

Supplementary Materials 1 E-mail correspondence between *Nature Biotechnology* and Irina Ermakova

Supplementary Materials 2 PDF of galley proof for Irina Ermakova

Supplementary Materials 1 E-mail correspondence between *Nature Biotechnology* and Irina Ermakova

From: Marshall, Andrew
Sent: Monday 25.06.2007 17:57
To: 'I_Ermakova@mail.ru'
Subject: Nature Biotechnology

Dr Irina V. Ermakova,

Vice President Russian National Genetic Safety Association

Institute of Higher Nervous Activity and Neurophysiology RAS,

Butlerov str.,

5a, Moscow,

Russia,

e-mail: I_Ermakova@mail.ru

Tel: +7 095 334-43-13

Dear Dr Ermakova

Nature Biotechnology (<http://www.nature.com/nbt/index.html>) is a journal of biotechnology research with a worldwide readership of over 109,000 that publishes papers on various products produced via recombinant DNA technology.

I am writing to you because the journal has been approached by a group of authors wishing to critique the results of your work that have been discussed in public forums. Given the attention given to your experiments on the viability of the offspring of female rats fed flour milled from soybeans genetically engineered with the 5-enolpyruvyl shikimate-3-phosphate synthase gene (originally presented on October 10, 2005 to the symposium on genetic modification in Russia, organized by the National Association for Genetic Security), the journal would, however, prefer to provide you with an opportunity to present your own findings and conclusions in your own words, rather than a critique from one side.

I was therefore wondering whether you be willing to answer (via e-mail or telephone) a set of questions about your work, with a view to their questions and answers being published as part of an article?

Let me know if you wish to participate

Best regards

Andrew Marshall, PhD

Editor

Nature Biotechnology

Tel: 212 726 9308

Fax: 212 696 9635

a.marshall@natureny.com

-----Original Message-----

From: Irina Ermakova [mailto:i_ermakova@mail.ru]
Sent: Tuesday, June 26, 2007 12:13 AM
To: Marshall, Andrew
Subject: Re: Nature Biotechnology

Dear Andrew,

Thank you very much for your letter. Can I present a whole paper about my experiments or it is better for you (and for me) to use my answers being published as part of an article?

Sincerely yours, Irina Ermakova

From: Marshall, Andrew
Sent: Thursday 28.06.2007 17:43
To: 'Irina Ermakova'
Subject: RE: Nature Biotechnology

Dear Irina

As your results have been widely reported but not yet published in the peer-reviewed literature, I envisage an article that would present the results and conclusions you previously discussed at the NAGS symposium on genetic modification in Russia, together with community feedback.

I think this would work best if you could provide the journal with a brief summary of the findings and conclusions of the work in your own words together with your responses to a set of questions about the work.

Would it be possible for me to send you the set of questions, and we take it from there?

Best regards

Andrew Marshall
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a.marshall@natureny.com

-----Original Message-----

From: Irina Ermakova [mailto:i_ermakova@mail.ru]
Sent: Thursday, June 28, 2007 11:23 PM
To: Marshall, Andrew
Subject: Re[2]: Nature Biotechnology

Dear Andrew,

My suggestion: I'll present you my paper, which you review and publicate in your journal. After that you open discussion of my paper and I'll answer questions.

Irina

From: Marshall, Andrew
Sent: Friday 29.06.2007 14:54
To: 'Irina Ermakova'
Subject: Nature Biotechnology

Hi Irina

I am afraid that because the results of your work have already been published publicly and discussed widely in the media, they are no longer eligible for peer review at Nature Biotechnology under our policies (see "pre-publicity" here: http://www.nature.com/authors/editorial_policies/confidentiality.html).

However, I am still keen to present a discussion of your paper that has your views/answers to the questions.

Let me know if you are willing to proceed and I can send over the questions.

All best

Andrew Marshall
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-----Original Message-----

From: Irina Ermakova [mailto:i_ermakova@mail.ru]
Sent: Friday, June 29, 2007 4:10 PM
To: Marshall, Andrew
Subject: Re: Nature Biotechnology

Dear Andrew,

I have different data. I have repeated experiments 5 times. So, I can prepare original paper.

