

# The Austrian experiment with mice fed with a hybrid GM maize from Monsanto

Did the Monsanto Hybrid Transgenic Maize lower the fertility of mice in a multi generation feeding experiment ?

The Austrian study published by the Austrian Ministry of Health in a preliminary report <sup>1)</sup> is summarized by the main scientific author Prof. Dr. Jürgen Zentek as follows:  
“Mice fed with GM maize had less offspring in the third and fourth generations, and these differences were statistically significant. Mice fed with non-GM maize reproduced more efficiently” Prof. Zentek himself declares his study as a preliminary draft which needs to be scrutinized and does only partially delivers conclusive results.

Comments by Prof. Klaus Ammann

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## Summary:

The Austrian study which has been prematurely published as a report by the Austrian government does not stand to the review of peer experts. The principal scientist Prof. J. Zentek himself declares the study as a preliminary draft with partially inconclusive results, which needs to be corrected in some points. He is not at all happy with the present situation and the premature press conference of the Austrian ministry, and particularly he is refuting the conclusions drawn by [Greenpeace](#), requesting the immediate retraction of GM maize in Europe, since there is a real risk that consumers of this maize could become sterile.

Andrew Apel, webmaster and author of [www.gmobelus.org](http://www.gmobelus.org), a new and excellent website, provides a lot of information, summarizing the case in several features and giving ample background [information](#) and lots of links. The study has been refuted by Monsanto in several statements, recently also by [EFSA](#) (European Food Safety Authority)

A German text can be found under the newpoints, written by Jan Lucht, on the [Internutrition website](#).

## 1. Background:

It is well known that the Austrian Government maintains for years a critical position regarding GM crops, this latest press conference in Vienna is not astonishing in the light of the transatlantic rift over risk perception and regulation of GM crops. Despite of hundreds of peer reviewed publications on risk assessment GM crops still have a bad reputation in Europe, and any kind of publication with seemingly negative results regarding environmental or food safety gets press attention way out of proportion, and activists with a negative agenda build up a culture of concern built on flawed data. [See link for more pages](#) on the launch of questionable publications, the transatlantic rift, European regulatory politics etc. [▶](#)

It has become sad routine that advocacy groups like [Greenpeace](#) publicize reports which have [not undergone peer review](#) using their typical inflammatory language:

“An explosive study published by the Austrian government, on Tuesday November 11, identified serious health threats of genetically engineered (GE) crops. Mice fed with GE maize were severely impaired in their fertility, and produced fewer children than mice fed with natural crops. This study shows how little we know about GM products and their long-term impacts on our health and the environment. It also shows how flawed the current EU risk assessment system is.”

It is also alarming to see, that the number of papers published in scientific journals grow steadily, reporting seemingly negative results about GM crops. A closer scrutiny reveals flaws and questionable conclusions. There are also a few reviewed papers which stress negative potentialities related to impact on environment and health of GM crops <sup>2)</sup>. However, those cases published in a peer reviewed journal are nevertheless highly controversial.

Information about the authors of the Austrian study, more details about the regulatory background and a series of recently published papers critical about GM crops can be seen [here](#) including a brief discussion.

A closer look at this preliminary, not yet peer reviewed Austrian report reveals serious mistakes in data analysis and methodology, putting into question all the negative conclusions drawn by Greenpeace.

## 2. Data analysis:

### 1.1. Data comparison GM- and non-GM feed

A comparison between the data for GM and non-GM feed does not show any effects on the mice used in the experiment.

However, there are considerable differences between the two non-GM lines used for the experiment: Several data sets (body mass in various generations) differed significantly, and the conclusion is simple: Non-GM maize seems to be an unhealthy feed for the mice in the experiment. Unfortunately, in some cases the two non-GM line data are lumped, a lost opportunity to study natural variation.

### 1.2. Confusion in terminology of pups

Table data comparison is hampered due to an inconsistent denomination of litter: Table 59: Despite the fact that the same terminology (pups per 'pair') was used as in Tables 36 and 40, most of the values in this table were appropriately expressed as numbers of pups per litter rather than per co-housed pair. Unfortunately, there appeared to be three major exceptions, all in the GM groups: pups at weaning for the 3rd litter, pups at birth for the 4th litter, and pups at weaning for the 4th litter. Resulting calculation errors and erroneous conclusions as a consequence under 3.2.

### 1.3. Organ weights: few conclusive results

The same picture for the organ weights: High variation, inconclusive results for the comparison between GM- and non-GM maize, but again the two non-GM maize lines demonstrated considerable differences, although this could not be corroborated by the microscopical comparison, which are in their methodology questioned by the [Monsanto-analysis](#) p. 6 paragraph 2.

### 1.4. Inconclusive immunochemistry results

The immunochemistry was inconclusive by the statements of the authors themselves: The differences were inconsistent between the two sexes and were not found in all segments, and specific immune populations did not show feed effects or there was too much individual variability or there were no statistically significant differences seen between the groups.

