CASE STUDY 1 – GM OIL SEED RAPE *(Brassica napus)*

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OVERVIEW

- Three levels of deliberate releases of LMOs
- Information requirements in notifications for food & feed (CPB, Annex II)
- The case study GM OSR HCN92
- Handling of notifications in the EU

DELIBERATE RELEASE INTO THE ENVIRONMENT

(1) Field trials

usually small scale, limited time period, for scientific or agronomic purposes, safety measures and conditions are case-dependant, inspection plan

(2) Food & feed

commercial purposes, limited consent?, focus on effects on human and animal health

(3) Cultivation

commercial purposes, limited consent?, focus on effects on the environment, potentially irreversible

INFORMATION REQUIREMENTS (CPB, ANNEX II)

- Name & contact details of the applicant
- Name & contact details of competent authority
- **Name & identity of LMO (e.g. unique identifier)**
- **Info on the genetic modification (e.g. method of transformation, donor organism)**
- **Info on the recipient plant (incl. centre of origin, biological characteristics)**
- **Info on the LMO (e.g. new characteristics, sequences inserted, expression of inserts)**
- **Approved uses of the LMO**
- Risk assessment report (consistent with CPB, Annex III)
- Indications for safe handling, transport and use (e.g. labelling, packaging)

THE CASE AT HAND

- LMO?
- Novel trait?
- The potential receiving environment?

GM Oil Seed Rape HCN92

- herbicide tolerance (HT)
- antibiotic resistance (AR)
- people and livestock consuming the LMO
- the environment of the Republic of Belarus in case of loss and spillage

GM OIL SEED RAPE HCN92

- Liberty-Link™ Innovator Canola (ACS-BNØØ7-1)
- Application for direct use as food and feed or processing (CPB, Art .11)
- herbicide tolerant canola produced by inserting
  - the *bar* gene expressing the phosphinothricin acetyltransferase (PAT) enzyme conferring tolerance to glufosinate (phosphinothricin) herbicides and
  - the *nptII* gene expressing neomycin phosphotransferase II enzyme conferring resistance to the antibiotics neomycin and kanamycin (for selection purposes)

INFORMATION ON THE GENETIC MODIFICATION

- Agrobacterium-mediated DNA transfer used for transformation
- Genetic elements in vector
  - CaMV 35S promoter
  - Phosphinothricin N-acetyltransferase gene
  - CaMV 35S terminator
  - Nopaline Synthase Gene Promoter
  - Neomycin Phosphotransferase II
  - Octopine Synthase Gene Terminator
- Donor organisms
  - For the *bar* gene: common aerobic soil actinomycete, *Streptomyces viridochromogenes*
  - For the *nptII* gene: *Escherichia coli* Tn5 transposon

INFORMATION ON THE RECIPIENT PLANT - 1

- Oilseed rape (*Brassica napus*) is a member of Cruciferae or Brassicaceae family
- Centre of origin: Mediterranean Europe,
  cultivated in Asia, Europe and NW Africa since ancient times
- *B. napus* (2n=38) is a allopolyploid hybrid of *Brassica rapa* with *Brassica oleracea*
- Reproduction
  - sexual, pollination by wind and insects
  - self- and cross-pollination occur naturally
    (average rate of cross-pollination 30%; varies between 12% - 55% depending on cultivar, pollen viability, distance & insect activity)
  - pollen deposition decreases rapidly with increasing distance from the source, long-distance pollination events mediated by insects (e.g. honeybees)
  - pollen viability varies with environmental conditions (e.g. temperature, humidity)
    and decreases over 3-5 days

INFORMATION ON THE RECIPIENT PLANT – 2

- Seed characteristics
  - small, light and produced in large quantities in seed pods
  - easily dispersed by wind, water, animals and humans
  - seed dormancy (5-10 years)
- *B. napus* is considered a ruderal species preferably colonising disturbed habitats (e.g. field margins, road verges, paths)
- Feral feral populations under northern-western European climatic conditions
- Volunteers in agricultural fields
- Hybridisation with closely related species (e.g. *B. rapa*, *B. oleracera, Raphanus raphanistrum, Hirschfeldia incana, Sinapis arvensis*)
- Fertile offspring (e.g. with *B. rapa*)

INFORMATION ON THE RECIPIENT PLANT - 3

- *B. napus* is an important oil crop cultivated in the temperate climate regions of the world
- Important trade commodity
- *B. napus* cultivars are available in winter and spring varieties
- The oil is used for human consumption and industrial purposes (e.g. biodiesel)
- Rape seed cake (remainder of the crushed seeds) is processed into ‘meal’, a high quality protein fraction used for feed
- Modern varieties of OSR are use for food and feed are low in erucic acid and glucosinolates (e.g. trademark ’canola’ - ’**Can**adian **o**il **l**ow **a**cid’)
- Meal and oil regulated:
  max. 30 µmol/g glucosinolates and max. of 2% erucic acid in USA and 5 % erucic acid in EU
- High erucic acid OSR varieties are suitable for biofuel and industrial purposes