Irina

-----Original Message-----

From: Marshall, Andrew
Sent: Friday 29.06.2007 16:27
To: 'Irina Ermakova'
Subject: RE: Nature Biotechnology

Irina

1. For the new data, perhaps you could send a short 'presubmission inquiry' through the online submission system <http://mts-nbt.nature.com/cgi-bin/main.plex?>

I would need a brief paragraph stating the interest to a broad scientific readership, a title, a fully referenced summary paragraph that describes what was done, what was found and what your conclusions are, as well as a list of the peer-reviewed references cited in the summary paragraph.

2. With regard to your previously published data, I am still wondering whether you would be willing to present your views/answers to the questions.

Let me know

Andrew Marshall
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-----Original Message-----

From: Irina Ermakova [mailto:i_ermakova@mail.ru]

Sent: Tuesday, July 10, 2007 9:20 AM

To: Marshall, Andrew

Subject: Re[2]: Nature Biotechnology

Dear Andrew,

I am going to send a short presubmission inquiry online.

According to your offer

> With regard to your previously published data, I am still wondering

> whether you would be willing to present your views/answers to the

> questions

I am waiting for the questions.

There is list of some my publications on GMO influence on animals:

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 (in English).

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 (In English)
(<http://www.oeko.de/oekodoc/277/2006-002-en.pdf>).

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. 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" } 18 September 2006 (in English) (www.eco-irina-ermakova.narod.ru; www.gmwatch.org; food.gov.uk/multimedia/pdfs/acnfp8008gmsoya.pdf)

5. Ermakova I.V. The effect of GM-soy on rats and their posterity. The first International Forum on Patient safety. January 23-24, 2006. p.30 (in English).

6. 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 (in English).

7. Ermakova I.V. Genetics and ecology. In: Actual problems of science. Moscow, 2005, pp.53-57 (in Russian).

8. Ermakova I.V. Conclusion to the Report about the feeding of rats by genetically modified potatoes Russet Burbank, resistant to the Colorado Beetle//Agrarian Russia, 4, 2005, pp.62-64 (in Russian);

www.gmwatch.org (in English).

Sincerely yours, Irina Ermakova

From: Marshall, Andrew
Sent: Thursday 19.07.2007 16:18
To: 'Irina Ermakova'
Subject: Nature Biotechnology

Dear Dr Ermakova

Many apologies for the delay in getting back to you.

I present below the list of questions concerning your work on studying the the influence of Ready Roundup soy beans (containing the transgene CP4 5-enol-pyruvylshikimate-3-phosphate synthase) on the health, behavior and mortality of rats and their offspring. I would be very grateful if you provide as detailed and substantive responses as you feel necessary.

Best regards

Andrew Marshall
Editor
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a.marshall@natureny.com

WHAT WAS DONE

1. Please briefly describe your experimental design
2. What was the origin and type of the control and test materials (transgenic and nontransgenic soybean) used in your feeding study? Please provide as much detail as possible.
3. How was the control and test material administered to the rats and how much of each was given to the animals each day?
4. How many animals were studied and how many experiments were pooled into your final results?
5. Please describe the conditions of animal housing and care of the animals in the study and what methods of observation were used?
6. What methods were used to assess animal health (whole body/organ weight, behavior)?

WHAT WAS FOUND

7. Please briefly describe your main findings (without conclusions) from the study
8. What was the level of mortality of the pups you found in the control and test group?
9. What information on the weights of the control and test group animals was found?
10. What observations were made of animal behavior?

WHAT WAS CONCLUDED

11. Please provide a description of what you conclude from your findings
12. What are the human health implications of your findings?
13. What mechanism do you think might underlie the health effects you observe in your study?
14. What, if any, caveats would you make about your conclusions?

