COMMITTEE PAPER FOR DISCUSSION **ACNFP/80/8**

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

THE EFFECT OF GM SOYA ON NEWBORN RATS

Issue

Dr Irina Ermakova has responded to the statement drawn up by the Committee in December 2005, concerning the effects of GM soya on newborn rats. The Committee is asked whether it wishes to revise its statement in the light of this further information.

Background

1. November 2005 the Committee considered a preliminary report from Dr Irina Ermakova, describing feeding studies carried out in Russia in rats given GM and non-GM soya flour (ACNFP/74/8). These studies indicated that the offspring of rats given GM soya had reduced body weights and increased mortality compared with rats given non-GM soy and with a control group.

2. The Committee concluded that there were a number of possible explanations for the differences observed between rats fed the two types of soya and was interested to see further information that might help in its evaluation of the results. The Committee's statement was published on 5 December 2005 (Annex 1) and the Secretariat wrote to Dr Ermakova with a request for further information, such as the quantities of conventional feed pellets and soya flour consumed by the animals, the nutrient composition of the soya flour, and the results of investigations into the causes of death.

New information

3. Dr Ermakova replied in September 2006 (Annex 2) and provided references to papers published as proceedings of conferences where these experiments have been presented. Two of these (in English) are attached (Annexes 3 and 4). These papers include results from a third experiment that data the results reported in 2005. Annex 4 describes a further treatment group, not mentioned in the earlier papers or in Annex 3, in which the animals were given "Protein-isolate GM soya".

4. The paper at Annex 3 provides some details of the source of the GM and non-GM soya flour used in the experiments, and indicates that the flour was mixed with water and presented to the animals as a paste. However, there is no information on the quantities of soya paste and standard laboratory feed consumed by the animals. The tables provide additional information on the rate of growth of the offspring and the times at which deaths occurred. Table 5 provides information on weights of preserved organs in 2 offspring from each group, sacrificed at 3 weeks of age.

5. Dr Ermakova's reply also refers to a paper "in press" reporting on pathological changes in testes and liver of male rats given the GM soya.

6. She has suggested three possible mechanisms for toxicity of GM soya, namely foreign DNA introduced into rat cells via plasmids derived from the GM soya; mutagenic effects of the transformation process that was used to derive the GM soya, or residues of the herbicide Roundup in the GM soya sample.

Committee action required:

7. The Committee is invited to consider the attached reply from Dr Ermakova. Members are asked if they wish to update their December 2005 statement, and whether they wish to await publication of the further paper mentioned in the reply.

Secretariat
January 2007

ACNFP STATEMENT ON THE EFFECT OF GM SOYA ON NEWBORN RATS

The Committee has examined a report provided to it by Dr Irina Ermakova containing preliminary results from a study of genetically modified (herbicide-tolerant) soya that was conducted in Russia. The report described reduced growth and increased mortality amongst pups born to rats given soya flour from GM soya beans, when compared with those born to rats given non-GM soya flour or a control group given no soya.

The report lacks detail essential to meaningful assessment of the results. In particular, it does not provide key information concerning the composition and nutritional adequacy of the test diets. Also, the Committee notes that these are preliminary results; the study has not been quality-controlled through the normal peer review process preceding scientific publication.

It is well known that rodents fed large quantities of raw soya will suffer various nutrient imbalances that cause reduced growth rates and other adverse effects. This would be expected whether the soya beans are from a GM or non-GM source. It is also well known that protein quality varies between varieties and geographical origins of soya, independently of whether they have been genetically modified. It is therefore essential to ensure that diets which contain a high proportion of different types of soya are carefully balanced and equivalent in terms of nutrients and anti-nutritional components. It is not known whether this was done in the present study.

Unusually, the soya flour was given to the animals alongside conventional feed pellets rather than incorporated into the feed. The mothers received up to 20g of soya flour per day during the study, which could have displaced a significant quantity of the conventional feed pellets which normally assure optimum vitamin and mineral intake. The quantities of soya consumed by each animal are not known and there are no data on the consumption of the conventional feed. Neither were any data on cause of death provided. The GM and non-GM soya samples were obtained from different sources and there is no information on the presence of potential contaminants, such as mycotoxins, resulting from contamination during transportation and storage. In conclusion, there are a number of possible explanations for the results obtained in this preliminary study, apart from the GM and non-GM origin of the test materials. Without information on a range of important factors conclusions cannot be drawn from this work. The Committee Secretariat is contacting Dr Ermakova to obtain further information on this study and the Committee will consider any further information that can be obtained and review the position if a full report of the study is published in the peer-reviewed literature.

