Conflicts of interests, confidentiality and censorship in health risk assessment: The example of the fate of Long Term Toxicity of a Roundup Herbicide and a Roundup-Tolerant Genetically Modified Maize

By Séralini et al., 2012-2014

Nicolas DEFARGE

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Berlin, 10th of September 2014

Plenary Symposium II
Committee for Independent Research and Information on Genetic Engineering

CRIIGEN is an independent non-profit organization of scientific counter-expertise to study GMOs, pesticides and impacts of pollutants on health and environment, and to develop non-polluting alternatives. It is particularly involved in the case of GMOs and is totally independent of biotechnological companies and governments.

Conflicts of interests, confidentiality and censorship in health risk assessment: the example of an herbicide and a GMO


The present opinion is a summary of the debate resulting in this retraction, as it is a historic example of conflicts of interest in the scientific assessments of products commercialized worldwide. Censorship of research into health risks undermines the value and the credibility of science; thus, we republish our paper.
I. Context

Why testing the safety of a Roundup tolerant GMO?

Why testing the safety of Roundup as a formulation?
Agricultural GMOs are modified only to tolerate or produce pesticides. (2-8 traits)

- 56.5% herbicide tolerant
- 26.5% absorb and produce pesticides
- 17% produce a modified insecticide

Source: ISAAA 2011
Pesticides used Worldwide

- **39%** Herbicides
- **18%** Insecticides
- **10%** Fungicides
- **33%** Others

2006 and 2007 estimates of EPA
Our studies have demonstrated Roundup toxicity on human cells. Roundup residues are found in 80% of edible GMOs.

Richard et al., EHP, 2005
Benachour et al., AECT, 2007
Benachour & Séralini, CRT, 2009
Gasnier et al., Toxicology, 2009
Gasnier et al., JOMT, 2010, 2011
Mesnage et al., JAT, Toxicology, 2012
Clair et al., Tox. in Vitro, Curr. Microb, 2012
Defarge et al., JTEH, 2012
Cassault-Meyer et al., ETAP, 2014
Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity

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\section*{ARTICLE INFO}

Article history:
Received 27 April 2012
Received in revised form 30 August 2012
Accepted 10 September 2012
Available online xxx

Keywords:
Pesticide
Glyphosate
POEA
Adjuvant
Roundup
Human cells

\section*{ABSTRACT}

Pesticides are always used in formulations as mixtures of an active principle with adjuvants. Glyphosate, the active ingredient of the major pesticide in the world, is a herbicide supposed to be specific on plant metabolism. Its adjuvants are generally considered as inert diluents. Since side effects for all these compounds have been claimed, we studied potential active principles for toxicity on human cells for 9 glyphosate-based formulations. For this we detailed their compositions and toxicities, and as controls we used a major adjuvant (the polyethoxylated tallowamine POE-15), glyphosate alone, and a total formulation without glyphosate. This was performed after 24h exposures on hepatic (HepG2), embryonic (HEK293) and placental (JEG3) cell lines. We measured mitochondrial activities, membrane degradations, and caspases 3/7 activities. The compositions in adjuvants were analyzed by mass spectrometry. Here we demonstrate that all formulations are more toxic than glyphosate, and we separated experimentally three groups of formulations differentially toxic according to their concentrations in ethoxylated adjuvants. Among them, POE-15 clearly appears to be the most toxic principle against human cells, even if others are not excluded. It begins to be active with negative dose-dependent effects on cellular respiration and membrane integrity between 1 and 3 ppm, at environmental/occupational doses. We demonstrate in addition that POE-15 induces necrosis when its first micellization process occurs, by contrast to glyphosate which is known to promote endocrine disrupting effects after entering cells. Altogether, these results challenge the establishment of guidance values such as the acceptable daily intake of glyphosate, when these are mostly based on a long term in vivo test of glyphosate alone. Since pesticides are always used with adjuvants that could change their toxicity, the necessity to assess their whole formulations as mixtures becomes obvious. This challenges the concept of active principle of pesticides for non-target species.
We have tested the human cellular toxicity of 9 Roundup formulations, their adjuvants, and glyphosate.

All formulations are more toxic than glyphosate whatever the cell type.
What is the agent of cytotoxicity?

Linearity of the toxicity of GBH containing glyphosate and POEA

Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity

Mesnage et al., 2013
Adding adjuvants increased glyphosate mediated endocrine disruption (Richard et al. 2005, Gasnier et al., 2009)

Adjuvants induced see urchin embryo death (Marc et al. 2005)

Adjuvants are more toxic than Roundup in various models: Microtox bacterium, microalgae, protozoa, and crustaceans (Tsui et al. 2003), even rats (Adam et al., 1997)

Toxicity is due to adjuvants rather than to glyphosate itself!
(Also according to a review of developmental and reproductive outcomes from Williams et al., 2012)
Is it generalizable for pesticides?

We have tested the toxicity of 9 pesticides (insecticides, fungicides, herbicides), comparing active principles and their formulations.

