

Monsanto Company Response to a Cii-Gen Report Relating to the NK 603 Maize Rat Feeding Study

- Eric Sachs, Bruce Hammond, Margaret Nemeth, and Kerstin Kramer, June 24, 2007

Subject: Controversial effects on health reported after subchronic toxicity test: 90-day study feeding rats (June 2007) by Seralini, Gilles-Eric, Cellier, Dominique, and Joel Spiroux de Vendomois

- A Cii-Gen [Internet] Report on NK603 GM maize produced by Monsanto Company

- Announced by Greenpeace in a press statement

- Restricted access to the critique, accessible only from Greenpeace or Gilles-Eric Seralini

Overview

Greenpeace issued on 14 June 2007 a press release (<http://www.greenpeace.org/eu-unit/press-centre/press-releases2/seralini-NK603>) highlighting 67 statistically significant differences between test and control treatments in the NK 603 maize 90-day rat feeding study submitted to European Union (EU) regulatory authorities prior to approval in the EU for import, feed and processing under Directive 2001/18/EC on 19 July 2004 and under the Novel Food and Novel Food Ingredient Regulation on 3 March 2005. The analysis by Cii-Gen (Committee for Independent Research and Genetic Engineering) focused on statistically significant differences between rats that received NK603 in their diet and those fed conventional maize. Greenpeace demanded the withdrawal of NK 603 maize and all other GMOs and a review of the European Union risk assessment system.

Importantly, the Cii-Gen report does not include any new data or statistical analysis of the original study data and has not been published in a peer-reviewed scientific journal. It is a critique of the analyses carried out by Monsanto scientists and the conclusions reached by regulatory authorities in the EU in support of the safety of NK 603 maize for use in feed and processed foods. The authors of the Internet report and Greenpeace are calling for further investigation and funding for Cii-Gen to conduct new experiments of longer duration on two generations of rats.

The principal claims of Cii-Gen and Greenpeace include:

- Differences in kidney, brain, heart and liver measurements, as well as weight differences, could be warning signs of toxicity, but were not further investigated by Monsanto or other independent researchers.

- Long term consumption of GMOs could lead to "alarming health anomalies."

- EU risk assessment procedures are not adequate and respective authorities are "just

rubberstamping company dossiers."

- The study conducted by Monsanto scientists "is likely to seriously impair the independence of the expertise involved"

The criticisms of the NK 603 maize rat feeding study by the Cii-Gen team led by Seralini are contradicted by the assessments by EU member state authorities, the European Food Safety Authority, and expert reviewers of the study published in a leading toxicology journal (Hammond et al. 2004, Food Chem. Toxicol.). All statistically significant differences were investigated and no biologically or toxicologically significant differences were observed. This study was published in a peer-reviewed scientific journal, Food and Chemical Toxicology, in 2004.

Hammond, B., R. Dudek, J. Lemen, and M. Nemeth. 2004. Results of a 13-week safety assurance study with rats fed grain from glyphosate tolerant corn. Food and Chemical Toxicology 42 (2004) 1003-1014.

Regulatory Process

The 90 day rat study with NK 603 maize was conducted in accordance with EU regulations and followed strict OECD guidelines for laboratory animal testing. The key steps to date in the EU regulatory process for NK603 include:

- The Spanish Comision Nacional de Bioseguridad reviewed the rat feeding study as Rapporteur as part of Monsanto's notification C/ES/00/01 under Directive 2001/18/EC. Its assessment report concludes that "there is no scientific evidence which indicate any risk for human and animal health or the environment of NK603 maize".

- The Committee on the Safety Assessment of Novel Foods of the Health Council of the Netherlands reviewed the rat feeding study as Rapporteur as part of Monsanto's notification under Regulation (EC) No 258/97. Its assessment report concludes that "the consumption of NK603 maize and foods and food ingredients derived from this is just as safe for humans as the consumption of non-genetically modified maize and maize products".