PUBLICATION OF RESULTS

15. What are your plans for future lines of investigation in your research?
16. Your original work was in Russian. Do you feel the translation or interpretation of your work in reports and other English language forum has been accurate
16. Some have commented on the fact that you forewent the normal process of publication via a peer-reviewed journal. What do you think is the value of peer-reviewed publication compared with publication by press conference, press release or on web sites? What reasons are there for a researcher to forego the process of peer review, which is the conventional and established method of publishing scientific findings?
17. Do you intend to publish in peer-reviewed journals in the future or are you planning to submit your work for peer-reviewed publication?

-----Original Message-----

From: Irina Ermakova [mailto:i_ermakova@mail.ru]

Sent: Tuesday, July 31, 2007 5:23 PM

To: Marshall, Andrew

Subject: One question

Dear Mr Marshall,

Sorry, but I don't understand question N14 from your list:

N14. What, if any, caveats would you make about your conclusions?

I would be very thankful if you explain it or use another form of question.

Excuse me for troubles, Dr.Ermakova

From: Marshall, Andrew

Sent: Tuesday 31.07.2007 17:33

To: 'Irina Ermakova'

Subject: RE: One question

The question relates to any reservations you may have concerning the conclusions reached in your work (for example, are the results generalizable to other animals and other types of GM plant as others citing your work have claimed? Did you use a sufficient number of animals used in your experiments to reach statistically robust conclusions? Would you use an additional controls in future experiments etc?)

Andrew Marshall

Editor

Nature Biotechnology

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-----Original Message-----

From: Irina Ermakova [mailto:i_ermakova@mail.ru]

Sent: Thursday, August 02, 2007 1:35 PM

To: Marshall, Andrew

Subject: My answers

Dear Mr. Marshall,

Thank you very much for your interesting questions. Please, find attached my answers. I can also send figures and photos, if it is necessary.

I have checked the text carefully. However I would be so much thankful for correction of my text if you find mistakes.

Sincerely yours, Irina Ermakova

From: Marshall, Andrew
Sent: Thursday, 02.08.2007 13:49
To: 'Irina Ermakova'
Subject: RE: My answers

Dear Dr Ermakova

Many thanks for your responses. I will be responding back to you in the next few days with any questions/outstanding issues I have and will provide you with a version edited for grammar so that you can verify any changes made.

Sincerely

Andrew Marshall
Editor
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a.marshall@natureny.com

From: Marshall, Andrew
Sent: Wednesday 08.08.2007 17:53
To: 'Irina Ermakova'
Subject: Nature Biotechnology

Dear Dr Ermakova

Please take a look over this edit of your text. You should check the text carefully for places where inaccuracies may have been introduced during editing and address any queries directed to you.

I would be very grateful if you could send back your text to me by Friday August 10.

Let me know if you need any more information

Andrew Marshall
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a.marshall@natureny.com

-----Original Message-----

From: Irina Ermakova [mailto:i_ermakova@mail.ru]
Sent: Friday, August 10, 2007 10:24 AM
To: Marshall, Andrew
Subject: Text

Dear Andrew,

I am sending you the text. Thank you very much for your corrections!
I added some new data by red colour. Is it OK?

Your question about soy additives: soy additives can include GM-soy or non-GM soy (trad soy).

With Thanks, Irina Ermakova

-----Original Message-----

From: Irina Ermakova [mailto:i_ermakova@mail.ru]
Sent: Monday, August 13, 2007 8:43 AM
To: Marshall, Andrew
Subject: Re: Nature Biotechnology

Dear Mr Marshall,

I have sent on Friday (10 of August 2007) the text and pictures. I hope that you have got them. When is it planned to publish?

Best Regards, Irina Ermakova

From: Marshall, Andrew
Sent: Monday 13.08.2007 20:20
To: 'Irina Ermakova'
Subject: RE: Nature Biotechnology

Dear Dr Ermakova

The piece is planned to be published in the September issue of Nature Biotechnology. I have two remaining requests before galley proofs are sent to you:

- 1) In your answer to question 6, do you have any information on how the study was conducted, what equipment was used, animals age, gender, or number of animals? Were measurements made of variables that can affect behavior such sound level, temperature, humidity, lighting, odors, time of day, and environmental distractions?
- 2) Do you have a picture of yourself we could use to accompany the article. It would be better if the picture were at least 300 dpi and supplied as a jpeg or tiff file. Let me know if you have a suitable picture.

