EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Institute for Health and Consumer Protection

# TRAINING COURSE ON

# THE ANALYSIS OF FOOD AND FEED SAMPLES FOR THE PRESENCE OF GENETICALLY MODIFIED ORGANISMS

**June 25-29, 2007**

***AgroBioInstitute (ABI)***
***Sofia, Bulgaria***

ABI

AgroBioInstitute (ABI)

Black Sea Biotechnology Association

# COURSE CONTENT

## Covered topics:

- Overview of EU legislation on GMOs and specific requirements
- Introduction on the general procedures for GMO detection
- Experimental planning and sample preparation
- DNA extraction
- Qualitative PCR for GMO analysis
- Real-time PCR for GMO quantification
- Sampling concepts and recommended EU protocol
- Laboratory implementation and conduction of a GMO detection laboratory
- Implementation of ISO 17025 for accreditation in a GMO testing laboratory
- Method validation criteria

## Experimental work:

- Sample preparation and DNA extraction
- Qualitative PCR
- GMO quantitative analysis by real-time PCR
- Data analysis, expression and interpretation of the results

The course will provide overall scientific and technical information on sampling and on the analytical approaches for GMO analysis as well as hands on experience on how the methods are performed in the laboratory. In addition, the course will prove information on the different theoretical and technical requirements for proper laboratory implementation and conduction of testing activity according to current legislative requirements.

# WORKPROGRAMME

## 1ST DAY – MONDAY, JUNE 25TH 2007

9:00 am Welcoming speech – opening of the course – **A. Atanassov, R. Batchvarova, A. Golikov, D. Ivanov (ABI, BSBA)**

9:30 am Introduction and course content, presentation of the organizers and of the participants – **M. Querci - JRC**

### PREPARATION OF SAMPLES: DNA EXTRACTION

10:10 am **Experimental**: DNA extraction following the CTAB method - Part 1

10:40 am Coffee break

11:00 am **Experimental:** Preparation of agarose gels
DNA extraction following the CTAB method - Part 2

1:00 pm Lunch

2:00 pm **Experimental:** DNA extraction following the CTAB method - Part 3

3:00 pm **Experimental:** Sample loading

3:45 pm Coffee break

4:00 pm **Theory:** Sample preparation and DNA extraction - **N. Foti – JRC**

5:00 pm **Experimental:** interpretation of the gels

5:20 pm End of the day

# 2ND DAY – TUESDAY, JUNE 26TH 2007

## QUALITATIVE PCR

9:00 am **Theory:** Introduction to the Polymerase Chain Reaction and to the use of PCR for GMO analysis – **M. Querci - JRC**

10:00 am **Experimental:** Qualitative PCR
Plant specific: detection of the **zein** and **lectin** genes

10:45 am Coffee break

11:00 am Preparation of agarose gels

11:20 am **Theory:** Characteristics of GM soybean and maize events and introduction to GMO specific nested PCR – **M. Querci - JRC**

12:20 pm Lunch

1:30 pm Sample loading

2:00 pm **Theory:** Serological approach for the detection of GMOs - **M. Ermolli – JRC**

3:00 pm Interpretation of the gels (*zein* and *lectin* specific PCR)

3:15 pm Coffee break

3:45 pm **Experimental:** screening PCR: detection of the **35S promoter**

4:45 pm End of the day

# 3RD DAY – WEDNESDAY, JUNE 27TH 2007

## QUALITATIVE PCR

9:00 am **Experimental:** nested PCR for the specific detection of Roundup Ready® soybean (1st PCR reaction)

10:00 am Preparation of agarose gels

10:30 am Coffee break

11:00 am **Theory:** General consideration on PCR laboratory set up and on quality system implementation – **M. Querci - JRC**

12:00 pm Sample loading (35S screening PCR)

12:30 pm Lunch

1:30 pm **Experimental**: nested PCR for the specific detection of Roundup Ready® soybean (2nd PCR reaction)

2:30 pm **Experimental**: preparation of agarose gels and interpretation of the 35S screening PCR

3:00 pm Coffee break

3:30 pm **Theory:** Introduction to Real-Time PCR for GMO detection and quantification – **M. Querci - JRC**