- The rat feeding study was circulated to all EU Member States as part of the above notifications, after the respective rapporteur reviews.

- The European Food Safety Authority later issued a scientific opinion on above applications for NK603: "The EFSA GMO Panel considered the information made available by the applicant as sufficient to evaluate the safety of NK603 maize and derived products, food and feed ingredients and to address all the specific questions raised by the Member States related to the risk assessment", and further concludes: "NK603 maize is as safe as conventional maize and therefore the placing on the market of NK603 maize for food or feed use of the grain or processing is unlikely to have an adverse effect on human or animal health or, in that context, on the environment."

- NK603 was approved in the European Union for import, feed and processing under

Directive 2001/18/EC on 19 July 2004 and under the Novel Food and Novel Food Ingredient Regulation on 3 March 2005.

Comments on the critique by Cii-Gen

Gilles-Eric Seralini and the co-authors of the Cii-Gen report are critical of the risk assessment process used by regulatory authorities to assess the safety of food and feed derived from GM crops. They do not understand, or are unwilling to accept, the science that underpins the safety assessment of biotechnology-derived crops. This safety assessment process has been articulated by experts (EC, 2003; OECD, 1993; 2002a; EFSA, 2006; FAO/WHO 1996, 2000, 2003; Kuiper et al., 2001, 2002) in Europe and published in numerous monographs. The authors of the report are not toxicologists, oppose the commercial use of GM crops, and frequently challenge the safety of GM crops without legitimate scientific basis. These views are not shared by an overwhelming majority of scientific and regulatory experts around the world and these views were published on the internet, not in a peer-reviewed scientific journal.

The authors remark that "All the scientific committees consulted agree with Monsanto that statistically significant differences were reported during the 90-day study..." Their concerns are based on the finding of 67 statistically significant differences among some 1050 comparisons of overall health, body weight, food consumption, clinical pathology parameters, organ weights, and gross and microscopic appearance of tissues between test and control rats during the duration of the feeding study. There were a large number of comparisons made in the study and a number of statistically significant differences were expected to occur at random. All statistically significant differences were investigated and no biological or toxicological differences were observed (Hammond et al., 2004). A statistically significant finding does not automatically constitute definitive evidence of an adverse or toxicologically important effect. The magnitude of departure from the normal range, the consistency of the out-of-range responses, and the relationships of the abnormal responses to the physiological, physical, biochemical, and metabolic well-being of an animal all have to be considered. These must be evaluated on a case-by-case basis, taking into consideration such factors as the direction of change (i.e., increase or decrease), evidence of a dose response, the presence of a similar effect in animals of the other sex, and the presence of other indicators of clinico-pathology including, specific organ toxicity (Chan et al., 1982; Gad, 2001; Lewis et al., 2002; SOT, 1982; Weil and gad, 1980; Wilson et al., 2001).

Importantly, scientific experts participating in OECD provided guidance on the interpretation of statistical findings in toxicology studies:

"Because of normal biological variation in inter-animal values, and the alterations in values in response to a variety of inputs, evaluators have to contend with much "noise" in this area; they are frequently presented with statistically significant but scattered effects, in the absence of any evidence of clinically significant relationships with specific toxicity endpoints....To deal with the noise it is necessary to examine whether an effect is within the normal range of variation, using concurrent and historical controls...Frequently these data show apparently random changes in individual groups, or, less commonly, trends in changes across groups that are unrelated to dose. If, as an

aid to evaluation, historical control data are used for comparison, it must be kept in mind that "normal" values in haematological and clinical chemistry measurements depend on the specific methods used to generate the data. Thus, only values obtained using identical methods at the same laboratory are valid in such comparisons." (OECD, 2002b)

The authors acknowledge the need to evaluate the biological relevance of statistical findings. They state "The statistical analysis must be completed with data being interpreted by biologists, toxicologists and physicians (pathologists) in order to correlate the statistically significant differences and the possible development of signs of toxicity, clinical symptoms or pathologies (Remark 6)." They also acknowledge that the majority of scientific experts on government scientific committees evaluated all of the data according to the principles stated in the previous paragraph and concluded they were "not biologically meaningful." In spite of this important understanding, the Cii-Gen authors conclude that a statistically significant finding is evidence of an adverse effect and recommend "additional statistical analysis in a serious and independent way."