Many thanks for your patience

Andrew Marshall
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From: Irina Ermakova [mailto:i_ermakova@mail.ru]
Sent: Tuesday 14.08.2007 18:13
To: Marshall, Andrew
Subject: Re[2]: Nature Biotechnology

Dear Mr Marshall,

Please, find attached additional information to N6, N10 and two my photos.

With great thanks, Irina Ermakova

From: Marshall, Andrew
Sent: Tuesday 14.08.2007 20:33
To: 'Irina Ermakova'
Subject: RE: Re[2]: Nature Biotechnology

Dear Dr Ermakova

Many thanks for the additional information that I'll incorporate into the article. Hopefully the proofs will reach you later this week or early next week.

Andrew Marshall
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----- Forwarded Message

From: <McNamara>, Ingrid <i.mcnamara@natureny.com>
Date: Mon, 20 Aug 2007 17:51:51 -0400
To: <i_ermakova@mail.ru>
Cc: Brandy Moyer <b.moyer@natureny.com>, "Silver, Amy"
<A.Silver@natureny.com>
Conversation: Urgent publication proof
Subject: Urgent publication proof

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Fax: 646-563-7060
e-mail: i.mcnamara@natureny.com, b.moyer@natureny.com

Urgent: Publication Proof

FROM: Ingrid McNamara, Senior Production Editor, Brandy Moyer, Production Editor

Attached is a PDF galley proof of your manuscript. Please review it carefully as this is your only opportunity for corrections. Check figures carefully as they may have been altered. Print out the PDF and mark your corrections directly on the proof by underlining the text to be corrected. Number each correction and summarize your directions in the form of an itemized list. Please email the list to either Ingrid McNamara or Brandy Moyer (see e-mail addresses above) and fax your corrected proof to 646-563-7060).

It is imperative that you email a Word document with a list of all corrections and comments, as handwritten corrections sometimes cannot be read or are easily missed. Please verify receipt of proofs via e-mail. Do not send annotated PDFs.

PLEASE RETURN PROOFS WITHIN 24 HOURS

Questions regarding the layout (i.e. Figures, etc.) should be addressed to Ingrid McNamara, i.mcnamara@natureny.com, 212-726-9202; or Brandy Moyer; 212-726-9274.

Questions regarding editorial content should be addressed to Teresa Moogan, t.moogan@natureny.com.

GM soybeans and health safety—a controversy reexamined

Irina V Ermakova

An unprecedented study claiming that transgenic soybeans compromise the fertility of rats and the survival and growth of their offspring has garnered widespread media and political attention but remains unpublished in the peer-reviewed literature. Here, an account of the work from the principal investigator, Irina Ermakova, is appended with comments from researchers in the field.

Neurologist Irina Ermakova of the Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences in Moscow made news headlines two years ago when she reported that rats fed diets containing glyphosate-tolerant genetically modified (GM) soybeans gave birth to pups with low survival rates or stunted growth¹. Though these findings have yet to appear in a peer-reviewed journal and contradict publications in the literature, they have been widely disseminated and discussed over the media and internet and already cited by >500 organizations as evidence of the potential toxicity of GM products. They've also prompted the American Academy of Environmental Medicine (Wichita, KS, USA) to call for additional independent studies of food safety for GM crops², been referred to in a state Australian parliamentary debate as a reason to ban GM crop cultivation³ and motivated regulatory agencies in several countries to review their approvals of GM organisms or to comment on the work^{4,5}.

Nature Biotechnology approached Ermakova to ask for a detailed account of her work in her own words. Her answers are presented below together with comments solicited from a group of researchers working in the field.

Briefly describe your experimental design and methods.

Irina Ermakova. My experiments were designed to study the influence of a diet containing genetically modified (GM) soy beans

Irina V. Ermakova is at the Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences, Butlerova Street 5A, Moscow 117485, Russia.



