Irina Ermakova: Influence of genetically modified soya on the birth-weight and survival of rat pups

Introduction

Four main sources of the hazards of GMO are discussed 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). GM crops contain material, which is not present in them under natural conditions, and they form a part of our daily diet. To understand what effect they can have on us and on our animals it is vitally important to study the influence of these GM plants in different organisms for several generations. At the present, these studies are lacking from the scientific literature. Several detrimental effects of GM crops had been showed on the metabolism of animals. The hazard of genetically modified organisms (GMO) was shown for animals and the environment in many investigations (Traavik 1995; Ho and Tappeser 1997; Pusztai 1999 and 2001; Kuznetcov et al. 2004 and others). Experiments, conducted by Pusztai showed that potatoes modified by the insertion of the gene of the snowdrop lectin (an insecticidal proteins), stunted the growth of rats, significantly affected some of their vital organs, including the kidneys, thymus, gastrocnemius muscle and others (1998) and damaged their intestines and their immune system (Ewen and Pusztai 1999). Similar effect of GM potatoes on rats was obtained at Institute of Nutrition in Russia (Ermakova 2005). In the researches of Malatesta with co-authors the significant modifications in the cells of liver, exocrine pancreas and testis of mice, fed by diet containing Roundup Ready GM soybean were described (Malatesta et al. 2002, 2003; Vecchio et al. 2004).

It is put forward in the risk assessment documents that the GM components of transformed plants are completely destroyed in the digestive tract of humans and animals, together with the other genetic material found in them. However foreign DNA plasmids are steadier against the digestion, than it was originally believed. Plasmid DNA and GM DNA were found in microorganisms of the intestine and in saliva (Mercer et al. 1998; Coghlan 2002). Experimental researches in mice showed that ingested foreign DNA can persist in fragmented form in the gastrointestinal tract, penetrate the intestinal wall, and reach the nuclei of leukocytes, spleen and liver cells (Schubbert et al. 1994). In another research of Schubbert et al. (1998) the plasmid containing the gene for the green fluorescent protein (pEGFP-C1) or bacteriophage M13 DNA were fed to pregnant mice. Foreign DNA, orally ingested by pregnant mice, was discovered in blood (leukocytes), spleen, liver, heart, brain, testes and other organs of foetuses and newborn animals. The authors considered that maternally ingested foreign DNA could be potential mutagens for the developing fetus. At the same time Brake and

Evenson (2004) analyzing the testis in mice as a sensitive biomonitor of potential toxic, didn't find negative effects of transgenic soybean diet on fetal, postnatal, pubertal or adult testicular development.

There is a lack of investigations on the influence of GM crops on mammals, especially on their reproductive function. Therefore, we decided to undertake a study to see the effect of the most commonly used GM crop on the birth rate, mortality and weight gain of rat pups, if the females were fed diets supplemented with Roundup Ready soya.

Materials and Methods

Diets and dietary components:

Roundup-Ready (RR) soya (40.3.2 line) was used in experiments. Since we had no access to the exact parent line, we bought a traditional (trad.) soya variety (Arcon SJ 91-330, ADM, the Netherlands), which had a similar composition and nutritional value to the RR soya.

The soya flour was prepared from these varieties by grinding the raw whole soya bean seeds mix with water (40 ml) to form a paste. Standard laboratory food was obtained from Moscow, Russia.

Animals:

Wistar rats from (Stolbovay, Russia) were used in the experiment. The animals were brought up to sexual maturity on laboratory rat feed. When their weight reached about 180 - 200 g, the female rats were divided into 3 groups, and housed in groups (3 rat/cage), and kept under normal laboratory conditions.

The feeding scheme was as follows. Females in every cage daily received dry pellets from a special container placed on the top of their cage. Those rats receiving soya flour supplement, were given the soya flour in a small container placed inside their cage (20g x 40 ml water) for three rats and, so 5 - 7g flour for each rat every day.