The procedures used by Monsanto statisticians followed internationally accepted guidelines recognized by regulatory agencies and other government authorities. Not surprisingly, the authors still recommend that the data be subjected to more statistical analysis using additional methods. There are many tests that can be used to evaluate biological data, and some are more appropriate than others, depending upon the design of the toxicology study. In consideration of the many kinds of statistical tests that are available, and to bring some order to way that toxicology data is evaluated statistically for regulatory submissions, regulatory agencies and government authorities have provided guidance on how data should be evaluated (FDA, 2000; OECD, 2002b; WHO, 1987).

Importantly, it does not matter how many ways the data is analyzed statistically, the data still remain the same. Expert toxicologists have concluded that the measured responses of rats fed NK 603 grain fall within normal limits. There is no basis for conducting more statistical tests since more testing will not change the conclusion that no meaningful differences were observed in rats fed NK 603 grain when compared to rats fed control corn grain.

Monsanto Responses to Specific Remarks in the Cii-Gen Report

Remark 1- Claim that the study conducted by Monsanto scientists "is likely to seriously impair the independence of the expertise involved"

The study design was adapted from OECD Guideline No. 408 (1981) and the study was conducted in general compliance with OECD Good Laboratory Practice guidelines at the Metabolism and Safety Evaluation-Newstead, toxicology laboratory. Toxicology studies conducted at Monsanto laboratories are subject to inspections by Federal Regulatory Agencies in the U.S. (Hammond et al., 2004).

Remark 2- Claim that there may have been toxic effects of glyphosate residues not assessed in the study design

Glyphosate has an excellent human health and environmental profile and a long history of safe use in more than 130 countries. This has been a key factor in the acceptance of glyphosate products as among the most widely used herbicides in the world. When used according to label directions, these products do not represent a hazard to human health and the environment. This is confirmed by extensive studies as well by the firsthand experience of millions of farmers and home gardeners who have used this product.

Glyphosate, the active ingredient in Roundup branded agricultural products, inhibits an enzyme that is essential to plant growth; this enzyme is not found in humans or other animals, contributing to the low risk to human health from the use of glyphosate according to label directions (Franz et al., 1997). Comprehensive toxicological studies in animals have demonstrated that glyphosate does not cause cancer, birth defects, mutagenic effects, nervous system effects or reproductive problems (U.S. EPA, 1993; Williams et al., 2000; European Commission, 2002; WHO/FAO, 2004). In fact, after a thorough review of all toxicology data available, the U.S. EPA concluded that glyphosate should be classified in Category E ("Evidence of Non-carcinogenicity in Humans"), the most favorable category possible (U.S. EPA, 1993). Glyphosate has favorable environmental characteristics, including tight binding to most soils, making it unlikely to move to groundwater or reach nontarget plants, and degradation over time in soil and natural waters (Giesy et al., 2000). Finally, glyphosate has been shown to have favorable environmental characteristics compared to other herbicides (Nelson and Bullock, 2003).

Remark 3- Recommendation for further investigations using two-way ANOVA with specific focus on interactions

The ANOVA used in the original analysis by Monsanto did take interaction into account when it compared the test to the control for each of the diets (11% and 33%). If another ANOVA were to be performed for only groups 1 to 4 and the interaction turned out to be significant then one would compare the test to the control at each of the diets which is exactly what was originally done. This second analysis for groups 1 to 4 would have less power than the original analysis since inclusion of the reference groups increased the degrees of freedom (information) for experimental error for comparing the test to the control.