Irina Ermakova, the author of controversial studies reporting soybeans genetically modified for resistance to glyphosate may be dangerous to newborns, agreed to provide details of her work to *Nature Biotechnology*.

conventional soy flour or seeds and a third group received protein isolated from RR GM soy. A fourth group of rats received only the laboratory chow and was considered to be a positive control. We analyzed the physiological state (weight, size and so forth), reproductive functions, rate of mortality and behavior of rats and their offspring. Experiments were repeated five times using soy flour, soy seeds, standard chow and chow mixed with GM soy (~14%) in different groups of rats.

Standard chow contained wheat, wheat bran, sunflower, meat flour, animal fat, barley, fodder yeast, microelements and vitamins. RR soy flour genetically modified with the transgene 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) obtained from *Agrobacterium* sp. strain CP4 (Monsanto; St. Louis, MO, USA), its protein isolate and conventional soy flour

(Roundup Ready (RR) line 40.3.2) on the physiological state and behavior of Wistar rats and their offspring. In addition to laboratory chow, one group of female rats were fed soy flour or seeds for 2 weeks before mating, during mating and pregnancy, and were fed an increased daily amount for every pup during lactation. At the same intervals, a second group of female rats receiving

(Arcon SJ 91-330), which has a similar composition and nutritional value to RR GM soy, were obtained from the Netherlands supplier of Archer Daniels Midland (ADM; Decatur, IL, USA). Analysis of soy flour by PCR showed the presence of the EPSPS transgene in all samples of RR GM soy.

The chow was administered as dry pellets from a special container placed on the top of their cages and the (GM, GM protein isolate or conventional) soy flour mixed with water (20 g soy paste in 40 ml water) in a small container placed inside their cage for three rats. Each rat thus received 6–7 g flour every day. A similar scheme was used for soy seeds, which were kept in water for 1 day before feeding and then put into a small container inside the cage: four seeds for one female and six seeds for one male.

How many animals were studied and how many experiments were pooled into your final results?

I.E. We repeated the experiments five times with different groups of animals and with the four RR GM soy supplementations (that is, GM flour, GM seeds, protein-isolate GM soy or chow with GM soy). Rats in control groups received conventional soy (as flour or seeds). In the first three repeats of the experiments, 30 females, 40 males and 221 pups were investigated. In total, for the five repeats of the experiments, we examined 48 females, 52 males and 396 rat pups. Similar results were obtained in all the different repeat experiments.

How were the animals housed and observed during the study?

I.E. Rats, weighing from 180 g to 200 g, were kept in a vivarium with a reversed light-dark

cycle (12 a.m. to 12 p.m.). Each day, females and males in every cage received dry pellets from a special container placed on the top of their cage. Animals were also provided with 200 ml of drinking water per rat per day. After 2 weeks on the different diets, three females from each group were mated with two healthy males of the same age, who had not been exposed to the soy flour supplements. First one male was placed with a female in the cage for 3 days, and then another for 3 days. To minimize infection risk to females, invasive tests to determine sperm count and quality were not determined. Upon delivery, all females were transferred to individual cages, and the amount of soy supplement was increased by an additional 1 g for every pup born. Laboratory chow and water were available *ad libitum* during the experimental period, for all animals. When rat pups could feed themselves, the daily dose of soy supplement was increased to 2–3g for each pup. All rats ate their soy portions well.

What methods were used to assess animal health and behavior?

I.E. Adult animals were weighed before feeding and 2 weeks following commencement of the feeding experiments. Weights and sizes of pups from the different experimental groups born at the same time (± 1 –2 days) were recorded 2 weeks after birth. We also determined the weight of some internal organs (e.g., brain, liver, spleen, heart, lungs, kidneys and testes) and analyzed the morphology of the liver and testes. We examined the explorative behavior in the open field, determined the level of anxiety using a light/dark test and observed rat behavior in home cages.

