An Animal Welfare Perspective on Animal Testing of GMO Crops

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Summary
The public discussion on the introduction of agro-genetic engineering focuses mainly on economical, ecological and human health aspects. The fact is neglected that laboratory animals must suffer before either humans or the environment are affected. However, numerous animal experiments are conducted for toxicity testing and authorisation of genetically modified plants in the European Union. These are ethically questionable, because death and suffering of the animals for purely commercial purposes are accepted. Therefore, recent political initiatives to further increase animal testing for GMO crops must be regarded highly critically. Based on concrete examples this article demonstrates that animal experiments, on principle, cannot provide the expected protection of users and consumers despite all efforts to standardise, optimise or extend them.

Zusammenfassung: Tierversuche für die grüne Gentechnik aus der Sicht des Tierschutzes
Die in der Öffentlichkeit geführte Diskussion um die Einführung der Agro-Gentechnik dreht sich vor allem um deren ökonomische, gesundheitliche und ökologische Auswirkungen. Weniger beachtet wird dabei, dass – noch bevor Mensch oder Umwelt geschädigt werden – die Leidtragenden zunächst die Versuchstiere sind. Denn für die Giftigkeitsprüfung und für die Zulassung gentechnisch veränderter Pflanzen in der Europäischen Union werden zahlreiche Tierversuche durchgeführt. Sie sind ethisch fragwürdig, weil sie Tod und Leiden von Tieren für rein kommerzielle Zwecken billigend in Kauf nehmen. Daher müssen jüngste politische Bestrebungen, die tierexperimentelle Untersuchung gentechnisch veränderter Pflanzen sogar noch auszuweiten, kritisch betrachtet werden. Anhand konkreter Beispiele zeigt dieser Artikel, dass Tierversuche, trotz aller Initiativen diese zu standardisieren, optimieren oder auszuweiten, aus prinzipiellen Gründen nicht die erhoffte Sicherheit für Anwender und Verbraucher herstellen können.

Keywords: genetic engineering, GM food, GM crops, animal testing, animal experiments

1 Introduction

In 2004, the legal requirements for bringing genetically modified (GM) plants to market were newly defined in the EU. Since then the authorisation of GM food and feed is carried out according to EU Regulation 1829/2003/EU (EC, 2003). Applications for such authorisation are submitted to the central authorising authority, the European Safety Authority (EFSA). Some GM foods and feeds have already been authorised on the basis of the new regulation; other authorisations persist due to older licenses that were granted before that date.

As of March 2008, 51 applications were pending in the EU (EFSA, 2008a). The majority of applications concern authorisation of the first generation of maize, rape, soy and cotton plants[1]. However, these mostly do not refer to fundamental innovations but to conventional crossbreeds between already accredited GM plants, for example to combine an insect resistance with an herbicide resistance or to combine two kinds of insect resistances. In some cases, for example a rice breed, the GM plant was combined with a conventional breed.

Only a few applications refer to the second generation of GM plants. For instance, BASF Crop Science has applied for the authorisation of a GM potato that, unlike conventional potatoes that contain the starches amylase and amylopectine, contains exclusively the easily soluble amylopectine[2]. This is an advantage in the paper-, textile-, cosmetics and adhesive industries, where the GM-potato is to be used as a resource. By-products are to be used in animal feed. In another application for authorisation of a second generation product, a GM-maize breeding line of the Dutch company Renessen LLC, a subsidiary company of Monsanto, is concerned. In this case a bacterial gene has been inserted that increases lysine production in the maize kernel. Lysine is an essential amino acid that is used to increase body growth in animal breeding.

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[1] 1st generation crops are resistant against pests or show increased resistance against a certain plant protection chemical. In 2nd generation crops it is not resistances but a change of the plants' ingredients that is of importance, such as a change in the patterns of fatty acids or amino acids.

[2] The European Food Safety Authority (EFSA) concluded that the potato is harmless and recommended its authorisation. In July 2007, The Council of Ministers did not reach agreement on whether to authorise it or not. According to the accreditation procedure it is now up to the EU-Commission to decide.

The same company has applied for authorisation of a conventional crossbreed between the above mentioned GM maize line and MON 810. The "lysine-maize" is to be protected from the European corn borer in this way.

2 Authorisation of GMO crops involves animal experiments

According to Regulation 1829/2300/EG regarding GM food and feed, GM products may be authorised if they

- do not show any harmful effects on the health of humans, animals or the environment;
- do not mislead or harm the consumer or user;
- are not different from other food and feed they are to replace to the extent that their normal consumption would lead to nutritional deficiencies in humans or animals.