<table>
<thead>
<tr>
<th>Pesticide class</th>
<th>Active Principle</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbicides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphonoglycine</td>
<td><strong>Glyphosate</strong></td>
<td>Roundup GT+ (450 g/L)</td>
</tr>
<tr>
<td>Urea</td>
<td><strong>Isoproturon</strong></td>
<td>Matin EL (500 g/L)</td>
</tr>
<tr>
<td>Synthetic auxin</td>
<td><strong>Fluroxypyr (ester 1-methylheptyl)</strong></td>
<td>Starane 200 (200 g/L)</td>
</tr>
<tr>
<td>Insecticides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbamate</td>
<td><strong>Pirimicarb</strong></td>
<td>Pirimor G (50%)</td>
</tr>
<tr>
<td>Neonicotinoid</td>
<td><strong>Imidacloprid</strong></td>
<td>Confidor (200g/l)</td>
</tr>
<tr>
<td>Neonicotinoid</td>
<td><strong>Acetamiprid</strong></td>
<td>Polysect Ultra (5g/L)</td>
</tr>
<tr>
<td>Fungicides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triazole</td>
<td><strong>Tebuconazole</strong></td>
<td>Maronee (250 g/L)</td>
</tr>
<tr>
<td>Triazole</td>
<td><strong>Epoxinazole</strong></td>
<td>Opus (125 g/L)</td>
</tr>
<tr>
<td>Imidazole</td>
<td><strong>Prochloraz</strong></td>
<td>Eyetak (450 g/L)</td>
</tr>
</tbody>
</table>

Mesnage et al., 2014
Fongicide: Maronee (250 g/L Tebuconazole)

Active principle: dotted line
Formulation: solid line

HepG2

HEK293

JEG3

Cellular respiration (%)

Concentration (ppm)

Another widely used triazole fungicide on cereals

~ 1056 times more toxic than its active principle

Mesnage et al., 2014
Insecticide: Confidor (200 gL/ Imidacloprid)

The major neonicotinoids, the largest selling insecticides worldwide

Debated for bees death, Human neurotoxicity Suspended by efsa
Differential toxicities between active principles and their formulations appear to be a general property of pesticide toxicology.

<table>
<thead>
<tr>
<th>Active principles</th>
<th>Formulations</th>
<th>Declared adjuvants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>Roundup GT+</td>
<td>Ethoxylated etherealylamine</td>
</tr>
<tr>
<td>Isoproturon</td>
<td>Matin EL</td>
<td>Unknown</td>
</tr>
<tr>
<td>Fluroxypyr (ester 1-methylheptyl)</td>
<td>Starane 200</td>
<td>Solvent naphtha; alkyl-aryl sulfonates</td>
</tr>
<tr>
<td>Pirimicarb</td>
<td>Pirimor G</td>
<td>Docusate sodium; benzenesulfonic acid</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Confidor</td>
<td>1-Methyl-2-pyrrolidinone</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>Polysect Ultra</td>
<td>1,2-Benzisothiazoline-3-one; ethanol</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>Maronee</td>
<td>N,N-Dimethyldecanamide</td>
</tr>
<tr>
<td>Epoxiconazole</td>
<td>Opus</td>
<td>Solvent naphtha; fatty alcohol ethoxylated</td>
</tr>
<tr>
<td>Prochloraz</td>
<td>Eyetak</td>
<td>Solvent naphtha; xylene; isobutanol</td>
</tr>
</tbody>
</table>

Mesnage et al., 2014

- In fact, hidden toxic active principle (Mesnage et al., 2013)
- High toxicity to larval bees (Zhu et al., 2014)
- Developmental toxicant in rats (Saillenfait, 2002)
- Developmental effects in rodents (McKee et al., 1990)
- Developmental toxicant in rodents (US EPA)
- Associated with cardiac and central nervous system diseases in humans (Langman et al., 1984)
What are the consequences of this non-scientific concept?

Regulatory authorizations of pesticides are based on long-term tests carried with the active ingredient alone.

This allows the Acceptable Daily Intake calculation

![Graph showing biological response with doses and calculations](image)

This justify the presence of pesticide residues in the food/feed

15
I. Context

Important scientific insufficiencies in health risk assessment of 19 agricultural GMOs by industries and agencies

Over 120,000 views in Springer Open (http://www.springeropen.com/mostviewed/alltime, 06/2014)
<table>
<thead>
<tr>
<th>Parameters in 15 GMOs in vivo studies of toxicity</th>
<th>Measured by organ (%) / Total (694-698)</th>
<th>Disturbed in each organ (%) / Total disrupted parameters (~ 9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Liver</td>
<td>23.3</td>
<td>23.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>24.8</td>
<td>24.9</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>32.5</td>
<td>32.7</td>
</tr>
<tr>
<td>The 3 organs</td>
<td>80.7</td>
<td>81.1</td>
</tr>
</tbody>
</table>

Meta-analysis of statistical differences in rat feeding trials with 19 GMOs. Parameters are classified per tissue according to Séralini et al. (2007). Statistical differences are reported according to the statistics of the authors. All these data revealed that the kidney is particularly reached, concentrating 42% of all parameters disrupted for males.
A Comparison of the Effects of Three GM Corn Varieties on Mammalian Health

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Received: 2009.07.23; Accepted: 2009.11.17; Published: 2009.12.10

