GM soybeans and health safety—a controversy reexamined

Andrew Marshall

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rats and the survival and growth of their offspring has garnered widespread media and political attention but remains unpublished in the peer-reviewed literature.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.

Neuroscientist 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 growth[1]. 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 crops[2], been referred to in a state Australian parliamentary debate as a reason to ban GM crop cultivation[3] 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.

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*.

Briefly describe your experimental design and methods.

Irina Ermakova. My experiments were designed to study the influence of a diet containing genetically modified (GM) soybeans (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 was fed soy flour or seeds for 2 weeks before mating, during mating and pregnancy, and was fed an increased daily amount for every pup during lactation. At the same intervals, a second group of female rats receiving chow was fed 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 (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.

Bruce M. Chassy, L. Val Giddings, Alan McHughen and Vivian Moses. Ermakova states that RR soybeans and protein isolate were purchased from ADM in the Netherlands. ADM does not sell (and has never sold) pure 100% RR soybean preparations. It is accordingly not possible for Ermakova to have obtained RR soybeans from this source as stated. The best that can be said is that commercial products sold by ADM would have been an indeterminate and variable mixture of conventional and non-GM soybeans. These most likely would also have comprised a mixture of commercial soybean cultivars rather than a single cultivar. ADM does supply identity-preserved non-GM soybeans; however, most of these too would be mixtures of non-GM cultivars. It is standard practice in feeding studies of this kind to compare the responses of test animals fed the GM variety with those

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Bruce Chassy of the University of Illinois at Urbana-Champaign says Ermakova's work illustrates the need for the public and media to be cautious of scientific claims that have not been reproduced or passed the rigor of peer review.

fed a conventional variety with similar genetic background (near-isogenic control).

Ermakova also indicates that Arcon SJ was fed to one control group of animals. She describes the material as a "GM-free" soy flour with a composition equivalent to RR-soybeans. The ADM product catalog states that Arcon SJ is a soy protein concentrate that contains 70% protein (as opposed to 40–45% protein found in soybeans). Arcon SJ is not nutritionally equivalent to soy flour. Ermakova provided no PCR evidence that the Arcon SJ product did not contain the CP4 EPSPS gene or the CP4 EPSPS protein it encodes. These assays are necessary to demonstrate that this control is in fact a non-GM-containing material.

In feeding studies of the kind reported by Ermakova, it is essential to determine the nutritional composition of test and control diets and to show that each provides balanced and equivalent nutrition. In addition, the content of soybean antinutrients (e.g., trypsin inhibitors) should be determined to establish equivalency because these can affect the outcome of studies. It is of particular importance to measure the isoflavone content as differences in this pseudo-estrogenic component could affect mating, reproduction and growth, as well as other parameters[6]. The inaccurate description of materials used in this study and the lack of data regarding diet composition fail to meet minimum standards for animal studies.

The rats were fed chow (apparently *ad libitum*; see below) and soy preparations were supplied separately as an aqueous paste at a rate of 20 gm/3 rats. No data are supplied on individual consumption by each rat. In this kind of study, animals are normally caged individually, the test material is typically incorporated into the chow and the chow consumption of each animal is noted every day. Using the multiple animals/cage design described by Ermakova, animals could have consumed soy or soy-derived ingredients in an amount ranging from 0% to 100% of their daily intake. It is therefore impossible to determine either the food intake or soy exposure for any of the animals in the study (dams, sires and pups). Ermakova states that males were not exposed to soy; however, they were placed into cages with females to which soy was provided every day. After 3 days, the males were moved to the cage of another female where they remained for three additional days. No precautions were taken to exclude the possibility that males could consume some of the soy test material intended for females and thus the males would have been exposed to soy, GM and/or non-GM. Consumption of soy by males would have also reduced the ration of soy available to the females.

Several internationally accepted standard protocols for animal testing could have been followed by Ermakova in the design of the feeding and data collection procedures of this study[7–10]. These protocols have been developed to ensure the conduct of valid studies that will be acceptable to the scientific community, including regulatory agencies. Studies that do not record the exact dietary composition and intake amount for each animal, including exposure to test substance, lack scientific validity.

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.

B.M.C., L.V.G., A.M. and V.M. Results from independent, but identically designed animal studies can be used to evaluate the reproducibility of an effect, but it is not standard practice to pool data from such studies due to potential differences in factors such as diet, housing conditions and variability between batches of animals. Ermakova states that in five trials a total of 100 animals have been studied, which translates to an average of 20 animals per study and approximately 5 for each experimental group. Although some types of feeding studies can be performed with as few as 10 animals/group, standard protocols for reproductive toxicology studies typically commence with 20–25 animals[7–10]. It can be expected that the results from five trials performed with fewer animals will exhibit greater variability than a single large-scale trial that employs the same number of animals.