Remark 4- Claim that the study analysis must include a comparative growth curve analysis

The Monsanto original analysis compared the test and control weight for each diet at each time point. While a growth curve analysis can be done, the most relevant and important end result is whether the body weights fall within normal biological limits. The body weight and weight gain were comparable for the male and female groups fed NK 603 maize, control, and reference control groups (Hammond et al., 2004).

Remark 5- Claim that Monsanto scientists should have used multivariate methods like principal components analysis, data mining, and MANOVA

For a similar rat feeding study, Monsanto scientists evaluated additional statistical tests, which included repeated measures analysis of body weight taking into account correlations across time and increasing variability over time and multivariate analyses (MANOVA, principal components, and canonical correlation) for clinical chemistry and organ weight measurements. None of these additional analyses changed any of the conclusions from the original analysis. There are many different types of statistical analyses which can be done. Statistical analyses provide scientific validity to the study conclusions but statistical significance does not equal biological significance. Use of statistically significant differences alone as the basis for claiming biological significance is scientifically inappropriate. Scientists must look at the whole picture, including the biological context, not just a few isolated statistical differences which can occur by chance alone; or because small experimental variability identifies small numerical differences as statistically significant.

Remark 6- Claim that statistical analysis must be interpreted by biologists, toxicologists, and physicians in order to correlate the statistically significant differences with biological findings

Monsanto scientists, regulatory agencies, and government authorities agree that it is necessary to evaluate the biological relevance of statistical findings. Statistically significant differences must not be considered a biologically relevant finding of an effect in isolation of other relevant biological data. Remarkably, in spite of the author's apparent understanding of this basic principle of analysis of experimental data, they conclude that a statistically significant finding is evidence of an adverse effect and recommend "additional statistical analysis in a serious and independent way."

The authors also criticize the use of historical control data, yet scientific experts recognize this information may be used when needed to assist in the interpretation of "noise," which occurs in these studies (OECD 2002; Deschl et al., 2002).

Monsanto Summary and Recommendations

The European Union and other authoritative guidelines for the safety and risk assessment of biotechnology-derived crops include sufficiently robust experimental and statistical methods to ensure the safety of food and feed derived from GM crops.

The NK 603 maize study was conducted in accordance with European Union regulations and following strict OECD guidelines for laboratory animal testing.

The European Food Safety Authority concluded, "NK603 maize is as safe as conventional maize and therefore the placing on the market of NK603 maize for food or feed use of the grain or processing is unlikely to have an adverse effect on human or animal health or, in that context, on the environment."

Monsanto published the results of the NK 603 maize 90-day rat study in a peer-reviewed, highly regarded toxicology journal and provides additional information on our website supporting the safety of NK 603 maize (see: http://www.monsanto.com/monsanto/content/products/technicalAndSafety/roundup_corn)

[/pss_NK603.pdf](#) .

There is no scientific basis for conducting additional, independent studies. The conclusion by Seralini and the co-authors of the Cii-Gen report that the absence of additional studies "may present a serious risk to human and animal health" is not supported by the overwhelming majority of scientific and regulatory experts around the world.

Regulatory agencies, media, and the general public should be wary of allegations released in press releases by activist groups and based on reports by anti-GM scientists placed on the Internet. These groups do not recognize the well-established safety assessment process for food and feed derived from GM crops that is supported by regulatory agencies and government authorities around the world.

Regulatory authorities should not consider unpublished claims by activist groups first communicated by press release and via the Internet as worthy of consideration; unless and until such time that their claims have been peer-reviewed by qualified, reputable experts in the appropriate field and published in a mainstream scientific journal.

References

Betz, F., Hammond, B.G., and Fuchs, R.L. 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. *Regul. Toxicol. Pharmacol.* 32, 156.

Chan, P. K., G. P. O'Hara, and A. W. Hayes. 1982. Principles and methods for acute and subchronic toxicity. In: Hayes, A. W. (ed.). *Principles and Methods of Toxicology*. Raven Press; New York, pp. 1-51.