Abstract

We present for the first time a comparative analysis of blood and organ system data from trials with rats fed three main commercialized genetically modified (GM) maize (NK 603, MON 810, MON 863), which are present in food and feed in the world. NK 603 has been
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Week</th>
<th>Males 11%</th>
<th>Males 33%</th>
<th>Females 11%</th>
<th>Females 33%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BONE MARROW</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute Lymphocytes</td>
<td>14</td>
<td>-12</td>
<td>29</td>
<td>-1</td>
<td>-23 *</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>14</td>
<td>13</td>
<td>-34 **</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>14</td>
<td>-3</td>
<td>8 **</td>
<td>0</td>
<td>-2</td>
</tr>
<tr>
<td>Eosinophils (p)</td>
<td>5</td>
<td>38 *</td>
<td>-19</td>
<td>43</td>
<td>-13</td>
</tr>
<tr>
<td>Lar Uni Cell</td>
<td>5</td>
<td>4</td>
<td>-6</td>
<td>33 **</td>
<td>6</td>
</tr>
<tr>
<td><strong>HEART</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Wt</td>
<td>14</td>
<td>6</td>
<td>11 **</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Heart % Body Wt</td>
<td>14</td>
<td>5</td>
<td>9 **</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Heart % Brain Wt</td>
<td>14</td>
<td>6</td>
<td>9 *</td>
<td>-2</td>
<td>4</td>
</tr>
<tr>
<td><strong>KIDNEY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine Phosphorus</td>
<td>5</td>
<td>-15</td>
<td>67 **</td>
<td>-1</td>
<td>40 *</td>
</tr>
<tr>
<td>Urine Phosphorus</td>
<td>14</td>
<td>-10</td>
<td>97 **</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>Urine Sodium (p)</td>
<td>14</td>
<td>23</td>
<td>44 *</td>
<td>-7</td>
<td>37</td>
</tr>
<tr>
<td>Urine Potassium</td>
<td>14</td>
<td>-6</td>
<td>34 *</td>
<td>4</td>
<td>-13</td>
</tr>
<tr>
<td>Urine Creatinine Clearance</td>
<td>5</td>
<td>20</td>
<td>42 **</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Blood Urea Nitrogen</td>
<td>5</td>
<td>-14 *</td>
<td>-13 *</td>
<td>13</td>
<td>-14</td>
</tr>
<tr>
<td>Creatinine</td>
<td>5</td>
<td>-25 *</td>
<td>-23 **</td>
<td>-6</td>
<td>-17</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>5</td>
<td>2</td>
<td>-7 *</td>
<td>2</td>
<td>-8</td>
</tr>
<tr>
<td>Potassium</td>
<td>14</td>
<td>4</td>
<td>-2</td>
<td>5</td>
<td>13 **</td>
</tr>
<tr>
<td><strong>LIVER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Wt</td>
<td>14</td>
<td>2</td>
<td>10 *</td>
<td>-4</td>
<td>1</td>
</tr>
<tr>
<td>Liver % Body Wt</td>
<td>14</td>
<td>1</td>
<td>5 *</td>
<td>-2</td>
<td>-2</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>14</td>
<td>2</td>
<td>3</td>
<td>29 *</td>
<td>16</td>
</tr>
</tbody>
</table>
Criteria imposed by Doull et al. (2007) to take into account significant effects:

- Similarity between both sexes
- Proportional effects for 2 doses chosen a priori
- Correlations with organ lesions
- Effects over undefined « historical » norm ??
- Biologic plausibility !
- Do we have here good scientific criteria ?
The insufficiencies of GMOs testing: 3 m on young adults—no chronic toxicity can be studied.
Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize

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ARTICLE INFO

Article history:
Received 11 April 2012
Accepted 2 August 2012
Available online xxxxx

Keywords:
GMO
Roundup
NK603
Rat
Glyphosate-based herbicides
Endocrine disrupting effects

ABSTRACT

The health effects of a Roundup-tolerant genetically modified maize (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.1 ppb in water), were studied 2 years in rats. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed GMOs. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls, the pituitary was the second most disabled organ; the sex hormonal balance was modified by GMO and Roundup treatments. In treated males, liver congestions and necrosis were 2.5–5.5 times higher. This pathology was confirmed by optic and transmission electron microscopy. Marked and severe kidney nephropathies were also generally 1.3–2.3 greater. Males presented 4 times more large palpable tumors than controls which occurred up to 600 days earlier. Biochemistry data confirmed very significant kidney chronic deficiencies; for all treatments and both sexes, 76% of the altered parameters were kidney related. These results can be explained by the non linear endocrine-disrupting effects of Roundup, but also by the overexpression of the transgene in the GMO and its metabolic consequences.
• The first most detailed life-long rodent (rat) feeding study investigating possible toxic effects rising from:

• A Roundup-tolerant GM maize (NK603), treated or not with Roundup (11, 22 and 33%),

• A complete commercial formulation of a Roundup herbicide (0.1 ppb; 400 ppm and 0.5% in water)
CHRONIC TOXICITY OF ROUNDUP AND A ROUNDUP-TOLERANT GMO, the main findings

- The main pesticide of the world, Roundup, provokes severe hepatorenal deficiencies and sex-dependent hormonal effects such as mammary tumors from very low environmental levels (0.1 ppb).

- Comparable results have been obtained during chronic consumption of an equilibrated diet containing a Roundup-tolerant GMO (maize). This was due to Roundup residues and to this specific genetic modification (NK603).

- Roundup formulations and Roundup-tolerant GMOs should be considered as endocrine disruptors and their present assessments on health are drastically deficient.

To be kept in mind:

- 80 % of agricultural GMOs are Roundup-tolerant ones. The remaining are Bt-toxins (pesticides) - producing GMOs.

