Effects of field crops on animals: Considerations with regard to design using Chlormequat-treated wheat crop as an example

Martin T. Sørensen[1], Søren Højsgaard[2]

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Abstract

There is concern whether consuming products based on crop from Chlormequat-treated fields can cause reproduction problems in animals and humans. An experiment is presently being conducted to investigate this using the pig as a model. Considerations with regard to experimental design when investigating whether differently treated crop can affect animal/human biology is discussed. Only about half of the data are presently available. A preliminary survey of these data does not show clear differences between Chlormequat-treated and organic non-treated wheat with regard to reproduction performance of pigs.

Introduction

Experiments have suggested that the plant growth regulator Chlormequat is detrimental to animal reproduction (Torner et al., 1999; Sørensen and Danielsen, 2006). In the QLIF project, a new experiment is presently being conducted to further investigate this. The prime question is: Is wheat grain with residues of the growth regulator "Chlormequat" detrimental to animal reproduction when fed to animals? This question is also relevant for human since there is public concern whether consuming food products based on wheat originating from Chlormequat treated crop can cause reproduction problems.

Considerations with regard to experimental design

Crop or animal experiment?

First of all it is important to clarify what type of experiment is needed to answer the experimental question:

'Is it an animal experiment or a crop experiment?'

In the former case the strength of the conclusions can be increased substantially by increasing the number of animals. In the latter case the animal can be considered as an instrument much the same way as for example a chromatograph, e.g., the animal gives a measure of reproduction performance and the chromatograph gives the concentration of Chlormequat residue. Hence, in the latter type of experiment, the strength of the conclusions is increased by increasing the number of batches of crop, i.e. by increasing the number of fields each contributing with a separate batch of crop. The experiment relevant for investigating the question above is both an animal and a

[1] Research Centre Foulum, Faculty of Agricultural Sciences, University of Aarhus, DK-8830 Tjele, Denmark, E-mail MartinT.Sorensen@agrsci.dk, Internet www.agrsci.dk

[2] as above except E-mail Soren.Hojsgaard@agrsci.dk

crop experiment – but it is primarily a crop experiment in the sense that it is the variation in crops which is of primary concern.

A naïve design could be to take one Chlormequat-treated field and one organic non-treated field, take the crop from each of these fields and feed to animals (say 10 animals per treatment). The only random variation in this design is the animal-to-animal variation. However, it is generally accepted that there is field to field variation in crop. These differences may be attributed to e.g. soil composition and precipitation. Hence what might seem as a comparison of the two treatments in the naïve design (e.g. by a t-test) is effectively a comparison of fields (including treatments, precipitation, soil composition etc). Hence one can not from this experiment draw any conclusion with respect to treatment effect.

It is therefore important to create a design that allows test of differences between differently treated crop and not differences between fields. This requires several fields for each of the treatments and the design of experiment literature gives different schemes for choosing these. (One option is to choose the fields "randomly from the population of fields". Another option is blocking: Choose a random set of fields and subdivide each of these into two lots and assign the treatments randomly to each of the lots. In the latter case, some of the external effects can be eliminated, e.g. the effect of precipitation.) The crucial point is that when investigating the effect of different crops we need to be able to quantify the field-to-field variation. How many fields are needed depends on the field-to-field variation. Yet, it is important to note that it would not help to use 100 animals instead of 10.

Conclusion: Fields/lots are the relevant experimental units in experiments with crop, and not animals or chromatographs.

Mixed heap of crops or separate crops?

Would it be adequate to use material from a heap of Chlormequat-treated and a heap of organic non-treated crop, respectively, originating from many fields? If so, we avoid going through the logistic troubles of obtaining crops from separate fields and feeding these to animals. The answer is no, as can be seen from the following argument. Suppose the animal-to-animal (or chromatograph-to-chromatograph) variation is small compared to the field-to-field variation. Imagine that the average outcome (in some response) of three Chlormequat treated fields is, 95, 110 and 125 (average=110) while the average outcome of organic fields are 85, 115 and 125 (average=108). The difference in averages 100-108=2 will come out as being significant if the animal-to-animal variation is small. But, by looking at the results from the fields, one can see that there is no clear evidence that the outcome from one treatment tend to higher than the output from another treatment; quite on the contrary. Again the problem is that we throw away the information about field-to-field variation, and again it would not help to use 100 animals instead of 10.

