Monsanto Comments
(Update 11/1/2012)

Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize.

Gilles-Eric Séralini, Emilie Clair, Robin Mesnage, Steeve Gress, Nicolas Defarge, Manuela Malatesta, Didier Hennequin, Joël Spiroux de Vendômois
Food and Chemical Toxicology (electronic ahead of press)
http://www.sciencedirect.com/science/article/pii/S0278691512005637

Associated website and videos:
http://www.ogm-alerte-mondiale.net/?lang=en
http://www.youtube.com/channel/UCktZ44yjV7cq0yFhQlrpyOg?feature=guide
http://www.dailymotion.com/Lieurac_Productions

Summary:

This study does not meet minimum acceptable standards for this type of scientific research, the findings are not supported by the data presented, and the conclusions are not relevant for the purpose of safety assessment. The study has been the subject of initial critical reviews by multiple regulatory agencies (links provided below). For example, the Federal Institute for Risk Assessment (BfR) in Germany states that *"the authors' main statements are not sufficiently corroborated by experimental evidence, due to deficiencies in the study design and in the presentation and interpretation of the study results."*

Toxicologists and public health experts also find fundamental problems with the study design. Critical information about how the research was conducted is absent, and the data presented do not support the author's interpretations. **Among the key shortcomings are:**

- Research protocol does not meet OECD standards.
- Source and quality of corn used is unclear.
- Critical details on diet preparation and dietary intake are absent.
- Complete lack of data pertaining to assertions of microscopically visible changes in liver or kidney tissues (abnormal histopathology) and laboratory testing results of blood and urine analyses.
- Lack of any statistical analysis for mortality or tumor incidence endpoints. Monsanto statistical analysis of deaths based on visual approximation of the graphical data indicates a lack of statistical significance even when liberal criteria ($p<.10$) are applied.
- Mortality rates and tumor incidence in all groups fall within historical norms for this strain of laboratory rats, which is known for a high incidence of tumors.
- Data presented are highly selective, using (for example) different methods for male and female animals, and are not sufficient to support conclusions drawn.

- There is a lack of dose-response relationship throughout the study.
- There is no plausible mechanism for the results reported with genetically modified maize, and the results are inconsistent with an extensive body of experience and scientific study.
- Extensive animal and *in-vitro* (test-tube) data has demonstrated that glyphosate does not cause cancer or tumors, nor is an endocrine disrupter. This study does not provide information which calls into question the extensive safety evaluations of glyphosate or Roundup herbicides. Plant biotechnology has been in use for over 15 years, without documented evidence of adverse effects on human or animal health or the environment. An extensive body of scientific evidence, reviewed by regulatory agencies around the globe, supports the safety of plant biotechnology in general as well as the specific safety of NK603 maize.

General Comments: Plausibility and Weight of existing Evidence

(Specific comments on study itself follow.)

Extensive animal data has demonstrated glyphosate does not cause cancer/tumors. Multiple lifetime cancer studies from multiple glyphosate registrants, performed independently over the past 35 years have demonstrated that glyphosate does not cause tumors/cancer in rodent species (see glyphosate resources, appendix to this document).

Multiple epidemiology studies do not support the author's health claims related to glyphosate. Published epidemiology results evaluating human health effects, including cancer and reproductive effects, reinforce the lack of evidence linking such endpoints to glyphosate use, (see glyphosate resources, appendix to this document).

Extensive *in-vitro* (test-tube) and animal data indicate glyphosate is not an endocrine disrupter. Although glyphosate was included in the EPA's initial substances for the endocrine disrupter screening program, EPA has stated that the basis of this inclusion is the high frequency of use, not the existence of data indicating endocrine effect (see glyphosate resources, appendix to this document).

Surfactant components are not expected to contribute to cancer or endocrine risks. The category of surfactants used in the Roundup™ formulation used in the study was evaluated by the US EPA in 2009 and was considered acceptable for this use in pesticide products based on the results of multiple repeat dose studies, including reproductive and developmental toxicology. Consumers have a regular exposure to surfactant materials in the form of shampoos, soaps, and cleaning products. These are not believed to present reproductive/endocrine risks. Further, exposure to surfactant residues as a result of pesticide exposures represents a very small portion of human surfactant exposures.

Lack of plausible mechanism for effect of GM. NK 603 contains a bacterially derived form of the enzyme (protein) EPSPS, which confers resistance to the herbicide, glyphosate. EPSPS is present in all plants as well as in the bacteria found in human and

animal gut flora. It is a readily digestible protein not known to have any adverse effect on any species. There is simply no plausible means by which EPSPS or the genetic material which encodes it can cause cancer- any more than there is for the tens of thousands of other dietary proteins. It is notable that we do not do chronic toxicology testing on non-toxic proteins in the human diet. Virtually none of the plant proteins in the diet (including EPSPS) have been tested, simply because there is no rational reason for doing so.