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.

Former Biotechnology Industry Organization (BIO; Washington, DC, USA) staffer and industry consultant L. Val Giddings believes Ermakova ignored the standard scientific practice of submitting research for peer review before publicizing her results.

B.M.C., L.V.G., A.M. and V.M. Ermakova notes that the ration of soy supplement "was increased to 2–3 g" per day when rat pups could feed themselves and adds that "all rats ate their soy portions well." Setting aside the fact that the statement may indicate that the normal ration was inadequate to meet the animals' needs, quantitative intake is again not reported. What's more, it is not clear whether pups were weaned and removed from the dams. It is also not stated whether the litters were balanced with regard to number of pups and gender. It is normal practice to compare results from litters adjusted to equal size (usually eight pups, four females and four males) to avoid differences in nursing.

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.

Behavioral 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 usually contained 9–10 animals. The open field test was represented by 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) 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 and number of instances 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, urinations, defecations and grooming were all recorded.

The University of California's Alan McHughen thinks that there are critical problems with Ermakova's experimental design and research techniques that throw doubt on the validity of her conclusions.

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.

B.M.C., L.V.G., A.M. and V.M. Parental animals should be weighed on the first day of dosing and each week after. Parental females should be weighed at a minimum on gestation days 0, 7, 14 and 21 and during lactation on the same days as the weighing of the pups. Pups should be weighed individually at birth, or soon thereafter, and on days 4, 7, 14 and 21 of lactation. Ermakova reports the weight of pups at 2 weeks of age. Normally weights are compared at weaning (3 weeks). This makes comparison with literature values difficult.

There are several difficulties with the behavioral studies as described. It is not clear that these experiments were performed in a double-blinded manner and, given the apparent differences in size and vitality between the groups, it is hard to imagine that handlers could not distinguish between the groups and would thus lose their objectivity. In addition, no information is provided about external variables that can affect behavior, such as sound level, temperature, humidity, lighting, odors, time of day and environmental distractions. Explicit, operationally defined scales for each measure of the battery is to be employed in the study should have been provided[11,12]. No actual data from behavioral studies are presented. We are therefore being asked to accept the subjective anecdotal claim that soy diets affected behavior. Taking into account the deficient experimental design, and signs of poor animal husbandry and unbalanced nutrition—as judged by high control-group mortality and poor growth performance—it should come as no surprise that deficient animal stewardship would lead to behavioral changes.

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 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.

According to the University of London's Vivian Moses, in the context of published peer-reviewed studies—as well as more than 10 years of real-world use of RR soybeans and the products derived from them—the claims of Ermakova seem implausible at best.

B.M.C., L.V.G., A.M. and V.M. No objective behavioral or morphological data are presented. Claims should not be made without presentation of evidence. Previous reports in the literature have shown no effects of RR soy on birth weights or pup mortality[13,16,17]; they have also not shown any effects of RR soy on the testis or in the livers of male rats fed RR soy[13,16,17]. What is more unusual, no methodology is given nor data reported for Ermakova's claimed measurement of testicular blood flow, an endpoint that is not routine in rodent toxicology studies. Ermakova's claim that mating was not possible in second-generation (F_2) males as a result of GM soy exposure contradicts a previous study[13] that found no reproductive effects in mice in a multigenerational feeding study with RR 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 **Tables 1** and **2**). Pups from rats that had been fed a GM soy diet died mostly 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.

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 $P < 0.001$[a]	8.1%
GM soy	64	33	51.6%
GM soy protein isolate	33	5 $P < 0.01$[a]	15%
Traditional soy	50	5 $P < 0.001$[a]	10%

[a]Compared with the GM-soy flour–supplemented group.

B.M.C., L.V.G., A.M. and V.M. Pup mortality is usually reported at day = 0 or day = 1 and day = 21. The timing and causes of death are not reported. The data in **Tables 1** and **2** show that 8.1% of pups died in the control group. The typical mean pup survival observed for Wistar rats is greater than 99% ± 2 at day = 1 and 99.5% ± 1 at day = 21 (ref. 14). The abnormally high incidence of pup mortality in the controls indicates poor animal stewardship possibly arising from poor animal husbandry and/or dietary deficiency. No valid scientific conclusions can be based on a study with such a poor performance in the control group. **Table 1** also reports 10% mortality on conventional soy; no conclusions should be drawn from a study in which the conventional soy control mortality is tenfold higher than that normally observed for Wistar rats. The details of the post-mortem examination of pups are not reported and no cause of death is offered for the observed high incidence of pup mortality.

The claim of 51.6% pup mortality in GM soy-fed groups defies credibility. It is not possible that such a strong lethal effect could have evaded researchers, regulatory agencies, health and agricultural agencies and animal producers for more than a decade. The more likely explanation for the observed health effects is poor experimental design and conduct as demonstrated by the exceptionally high mortality observed in the controls.