- Roundup does not equal glyphosate, it contains adjuvants more toxic than this declared active principle, like other pesticides.
**III. Results**

Males died mostly from pathologies in **liver** and **kidneys**

**Liver**: congestions, macroscopic spots, necrotic foci

**Kidney**: chronic progressive nephropathies more severe and earlier than in controls

Kidney biochemistry is disrupted in females in our study.

*Table 3*

Percentage variation of parameters indicating kidney failures of female animals.

<table>
<thead>
<tr>
<th>Discriminant variables</th>
<th>GMO 11% + R</th>
<th>GMO 22% + R</th>
<th>GMO 33% + R</th>
<th>R (A)</th>
<th>R (B)</th>
<th>R (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary decrease</td>
<td>-4</td>
<td>-11</td>
<td>-20</td>
<td>-20</td>
<td>-24</td>
<td>-40</td>
</tr>
<tr>
<td>Creatinine</td>
<td>-5</td>
<td>-32</td>
<td>-37</td>
<td>-43</td>
<td>-23</td>
<td>-1</td>
</tr>
<tr>
<td>Creatinine ex</td>
<td>-5</td>
<td>-11</td>
<td>-19</td>
<td>-21</td>
<td>-22</td>
<td>-39</td>
</tr>
</tbody>
</table>

(Analysis at month 15th)
Rats treated with Roundup had more tumors

![Mammary fibradenoma](image)

Séralini et al. (2012) FCT, 50: 4221 - 4231
III. Results

**MALES**

- **GMO**
- **GMO + Roundup**
- **Roundup**

**FEMALES**

- **GMO**
- **GMO + Roundup**
- **Roundup**

<table>
<thead>
<tr>
<th>Controls</th>
<th>11% or A</th>
<th>22% or B</th>
<th>33% or C</th>
</tr>
</thead>
</table>

Time (days)
Females died mostly from mammary tumors

93% tumors are in mammary glands

...and pituitary dysfunctions
III. Results

These pathologies arise at very low levels of Roundup

0.1 ppb
MRL in tap water

0.3 ppm
ADI

22 ppm
Residues in food

1%
Occupational exposure

1E-05
0.001
0.1
10
1000

Dose R(A)
0.1 ppb

Dose R(B)
400 ppm

Dose R(C)
0.5%

At the lowest dose of Roundup:

Important and saturating effects
Disturbance of the testosterone/estradiol ratio in females
2.5 times more mammary tumors

Table 3
Percentage variation of parameters

<table>
<thead>
<tr>
<th>Discriminant variables</th>
<th>R (A)</th>
<th>R (B)</th>
<th>R (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonads</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol</td>
<td>-26</td>
<td>-73</td>
<td>39</td>
</tr>
<tr>
<td>Testosterone</td>
<td>97</td>
<td>-72</td>
<td>10</td>
</tr>
</tbody>
</table>

(Analysis at month 15th)
Glyphosate induces human breast cancer cells growth via estrogen receptors

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A R T I C L E    I N F O

Article history:
Received 9 October 2012
Accepted 29 May 2013
Available online 10 June 2013

Keywords:
Glyphosate
Estrogenic effect
Genistein
Human breast cancer
T47D
T47D-KBluc

A B S T R A C T

Glyphosate is an active ingredient of the most widely used herbicide and it is believed to be less toxic than other pesticides. However, several recent studies showed its potential adverse health effects to humans as it may be an endocrine disruptor. This study focuses on the effects of pure glyphosate on estrogen receptors (ERs) mediated transcriptional activity and their expressions. Glyphosate exerted proliferative effects only in human hormone-dependent breast cancer, T47D cells, but not in hormone-independent breast cancer, MDA-MB231 cells, at $10^{-12}$ to $10^{-6}$ M in estrogen withdrawal condition. The proliferative concentrations of glyphosate that induced the activation of estrogen response element (ERE) transcription activity were 5-13 fold of control in T47D-KBluc cells and this activation was inhibited by an estrogen antagonist, ICI 182780, indicating that the estrogenic activity of glyphosate was mediated via ERs. Furthermore, glyphosate also altered both ER\textalpha and \beta expression. These results indicated that low and environmentally relevant concentrations of glyphosate possessed estrogenic activity. Glyphosate-based herbicides are widely used for soybean cultivation, and our results also found that there was an additive estrogenic effect between glyphosate and genistein, a phytoestrogen in soybeans. However, these additive effects of glyphosate contamination in soybeans need further animal study.

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• The first arrived in 24h
• Published by FCT: 75% from plant biologists, some holding patents on GMO technology + R.Goodman, former employee of Monsanto
• From agencies having allowed NK603 and Roundup
• And insults: Arjo (2013), Parott (ILSI) and Christou (inventor of patents on GM crop technology owned by Monsanto)
V. Answers to the critics

Answers to critics: Why there is a long term toxicity due to a Roundup-tolerant genetically modified maize and to a Roundup herbicide

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ABSTRACT

Our recent work (Séràlìnì et al., 2012) remains to date the most detailed study involving the life-long consumption of an agricultural genetically modified organism (GMO). This is true especially for NK603 maize for which only a 90-day test for commercial release was previously conducted using the same rat strain (Hammond et al., 2004). It is also the first long term detailed research on mammals exposed to a highly diluted pesticide in its total formulation with adjuvants. This may explain why 75% of our first criticisms arising within a week, among publishing authors, come from plant biologists, some developing patents on GMOs, even if it was a toxicological paper on mammals, and from Monsanto Company who owns both the NK603 GM maize and Roundup herbicide (R). Our study has limits like any one, and here we carefully answer to all criticisms from agencies, consultants and scientists, that were sent to the Editor or to ourselves. At this level, a full debate is biased if the toxicity tests on mammals of NK603 and R obtained by
Statistical significance for the number of tumors in GMO 11% females