Deschl, U., Kittel, B., Rittinghausen, S., Morawietz, G., Kohler, M., Mohr, U., and Keenan, C. 2002. The value of historical control data-scientific advantages for pathologists, industry and agencies. *Toxicol. Pathol.* 30, 80-87.

European Commission. 2002. Report for the Active Substance Glyphosate, Directive 6511/VI/99, January 21, 2002.

http://europa.eu.int/comm/food/fs/ph_ps/pro/eva/existing/list1_en.htm

European Commission. 2003. Genetically Modified Crops in the EU: Food Safety Assessment, Regulation, and Public Concerns. The European Network on Safety Assessment of Genetically Modified Crops (2000-2003). European Commission, Directorate-General for Research Biotechnology, Agriculture and Food Research; Brussels, Belgium.

EFSA. 2006. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. *EFSA J*, 99, 1-94, 2004 (Final edited version May, 2006). Available from: <http://www.efsa.eu.int/cf/consultation.cfm>.

FAO/WHO. 1996. Biotechnology and Food Safety. Report of a Joint FAO/WHO Consultation, Sep. 30-Oct. 4, 1996, Rome, Italy. Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO). FAO Food and Nutrition Paper 61.

FAO/WHO. 2000. Safety Aspects of Genetically Modified Foods of Plant Origin. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, May 29-June 2, 2000, Geneva, Switz. Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO).

FAO/WHO. 2003. Report of the Fourth Session of the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology, Mar. 11-14, 2003, Yokohama, Japan to the Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Twenty-sixth Session, June 30-July 7, 2003, Rome, Italy. Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO) [Alinorm 03/34A]. Available from: <http://www.codexalimentarius.net/web/archives.jsp?year=03>.

FDA. 2000. Toxicological Principles for the Safety Assessment of Food Ingredients: Redbook 2000. Food and Drug Administration, U.S. (FDA), Center for Food Safety and Applied Nutrition (CFSAN); Washington, DC.

Federici, B. 2002. Case study: Bt crops. In: Atherton, K. (ed.). Genetically Modified Crops, Assessing Safety. Taylor and Francis; London.

Franz, J.D., M.K. Mao, J.A. Sikorski. 1997. Glyphosate: A Unique Global Herbicide. ACS Monograph No. 189. American Chemical Society, Washington, D.C.

Gad, S.C. 2001. Statistics for toxicologists. In: Hayes, A.W. (ed.). Principles and Methods of Toxicology, 4th ed. Taylor and Frances; Philadelphia, Penn, pp. 285-364.

Giesy, J.P., Dobson, S., K.R. Solomon. 2000. Ecotoxicological risk assessment for Roundup herbicide. Rev. Environ. Contam. Toxicol. 167:35-120.

Hammond, B., R. Dudek, J. Lemen, and M. Nemeth. 2004. Results of a 13-week safety assurance study with rats fed grain from glyphosate tolerant corn. Food and Chemical Toxicology 42 (2004) 1003-1014.

Kuiper, H.A., Kleter, G.A., and Kok, E.J. 2001. Assessment of the food safety issues related to genetically modified foods. Plant J. 27, 503-528.

Kuiper, H.A., Kleter, G.A., Hub, P.J.M., and Kok, E.J. 2002. Substantial equivalence? an appropriate paradigm for the safety assessment of genetically modified foods. Toxicology 181-182, 427-431.

Lewis, L.W., Billington, R., Debryune, E., Gamer, A., Lang, B., and Carpanini, F. 2002. Recognition of adverse and non-adverse effects in toxicity studies. Toxicol. Pathol. 30, 66-74.

McClintock, J. T., Schaffer, C.R., and Sjoblad, R.D. 1995. A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. *Pestic. Sci.* 45, 95-105.