Specific Comments on Study Design, Conduct and Interpretation:

Study does not meet OECD standards. Despite author's reference to OECD Testing Guidelines, the study design does not meet OECD standard for number of animals in a chronic study design (50 per group), and GLP (Good Laboratory Practice) status of laboratory and analytical facilities is not clear. Doses selected for GMO and Roundup™ are not based on standard approaches for dose setting.

Statistical analysis varies markedly from any OECD or other recognized approach, and does not establish toxicological relevance. The authors used a complex statistical technique to investigate the relationship among 48 blood and urine measurements in different treatment groups. This technique can be used to identify patterns in the data and to develop a mathematical function which can be used to discriminate between groups. However, just because you can discriminate between the groups mathematically does not make the result toxicologically relevant. In fact, nearly all laboratory values in all groups appear to be within the normal range established by variation within the study.

Further, fundamental data regarding laboratory results are either absent or, if present, are not presented in the typical format of mean values and standard deviations, which prevents the results from being evaluated appropriately. Data were obtained at multiple time points in both male and female animals, but only data from a single time point in one sex are provided. It is certainly expected that a toxicology study will attempt to address the cause of consistently observed abnormalities- indeed this is their purpose. Statistical comparisons of variations within the normal range do not generally address meaningful toxicological endpoints.

Source of control maize and applied pesticide is unclear. The test and control GM material was obtained from Canada, and it is unclear how the investigators could have identified or obtained the correct isoline for this particular genetic event. Further, growth conditions are not specified and there are no data as to mycotoxin (fungal toxin) content or anti-nutrient content of GM vs. control dietary components.

Critical detail on diet preparation is absent. For GM diet (11, 22, or 33% of diet as GM, 33% only for control), it is unclear whether all test diets contained the same quantity of maize- i.e.- does the 11% GM diet also contain 22% control maize so that total maize content is consistent. If this is not the case, dietary composition across the

study is not equivalent and differences observed may well be due to dietary compositional differences.

Critical data on dietary intake are also absent. Data on food/water intake are generally regarded as essential in order to understand actual delivered dose, animal health and susceptibility to certain tumor types. Dehydration (from aversion to drinking soapy herbicide formulation) will alone give rise to unintended biological outcomes.

Blinding of pathologists in reading data is not indicated. OECD Test Guidelines refer to published best practice guidelines which includes blind reading (i.e.- not knowing which group a specimen came from) to avoid observational bias. While this may have been the case, this is not clearly indicated.

Data presented are highly selective and are not sufficient to support conclusions drawn. While we understand that all data and all data analysis cannot be included in any publication of this nature, one would normally, for example, present data for both sexes rather than presenting data via one approach for male kidney outcomes and another for female kidney outcomes. This is important as it allows for determination of consistency of effect. Today, additional data sufficient to support conclusions can easily be provided in supplemental online data tables.

Mortality rates and tumor rates fall within historical norms for this strain of laboratory rats. The percent survival to study termination in SD rats (Charles River Laboratories) ranges from 17-62.9% in males and from 20-62% in females. Findings by Seralini are within this historical range. While it is certainly acceptable to use this rat strain in toxicology studies (industry studies often do), it is essential to use an appropriate number of animals and to apply appropriate statistical analysis against control groups, and to consider background incidence of tumors or death in untreated animals. Primary tumors observed are common in this rodent strain and observed frequencies are consistent with historical observations. For example- in female SD rats, the frequency of mammary adenocarcinoma (frequency based on single diagnosis of a particular tumor type per number of total organs examined) ranges from 8.6 - 58.3% and fibroadenoma plus fibroma ranges from 13.3 - 61.3%. (To put the actual numbers in the paper is perspective; a rat has up to 12 mammary glands. Number of observed tumors thus may exceed number of affected animals.) Pituitary tumors are common in male (adenoma 0.77-70%, carcinoma 0.77-36%) and female (adenoma 26-92.9%, carcinoma 1.43-58%) SD rats (1 pituitary per animal).

Statistical analysis is lacking on mortality and tumor incidence data. While cumulative mortality and tumor incidence plots are provided, no statistical analysis of these data is provided (i.e. – Mantel-Hansel survival statistic for mortality data). As group numbers are small (n=10) it does not appear that statistical significance is likely for the majority of graphical analyses in figures 1 and 2. As conclusions regarding tumor incidence are among the major conclusions of this paper, this lack of statistical analysis is remarkable. Monsanto has undertaken an approximate statistical analysis of animal deaths based upon the graphical data presented. This is necessarily an approximation

as we do not have access to raw data. This analysis indicates no statistical significance at the p=0.1 level (more liberal than the usually applied p=0.05). It appears unlikely that any of the observed death and tumor incidence data reach statistical significance.

Statements made in the paper do not comport with proper statistical analysis. Specifically, a proper analysis would statistically evaluate the incidence (or timing) of death or tumor frequency in a single group vs control (comparing, for example 10 males in a test group to 10 male controls). An alternative is to look simultaneously at controls and multiple dose groups to demonstrate a dose-response (increasing response as dose increases)- but as the authors have noted, no dose-response is evident. Instead, statements are made to the effect that the first occurrence of death or a tumor occurred in test rather than control animals. While this (especially when accompanied by unattractive pictures of rodent tumors) is designed to raise concern about GM safety, the reality is that, given a test regimen in which ten control animals (per sex) are compared with ninety test animals (all doses combined), it is far more likely that rare events such as very early tumors will be seen in the far larger test population.