Choice of instrument (i.e., which animal species, gender, experimental period)

With regard to the concern for detrimental effects on human reproduction, it is not possible to verify this in an experiment with human subjects. We have to rely on experiments with animals. Different species are apparently not equally sensitive to Chlormequat. It seems that the rat is relatively insensitive compared to the mouse. There are indications that the pig is more sensitive than the mouse (Sørensen and Danielsen, 2006), which was one of the reasons for choosing the pig. Another reason was that the pig is an important livestock species and that farmers avoid feeding

Chlormequat-treated grain to pigs (at least in Denmark) due to concern for their reproduction performance. Generally the male is considered more sensitive to environmental exposure than the female with regard to reproduction. This is in line with results obtained in mice exposed to Chlormequat: Torner et al. (1999) found that reproduction was affected in male mice while no effects were observed in the female (Langhammer et al., 1999).

A major part of the development of reproduction organs takes place during foetal life. Thus, it cannot be ruled out that Chlormequat may have serious detrimental effects during this period of life. In order to exclude the risk of not including a period in which Chlormequat potentially have chronic detrimental effects on subsequent reproduction performance, it was decided that the experimental period should be lifelong starting from the foetal stage. Excluding a critical period in life from the experimental period may lead to a false negative result.

Conclusion: It was decided to expose male pigs to Chlormequat (and Control) treatments from the initiation of life, i.e. from the beginning of foetal life.

Choice of measure (i.e., which reproduction trait)

When we want to test an effect on reproduction there are choices to make as to which reproduction trait to focus on. This is not a trivial matter. In the experiment of Torner et al. (1999), some reproduction measures were affected by Chlormequat while others were not. Thus a general conclusion with regard to the effect of Chlormequat on reproduction would be in one direction if in vitro fertilization was measured and in another direction if only testicle tissue had been analysed. Thus there is always the risk of a false negative result dependent on the choice response variable. We chose several variables among which was the ability of a male to make a female pregnant.

Conclusion: Fertilization competence of semen was chosen as the prime reproduction trait.

Size of the experiment

Size of an experiment can be determined based on knowledge to relevant variable variances and to the difference in trait that the investigator wants to be able to detect. If the variances are not available one has to rely on guesses/estimates. When this is done there is always, it seems, the trouble of adjusting the whole thing to the available budget. We ended up with a design that included wheat crop from 10 Chlormequat-treated and 10 organic non-treated fields. Three boars were then raised from foetal life (i.e., via three different mothers) to adulthood on wheat from each of these fields. Finally the three boars per field then delivered semen for the experiments.

Conclusion: Size of the experiment was determined based on estimates of relevant variable variation and adapted to the available resources.

The experiment

The experiment is well under way, but due to a long lag time between initiation of the experiment and obtaining data, only about half of the pig reproduction data are presently available. A preliminary survey of these data does not support to the results of Torner et al. (1999), i.e., no clear differences in reproduction performance are found between Chlormequat-treated and organic non-treated wheat. The average Chlormequat residue in the treated wheat was 0.333 mg/kg with a max/min of 0.525 and 0.084 mg/kg, respectively.

Closing remarks

If the trend in results for the first half of the data is maintained for the coming second half of the data, the conclusion will be that Chlormequat can not be proven to be detrimental to pig reproduction. In the mouse experiment the conclusion was the opposite (Torner et al., 1999). This leaves us with the question: Is human reproduction mostly comparable to mouse or to pig reproduction? It also leaves us with a need to repeat the "Torner-experiment" in a laboratory where differences could not be found in the pig when using comparable techniques, or that the "Torner-lab" repeats our pig experiment. Until this is done there will be much uncertainty and confusion in the public as to whether food based on Chlormequat-treated crop can be anticipated to be detrimental to human reproduction or not.

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Disclaimer

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