

## 附录 1： 研究报告样本

### **Influence of Genetically Modified Soya on the Birth-Weight and Survival of Rat Pups**

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#### **ABSTRACT**

Investigation of the influence of GM soya on the birthrate and survival of the offspring of Wistar rats were performed. A group of female rats were fed GM soya flour before mating and pregnancy. The control group of females were fed traditional soya and the third group of females ,the positive control group, received feed without any soya. The weight and the mortality rate of the newborn pups were analyzed. The study showed that there was a very high rate of pup mortality(55.6%) in the GM soya group in comparison with the control group and the positive control group (9% and 6.8% respectively). Moreover, death in the first group continued during lactation, and the weights of the survivors are lower those from the other two groups. It was revealed in these experiments, that GM soya could have a negative influence on the offsprings of Wistar rats

#### **INTRODUCTION**

It is well accepted by scientists worldwide that four main sources of the hazards of genetically modified organisms (GMO): 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).

To understand what effect they can have on us and on our animals and whether their risks may outweigh the benefits it is vitally important to study the influence of these GM plants in different organisms for several generations. The hazard of GMO was shown for animals in extensive investigations (Traavik 1995; Ho and Tappeser 1997; Pusztai 1999 and 2001; Kuznetcov et al. 2004 and others). Earlier it was shown that consumption of GM food by animals led to the negative changes in their organisms. 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 the Institute of Nutrition in Russia (Ermakova 2005). In another research of Shubbert et al. (1998), 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. They considered that maternally ingested foreign DNA could be potential mutagens for the developing fetus. However, Brake and Evenson (2004) analyzing the testis in mice as a sensitive biomonitor of potential toxic, didn't find adverse effects of transgenic soybean diet on fetal development. From the literature review, there seems a lack of investigations on the influence of GM crops on mammals, especially on their reproductive function. Therefore, the objective of the study we undertake is to see the effect of the most commonly used GM crop on the birth rate, mortality and weight gain of rat pups, whose mother were fed diets supplemented with Roundup-Ready soya, a kind of GM food.

#### **METHODS**

##### *Animals:*

Wistar rats were used as the subjects 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, 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.

*Experiment:*

One group of female rats of 180 - 200 g weight was allocated to the experimental group, and received 5-7 soy a flour/rat/day prepared from Roundup-Ready soya, added to the rat feed for two weeks. Another group females(3) were allocated to the control group, but their diet was supplemented with the same amount of soya flour, prepared from the traditional soya in which only traces (0.08+ 0.04%) of the GM construct was present, most likely resulting from cross-contamination. We also introduced a positive control group (in two cages:3x3), which had not been exposed to soya flour. Therefore females 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, which had never been exposed to soya flour supplements. In order to avoid infection of females, the sperm count and quality had not been determined. We carried on 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 during the experimental period. When the 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 the experiment was finished the organs of some pups were taken out and weighed. The level of mortality was analyzed by the one-way ANOVA, using the 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**

By the end of the experiment, from the 15 females included in the experiment, 11 gave birth and produced a total of 132 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/mother).

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 seen in Table 1. High pup mortality was generally characteristic for females fed the GM soya flour(Table2). 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.

**Table 1Mortality 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=0,0001 18)*	6.8 %
Trad. Soya	33	3 (p=0,0001 03)*	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 3The 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)

In two weeks after their birth the weight of pups from the GM soya supplemented group was less (23.95g ±1.5 g) than that of the pups of the positive control group (30.03g±1.1 g; p<0.005), or from the traditional soya flour supplemented group (27.1 g± 0.9 g; p< 0.1). Since the number of surviving pups was so different, the weigh distribution of the pups was 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 6% in the positive control group, and with 6.7% found in the traditional soya supplemented diet group (Table 4). The 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 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 mortality of females and survived young pups eating the GM soya flour supplemented diet was observed.

**Table 4Weigh 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.**

NN	Body	Liver	Lungs	Heart	Kidneys	Spleen	Testes	Brain
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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

## DISCUSSION

The reproductive behaviour of female rats fed on standard laboratory feed supplemented with soya flour prepared from either genetically modified soya or traditional soya was studied to see the effect of the diet on pregnancy, lactation and the growth of the rat pups. Upon delivery, very unexpectedly a very high rate of pup mortality (55.6%) was observed in the group of females whose diet was supplemented with the GM 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 explained by two possible factors. Firstly, it can be the result of transformation, and insertion of the foreign genes, which could penetrate into the sexual/stem cells, or/and into cells of the fetus, as it was observed by Schubbert et al. (1998). Secondly, the negative effect could be caused 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 is supposed that the effect could be caused by the first factor. (2005)

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