In September 2004, the EFSA issued a comprehensive guidance document for the risk assessment of genetically modified plants and derived food and feed in Europe, in which animal data are explicitly required for the evaluation of toxicity towards man and animals (EFSA, 2006). The type and quantity of the required animal experiments *inter alia* depend on the extent to which the GM plant differs from the conventional type (and possibly also from similar GM plants) and which type of usage is intended. The applications are not publicly available. Only miscellaneous summaries of the applications are accessible on the EFSA website and partly also the corresponding authority's comments. Generally, the following animal experiments are to be carried out for the evaluation of toxicity and tolerability:

- *28-day rodent repeated dose oral toxicity study of the isolated GM protein according to OECD-guideline 407 (protein in feed)*
  The standard procedure for this test uses 40 rats or mice, sometimes 100 and more animals are used. In this test, the protein is fed to several groups of animals in different dosages for a period of up to 28 days. Depending on the test performance, half of the animals are killed and dissected approximately after 14 days and the rest after the complete feeding period (OECD, 1995). A 28-day toxicity study using rodents is explicitly expected for newly expressed proteins for which no comparative data exist. Depending on the outcome of the 28-day toxicity study, additional targeted investigations may be required, including an analysis of immunotoxicity.
- Repeated Dose 90-Day Oral Toxicity Study in Rodents *according to OECD-guideline 408*
  The test procedure is similar to the one mentioned above (OECD, 1998). However, usually at least 80 animals are used, in most cases significantly more. In the controversial case of MON 863, which will be discussed further below, 400 rats were used. Half of the animals were killed and dissected in the 5th week, the others after 13 weeks (Lemen et al., 2002).
- *Feeding studies with target species to assess tolerance for the whole GM plant*
  In specific cases, feeding studies with the target animal species in different phases of growth and use (for example with cows or pigs) are performed alternatively or in addition to the experiments mentioned above. Testing protocols and number of animals used vary significantly. Details are not mentioned in the available documentation. Depending on the approach normally dozens up to a few hundred chickens are used and, if used, 80-100 cows on average (ILSI, 2003).
- *90-day feeding studies in rodents*
  If the composition of the GM plant is modified substantially or if there are any indications of the potential occurrence of unintended effects, 90-day feeding studies in rodents have to be considered. New constituents and also the whole GM food/feed are expected to be tested. The testing scheme for this is to include at least a 90-day toxicity study in rodents. At least two dose levels of the GM and parental test food are to be included in the diet. These animal data are submitted on a general basis.
- *Feeding studies with other species*
  For the investigation of unexpected side effects, feeding studies with other rapidly growing animal species, particularly chickens, have to be considered (broiler chicks as animal model for non-ruminants; lambs for ruminants; or other rapidly growing species). These animal data are also submitted on a general basis.
- *Feeding studies using diverse farm animal species*
  In case the genetic manipulation leads to a change in the bioavailability (for example increased water solubility of carbohydrates, starch enriched with amylopectines), feeding studies using several farm animal (target) species are to be carried out for a time period representing the respective production cycle. For feedstuffs intended only for aquaculture, growth studies with fish species are foreseen.

In all the above-mentioned feeding trials the animals are usually killed and dissected after completion of the studies. Consequently, for one single application several hundreds of animals have to die. This adds to the vast number of animal experiments that are used for the elucidation of fundamental problems before the application process is even started, or for the toxicity testing of GM plants for which in the end no application will be filed at all.

3 Lack of explanatory power in animal experiments

As was to be expected, no significant adverse effects towards animal health or abnormalities were reported either in the applications or in the summaries. It is always emphasised that all ingredients and nutritional substances are equivalent to those of the conventional plant and that therefore potential risks for use in practice are negligible.

However, when details of feeding studies on occasion do become public, be it in the context of the EU authorisation or in another context, they raise doubts concerning this opinion. Even more doubts emerge concerning the question whether animal experiments are at all useful tools for risk assessment, particularly when it comes to possible effects on humans. The interpretation of experimental approaches and data is highly

controversial as can be seen in the following examples:

- *Example MON 863*
  In January 2006, the European Commission allowed the import of the GM maize breed MON 863 as food or food ingredient. Before that date, French scientists had observed irregularities within the submitted 90-day feeding study with adolescent rats (Séralini et al., 2007). According to them "rats showed slight but dose-related significant variations in growth for both sexes […]. Chemistry measurements reveal signs of hepatorenal toxicity, marked also by differential sensitivities in males and females." The EFSA and other institutes were of the opinion that the abnormalities were within the statistical random range and that the danger of inflammation caused by the GM maize could not be confirmed. The breed was accredited.
- *Example lectine*
  Scientists at the Scottish Rowett Research Institute in Aberdeen fed raw potatoes containing a lectine gene from snowdrops to rats (Ewen and Pusztai, 1999). They observed negative effects on growth, organ development and the immune system of the rats. Other scientists, however, criticised, among other things, the experimental design: The diet had exclusively consisted of potatoes and therefore contained insufficient amounts of protein needed to maintain health. Too few animals had been used per experimental group and at least two important controls had been omitted: The feeding of a standard rodent diet with 15% protein content and the feeding of transgenic potatoes containing an empty vector.
- *Example alpha-amylase-inhibitor*
  In Australia, in 2005, a multi-year study with genetically engineered peas containing an alpha-amylase-inhibitor-gene from beans was discontinued. Negative results from feeding studies with mice were the reason (Prescott et al., 2005): Mice that were fed GM peas showed allergic reactions and developed pneumonia after two weeks. Animals from the control group that were either fed beans or enzymes from the beans did not show any adverse effects. This is probably due to the fact that the same genetic blueprint results in different products in peas and beans and consequently in different reactions of the immune system. The Australian scientists and authorities interpreted these results as evidence that the tests, carried out like those for EU applications, actually work.