Experiments were performed with male and female rats 2 weeks after commencement of feeding and when pups were 2 months old. All experiments were conducted in the second half of the day when rats were more active (starting at 5 p.m.). Each group contained 9–10 animals. The open field was represented by [AU:OK?] a round platform, 100 cm, in diameter divided into zones restricted by sector rays and concentric circles. The platform was surrounded by a wall, 30 cm high. The center of the open field was illuminated by a frosted bulb (40 W). The session was conducted in a sound- and light-proof room. A rat was placed in the center of the open field and the number of horizontal translocations, vertical positioning, grooming, number of boluses (defecation) and freezing were recorded over 6 min. For each parameter, the relative value of extinction was estimated as the following ratio: difference in activity between the second and the first 3-min intervals divided by integral activity. The level of anxiety was investigated using a light-dark test

(Intertex, Multiscreen [AU: Location] for 5 min. This model included two boxes: dark and light (four 3.5-W lamps). The number of rat entries into the light box, time spent in the light box, duration of rat rearing on hind legs in the light box, the latency before a rat first entered the light box, the number of times a rat looked out from the dark box, the vertical activity of rats in the light box, urinations, defecations and grooming were all recorded.

We analyzed the level of mortality in each of the test groups using one-way ANOVA verified using Newman-Keuls share distribution test. Pup weight was analyzed by Mann-Whitney and its distribution by Chi-square using StatSoft (Moscow) Statistical version 6.0.

Briefly describe the main findings from your study.

I.E. Our data demonstrate a high level of mortality in pups born to mothers receiving RR GM soy-supplemented diets during the 3 weeks following birth compared with pups from control groups over the same period. Many (more than one-third) of the surviving pups born to mothers receiving GM soy had a stunted size and low weight compared with pups born to mothers from controls. A similar number of pups were born to mothers receiving GM soy, traditional soy and control groups (10–11 pups per female) but fewer pups were born to rats receiving soy protein isolate (8 pups per female). Behavioral studies indicated a high level of anxiety and aggression in males, females and young pups fed on the different groups fed GM material. [AU: Word/words missing? Or, should “fed GM material” be “of GM material”?] Morphological analysis of internal organs indicated marked pathological changes in the blood supply to testes and vacuolization in the livers of male rats fed GM soy seeds. We also failed to breed second-generation (F_2) pups from matings of first-generation (F_1) females and males fed material based on GM soy.

What was the level of mortality of the pups you found in the control and test groups?

I.E. In first three repeats of experiments, up to five times higher mortality was observed in newly born pups whose mothers had received the GM soy flour supplementation compared with pups from rats receiving GM soy protein isolate, traditional soy or laboratory chow (controls) (see Table 1). Pups from rats that had been fed a GM soy diet died during the 3 weeks following birth; pups from rats fed laboratory chow (positive control) died during the 2 weeks postpartum; and pups from those fed traditional soy died during the first week after birth.

What was the weight of the control and test group animals?

I.E. We did not find any significant differences in the weights of adult rats fed the different diets. Even so, for 2 weeks following birth, the weights of pups from mothers fed GM soy supplement were lower than those of pups from rats in the positive control (laboratory chow) group or from the conventional soy flour-supplemented group. We also found that 33% of pups from rats fed GM soy had smaller sizes and lower weights than pups from rats fed laboratory chow, traditional soybeans or soybean protein isolate (Table 3). A crude anatomical analysis revealed that the organs of pups from rats fed GM soy were much smaller (except the brain mass) than those from pups born to rats fed other diets (Table 4). Thus, age-matched pups in the test and control groups show differences in the development of internal organs.

How was animal behavior and fertility affected?

I.E. Behavioral experiments showed very slight differences between groups in open field behavior. Even so, both anxiety in the ‘light-dark’ test and aggression were higher in females, males and offspring receiving GM soy in their home cages than in rats from other groups. Aggression was more frequent in females and pups; not only toward one another, but also toward the laboratory personnel caring for them. Some (~20%) of the females, fed by GM soy, failed to care for their pups (instead scattering them around the cage without nesting). For rats fed GM soy, we failed to breed second-generation pups from F_1 males ($n = 24$) and females ($n = 24$). In marked contrast, the crossing of F_1 females ($n = 12$) receiving the GM soy diet with F_1 males ($n = 12$) from the positive control group (laboratory chow) resulted in 72 pups (Table 5). Even here, however, the number of pups per female was fewer than in the other groups (8 pups per female instead of 10–11 pups per female) and 25% of females didn’t deliver pups at all. These results indicate that GM soy had a deleterious effect on the reproductive function especially of F_1 males, but also female rats.