## 3. Methodological flaws

### 3.1. Unusual high mortality of mice populations used

Data analysis shows that the experiment suffers under an unusual high mortality of the mice used: Instead of the 1% mortality you can see a mortality of 8%. And, if you look closely in the tables, you can discover that the GM mice survived better than the non-GM mice. This means in fact, that the GM mice used in the Austrian experiment died 8 times more often than mice used for correct experimentation schemes. More details, explanation and graph [here](#) in the GMO Pundit of David Tribe:

There is no explanation given about the fact, that there were five instances of total litter loss after birth in the 3<sup>rd</sup> litter and one instance in the 4<sup>th</sup> litter. The reason for the total litter loss was not discussed, but it would probably be most appropriate to use the total number of surviving litters as the denominator when expressing number of pups at weaning, not the numbers of litters at birth. This is – among other reasons, important for the explanation of the lowering of fertility in the whole experiment.

### 3.2. Calculation errors

There are some calculation errors in the tables of the report <sup>1)</sup>, this has been found out and recalculated by Dr. James Lamb, who [reviewed](#) the study as an experienced independent scientist from a consulting company on request of Monsanto, the major statements:

“The statistics cannot be tested appropriately without the individual animal data. The computational errors in such critical tables (Tables 36 and 59) raise serious questions

about the other data in the report and the quality assurance methods that were or should have been applied before the conclusions were drafted and the report was released. When properly analyzed, **these data do not appear to support an effect on fertility or reproduction from consumption of GM corn.**"

Details about the calculation and the two tables separately reproduced [here](#).

Dr. Lamb is, together with his colleagues Morrissey, Chapin and Gulati, a leading and independent expert on mice studies in toxicology, he co-authored important methodological papers on long-term mice experiments, which have contributed in an important way to the standard procedure later <sup>3)</sup>.

#### 4. Study authors not specialized in experimentation with mice

Prof. Jürgen Zentek is according to his [bibliography](#) extracted from the Web of Knowledge a specialist on live stock feeding, with a prolific publication activity in excellent peer reviewed journals on animal feed science. The first author A. Velimirov is a specialist on organic food with two pertinent publications available on the Web of Knowledge <sup>4, 5)</sup>, C. Binter has one conference paper on animal feeding <sup>6)</sup>. So it is fair to say that the authors have no extensive experience in experimenting with mice. This is why, although following with minor deviations the usual procedures of such studies with mice, their data calculation and interpretation is flawed due to lack of experience and leads to the wrong conclusions, see the [Monsanto analysis](#) p.2 second paragraph.

#### 5. Specific long term problems in experimentation with mice

**Reproductive Assessment by Continuous Breeding (RACB)** have their own rules: RACB involves a set of parent mice who give birth to a series of 'litters' of baby mice. The mice in each litter from these parents are counted, measured, and evaluated in other ways. Guidelines are published by [EPA](#) <sup>7)</sup> in all details in Chapter 3.1.5, p.8: (not cited in <sup>1)</sup>)

"Because the parental and subsequent filial generations have different exposure histories, reproductive effects seen in any particular generation are not necessarily comparable with those of another generation. Also, successive litters from the same parents cannot be considered as replicates because of factors such as continuing exposure of the parents, increased parental age, sexual experience, and parity of the females."

Also other documents point to the same 'normal' long term fertility problems. According to [Chapin](#) <sup>8)</sup>, also given in a website of the [National Toxicology Program](#) of the USA stress the following facts:

With RACB, the successive generations of mice born to one set of parents, are necessarily born to parents of increasing age. As a result, a decline in the number of mice born in later generations is expected as a matter of course.

Unless offspring were allowed to grow and reproduce (as they are routinely in the more recent version of the RACB protocol) <sup>9)</sup>, little or no information will be available on postnatal development or reproductive capability of a second generation.

Differences in the ages of the parent mice at the beginning of the experiment can have a significant impact. According to the University of North Carolina, on the very informative website '[Mouse Breeding Advice](#)' it is confirmed again that there are long term/multigeneration effects which need to be considered:

"Delayed breeding was associated with smaller litter sizes, both at birth and at weaning, a higher bodyweight of pups at weaning, a higher percentage of litters with at least one newborn pup cannibalized, earlier cessation of female reproductive life and a higher mortality rate of dams during the breeding period."

The Austrian study duly cites all advice of authorities related to Bt toxicity biosafety assessment, but seems to ignore the long term problems of multigeneration experiments with mice displayed in many websites and scientific papers, last but not least detailed also in comprehensive reviews of ILSI <sup>10, 11</sup>).

There is an additional problem with mice studies in general: there are considerable differences in the performance of various mouse stocks used for breeding, as is again shown by the ['Mouse Breeding Advice'](#), the differences in breeding performance are remarkable and need to be taken into account in the study.

The summary of another study from Dr. John DeSesso, Senior Fellow at the non-profit group Noblis and editorial board of the journal Reproductive Toxicology, commissioned by Monsanto, comes to the same [conclusions](#):

"The report by Velimirov et al describes investigations into potential health effects in mice after long-term feeding of diets that contain several sources of corn. The studies have not been peer-reviewed and have not been published in the open literature. The methods and description of the electron microscopic findings are presented in insufficient detail to support the authors' claims. The reproductive studies (multigenerational versus continuous breeding) resulted in conflicting results. The results from only the continuous breeding study had findings that appeared to indicate an effect on reproduction. However, the continuous breeding study used a non-standard study design, collected data that are not typically measured in these types of studies, and presented data in displays that are both difficult to understand and have mathematical errors. If data for individual animals were available for inspection, it may be possible to re-analyze the results and draw defensible conclusions. In the absence of such a re-analysis, the data are inconclusive, at best and provide no evidence of an adverse effect on reproductive performance in mice."

It is interesting to note that both experts J.C. Lamb and Dr. DeSesso are not mentioning the long term problems in RACB studies, the results can be debunked by pointing to the calculation errors and other interpretation flaws.

Notable is the critique that the mice experiments have been conducted under rather poor health standards and questionable procedures have been applied related to the control of litters.

## 6. EFSA opinion on Austrian mice study Dec. 4, 2008

Finally, EFSA also published, as part of their minutes from the 46<sup>th</sup> plenary meeting on December 4, 2008 the following [statement](#), which is given here in extenso:

**Adopted part of the minutes of the 46<sup>th</sup> plenary meeting of the Scientific Panel on Genetically Modified Organisms held on 3-4 December 2008 GMO Panel deliberations on the Austrian report "Biological effects of transgenic maize NK603 x MON 810 fed in long term reproduction studies in mice" as adopted at the plenary meeting of 3-4 December 2008.**

On 11 November 2008 the Austrian Federal Ministry of Health, Family and Youth released a research report on studies in mice, which were conducted to assess the impact of genetically modified (GM) maize NK603 x MON 810 on reproduction (Biological effects of transgenic maize NK603 x MON 810 fed in long term reproduction studies in mice, Dr. Alberta Velimirov, Dr. Claudia Binter, Univ. Prof. Dr. Jürgen Zentek).

The report includes three studies, a life-time study, a multigeneration study (MGS), and a reproductive assessment by continuous breeding study (RACB). According to the authors the life-time study showed no statistically significant differences in survival between mice fed with kernels of maize NK603 x MON 810 and the controls. They also reported that, in the MGS study, no significant differences in reproductive traits were found between mice fed with kernels of maize NK603 x MON 810 and the controls. In the RACB study, the authors used a modified protocol of the original RACB study developed at the U.S. National Toxicology Program (NTP) for the testing of chemicals. Male and female mice were housed as breeding pairs for approximately 20 weeks and allowed to produce litters continuously throughout the cohabitation period. The authors identified differences in reproductive parameters between mice fed with the GM maize and the controls. They reported that there were statistically significantly fewer pups born in the GM group in the 3<sup>rd</sup> and 4<sup>th</sup> delivery and fewer pups weaned in the 4<sup>th</sup> litter compared with the control group. The GMO Panel considered this report and came to the following conclusions. Regarding the RACB study, the summary Table 59 contains calculation errors and inconsistencies in the treatment of the data regarding the 3<sup>rd</sup> and 4<sup>th</sup> litters. In addition, it seems that the authors have calculated the number of pups at birth per pair and not per delivering pair, which is standard practice. Also, there appears to be methodological deficiencies in the statistical analysis that seriously compromise the interpretation of the data. For the reasons stated above, individual data are required for a proper assessment. In addition, more detailed information regarding the breeding scheme is needed. In particular, it should be clarified whether in the 3<sup>rd</sup> and 4<sup>th</sup> pairing the same or different pairs failed to reproduce.

Information regarding the normal variation of the parameters examined in this study for the mouse strain used (historical control data) is required before any conclusion may be drawn on possible alterations in reproductive performance. In addition, further information on the estrous cycle and histopathological parameters including spermatogenesis, follicle and oocyte counts is essential for assessing the claims of reduced fertility.

The GMO Panel also notes that information on the genetic identity and characteristics of the tested materials is not sufficient.

On the basis of the data presented the GMO Panel is of the opinion that *no conclusions can be drawn from the report*.

Published at [http://www.efsa.europa.eu/EFSA/efsa\\_locale-1178620753812\\_1211902199319.htm](http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902199319.htm). The complete minutes will be adopted at the 47<sup>th</sup> plenary meeting (28-29 January 2009) and will be published shortly afterwards. *EFSA/GMO/457 – part of the Minutes 46<sup>th</sup> Plenary Meeting of the GMO Panel*

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