The Committee also notes that Dr Ermakova's findings are not consistent with those described in a peer-reviewed paper published in 2004.[1] In a well controlled study no adverse effects were found in mice fed on diets containing 21% GM herbicide-resistant soya beans and followed through up to 4 generations.

5 December 2005

[1] "A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development" Brake DG and Evenson DP; Food and Chemical Toxicology 42 (2004) 29-36.

ANNEX 2 to ACNFP/80/8

RESPONSE RECEIVED FROM Dr ERMAKOVA 18 September 2006

Genetically modified organisms could be real threat to the life.
(Reply to ACNFP on the "Statement on the effect of GM soy on newborn rats").

Irina Ermakova

On November 2005 I got a letter from the Food Standards Agency in London, the government department that has responsibility for food safety issues in the UK with the request to send them information about my experiments. I sent them the text, indicating that there was a short version of the paper with some results, which were described already, and that I was preparing a big paper with more data. At that moment I was so shocked by the results of my own experiments that appealed to scientists of different countries to repeat my experiments or to help us to continue the researches. I indicated this request also in my answer to Food Standards Agency. After that the "Statement on the effect of GM soy on newborn rats" of Advisory Committee of Novel Foods and Processes (ACNFP) has appeared. The Statement of ACNFP on my results surprised me very much. Committee did not pay real attention to possible danger of genetically modified organisms (GMO) obtained in my experiments, but concentrated on details of their realization.

The hazard of genetically modified or transgenic organisms was described for humans, animals and the Environment in many scientific investigations (Ho and Tappeser, 1997; Traavik, 1999; Chirkov, 2001; Wilson et al., 2004; Kuznetcov and Kulikov, 2006 and many others). Four main sources of the hazards of GMO are accepted by scientists worldwide: 1) those due to the new genes, and gene products introduced; 2) unintended effects inherent to the technology; 3) interactions between foreign genes and host genes; and 4) those arising from the spread of the introduced genes by ordinary cross-pollination as well as by horizontal gene transfer (World Scientists' Statement, 2000). Experimental researches showed negative effects of GMO on insects (Birch et al., 1996; Losey, 1999; Zangerl et al., 2001). It was found that consumption of GM-food by animals led to the negative changes in their organs (Pusztai, 1998, 2001; Ewen and Pusztai, 1999; Malatesta et al., 2002, 2003; Vecchio et al., 2003; Prescott et al., 2005 and others). However there is great lack of investigations concerning the influence of GMO on physiological state and behavior of rats and their offspring. It was the reason why I started my own experiments directed on this kind of investigations.

Our experiments showed a danger of Ready Roundup soy-bean (line 40.3.2), modified by the transgene CP4 EPSPS, for rats and their offspring. Supplementation of the diet of the females with GM soy led to the higher mortality of rat pups (more than one half) in comparison with the pups from control groups. High pup mortality was observed for every litter from mothers fed by the GM soy flour. Third of pups were sick and weighed several times less, than pups from the control groups. The obtained data showed a high level of anxiety and aggression in rats from the GM-soy group: females and rat pups attacked and bit each other and the worker who took care about them. Pathological changes were found in

testes and in liver of males fed by GM-soy seeds (Ermakova, Barskov, 2006, in press). In our experiments we did not succeed to get the second generation (F2).

Our data allow us to suppose that the negative effect of the GM-soy on newborn pups could be a result of transformation of foreign genes, which could penetrate into the sexual/stem cells or/and into cells of the fetus, as it was observed by Schubbert and colleagues (1998). In their experiments the plasmids containing the green fluorescent protein (pEGFP-C1) gene, or the bacteriofaphage M13 DNA was fed to pregnant mice. Using the polymerase chain reaction (PCR) or the fluorescent in situ hybridization (FISH) method, foreign DNA, orally ingested by pregnant mice, was discovered in various organs of fetuses and of newborn animals. GM-soy is one of the GM-plants, created by the help of bacterial DNA plasmids *(Agrobacter tumefaciensis method)*. So, we can assume that plazmids able for replication are kept in the cells of GM-plants (in our case in the GM-soy). The affect on sexual cells and reproductive organs of rats by plasmids with foreign DNA from GM soy could be occurred. So, we can have "**plazmid effect**", that is more dangerous than virus infection, because plasmids can affect bacteria, plants, animals and human.