INFORMATION ON THE LMO

- size & structure of the insert:
  the 2 cassettes were inserted in an inverted arrangement
- Location of the insert & stability:
  at a single insertion site in OSR nuclear genome
- Copy number:
  - Southern blot analysis indicated that 2 copies of the T-DNA cassette were integrated into the host genome
  - One *nptII* gene was completely, the other one only partially integrated
- Expression:
  - PAT protein (green tissues)
  - neomycin phosphotransferase II enzyme

NEW CHARACTERISTIC – HT TRAIT

- Tolerance to glufosinate ammonium conferred to by *bar* gene
  (isolated from the same organisms as glufosinate was originally isolated from)
- glufosinate ammonium, the active ingredient in phosphinothricin herbicides
  (e.g. Basta®, Rely®, Finale®, and Liberty®)
- Mode of action:
  glufosinate-ammonium inhibits the glutamine synthetase, an enzyme responsible for the detoxification of ammonia. The application of this herbicide disturbs the nitrogen metabolism, ammonia accumulates in plant tissues resulting in withering & plant necrosis
- The PAT enzyme expressed by the *bar* gene catalyses the acetylation of glufosinate (phosphinothricin), detoxifying it into an inactive compound.

APPROVED USES OF GM OSR HCN92

- authorized for **food & feed use** in Australia, USA, Canada, Japan, Mexico, New Zealand, South Africa and South Korea
- authorized for **cultivatio**n in Australia, USA, Canada and Japan
- authorization expired in the EU and China

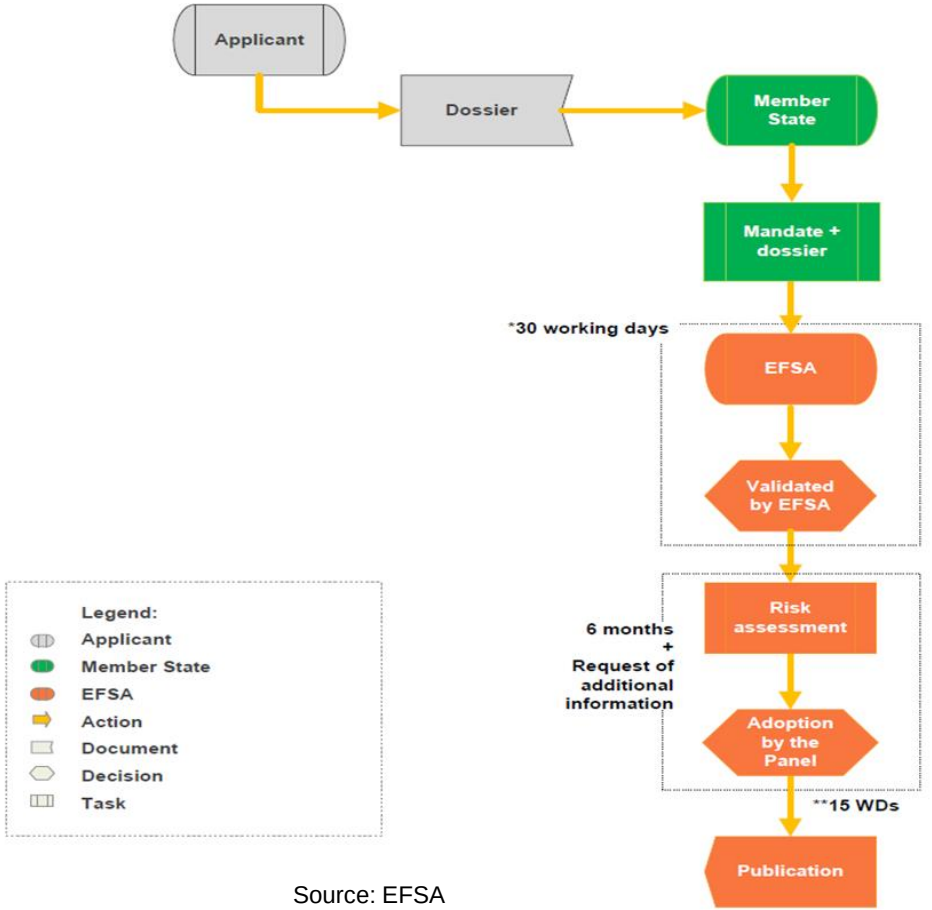
source: http://www.isaaa.org/gmapprovaldatabase

POINTS TO CONSIDER FOR THE RA OF GM OSR

- **Survivability, persistence and invasiveness as GM HT *B. napus…***
  - is capable of forming feral populations (e.g. along distribution routes and shipment areas), which can persist for several years
  - often occurs as volunteer in crop fields
  - seed persists in seed the bank for several years
  - may out-cross and hybridize with closely related species
  - has a fitness advantage in environments where glufosinate is applied
- **Horizontal gene transfer**
  - *nptII* gene may compromise antiobtic therapy if transferred to symbiotic gut bacteria

