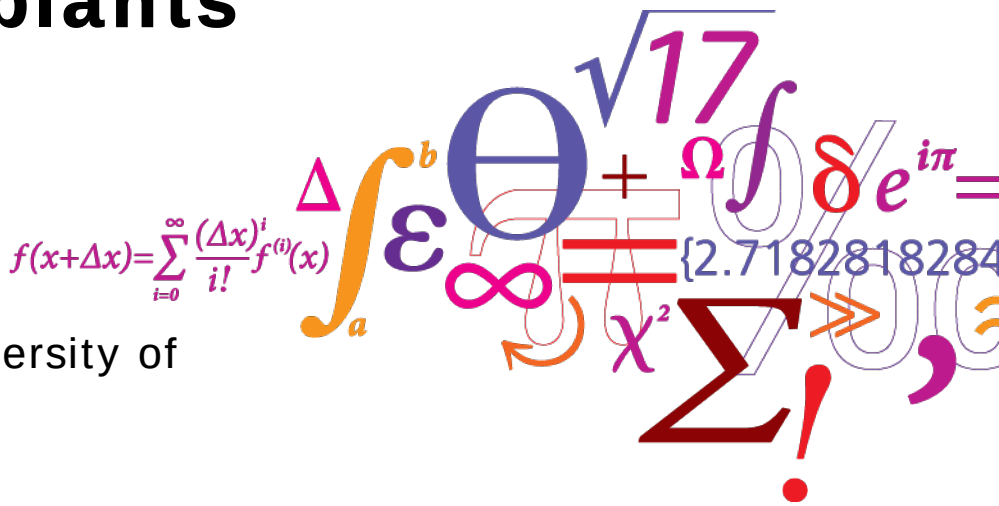
# GMASSURE

## Feeding studies for the safety and nutritional assessment of food / feed derived from GM plants




Morten Poulsen, Technical University of Denmark

DTU Food
National Food Institute

# Aim of the session

- Understand the basic principles of animal studies
- Introduction to the importance of study design
- Understand the strengths and limitations of animal studies (90-day feeding study)

# Assessment of GMOs – where are we?!

Molecular characterization of the genetic modification event
Characterization of donor and host organism

- Methods
- Inserted genes
- Gene expression

Analysis of agronomical and compositional proprieties
**Toxicity / allergenicity / nutritional testing**
Post market monitoring
Environmental risk assessment
Environmental monitoring /surveillance

# What do we want to test in the animal feeding studies and why?

Testing the safety of GM food in animal experiments in two steps:

**whole GM food and**
**proteins / other constituents**

- Is the feeding study requested / needed?
- Purpose of testing - what do we expect to see?
- Laboratory animals vs. Target animals

# Toxicological studies af GM Food/Feed

**Toxicity testing of newly expressed proteins**
Protein equivalency, homology, stability etc.
Repeated dose toxicity (28-days) and if needed additional studies (immunotoxicity)

**Toxicity testing of constituents other than proteins**
Core set studies on metabolism/kinetics, genotoxicity, subchronic toxicity, reproduction and developmental toxicity

**Testing whole GM food/feed**

90-day study

# Why animal feeding studies

- Used to predict the possible effect in humans
- Standardised test conditions – world wide
- Well defined exposure concentrations or doses
- Most effects are picked up
- A sentinel study

# Test guidelines and guidance

Good Laboratory Practice (GLP)

OECD Test guideline

The 90-day feeding study in rodents (OECD 408)

Other types of feeding studies

# Test guidelines and guidance

Are OECD test guidelines suitable for testing GMO?

OECD TG designed for chemicals

Be aware of the differences !

# Test guidelines and guidance

EFSA guidance (2011) on how to test whole food/feed (GMOs) in the 90-day study

EFSA explanatory statement (2014) for the applicability to conduct the 90-day study

Scenario 1 and 2 are introduced – based on hazards in previous analysis

# How to test a new GM plant

We have been asked to perform an animal study to assure the safety of a new GM plant –

How do we proceed?

# Pre-test considerations (if not mandatory!)

Is it necessary ?

Will it answer our question ?

Can we do it in another way ?

# Pre-test considerations

## - Some of these will more or less be determined by OECD guidelines

Choice of  test guideline (duration of study)

Design of study (dose groups, controls etc.)

Choice of animal species

Compositional analysis of the GM food

# Pre-test considerations

Food/feed for animals (route of administration, type of feed and in what amounts)

Should special endpoints /parameters be included

Statistically considerations

Do we need to perform a pilot study?

# Study endpoints

Clinical observations /behaviour

Body weight / feed and water intake

Haematology and clinical biochemistry

Pathology (organ weight - relative and absolute)

Histopathology

Data and reporting

# Quick guide to animal study reports

Imagine that you have been given an animal study report of more than 700 pages and you are expected to deliver an opinion of that report by the end of the week!