Abstract

In this paper, we investigate the use of precedence and exceedance tests based on equal size random samples for testing stochastic dominance. We derive exact distributions of the statistics as well as the corresponding limit laws for large sample sizes, which are shown to be negative binomial. To illustrate the usefulness of these methods, we apply these tests to an experimental data set composed of two paired samples of size $n = 10$, due to Seralini et al. [20]. We show that exceedance tests compare well with other classical tests (Kolmogorov-Smirnov, Mann-Whitney and Wilcoxon). For either of these methods, in our example, the assumption that the samples are equally distributed is rejected with a level of significance varying between 1.4% and 2.9%.

Keywords and phrases: Slippage tests, Rank tests, Precedence tests, Non-parametric tests, distribution-free methods, limit laws, negative binomial distribution.

1 Introduction.

Let $X := X_1, \ldots, X_{n_A}$ (Sample (A)) and $Y := Y_1, \ldots, Y_{n_B}$ (Sample (B)) be independent random samples, each composed of a sequence of independent and identically distributed [iid] random failure-times, with respective continuous distribution functions [df’s] $F(x) = \mathbb{P}(X \leq x)$ and $G(x) = \mathbb{P}(Y \leq y)$, for $x \in \mathbb{R}$. Our aim is to investigate distribution-free tests of the hypotheses $H_0 : F = G$ versus $H_1 : F > G$ (the stochastic dominance of $F$ over $G$). By distribution free is meant the fact that the null distribution of the test statistics in use must
SCIENTIFIC REPORT OF EFSA

Considerations on the applicability of OECD TG 453 to whole food/feed testing

European Food Safety Authority

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Upon request from the European Commission, the European Food Safety Authority prepared a scientific report that would aid the future establishment of protocols for chronic toxicity and/or carcinogenicity studies in rodents with whole food/feed. This scientific report provides a commentary on OECD TG 453 with considerations on its applicability to support the safety assessment of long term consumption of a given food with respect to its chronic toxicity or carcinogenicity potential. The decision to conduct chronic toxicity and/or carcinogenicity studies with whole food/feed should be taken on a case-by-case basis. It should be based on the evaluation of all the available information on the whole food/feed resulting from compositional analyses and any other available

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KEY WORDS

2-year feeding study, whole food/feed, carcinogenicity, chronic toxicity, experimental design, statistical analysis
Commentary

New EU legislation for risk assessment of GM food: no scientific justification for mandatory animal feeding trials

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Keywords: risk assessment, GM foods, EU legislation, animal feeding trials, European food safety authority.

Summary
This commentary focuses on the potential added value of and need for (sub)-chronic testing of whole genetically modified (GM) foods in rodents to assess their safety. Such routine testing should not be required since, due to apparent weaknesses in the approach, it does not add to current risk assessment of GM foods. Moreover, the demand for routine testing using animals is in conflict with the European Union (EU) Commission’s efforts to reduce animal experimentation. Regulating agencies in the EU are invited to respect the sound scientific principles applied to the risk assessment of foods derived from GM plants and not to interfere in the risk assessment by introducing extra requirements based on pseudo-scientific or political considerations.
ABOUT ILSI

The International Life Sciences Institute (ILSI) is a nonprofit, worldwide organization whose mission is to provide science that improves public health and well-being.

Member companies

The ILSI has over 400 companies as members, but institute’s main member companies (those that can influence directions) include:

- Bayer AG
- Coca-Cola
- Dow Agrosciences/Dow Chemical
- DuPont
- ExxonMobil
- General Mills
- Hershey Foods
- Kellogg
- Kraft
- McDonald’s
- Merck & Co.
- Monsanto
- Nestle
- Novartis
- PepsiCo
- Pfizer
- Procter & Gamble
Stakeholder conference - Transparency in Risk Assessment

Parma, 3 October 2013

* Draft programme

The European Food Safety Authority (EFSA) is holding a conference on Transparency in Risk Assessment on 3 October 2013 in Parma, Italy, from 09:00 to 16:15.

Press Release

10th September 2013

Brussels

Can a science director at food authority EFSA with an industry background be neutral on science?

The European Food Authority EFSA promoted Ms. Juliane Kleiner to the crucial position of science director just before the summer. Ms. Kleiner used to work for industry lobby group ILSI (International Life Sciences Institute) for over 7 years and defended a long range of industry positions and industry tools such as the "thresholds" for genotoxic carcinogens, TTC, the threshold of toxicological concern for all chemicals, the "human relevance"-tool to disqualify relevant data from animal studies, all initiatives of research and reviewing EFSA’s policy in favour of industry. Shorty after her promotion to science director she was appointed as Chair of the European Food Safety Authority EFSA’s Scientific Committee on Food (SCF) which is the major forum for science decisions in the EU. It is astonishing that such a person is promoted as an independent voice for science and the public health in the EU and is responsible for the setting of thresholds which are supposed to increase the consumer confidence in food.
Meanwhile, Richard Goodman entered the editorial board of FCT for biotechnologies