4:45 pm **Experimental:** sample loading (nested PCR products)

5:15 pm End of the day

# 4TH DAY – THURSDAY, JUNE 28TH 2007

## QUANTITATIVE REAL-TIME PCR

9:00 am **Experimental:** Interpretation of the gel Roundup Ready soybean specific nested PCR

9:15 am Preparation of samples for the Real-Time PCR for the specific detection of Roundup Ready® soybean and samples loading

10:30 am Coffee break

11:00 am **Theory:** Real-Time PCR for GMO quantification – Part 2. – **M. Querci - JRC**

12:15 pm Lunch

1:15 pm **Experimental:** Experimental design, data analysis and interpretation – **N. Foti, M. Ermolli – JRC**

2:45 pm ***Seminar:*** Novel methodological approaches for GMO analysis – **M. Ermolli - JRC**

3:30 pm Coffee break

3:45 pm ***Seminar:*** Sampling: basic principles – **M. Querci - JRC**

4:45 pm End of the day

# 5TH DAY – FRIDAY, JUNE 29TH 2007

9:00 am ***Seminar*:** Introduction to the European legislation on GMOs – **M. Querci - JRC**

10:00 am Coffee break

10:30 am ***Seminar:*** The JRC and its mandate in the European Community – **M. Querci - JRC**

11:30 am ***Seminar*:** Regional experience in GMO regulation – **N. Alexandrova - ABI**

12:30 pm ***Round table*:** Troubleshooting, data interpretation and practical experimental issues. Questions and answers session

1:30 pm Lunch

2:30 pm General discussion and conclusion of the course

3:00 pm Transport to the hotel or airport

# Samples used during the course

During the course we will use different methods to detect the presence of MON810 maize and Roundup Ready® soybean in different materials. For this purpose, we will analyse non-GM and GM maize and soybean samples at different concentrations. Two types of materials will be used:

- Certified Reference Materials (see User Manual Session 3) as positive and negative controls
- Raw and processed materials distributed to the participants

## Composition of samples distributed to participants:

### WT Soybean Flour (0%); Soybean Flour containing Roundup Ready® soybean (RR)

The material was obtained from GMO proficiency testing Gemma Scheme and tested at the JRC Biotechnology and GMOs Unit, resulting either negative or positive for the presence of Roundup Ready soybean.

### WT Maize Flour (0%); MAize Flour containing MON810 maize

The material was obtained from GMO proficiency testing Gemma Scheme and tested at the JRC Biotechnology and GMOs Unit, resulting either negative or positive for the presence of GM maize.

### Baby Quaker®

This sample was purchased from a supermarket in Aruba. Baby Quaker® is a pre-cooked cereal-based infant food produced by Quaker® S.A, Colombia and distributed by Supermercados La Favorita C.A., Ecuador. The ingredients as from label are: oats, rice flour, wheat flour, sugar, soybean flour, lecithin, Vitamin A, Vitamin E, Amylase Vitamin B12, Vitamin B6, Vitamin B2, Biotin.

The sample was analysed at the JRC Biotechnology and GMOs Unit and resulted positive for the presence of Roundup Ready soybean.

## List of samples distributed during the course

| Sample | % GMO (specific ingredient) | |
|---|---|---|
| | RR soybean | MON810 maize |
| Soybean Flour (wt) | 0% | - |
| Soybean Flour (RR) | ~2% | - |
| Baby Quaker | ~ 8 % | - |
| Maize Flour | - | 0.1% |
| Maize Flour | - | 1% |

## Expected results by PCR

| Sample | ***zein*** | ***lectin*** | **35S** | ***nos*** | ***CryIA(b)*** | **CTP/EPSPS** |
|---|---|---|---|---|---|---|
| Soybean Flour (wt) | - | + | - | - | - | - |
| Soybean Flour (RR) | - | + | + | + | - | + |
| Baby Quaker (RR) | - | + | + | + | - | + |
| Maize Flour (MON810) | + | - | + | - | + | - |
| Maize Flour (MON810) | + | - | + | - | + | - |
| Extraction control | - | - | - | - | - | - |