What do you conclude from your findings and what are your plans for future research?

I.E. As it is well established that raw soybean contains several antinutrients (e.g., lectins and trypsin inhibitors)¹ and female hormone-like substances (e.g., phytoestrogens), our experiments both used a positive control (laboratory chow alone) and fed rats experimental and control diets 2 weeks before mating, during mating, through pregnancy and until the litters were weaned. The very high rate of pup mortality

in litters of mothers on a diet supplemented with RR GM soy flour was very unexpected. The lower weight of surviving pups from rats receiving GM soy was also notable, particularly because the higher mortality resulted in (~50%) smaller litters, which should have doubled the amount of milk available. These pups should have had a better chance to grow than pups from other groups with larger litters, unless the amount and/or the quality of the milk is deleteriously affected by consumption of GM soy flour.

We concluded that RR GM soy appears to have a strong negative influence on Wistar rats and their offspring, causing high levels of pup mortality, infertility in surviving pups, decreased weight gain in some pups, pathological changes in internal organs and deleterious effects on behavior. My opinion is that GM soy's effect on Wistar rats and their offspring should be relevant to all mammals, including humans.

It would have been instructive to compare the effect on rats and their offspring of RR GM soy with another GM soy line or with a completely different kind of GM plant. I hope to perform these experiments in future. We plan to compare the influence of different GMOs [genetically modified organisms] (not only RR soybeans) on the physiological state and behavior of rats and their offspring. We are also planning to analyze the reason of pup's death and attempt to detect the presence of foreign DNA in white blood cells, brain, liver and other internal organs of adult animals and pups.

Do you feel that the translation/interpretation of your work has been accurate?

I.E. My experiments were published first in Russian and then in English. There were several incorrect (some even funny) interpretations of my work. One of the most serious critiques of

my work was published in the "Statement on the effect of GM soy on newborn rats" from the UK's Advisory Committee of Novel Foods and Processes (ACNFP; London)². The Committee compared my research with only one (!) published article by Brake and Evenson³. But my study is not comparable with the work by Brake and Evenson for several reasons. First, the focus of the two investigations was completely different. Our experiments analyzed the effect of GM soy on mortality, physiological state and behavior of pups; in contrast, the studies of Brake and Evenson investigated the effect of GM soy on fetal, postnatal, pubertal and adult testicular development. Second, we used several different schemes of feeding; we commenced feeding 2 weeks before mating, which suggests that foreign genes ingested by these animals can penetrate and affect the sexual cells and/or organs. In the experiments of Brake and Evenson "pregnant mice were fed a transgenic soybean or a nontransgenic (conventional) diet through gestation and lactation....Multi-generational studies were conducted in the same manner." Thus, in their study, foreign genes could influence only embryonic cells in the womb and not sexual cells or organs before mating. And third, Brake and Evenson used only a very small number of pups in their study: "At each point, three male mice were killed, the testes surgically removed and the cell populations measured by flow cytometry." And they also mated a smaller number of animals: "Two C3H/HeJ males and two C3H/HeJ females were bred to keep that strain pure." In our experiments, more females and males were mated and 10–20 times more pups were obtained in each group. Thus, it is clear that my investigation and that of Brake and Evenson's are quite different and should not be compared.

Why have you so far forgone publishing your work in a peer-reviewed journal?

I.E. I first presented the data at the 11th Russian Gastroenterological Week (in a section on Nutrition and GMOs organized by the Moscow-based National Association for Genetic Safety) at the Russian Academy of State Service in Moscow, October 10–12, 2005. I was perplexed by my data and I appealed to scientists at this conference to repeat my experiments. This drew the attention of a journalist, Dmitry Starostin, and a note was published by the Russian federal news agency Regnum⁴. In December 2005, I spoke at a conference "Epigenetics, Transgenic Plants and Risk Assessment" in Frankfurt am Main, Germany. The paper detailing my preliminary results was published in the Proceedings of this conference¹. Several papers have subsequently been published in different journals and proceedings. I have submitted a paper to a Russian peer-reviewed journal and am currently preparing other papers for consideration by peer-reviewed scientific journals in English.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/naturebiotechnology/>.