NOTIFICATIONS IN THE EU - 1

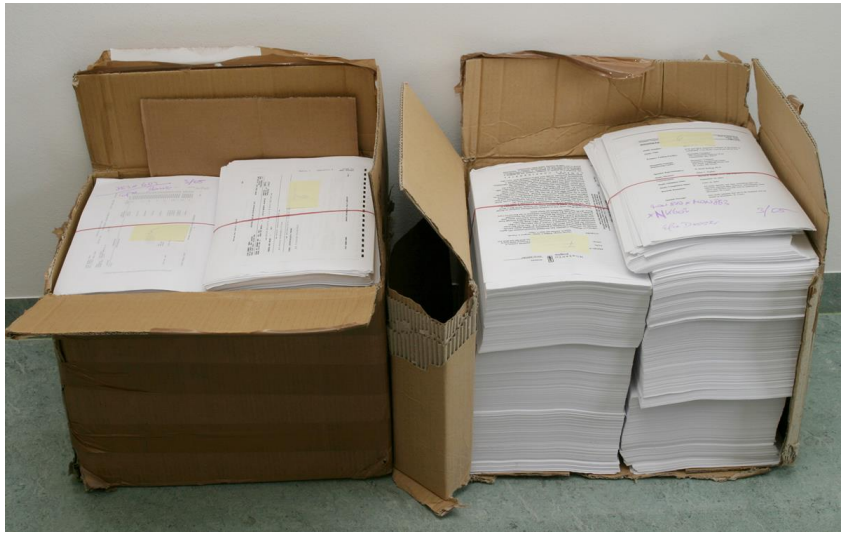
- EU Member States may transmit comments within 3 months



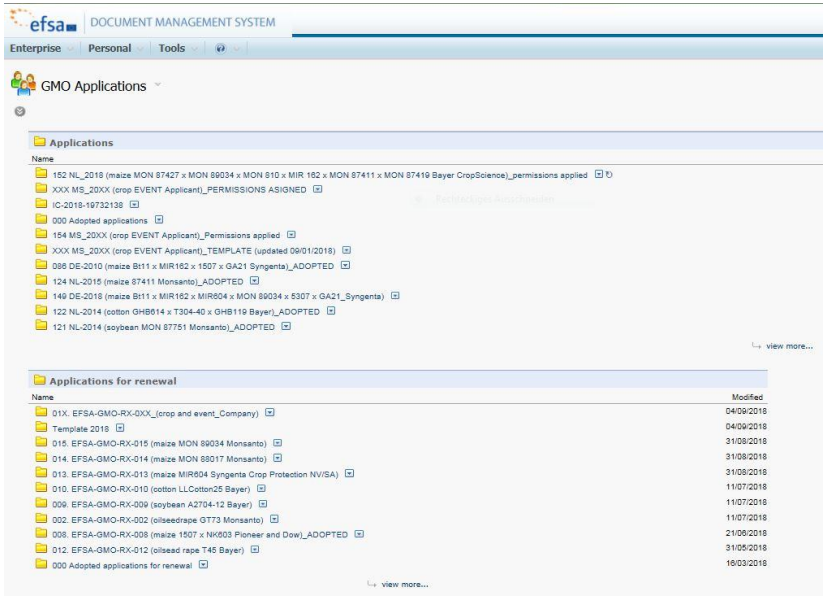
Source: EFSA

NOTIFICATIONS IN THE EU - 2

15 years ago - parcels sent by post



today - national authorities have online access to document management system at EFSA



INFO IN THE DOCUMENT MANAGEMENT SYSTEM

- Competent authorities have access to DMS (document management system) at EFSA
- Applications & applications for renewal (pending & adopted):
  - Technical dossier & overview tables
  - non-CBI and CBI information
  - studies conduced by or on behalf of the applicants
  - raw data of all studies
  - statistical analysis reports
  - production plans of field trials
  - scientific literature cited
- Correspondence between EFSA and applicants
- Additional information received upon request by EFSA
- Scientific Opinions issued by EFSA (i.e. RA report)

INFORMATION IN THE TECHNICAL DOSSIER

- Information on the recipient plant or parental plants
- Molecular characterisation
  (e.g. genetic modification, information on the GM plant, expression)
- Comparative analysis
  (e.g. experimental design of field trials, compositional analysis, agronomic & phenotypic characterisation)
- Toxicological assessment (information on the newly expressed proteins: e.g. heat stability, digestibility, acute toxicity, feeding studies)
- Allergenicity assessment (e.g. homology to known allergens)
- Nutritional assessment
- Environmental risk assessment (e.g. potential effects resulting from gene transfer, potential effects on (non-) target organisms, changes in cultivation and management practices)
- