizen's Guide to the Evidence on the Safety and Efficacy of Genetically Modified Crops and Foods, 4th Edition,** from Amazon or Chelsea Green Publishing.