Experimental protocol:

One group of female rats of 180 - 200 g weight was allocated to the experimental group, and received flour/rat/day prepared from Roundup-Ready soya, added to the rat feed for two weeks. Another group females were allocated to the control group, but their diet was supplemented with the same amount of soya flour, prepared from the trad. soya. We also introduced a positive control group, which had not been exposed to soya flour. Therefore females have only got the standard laboratory feed without any supplementation, although it is acknowledged that the energy and protein content of this diet was less than in the other two groups. After two weeks on the diets all groups of 3 females were mated with two healthy males of the same age, who have never been exposed to soya flour supplements. First one than the other male was put into the cage for 3 days. In order to avoid infection of females, the sperm count and quality has not been determined. We carried on with feeding the respective diets to all females during mating and pregnancy. Upon delivery, all females were transferred to individual cages, and the amount of soya supplement was increased by an additional g for every pup born. Lab feed and water was available for all animals *ad libitum* during the experimental period. When rat pups opened their eyes and could feed themselves (from 13-14 days of age), the daily dose of soya supplement was increased till 2 - 3g for every pup, although all rats had free approach to the soya. All rats ate their soya portions well. After finishing of experiments organs of some pups were taken out and weighed.

Statistical analysis:

The level of mortality was analyzed by the one-way ANOVA, using of Newman-Keuls test for share distribution. The pup's weight and its distribution were checked by Mann-Whitney test and Chi-square in StatSoft Statistica v6.0 Multilingua (Russia).

Results

Quantitative analysis of RR soya by using the "CP4-LEC-RT-PCR" construct confirmed the presence of genetic modification in 100% of the flour. In the traditional, non-GM soya flour only traces (0.08+ 0.04%) of the same construct was present, most likely resulting from cross-contamination.

By the end of the experiment, from the 15 females included in the experiment, 11 gave birth and produced a total of 122 rat pups. The 4 rats who became pregnant from 6 females on the positive control diet gave birth to 44 pups (an average of 11 pups/female), while the four females, from the six on GM soya flour supplemented groups gave birth to 45 (11.3 pups/female), and 3 from traditional soya group - 33 pups (11 pups/female).

Supplementation of the diet of the females with GM soya led to the death of 25 pups, out of the 45 born by the end of the third week of lactation, while during the same period on the traditional soya supplemented diets only 3 pups died from 33. The mortality in the positive control group was also 3, but from the larger number of pups born, as it seen in Table 1.

High pup mortality was generally characteristic for females fed the GM soya flour (Table 2).

Among the pups from the females fed the positive control diet, 2 pups died during the first week, and 1 during the second week after delivery. All pups from females fed traditional soya flour died during the first week after birth. However, pups from females fed the GM soya flour supplemented diet kept dying during lactation period as it is evident from Table 3.

In two weeks after their birth the weight of pups (with SE) from the GM soya supplemented group was less (23.95g $\pm$ 1.5 g) than that of the pups of the positive control group (30.03g $\pm$ 1.1 g; p<0.005), or from the traditional soya flour supplemented group (27.1 g $\pm$ 0.9 g; p< 0.1). Since the number of surviving pups were so different, the weigh distribution of the pups were compared in Table 4. From the data it is evident, that 36% of the pups from the GM soya group weighed less than 20 g, in comparison with the 6% in the positive control group, and with the 6.7% found in the traditional soya supplemented diet group (Table 4). Study of pup's organs mass showed that the organs of small pups from GM group were tiny in comparison with the same of other groups except the brain mass (Table 5). This fact indicated that the pups from the GM group were the same age as others, but changes were occurred with the development of internal organs. Slight negative effect was found in the group, which received the traditional soya, but this effect was not significant. No lethality of females and survived young pups eating the GM soya flour supplemented diet was observed.

Discussion

The reproductive behaviour of female rats fed on standard laboratory feed supplemented with soya flour prepared from either genetically modified (RR) or traditional soybean seeds was studied to see the effect of the diet on pregnancy, lactation and the growth of the rat pups. Since it is well established, that raw soybean contains a number of anti-nutrients (such as the lectins, trypsin inhibitors, etc. (Pusztai et al. 1998), and also female hormone-like substances, it was thought to be necessary to compare these data also with those from a positive control group when animals were not exposed to any soya flour supplementation.