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 in two weeks after beginning of feeding. 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 and weighed less (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.

B.M.C., L.V.G., A.M. and V.M. Animal weights are normally recorded for individual animals in a litter and then averaged as mean for females and mean for males to account for gender differences. **Table 3** does not segregate animals by gender, despite the likelihood of males being ~2–3% larger than females at this age. More importantly, under carefully controlled conditions, 14-day pup weight (~38g ± 3g) will vary by no more than ±10% (ref. 14). The data in **Table 3** are presented in an unconventional manner that makes it difficult to determine the exact mean and standard deviation among groups. **Table 3** states that 53% of control pups are below 30 g, which is abnormally small for two-week-old Wistar rat pups. More than 90% of rat pups fed conventional soy are >20% below normal weight; GM soy (79% below typical weight) and GM soy protein isolate–fed pups (78% below typical weight) fared somewhat better. The wide variance of data in **Table 3** and the high percentage of low-weight animals are clear indicators of malnutrition and/or poor environmental conditions. No conclusion can be made about abnormal development unless the controls conform to internationally observed norms.

Table 4 reports "examples" of body and organ weights (with no units specified). Means are normally reported for all the animals in a control or experimental group and the values are normalized to both body weight and to brain weight. As presented, **Table 4** is meaningless.

Table 2 Comparison of different kinds of chow on rat pup mortality[a]

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%

[a]By end of the 3rd week of lactation.

How were animal behavior and fertility affected?

I.E. Behavioral experiments showed very slight differences between groups in open field test. 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.

B.M.C., L.V.G., A.M. and V.M. The results reported in **Table 5** are unique and without precedent in whole food feeding studies with rats. Although no objective behavioral data are presented, a total failure of adult animals to produce offspring would be remarkable. It is not clear whether the animals failed to achieve estrus, whether they mated but were infertile or whether pregnancy was aborted. The more significant problem with the data as presented is that there are no data for conventional soybeans. There is no way to determine if soybeans *per se* produced this effect or whether

the effect is restricted to GM soybeans. We would be skeptical of the latter claim.

We find the claim (**Table 5**) that 12/12 dams fed non-GM soy produced a total of 72 pups whereas none of the GM-soy fed groups produced a single pup, even less plausible when we consider Ermakova's previous reports. The Wistar rat has a typical litter size of approximately 12 ± 1 (ref. 14), whereas in **Table 5** the average litter size is six pups. This is a clear caution flag of poor animal health, nutrition and/or stewardship. On her website (http://www.irina-ermakova.by.ru/eng/articles.html) and in published conference proceedings[1] Ermakova reported a gestation rate of 73.3% (11/15 dams), with control, soy fed and GM soy fed groups having comparable fertility. These numbers are far below the gestation results typically observed for Wistar rats (>98.5%), which once again points to poor stewardship[14]. These numbers are also inconsistent with the present claim of 100% fertility in control groups. It is remarkable that such a strong effect would be seen in the F_1 generation whereas no effects were noted in comparably treated dams of the F_0 generation. We are at a loss to explain these sudden changes in reported fertility.

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% ($P < 0.05$)[a]
Traditional soy	0%	9.7%	77.4%	12.9% ($P < 0.05$)[a]
GM soy protein isolate	0%	21%	72%	7.0% ($P < 0.05$)[a]
GM soy	0%	26%	40.7%	33.3%

[a]In comparison with GM soy.

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 and size 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.

B.M.C., L.V.G., A.M. and V.M. In contrast to Ermakova, we conclude that no meaningful inferences can be drawn from these results. The experimental design does not follow internationally recognized protocols that were developed to guide researchers in proper design[7–10]. The nature of the source material is unknown, the consumption by each animal is unknown and the composition of the diet is unknown. Too few animals were studied and gender differences were not recorded. The abnormally high mortality and low growth rates of the control groups point to poor animal stewardship.

Considering the control results were consistently outside of the range of norms observed for Wistar rats, we have broader questions as well. Is the animal care facility in which these experiments were done a certified facility that meets contemporary standards, such as those described in the guide published by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC; Frederick, MD, USA; http://www.aaalac.org/resources/theguide.cfm)? Were all conditions in the environment appropriately controlled?

It is also of concern to us that Ermakova appears never to have published a peer-reviewed paper describing an animal study of this kind nor does training for such studies appear to be in her academic background. We are not suggesting that trained investigators cannot teach themselves to perform a proper study, but this lack of prior experience may explain why Ermakova failed to heed published international protocols for laboratory animal studies (or was unaware of them). It is noteworthy that, like Ermakova, none of us has performed or published a reproductive toxicological animal study. We have educated ourselves on the proper performance of such studies by reviewing the literature and the readily available standard protocols[7–11,13,14,16–19]. It has been reported that animal studies cost $300,000–$845,000 (ref. 15). Did Ermakova have the required level of funding and resources to carry out the experiments judiciously? And if she had external funding, why are we not told who provided such significant funding?