Also a negative effect of GM-soy on rats could be mediated by the highly mutagenic nature of the GM transformation process, described by Windels et al. (2001) and Wilson et al., (2004) or/and by accumulation of Roundup residues in the GM-soy shown by Richard et al., 2005.

We repeated similar experiments three times in four groups: "GM-soya" group, "Trad-soya" group, "Protein-isolate GM-soya" group and "Control" group. Committee analyzed preliminary study of the first two experiments in three groups, comparing my draft paper with the published paper of D.G. Brake, D.P. Evenson "A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development" (B& E).

I believe that our researches are so various, that cannot be compared:

1. *Different scheme of feeding.* In my experiments I started to feed animals before mating, suggesting that foreign genes penetrate and effect the sexual cells and/or organs. In the experiments of B&E "pregnant mice were fed a transgenic soybean or a non-transgenic (conventional) diet through gestation and lactation... Multi-generational studies were conducted in the same manner". Thus genes could influence only on embryonic cells protected by the mother's organism, not on sexual cells or organs before mating.
2. *Different subjects of investigations.* In my experiments I analyzed the mortality, physiological state and behaviour of pups, B&E - fetal, postnatal, pubertal and adult testicular development.
3. *B&E used very small number of pups* for the study at each point "At each point three male mice were killed, the testes surgically removed, and the cell populations measured by flow cytometry" and for mating "Two C3H/HeJ males and two C3H/HeJ females were bred to keep that strain pure". In my experiments I used more females and males for mating and 10-20 times more pups in each group.
4. *Different species of animals*: in my experiments – rats, in B&E – mice.
5. I presented to Food Standards Agency the *draft version* but not the final one as paper of B&E.

So, it was clear that the investigations of B&E and mine were quite different and both researches were incomplete. So, it is necessary to perform complex researches, including histological, genetical, and embryo-toxicological investigations by different scientific groups (including international ones).

Scientists should be responsible for the obtained data, but are even more responsible for concealment of the received data, especially if somebody's life depends on them. A lot of

independent investigations showed hazard of GMO for alive organisms. I hope very much that ACNFP will help us to perform detailed and complex investigations and to stop uncontrolled distribution of and contamination by imperfect genetically modified organisms that can cause such human diseases as cancer, allergy, brain and heart diseases, can lead to disappearance of a great number of different species of useful bacteria, plants and animals and cause destruction of the nature and the biosphere.The results of my researches were published in English and in Russian:

1. Ermakova I.V. Genetically modified organisms and biological risks. Proceedings of International Disaster Reduction Conference, Davos, Switzerland, August 27 – September 1, 2006, pp.168-171.
2. Ermakova I. Influence of genetically modified soya on the birth-weight and survival of rat pups// Proceedings “Epigenetics, Transgenic Plants and Risk Assessment”, 2006, pp.41-48.
3. Ermakova I.V. Genetically modified soy leads to the decrease of weight and high mortality of rat pups of the first generation. Preliminary studies" EcosInform 1, 2006, pp.4-9 (in Russian).
4. Ermakova I.V. The effect of GM-soya on rats and their posterity. The first International Forum on Patient safety. January 23-24, 2006. p.30.
5. Ermakova I.V. Diet with the food, modified by gene EPSPS CP4, leads to the anxiety and aggression in rats. 14th European Congress of Psychiatry. Nice, France, March 4-8, 2006.
6. Ermakova I.V. Mine field of genetics//State management of resources. 2006, N2, pp.44-52 (in Russian).
7. Ermakova I.V. Genetics and ecology. In: Actual problems of science. Moscow, 2005, pp.53-59 (in Russian).

ANNEX 3 to ACNFP/80/8

Proceedings of the Conference
"Epigenetics, Transgenic Plants and Risk Assessment"

1 December 2005
in Frankfurt am Main, Germany

Pages 41-47

ANNEX 4 to ACNFP/80/8

Proceedings of
International Disaster Reduction Conference

27 August 27 – 1 September 2006
Davos, Switzerland

Pages 168-172
“Genetically Modified Organisms and Biological Risks”
I Ermakova

(text taken from http://irina-ermakova.by.ru/eng/art/art16.html)

Note:
Slides from the author’s presentation are published at:
http://222.davos2006.ch/pres.html