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Comments to the feeding studies with MON810xNK603

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It was my part in the project “Biological effects of transgenic maize NK603 x MON810 fed in long term reproduction studies in mice” (project leader Prof. Zentek) to perform the feeding studies. From a legal point of view none of the feeding studies was an animal experiment (subject of authorisation). These studies were animal husbandry systems with different feeds (subject of registration only). This means that the animals are not manipulated in any way. The hypothesis of the feeding studies was that GM could compromise food quality. Multigenerational studies are a simple and effective way to show up food differences and should be routine in risk assessments of novel foods.

Study designs

The study included 2 harvests and 2 test designs. A multigenerational study (MGS) performed with MON810xNK603 from 2005 and a modified reproductive assessment by continuous breeding (RACB) with MON810xNK603 from 2007. In a MGS the parents of each new generation are randomly chosen from the offspring of the previous generation. In this way 4 generations were bred. In the RACB the same pair is allowed to produce litters during a defined period of time, in this project 20 weeks. The RACB design is routinely used for risk assessment processes e.g. by the US National Toxicology Program (NTP) since 15 years, so far to establish any possible adverse effects of chemical compounds on the reproductive performance of the test animals. According to the Environmental Protection Agency (EPA), risk assessments of the plant pesticide Bt delta-endotoxin needs no threshold regulation, since it revealed no toxic effects in acute toxicity testing and its exposure level is very low. Therefore no dose-related diets have been tested and the design was adapted to assess whole feed effects. The adaptation concerned the keeping of the litters until weaning (3 weeks). Since the study was a research project, not a routine risk assessment, new approaches have to be tested for potential future applications.

Animals

The animals were an outbred mouse strain (OF1). These mice are good breeders with relatively large litters and the results of outbred strains might be more meaningful with a wider genetic background than inbred lines. According to the breeders a pup loss up to 10% is “normal” for this strain. Breeding loss is generally higher in outbred mice and is influenced by season, female weight, litter size and birth weight¹. The mice were kept in macrolon cages III with an average room temperature of 23°C and a light program for a 12 hour day/night cycle. The health status of the mice was checked daily by a veterinarian. The cages were furnished with dust-free litter and cleaned weekly. Furthermore the mice had mouse houses, soft paper for nesting and paper roles, especially for pups. Fresh tap water and test feed were offered ad libitum.

¹ Bell M.A. (2004): Zuchtdate zu Körpergewicht, Fruchtbarkeit und Aufzuchtleistung der Schleißeheimer Mäusestämme zwischen 1990 und 2001. Inaugural-Dissertation zur Erlangung der tiermedizinischen Doktorwürde der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München,

The animal studies were approved by the ethics committee of the University of Veterinary Medicine of Vienna and the national ethics committee for animal experiments (GZ: 68.205/0042 – BrGT/2006).

Diets

A standardized diet for laboratory mice in reproduction in accordance with Nutrient Requirements of Laboratory Animals (National Research Council, NRC, 1995) with 33.0% maize content – added according to group - was chosen.

Results

Generally speaking feed uptake and parental weight development were comparable throughout the studies.

In the MGS the parameters litter size, litter weight and number of weaned pups were higher in the group fed with the non-gm maize diet in all 4 generations, but not on a significant level. The pup loss was lower in this group (n.s.). There was a considerable reduction of females with litters in the 4th generation: out of the 22 females per group only 16 of the non-gm maize group and 14 of the GM maize group had litters, whereas 20 litters were produced in the control group with an Austrian maize variety, constituting the potential base line.

The same parameters were also measured in the modified RACB. In this case there were statistically significant differences in litter size and weight in the 3rd and 4th litters in favour of the non-gm maize group. But this time pup loss was higher in this group (sign. only in the 3rd litters).

Statistical evaluation

It has been suggested, that the statistical methods used to evaluate the results were not state of the art. Especially it was criticised that pairs without litters or lost litters were included in the evaluation. Thus it was recommended to strictly relate all data to the number of real litters and not to the number of breeding pairs. Breeding pairs without litters or without live pups at delivery should be excluded from the evaluation.

But the type of statistical method used clearly depends on the question to be answered. The aim of the project was to compare the reproductive performance between differently fed groups. Thus perished litters as well as pairs without litters but having already delivered litters (not sterile pairs without litters throughout the experiment) have to be included when evaluating group performance.

A new statistical approach summarizing the reproductive performance of each female during the test period of the RACB including the co-variate female weight at the beginning of the experiment has been applied and will be evaluated as to its appropriateness (for peer-reviewed publication). The new results show that in the group with the non-gm maize significantly more pups were born in the overall comparison. But the number of pups weaned was only tendentially different in the overall comparison due to the higher pup loss in this group. The litter weights again were significantly higher in the same group.

But whatever statistical analyses might or might not be used, the data are always the same and open for everybody to see. This study gives the first indication that the stacked event MON 810xNK603 led to a reduced reproductive performance as compared to the non-gm line. This result should not be dismissed as “bad science” but discussed on a scientific level and taken up as incentive to continue investigations in this direction with other GM crops.