- Had written a letter to FCT:
  « The implications and the impacts of this uncontrolled study is having HUGE impacts, in international trade, in consumer confidence in all aspects of food safety, and certainly in US state referendums on labelling. »
- Asked for ‘an evaluation by an independant set of toxicologists’
- Mezzomo et al. withdrawn, directly published elsewhere

and we had to send our raw data to FCT for a post-publication reviewing

An exceptional post-publication panel was first led by Richard Goodman, who entered the editorial board of FCT after complaining about the study. He is a Monsanto former employee, a member of the International Life Science Institute (ILSI, a pressure group acting in favour of GMOs that includes the largest global agrochemical companies, including Monsanto) and whose research at the University of Nebraska are also funded by the Biotech industry. It took the involvement of the parent company (Elsevier), which was aware of the serious conflicts of interest of Mr. Goodman in order for him to be pulled off the post-publication review panel, while remaining in his position as the Editor on Biotechnologies at FCT.
data were “not incorrect”, “there was no misconduct”, and that “Unequivocally, the Editor-in-Chief found no evidence of fraud or intentional misrepresentation of the data”

- Given the "small size sample" and “high incidence of tumors in the Sprague Dawley rats, normal variability cannot be excluded”

- “To be very clear, it is the entire paper, with the claim that there is definitive link between GMO and cancer that is being retracted”

- The word “cancer” never appears in our 2012 paper!!!
Inconclusive Findings: Now You See Them, Now You Don’t!

The environmental health literature is rife with controversial papers that evoke criticism, support, and, most importantly, a desire to better understand the findings put forth by the authors. A research article by Séralini and colleagues (Séralini et al. 2012), published in the journal Food and Chemical Toxicology, resulting in e-2013, Olivier

not reach that threshold. The COPE guidelines for retracting articles (Committee on Publication Ethics 2009) provide four reasons for retraction: scientific misconduct/honest error, prior publication, plagiarism, or unethical research. None of these reasons apply to this particular article, and yet Elsevier, a member of COPE, chose to retract the paper.

The nature of science is such that individual studies are rarely, if ever, conclusive. Numerous published studies have later been found to be deeply flawed through further scientific investigation, as may well be the study by Séralini et al. To our knowledge, there is no precedent for “inconclusive data” being a reason for retraction for Elsevier or other publishers, or elsewhere in the scientific literature. To single out this one study for retraction is almost certainly due to the controversy following its publication. The repercussions of this directed action extend well beyond this single publication and raise several larger scientific questions. Will these data, which could well have been accepted by the scientific community, give rise to unending debates or what?
Conclusiveness of toxicity data and double standards

We would like to comment on your answers (Hayes, 2014a) concerning the retraction of our study (Seralini et al., 2012, Hayes, 2014b) by Food and Chemical Toxicology (FCT). Our study investigated the long-term effects in rats of consumption of two Monsanto products, a genetically modified (GM) maize and its associated pesticide, Roundup, together and separately. The decision to retract the paper was reached a few months after the appointment of a former Monsanto employee as “editor for biotechnology”, a position created for him at FCT (Robinson and Latham, 2013). In a recent editorial, Portier and colleagues express concern about the “dangerous erosion of the underpinnings of the peer-review process” in the case of our study (Portier et al., 2014).

In contrast with our study, Zhang and colleagues performed anatomopathology on an interim group of 10 rats analyzed at 52 weeks, though the results are not detailed in the paper. Zhang and colleagues also measured the mortality and tumour incidence of the remaining rats at the end of the experiment. This omits the chronological data provided in our experiment, in which the differential development of tumours in the treatment groups was traced through bi-weekly recording.

The criticism of the relatively low number of rats used in our experiment relies entirely on the misconception that it is a carcinogenicity study. It was not the case, as we stated clearly in the title and introduction. It was a long-term (chronic) toxicity study, which unexpectedly found increased rates of tumorigenesis and mortality in some treatment groups that we had to report.
Long-term toxicity study on transgenic rice with Cry1Ac and sck genes
Min Zhang, Qin Zhuo*, Yuan Tian, Jianhua Piao, Xiaoguang Yang*

2.5. Hematology

Hematological examinations were carried out at 13, 26, 52 and 78 weeks. Briefly, 10 male and 10 female rats were randomly selected from each group and fasted for 16 h. Blood was collected via the tail vein and placed in tubes containing

Professor Séralini commented:

“We are forced to conclude that the decision of the journal FCT is not conditioned by the rigour of the protocol and of the scientific method, but by our results. This case of "double standards" can only be explained by compromises on publications offered to the Biotech industry, in order to force the acceptance of GMOs and Roundup.”
Rat feeding studies with genetically modified maize - a comparative evaluation of applied methods and risk assessment standards

Hartmut Meyer¹* and Angelika Hilbeck¹,²

Abstract

A 2-year rat feeding study with genetically modified NK603 maize sparked an international scientific and public debate as well as policy responses by the European Commission. The European Food Safety Authority (EFSA) evaluated the study as defective based on conceptual and methodological shortcomings by retroactive application of the recommendations of its recent guidance on 90-day feeding studies. Our comparative analysis of the three relevant NK603 publications, including a 90-day feeding study of Monsanto, showed that all of them satisfy or fail to satisfy the EFSA evaluation criteria to a comparable extent; the rejection of only one of the papers is, thus, not scientifically justified. We also show that EFSA’s criteria are not standard practice in 21 other rat feeding studies lasting at a minimum of 12 months. The review reveals critical double standards in the evaluation of feeding studies submitted as proof of safety for regulatory approval to EFSA. We specifically argue that the current approach to declare statistically significant differences between genetically modified organisms and its parents as ‘biologically irrelevant’ based on additional reference controls lacks scientific rigor and legal justification in the European Union (EU) system. Only recently, the EU authorities started building up an implementing system based on its own legislation and supportive of the EU approach to risk assessment in the context of technology assessment. Until these issues are resolved, we do not expect that neither the public nor the scientific debate will subside.
And to « safety » agencies!