In most cases such a report will for GMO be a 90-day study report

# How to interpret the study

Pre-test considerations including OECD guideline requirements should have been taken

Look at summary tables and graphs

Look for statistically significant findings and biological relevant findings

Look for trends and patterns

If necessary look at the individual data

# How to interpret the study

A small number of significant effects are expected

Does the provided explanations for significant differences make sense

Examples of explanations (often provided by the applicant):

- Seen in only one gender
- Within historical control data
- Within commercial control data in the same study
- No clear dose-response

# How to interpret the study

Could some af the observed significant differences be due to or lead to an adverse effect

Do not copy-paste the Conclusion!

Draw your own conclusion based on the advices given above

# Adverse vs non-adverse effects

Often the essential question

No clear answer

Judgement based on pre-knowledge, historical data, experience and communication with the company

# Adverse effects – a definition

Change in the morphology, physiology, growth, development, reproduction or life span of an organism that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (OECD 2003)

# Examples of adverse effect

Cancer

Damage/(weight) changes on organs (liver, kidney, lung, heart....etc)

Damage on the central nervous system (neurotoxicity)

Damage on the reproductive system

Body weight if not explained by a reasonable physiological expl.

Number of non-adverse effects in a physiological pattern

Effects that cannot be explained

# Examples of non-adverse effect

- Most changes in gene expression (if measured)
- Fluctuations enzyme levels / biochemical parameters
- Induction of enzymes involved in metabolism of test chemical
- Decreased body weight gain if due to lower feed intake
- Discolouration of organs / tissues
- Effects which are not statistical significance (but…)

# What can be concluded from an animal feeding study

For the vast majority of studies performed, the NOAEL is the highest dose level

A NOAEL is expressed as the intake of X g/kg body weight/day

A Margin of exposure (MOE) can be calculated

Safety factors can be used to estimate an Acceptable Daily Intake (ADI) but often not the case

# What can be concluded from feeding studies in broilers / salmon and other target animals

Absence of toxicity endpoints

A sensitive study due to the high growth rate

Assessment of nutrition endpoints

Not suitable for calculating MOE, ADI and etc.

# Other testing strategies – The SAFOTEST approach

Developed in an EU-project

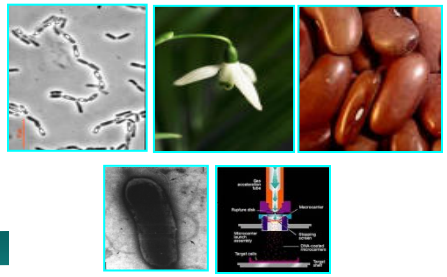
Purpose to increase the sensitivity and specificity of the 90-day feeding study

Useful in some but not all cases

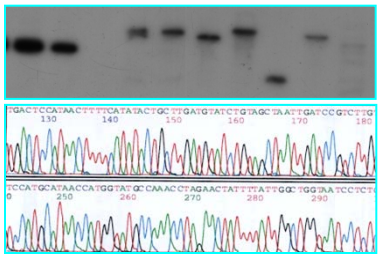
# SAFOTEST in Brief

Bt GNA PHA-E

## Plant Transformation



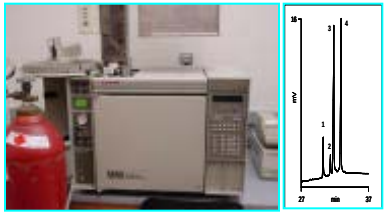
## Rice Genomics



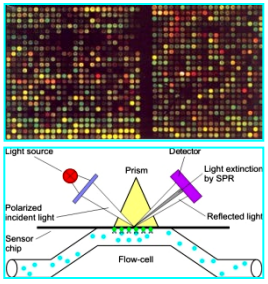
## Production and Purification of Proteins

## Seed Bulking up

## Compositional Analysis



## In-Vitro Tox and Genomics




## In-Vivo Toxicity Studies (DTU)

## Conclusion

The use of spiking was tested and has as a consequence hereof in recent guidelines been suggested as a possible tool in future risk assessment of GM food

- Carefully designed by the use of nutritional well-balanced diets
- Increased sensitivity by the use of targeted parameters and in vitro studies
- Check of sensitivity and specificity by use of spiking (positive control)

# Next generations of GMOs – New challenges for the testing strategies

Introduction of nutritionally enhanced GM food

Keep focus on the safety issue (risk-benefit assessment is a posibility)

Additional test groups and more focuss on the design of the feed

# Check list for animal study reports

Performed under GLP and an international accepted guideline

Is the test compound comparable to the product to be marketed

Are all data present and presented in a clear way

Are statistical significantly results properly explained

Are biological relevant differences explained

# Check list for animal study reports

Biological variance is expected

Do not only read the conclusion of the study report !

Ask experts if you are in doubt

# Thank you

# Questions?