In order to understand the mechanism how this widely consumed GM crop exerts its influence on the reproductive performance of mammals and their offspring, it would be necessary to perform complex researches, including histological, genetic and embryo-toxicological investigations. However, we had to restrict our experiments only for a short time-span, and starting to feed the female rats two weeks before mating. However, unlike the experiments of Brake and Evenson (2004), who started to feed pregnant mice, in our experiments the diets supplemented with GM or traditional soya flours were already given to the female rats 2 weeks before mating already, and we continued to treat them with their respective diet until the pups were weaned.

Upon delivery, very unexpectedly a very high rate of pup mortality (~ 55,6%) was observed in among pups from females, whose diet was supplemented with the Roundup Ready soya flour in comparison with the pups of both the positive control (6,8 %) and the traditional soya flour supplemented (9%) groups. Also, in this group the pups continued to die over the period of lactation, which occurred only in the GM soya fed group. At the same time, the weights of the surviving rat pups were also lower. It is the more surprising, since the pups were smaller, about half, therefore more milk should have been available for the individual pups. They should have a better chance to grow optimally, unless the amount, and/or the quality of the milk were not affected by consuming the GM soya flour.

Our data allow us to speculate and presume that the negative effect of GM soya on the newborn pups could be mediated by several possible factors. Firstly, it can be the result of transformation, and insertion of the foreign genes, which could enter into the sexual/stem cells, or/and into cells of the fetus, as it was observed by Schubbert et al. (1998). In their experiments the plasmid containing the green fluorescent protein (pEGFP-C1) gene, or the bacteriophage M13 DNA was fed to pregnant mice. The presence of the foreign DNA was detected in the cells of the mice in both cases. Also, the instability of gene constructs was described for GM-soya (Windels et al. 2001) and rice (Yang et al. 2005). Secondly, negative effect could be result of the highly mutagenic nature of the GM transformation process as illustrated by Wilson et al. (2005). Finally, the negative effect of GM soya could be mediated by the accumulation of Roundup residues in GM soya. However, no mortality was observed with female rats, nor with the young pups survived, although they also began to eat the GM soya, it was supposed that the effect could be mediated by the two first factors.

References

Brake DG and Evenson DP (2004): A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. Food Chemistry and Toxicology 42: 29-36.

Coghlan A (2002): GM crop DNA found in human gut bugs. NewScientist. 2002.

Ermakova IV (2005): Conclusion to the report about feeding of rats by genetically modified potatoes Russet Burbank Agrarian Russia 2005: 62-64.

Ewen SW, Pusztai A (1999): Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. Lancet 354 (9187).

Ho MW and Tappeser B (1997): Potential contributions of horizontal gene transfer to the transboundary movement of living modified organisms resulting from modern biotechnology. In Transboundary Movement of Living Modified Organisms Resulting from Modern Biotechnology: Issues and Opportunities for Policy-Makers (K.J. Mulongoy, ed.) International Academy of the Environment, Switzerland: 171-193.

Kuznetcov VV, Kulikov AM, Mitrohin IA and Cidendambaev VD (2004): Genetically modified organisms and biological safety. Ecos 2004: 3-64.

Malatesta M, Biggiogera M, Manuali E, Rocchi MBL, Baldelli B, Gazzanelli G (2003): Fine structural analyses of pancreatic acinar cell nuclei from mice fed on GM soybean. Eur. J. Histochem. 47: 385-388.

Malatesta M, Caporalony C, Gavaudan S, Rocchi MBL, Tiberi C, Gazzanelli G (2002): Ultrastructural, morphometrical and immunocytochemical analysis of hepatocyte nuclei from mice fed on genetically modified soybean. Cell Struct. Funct. 27: 173-180.

Mercer DK, Scott KP, Bruce-Johnson WA, Glover LA and Flint HJ (1999): Fate of free DNA and transformation of oral bacterium Streptococcus gordonii DL1 plasmid DNA in human saliva. Applied and Environmental Microbiology 65: 6-10.

Pusztai A (1998): Report of Project Coordinator on data produced at the Rowett Research Institute. SOAEFD flexible Fund Project RO 818. 22 October 1998.