ENSSER Comments on the Retraction of the Séralini et al. 2012 Study

Journal's retraction of rat feeding paper is a travesty of science and looks like a bow to industry
Glyphosate induces human breast cancer cells growth via estrogen receptors

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\section*{ARTICLE INFO}

Article history:
Received 9 October 2012
Accepted 29 May 2013
Available online 10 June 2013

Keywords:
Glyphosate
Estrogenic effect
Genistein
Human breast cancer
T47D
T47D-KB1uc

\section*{ABSTRACT}

Glyphosate is an active ingredient of the most widely used herbicide and it is believed to be less toxic than other pesticides. However, several recent studies showed its potential adverse health effects to humans as it may be an endocrine disruptor. This study focuses on the effects of pure glyphosate on estrogen receptors (ERs) mediated transcriptional activity and their expressions. Glyphosate exerted proliferative effects only in human hormone-dependent breast cancer, T47D cells, but not in hormone-independent breast cancer, MDA-MB231 cells, at \(10^{-12}\) to \(10^{-6}\) M in estrogen withdrawal condition. The proliferative concentrations of glyphosate that induced the activation of estrogen response element (ERE) transcription activity were 5-13 fold of control in T47D-KB1uc cells and this activation was inhibited by an estrogen antagonist, ICI 182780, indicating that the estrogenic activity of glyphosate was mediated via ER\(\alpha\). Furthermore, glyphosate also altered both ER\(\alpha\) and \(\beta\) expression. These results indicated that low and environmentally relevant concentrations of glyphosate possessed estrogenic activity. Glyphosate-based herbicides are widely used for soybean cultivation, and our results also found that there was an additive estrogenic effect between glyphosate and genistein, a phytoestrogen in soybeans. However, these additive effects of glyphosate contamination in soybeans need further animal study.

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The answer by german BfR in charge of the reassessment of glyphosate

surrounding this research include irrelevant routes of exposure as well as excessively high and environmentally unrealistic doses.

Thongprakaisang et al., (2013, ASB2013-11991) submitted a study on the effects of pure glyphosate on estrogen receptors mediated transcriptional activity and their expressions. The following cell lines have been used: a hormone-dependent breast cancer, T47D, a stably EREC-luc construct transfected hormone-dependent breast cancer T47D-KBH luc and a hormone-independent human breast cancer, MDA-MB231. Glyphosate (purity ≥98 %) was tested in concentrations from 10^{-12} to 10^{-6} M. Glyphosate exerted proliferative effects on human hormone-dependent cell lines but not in hormone-independent cell lines. Furthermore, an additive estrogenic effect between glyphosate and genistein, a phytoestrogen, was reported. The authors conclude that these in vitro results need further investigation in an animal study. It must be emphasised that no increase in mammary tumours was reported in any of the numerous long-term studies in rats or mice (see Vol. 3, B.6.5 and Vol. 1, B.2.6).

**In Vivo Glyphosate DART/ED Publications**

Relatively few in vivo publications on glyphosate DART and ED exist in comparison with the list of in vitro publications. Some lack appropriate interpretation of basic toxicology; e.g. Panitch et al. (2001, ASP2013-12089) and Herbst et al. (2012, ASP2013-12090) analyzed...
The republication of the study will be accompanied not only by a new article by the Séralini team on the influence of conflicts of interest in the world of publishing and of health expertise (paper joined), but also by a message from the Editor explaining his approach:

“Empirical natural and social sciences produce knowledge (in German: Wissenschaften schaffen Wissen) which should describe and explain past and present phenomena and to estimate their future development. To this end quantitative methods are used. Progress in science needs controversial debates aiming at the best methods as basis for objective, reliable and valid results approximating what could be the truth. Such methodological competition is the energy needed for scientific progress. In this sense, ESEU aims to enable rational discussions dealing with the article from G.-E. Séralini et al. (Food Chem Toxicol 2012, 50:4221-4231) by re-publishing it. By doing so, any kind of appraisal of the paper’s content should not be connoted. The only aim is to enable scientific transparency and, based on this, a discussion which does not hide but focus methodological controversies.”
Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize

Gilles-Eric Séralini¹*, Emilie Clair¹, Robin Mesnage¹, Steeve Gress¹, Nicolas Defarge¹, Manuela Malatesta², Didier Hennequin³ and Joël Spiroux de Vendômois¹

Abstract

Background: The health effects of a Roundup-tolerant NK603 genetically modified (GM) maize (from 11% in the diet), cultivated with or without Roundup application and Roundup alone (from 0.1 ppb of the full pesticide containing glyphosate and adjuvants) in drinking water, were evaluated for 2 years in rats. This study constitutes a follow-up investigation of a 90-day feeding study conducted by Monsanto in order to obtain commercial release of this GMO, employing the same rat strain and analyzing biochemical parameters on the same number of animals per group as our investigation. Our research represents the first chronic study on these substances, in which all observations including tumors are reported chronologically. Thus, it was not designed as a carcinogenicity study. We report the major findings with 34 organs observed and 56 parameters analyzed at 11 time points for most organs.