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2. The Advisory Committee on Novel Foods and Processes. ACNFP statement on the effect of GM soya on newborn rats. *ACNFP* <<http://www.acnfp.gov.uk/acnfpapers/gmissues/acnfpgmsoya>> (2005).
3. Brake, D.G. & Evenson D.P. A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. *Food Chem. Toxicol.* **42**, 29–36 (2004).
4. Genetically modified soy affects posterity: results of Russian scientists' studies. *Regnum [online]* published online 10 December 2005 <<http://www.regnum.ru/english/526651.html>>.

Table 1 Mortality of rat pups by the end of the 3rd week of lactation

Groups	Number of newborn pups	Number of dead pups	Dead pups/total born (%)
Control	74	6 <i>P</i> < 0.001 ^a	8.1%
GM soy	64	33	51.6%
GM soy protein isolate	33	5 <i>P</i> < 0.01 ^a	15%
Traditional soy	50	5 <i>P</i> < 0.001 ^a	10%

^aCompared with the GM-soy flour-supplemented group.

Table 2 Comparison of different kinds of chow on rat pup mortality^b

Groups	Number of pups born per female	Number of pups born	Number of dead pups	Dead pups/total born (%)
Usual chow	~ 11	74	6	8.1%
Chow containing 14% GM soy content	~ 10	72	24	33.3%
Usual chow plus GM soy	~ 11	64	33	51.6%
Chow containing 14% GM soy content plus GM soy	~ 10	89	46	51.7%

^bBy end of the 3rd week of lactation.

Table 3 Distribution of weights of pups in 2 weeks after birth

Groups	50–40 g	40–30 g	30–20 g	20–10 g
Control	8.2%	38.8%	40.8%	12.2% (<i>P</i> < 0.05) ^a
Traditional soy	0%	9.7%	77.4%	12.9% (<i>P</i> < 0.05) ^a
GM soy protein isolate	0%	21%	72%	7.0% (<i>P</i> < 0.05) ^a
GM soy	0%	26%	40.7%	33.3%

^aIn comparison with GM soy.

Table 4 Examples of absolute values of organ mass^a in pups 3 weeks after birth

Experiment	Body	Liver	Lungs	Heart	Individual kidney	Spleen	Testes	Brain
Control	69	3.80	1.20	0.37	0.44 and 0.44	0.52	0.34/0.34	1.67
Control	72	4.63	1.55	0.38	0.52 and 0.42	0.81	0.3/0.3	1.6
GM soy	35	1.83	0.6	0.19	0.28 and 0.28	0.21	0.13/0.14	1.60
GM soy	30	1.68	0.5	0.20	0.2 and 0.19	0.19	0.14/0.18	1.54
Conventional soy	62	4.28	0.95	0.36	0.38 and 0.38	0.24	0.22/0.26	1.76
Conventional soy	63	4.35	0.94	0.39	0.42 and 0.42	0.32	0.23/0.22	1.66
GM soy protein isolate	63	3.71	1.04	0.47	0.44 and 0.44	0.36	0.2/0.19	1.62
GM soy protein isolate	63	3.46	1.42	0.41	0.43 and 0.33	0.38	0.23/0.24	1.74

^aOrgans fixed in formaldehyde, 0.1 M PBS, pH 7.2.

Table 5 Success of mating of first-generation (F₁) offspring receiving GM soy

Females (number)	Males (number)	GM soy feeding scheme	Mating scheme	Number of rat pups F ₂
12 F ₁	12 F ₁	Continuation of GM soy additives for females and males	3 females × 3 males (in turn) <i>n</i> = 36	0
12 F ₁	12 F ₁	Feeding by GM soy was stopped before mating for females and males	3 females × 3 males (in turn) <i>n</i> = 36	0
12 F ₁	12 controls (from mothers that didn't receive any soy additives)	Stopping of GM-soy additives before mating for females	3 females × 3 males (in turn) <i>n</i> = 36	72