Pusztai A (2001): Genetically Modified Foods: Are They a Risk to Human/Animal Health. Biotechnology: genetically modified organisms. 2001.

Schubbert R, Lettmann C and Doerfler W (1994): Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the blood stream of mice. Molecules. Genes and Genetics 242. 495-504.

Schubbert R, Hohlweg U, Renz D and Doerfler W (1998): On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission in the fetus. Molecules. Genes and Genetics 259: 569-576.

Traavik T (1999): Too Early May Be Too Late. Ecological Risks Associated with the Use of Naked DNA as a Biological Tool for Research, Production and Therapy (Norwegian). Report for the Directorate for Nature Research Tungasletta 2, 7005 Trondheim. English translation, 1999.

Yang L, Ding J, Zhang C, Jia J, Weng H, Liu W, Zhang D (2005): Estimating the copy number of transgenes in transformed rice by real-time quantitative PCR. Plant Cell Rep. 23, 10-11: 759-763.

Vecchio L, Cisterna B, Malatesta M, Martin TE, Biggiogera B (2003): Ultrastructural analysis of testes from mice fed on genetically modified soybean. Eur. J. Histochem. 48: 449-453.

Wilson A, Latham J, Steinbrecher R (2004): Genome Scrambling – Myth or Reality? Transformation - Induced Mutations in Transgenic Crop Plants. EcoNexus, 2004, 35p.

Windels P, Taverniers I, Depicker A, Van Bockstaele E, De Loose M (2001): Characterisation of the Roundup Ready soybean insert. Eur. Food Res. Technol. 231: 107–112.

World Scientists Statement. Supplementary Information of the Hazards of Genetic Engineering Biotechnology. Third World Network. 2000.

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Table 1: Mortality of rat pups by the end of the 3rd week of lactation; *compared to the GM soya flour supplemented group

Groups	Number of pups born	Number of dead pups	Dead pups/total born (%)
Positive control	44	3 (p=0,000118)*	6.8 %
Trad. Soya	33	3 (p=0,000103)*	9 %
GM soya	45	25	55.6 %

Table 2: Number rat pups died from the litter of individual females on the GM soya flour supplemented diet

Females	Number of newborn rats	Number of pups died	Number of dead pups/born (%)
Female No. 1	11	7	64 %
Female No. 2	8	4	50 %
Female No. 3	13	6	46 %
Female No. 4	13	8	62 %

Table 3.The number of dead pups (number and as %) from the treatment groups at different times after birth

Groups	1st week	2nd week	3rd week
Positive control	4.5 % (2)	2.3 % (1)	0
Trad. Soya	9 % (3)	0	0
GM soya	31,1 % (14)	13,4 % (6)	11,1% (5)

Table 4. Weigh distribution of rat pups by 2 weeks of age on different diets; * – in comparison with GM-group

Group:	*50-40 g*	*40-30 g*	*30-20 g*	*20-10 g*
Positive control	12.5 %	37.5 %	44 %	6 % * ($p<0.01$)
Trad. soya	0 %	20 %	73.3 %	6.7 % * ($p<0.05$)
GM soya	0 %	23 %	41 %	36 %

Table 5: Examples of absolute values of organ mass in pups in three weeks after their birth. Fixation in formaldehyde 0.1M PBS, pH7.2.

NN	Body	Liver	Lungs	Heart	Kidneys	Spleen	Testes	Brain
N26; control	69	3.80	1.20	0.37	0.44/0.44	0.52	0.34/0.34	**1.67**
N27; control	72	4.63	1.55	0.38	0.52/0.42	0.81	0.3/0.3	**1.6**
N28; GM soya	35	1.83	0.6	0.19	0.28/0.28	0.21	0.13/0.14	**1.60**
N29; GM soya	30	1.68	0.5	0.20	0.19/0.20	0.19	0.14/0.18	**1.54**
N30; trad. soya	62	4.28	0.95	0.36	0.38/0.38	0.24	0.22/0.26	**1.76**
N31; trad. soya	63	4.35	0.94	0.39	0.42/0.42	0.32	0.22/0.23	**1.66**