Nelson, G.C. and D.S. Bullock. 2003. Environmental effects of glyphosate resistant soybeans in the United States. In: *The Economic and Environmental Impacts of Agbiotech: A Global Perspective*. Kalaizandonakes, N. (ed.). Kluwer Academic/Plenum Publishers, New York.

OECD. 1993. *Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts And Principles*; Organization for Economic Cooperation and Development (OECD); Paris.

OECD. 1998. Repeated Dose 90-Day Oral Toxicity Study in Rodents. OECD Guideline for the Testing of Chemicals. Guideline 408. Adopted 21 September 1998. Organization for Economic Cooperation and Development (OECD); Paris.

OECD. 2002a. Task Force for the Safety of Novel Foods and Feeds. 6th Session of the Task Force on the Safety of Novel Foods and Feeds, Chateau de la Muette, 17-19 June 2002. Organization for Economic Cooperation and Development (OECD); Paris, ENV/JM/FOOD(2001)8/REV1.

OECD. 2002b. Environmental Health and Safety Publications. Series on Testing and Assessment No. 35 and Series on Pesticides No. 14. *Guidance Notes for Analysis and Evaluation of Chronic toxicity and Carcinogenicity Studies*. Organization for Economic Cooperation and Development (OECD); Paris, ENV/JM/MONO(2002)19, 04-Sep-2002, pp. 37-38.

Siegel, J.P. 2001. The mammalian safety of *Bacillus thuringiensis*-based insecticides. *J. Invertebr. Pathol.* 77, 13-21.

Sjoblad, R.D., McClintock, J.T., and Engler, R. 1992. Toxicological considerations for protein components of biological pesticide products. *Regul. Toxicol. Pharmacol.* 15, 3-9.

Task Force of Past Presidents. 1982. Animal data in hazard evaluation: paths and pitfalls. *Fundam. Appl. Toxicol.* 2, 101-107.

U.S. EPA. 1998. Reregistration Eligibility Decision (RED) *Bacillus thuringiensis*. U.S. Environmental Protection Agency (U.S. EPA), Prevention, Pesticides and Toxic Substances (7508W); Washington, DC, EPA738-R-98-004. Available from: <http://www.epa.gov/oppsrrd1/REDs/0247.pdf>.

U.S. EPA. 1993. R.E.D. Facts: Glyphosate. EPA-738-F-93-011. Environmental Protection Agency, Washington, D.C.

Weil, C.S. and Gad, S.C. 1980. Applications of methods of statistical analysis to efficient repeated- dose toxicologic tests: 2. Methods for analysis of body, liver, and kidney weight data. *Toxicol. Appl. Pharmacol.* 52, 214-226.

WHO. 1987. Principles for the Safety Assessment of Food Additives and Contaminants in Food. World Health Organization (WHO), International Programme on Chemical Safety (IPCS); Geneva, Switz. Environmental Health Criteria, No. 70. Available from: <http://www.inchem.org/documents/ehc/ehc/ehc70.htm>.

WHO. 1999. Microbial Pest Control Agent: *Bacillus thuringiensis*. World Health Organization (WHO), International Programme on Chemical Safety (IPCS); Geneva, Switz. Environmental Health Criteria, No. 217. Available from: <http://www.inchem.org/documents/ehc/ehc/ehc217.htm>.

WHO/FAO. 2004. Pesticides residues in food. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticides Residues (JMPR). Rome, Italy, September 20-29, 2004. FAO Plant Production and Protection Paper 178. World Health Organization and Food and Agriculture Organization of the United States. http://www.fao.org/ag/agp/agpp/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf

Wilson, N.H., Hardisty, J.F., and Hayes, J.R. 2001. Short-term, subchronic, and chronic toxicology studies. In: Hayes, A.W. (ed.). Principles and Methods of Toxicology, 4th ed. Taylor and Frances; Philadelphia, Penn., pp. 917-957.

Williams, G.M., R. Kroes, and I.C. Munro. 2000. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Reg. Toxicol. Pharmacol. 31(2):117-165.