The mentioned examples raise a couple of questions:

- In which case can an incident be regarded as significant or not? Is, for example, the increase in the number of lymphocytes after feeding MON 863 significant?
- What is the actual cause of this incident? Are, for example, the effects on the health of rats in experiments with lectine-potatoes caused by the GM protein or by the experimental design?

And particularly:

- Can the results of the experiments be transferred to humans at all? If, as in the case of MON 863, male and female mice react differently, how can a reliable statement be made for humans?

It is absurd to regard the outcome of the Australian experiments with the GM peas as evidence for the functioning of the approval system or of the animal testing approach. The only sound result is that mice develop allergic reactions to the GM protein. It was neither demonstrated that humans also develop allergic reactions nor, *vice versa*, that proteins not provoking allergic reactions in mice are safe for humans, too. In fact, the opposite is the case. It was solely proven that mice become allergic to the GM protein: Particularly concerning allergenicity, the reactions of humans and animals differ significantly. Even the EFSA is convinced that there are no appropriate animal models to detect allergenicity in humans (see box).

Even if a crop is "only" used as a feed for animals in agriculture, animal studies do not allow for reliable conclusions on how GM feedstuff could affect those animals in agricultural practice. There are no long-term studies, and the basic data that have been collected using experiments with mice or rats cannot unquestioningly be transferred to chickens, pigs or cows.

4 Conclusions

Regardless of the evaluation of the risks of GM crops and products, in any case the animals are the ones who suffer. Those demanding additional animal experiments, among them Austria and other EU Member States (Council of the European Union, 2007) suggest that these types of tests are reliable and that they make GM products more safe. In a recently published report the EFSA concludes that animal experiments generally are suitable to examine health effects of genetically modified crops. At the same time it regards as necessary a better standardisation of *in vivo* testing strategies and the development of new animal testing strategies in specific areas such as allergenicity testing (EFSA, 2008b).

Reliance on animal experimentation for the risk assessment of GMOs gives consumers a false sense of security and at the same time improves the acceptance of GM products by the public (because these are supposed to be regarded as "safe"). In light of the named risks and in light of the fact that the use of GM plants is more than questionable and discussed highly controversially in society, animal experiments for GM plants generally cannot be justified.

Next to the scientific problems connected to the safety assessment using *in vivo* animal studies, animal testing of GMO crops carries additional ethical problems. There should be a clear necessity to provide evidence that the product to be developed and tested is really needed before animal experiments are even considered. The German Animal Welfare Federation has been demanding for many years that such a proof of need is introduced into the national and international regulations concerning animal welfare or animal experiments for all products that are to be developed and accredited on the basis of animal tests.

References

Council of the European Union (2007). Note from General Secretariat to Delegations. Risk Assessment of GMOs, in particular GM maize MON 863. 10430/07, ENV 317, *AGRILEG 78*, 1-5.

EC (2003). Regulation (EC) 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. *Off. J. Eur. Communities L268*, 1-23.

EFSA (2006). Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. *EFSA Journal 99*, 1-100

EFSA (2008a). http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications

EFSA (2008b). Safety and nutritional assessment of GM plants and derived food and feed: The role of animal feeding trials. Report of the EFSA GMO Panel Working Group on Animal Feeding Trials. *Food Chem. Toxicol. 46*, S2-S70

Ewen, S. W. B. And Pusztai, A. (1999). Effect of Diets Containing Genetically Modified Potatoes Expressing Galanthus Nivalis Lectin on Rat Small Intestine. *Lancet 354(9187)*, 1353-1354.

ILSI (2003). *Best practices for the conduct of animal studies to evaluate crops genetically modified for input traits*. Washington, D.C.: International Life Sciences Institute. http://www.ilsi.org/file/bestpracticescas.pdf

Lemen, J. K., Hammond B. G., Riordan, S. G. Et al. (2002). (Monsanto): Summary of Study CV-2000-260: 13-Week Dietary Subchronic Comparison Study with MON 863 Corn in Rats Preceded by a 1-Week Baseline Food Consumption Determination with PMI Certified Rodent Diet #5002. 12/2002.

Prescott, V. E., Campbell, P. M., Moore, A. Et al. (2005). Transgenic Expression of Bean r-Amylase Inhibitor in Peas Results in Altered Structure and Immunogenicity. *J. Agric. Food Chem. 53*, 9023-9030.

OECD (1995). OECD Guidelines for the Testing of Chemicals. Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents (adopted 27th July 1995).

OECD (1998). OECD Guidelines for the Testing of Chemicals: Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents (adopted 21st September 1998)

Séralini, G. E., Cellier, D. And de Vendomois, J. S. (2007). New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. *Arch. Environ. Contam. Toxicol.*, 596-602.

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