Last, but by no means least, the adverse effects on reproduction, survival and growth rate observed by Ermakova when RR soybeans are combined in animal diets contrast sharply

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	1.60
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

[a]Organs fixed in formaldehyde, 0.1 M PBS, pH 7.2.

Table 5 Success of mating of first-generation (F_1) 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) $n = 36$	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) $n = 36$	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) $n = 36$	72

with the results of all previous studies. Brake and Evenson[13] conducted a multi-generational feeding study in which mice were fed a diet containing glyphosate-tolerant soybeans. These authors observed no differences in litter size over four generations between mice eating a diet containing 21% RR soy versus conventional soy. Most notably, and markedly different from what Ermakova reports, Brake and Evenson state "in all generations we noted no deaths of the progeny." In other studies where rats and mice were fed meal from conventional and RR soy for 15 weeks at dietary levels of 30% by weight, there was no evidence of any changes in survival, growth, food consumption, organ weights or histological appearance of tissues in animals fed RR soy soybean meal compared with those fed conventional soy[16]. When rats were fed meal from conventional soy and RR soy (up to 90% of the diet by weight) for 13 weeks, no evidence was obtained of reduced survival, growth retardation, changes in clinical pathology or microscopic appearance of tissues[17]. Neither was any difference observed in the growth of young rats, catfish and chickens that were fed GM glyphosate-tolerant soybeans in the diet for 4 to 10 weeks[18]. None of these studies reported unusual mortality or changes in growth rates in the presence of RR soybeans. Finally, in a study involving swine, which have cardiovascular and digestive systems more similar to humans than rats, no evidence has been found for growth retardation or reduced survival when RR soybeans are added to the diet[19].

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 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)[20]. The Committee compared my research with only one (!) published article by Brake and Evenson[13]. 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....Multigenerational 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 and during 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.

B.M.C., L.V.G., A.M. and V.M. Ermakova refers to the comparison by the ACNFP of her research with that of Brake and Evenson[13] as "funny" because the latter investigators focused solely on reproductive physiology and did not feed rats before mating. But she overlooks the fact that her study can be viewed as a subset of the Brake and Evenson study because these authors measured mortality and growth in addition to numerous other parameters; it should be noted that the Brake and Evenson study conforms to internationally accepted norms for animal studies. In stark contrast to Ermakova's observations, they observed not a single mortality in four generations of pups fed GM soybeans at 14% of their dietary intake! Ermakova correctly notes that there is a difference in the timing of the exposure to soybeans in the feed of the dams (2 weeks prior and during, as opposed to only during, pregnancy). The assertion that Brake and Evenson missed the mutagenic effect of GM soy to the germ cells of the parents *per se* because they did not feed for 2 weeks prior to mating ignores the fact that there is no evidence that DNA is mutagenic; indeed, years of study suggest it is not[21,22]. Finally, Ermakova is somewhat disingenuous in claiming Brake and Evenson used a small number of animals because fewer animals were used in each of the five repetitions she reports. Brake and Evenson began with 16 animals (10 female, 6 male) in each group and then analyzed three offspring at 8, 16, 26, 32, 63 and 87 days of age for a total of 18 animals in each group. The numbers are therefore roughly comparable. Perhaps the biggest difference between the two studies is that Brake and Evenson used soybeans of known identity that were specifically grown under their control for the study, and they report a complete compositional analysis of the diets. Additionally, analysis of changes in the reproductive system parameters measured by Brake and Evenson are generally far more sensitive at revealing potential toxic effects than the weight gain and mortality observations of Ermakova. That notwithstanding, they report no pup mortality or other adverse effects over four generations of feeding RR soybeans.

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 Regnum[23]. 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 conference[1]. 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.

B.M.C., L.V.G., A.M. and V.M. Ermakova does not answer the question. She has widely publicized her work at various congresses, meetings, press conferences and on the internet—this is not necessarily uncommon for major new findings. She strays, however, by announcing striking definitive conclusions from her experiments while at the same time claiming to entertain doubts in her own mind about her results. Her results depart so dramatically from previously reported findings as to be remarkable, and remarkable results demand remarkable support that Ermakova fails to provide.

We would add that even publication in a peer-reviewed journal does not *per se* validate scientific claims. It is up to the scientific community to weigh all reports against the best currently available evidence, including prior literature. Science needs to be repeated and to stand the test of time. When scientists circumvent peer review, they not only undermine science, they also undercut the credibility of science in the eyes of the general public[24]. If she had questions about her own results, as she says she did, she should not have devoted so much time to publicizing what are demonstrably flawed studies.

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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