Lack of effect on body weight parameters. Given claimed degree of illness in test populations, the absence of a difference in weight gain among the control and experimental groups (data not shown, but stated by authors) is surprising.

Lack of tumors or tumor precursors in 90-Day studies. While we concede that a 90-day study is not the same as a lifetime study in purpose or interpretation, the authors of this paper suggest that palpable tumors are occurring as early as 4 months into the protocol. As tumors take considerable time to grow to palpable size, and as only a minority of tumors generally grow to large size, tumors (even if not palpable) should have been evident in the 90 day studies performed with NK-603. This was not observed.

General lack of dose-response on critical endpoints: While the authors argue for some type of low-dose phenomenon or maximal response phenomenon in which maximal response is reached at the lower dose levels, it should be noted that a) the phenomenon of low dose response is highly contentious in the scientific community and that b) when accepted, is usually argued for endocrine effects. General systemic effects like mortality, as well as the occurrence of tumors (especially nonendocrine tumors) are expected to follow a dose-response pattern. This response may not be simple, but higher dose should reliably produce greater response. The Vandenberg paper cited in support of low-dose or non-dose-response effects is entirely about endocrine effects as is the recent EC Workshop and the existence of this phenomenon has been questioned.

Complete lack of data regarding purported liver or kidney histopathology, liver function tests, and cytochrome activity. While stated in the paper, no data at all, let alone any summary statistics, are provided. The selected images presented are insufficient to support any analysis or conclusions. Male animals are said to have died primarily from “severe hepatorenal (i.e.- liver and kidney) insufficiencies,” but no data are provided in support of this observation.

Lack of documented consistency or of clinical significance for chemical parameters. While the non-OECD analysis technique used does find some parameters which are statistically significant, findings in males and at time points other than 15 months are not provided or discussed to allow evaluation for consistency, nor are values for all test doses provided to allow for assessment of dose-response. More importantly, although the application of this statistical technique will drive the creation of a predictor for the observed data, the clinical and toxicological relevance of the observed data need to be considered. Virtually all data points are within the general normal range within the study. [Exceptions - one urinary sodium (test animal) and one estradiol level (control animal)].

Reviews of long term studies by scientists, physicians, and regulators have reached the conclusion that GM crops are safe.

- Scientific review:
http://www.sciencedirect.com/science/article/pii/S0278691511006399

- European reviews:
http://www.gmo-safety.eu/news/1410.long-term-studies-safety-gm-food.html

http://www.gmo-safety.eu/news/1378.genetic-engineering-feeding-experiments-meta-study.html

- American Medical Association (2012):
https://ssl3.ama-assn.org/apps/ecomm/PolicyFinderForm.pl?site=www.ama-assn.org&uri=%2fresources%2fdoc%2fPolicyFinder%2fpolicyfiles%2fHnE%2fH-480.958.HTM

- Swiss National Science Foundation
http://www.snf.ch/e/media/pressconferences/pages/default.aspx?NEWSID=1772&WEBID=F6B532FB-64ED-466F-8816-193D4DE8DC94

APPENDIX: Glyphosate resources

Three new reviews on glyphosate and human safety published within the last year (available online from publisher website):

- Pamela J. Mink, Jack S. Mandel, Bonnielin K. Sceurman, Jessica I. Lundin. Epidemiologic studies of glyphosate and cancer: A review. http://www.sciencedirect.com/science/article/pii/S0273230012000943

- Pamela J. Mink, Jack S. Mandel, Jessica I. Lundin, Bonnielin K. Sceurman. Epidemiologic Studies of Glyphosate and Non-Cancer Health Outcomes: A Review.
http://www.sciencedirect.com/science/article/pii/S0273230011001516

- Amy Lavin Williams, Rebecca E. Watson, John M. DeSesso. Developmental and Reproductive Outcomes in Humans and Animals After Glyphosate Exposure: A Critical Analysis. http://www.tandfonline.com/doi/abs/10.1080/10937404.2012.632361

The most recent review was conducted by the European Commission's Health and Consumer Protection Directorate-General, 2002 (Compounds are reviewed every 10 years and a review is in progress now.) http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1_glyphosate_en.pdf

WHO/FAO. (2004) Pesticides residues in food -- 2004.
http://www.fao.org/ag/agp/agpp/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf

Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans" (Williams et al., 2000): http://dx.doi.org/10.1006/rtph.1999.1371

WHO Environmental Health Criteria 159: Glyphosate (1994):
http://www.inchem.org/documents/ehc/ehc/ehc159.htm

EPA Reregistration Eligibility Decision: Glyphosate (September 1993):
Fact Sheet: http://www.epa.gov/oppsrrd1/REDs/factsheets/0178fact.pdf
Full RED: http://www.epa.gov/oppsrrd1/REDs/old_reds/glyphosate.pdf