Results: Biochemical analyses confirmed very significant chronic kidney deficiencies, for all treatments and both sexes; 76% of the altered parameters were kidney-related. In treated males, liver congestions and necrosis were 2.5 to 5.5 times higher. Marked and severe nephropathies were also generally 1.3 to 2.3 times greater. In females, all treatment groups showed a two- to threefold increase in mortality, and deaths were earlier. This difference was also evident in the weights and ratios of the kidney, brain, liver, heart, lungs, and kidneys.
Republish the study in open source in order to advance science

Séralini’s study has been republished in open access and its raw data is now made public

With the opportunity of republication by Springer Open, the Séralini team will also provide the scientific community with the raw data of its study, something that the industry has always refused to do under the premise of "trade secrets" or "intellectual property". The team believes that the industry has never achieved adequate testing, and has some data which indicates that there has never been blood analysis on rats contaminated by Roundup.

In January 2013, ANSES (Agence nationale de sécurité sanitaire de l’alimentation, de l’environnement et du travail - National Agency for Food, Environmental and Occupational Health Safety), in the person of its director Marc Mortureux, confirmed to Prof. Séralini in writing that there is no toxicological analysis of Roundup in its most complete formulation on animals over a two year period, adding that there are only a few studies on acute toxicity (from a few days to 3 weeks) without any blood testing.
and in females six times greater mortality by the 21st month on the 22% GM maize diet with and without R. In the female cohorts, there were two to three times more deaths in all treated groups compared with controls by the end of the experiment and deaths occurred earlier in general. Females were more sensitive to the presence of R in drinking water than males, as evidenced by a shorter lifespan (Figure 6, panels R). The general causes of death represented in histogram format within each of the panels in Figure 6, are linked mostly to mammary tumors in females and to problems in other organ systems in males.

**Additional file 1.** Biochemistry M15  
Format: XLS Size: 140KB [Download file](#)  
This file can be viewed with: [Microsoft Excel Viewer](#)  

**Additional file 2.** List of blood and urine parameters with sampling and unit detail  
Format: PDF Size: 172KB [Download file](#)  
This file can be viewed with: [Adobe Acrobat Reader](#)  

**Additional file 3.** Mortality and tumors raw data  
Format: XLSX Size: 122KB [Download file](#)  

**Additional file 4.** Rats identification  
Format: PDF Size: 90KB [Download file](#)  
This file can be viewed with: [Adobe Acrobat Reader](#)  

**Additional file 5.** Raw data legends  
Format: DOCX Size: 14KB [Download file](#)
Conflicts of interests, confidentiality and censorship in health risk assessment: the example of an herbicide and a GMO

Gilles-Eric Séralini\textsuperscript{1,2*}, Robin Mesnage\textsuperscript{1,2}, Nicolas Defarge\textsuperscript{1,2} and Joël Spiroux de Vendômois\textsuperscript{2}

Abstract

We have studied the long-term toxicity of a Roundup-tolerant GM maize (NK603) and a whole Roundup pesticide formulation at environmentally relevant levels from 0.1 ppb. Our study was first published in \textit{Food and Chemical Toxicology} (FCT) on 19 September, 2012. The first wave of criticisms arrived within a week, mostly from plant biologists without experience in toxicology. We answered all these criticisms. The debate then encompassed scientific arguments and a wave of \textit{ad hominem} and potentially libellous comments appeared in different journals by authors having serious yet undisclosed conflicts of interests. At the same time, FCT acquired as its new assistant editor for biotechnology a former employee of Monsanto after he sent a letter to FCT to complain about our study. This is in particular why FCT asked for a \textit{post-hoc} analysis of our raw data. On 19 November, 2013, the editor-in-chief requested the retraction of our study while recognizing that the data were not incorrect and that there was no misconduct and no fraud or intentional misinterpretation in our complete raw data - an unusual or even unprecedented action in scientific publishing. The editor argued that no conclusions could be drawn because we studied 10 rats per group over 2 years, because they were Sprague Dawley rats, and because the data were inconclusive on cancer. Yet this was known at the time of submission of our study. Our study was however never attended to be a carcinogenicity study. We never used the word ‘cancer’ in our paper. The present opinion is a summary of the debate resulting in this retraction, as it is a historic
In the feed of Control rats:
18% NK603
15% MON810
310 ppb Glyphosate + AMPA
Food and Chemical Toxicology Editorial Board

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GMO toxicity tests cannot be conclusive because the diets used are not monitored for contaminations by the tested substances
In conclusion:

Regulators appear to ignore that agricultural GMOs have been modified to contain new pesticide residues, enabling them to escape their long-term toxicity testing.

Recommendations:

1/ Transparency of, and access to, all the raw data

2/ Building of new data by independent laboratories
THANK YOU FOR YOUR ATTENTION

Part of the CRIIGEN research team, Professor Gilles-Eric Séralini, Dr. Robin Mesnage, Dr. Joël Spiroux de Vendômois (Chairman of CRIIGEN) and Nicolas Defarge.

www.criigen.org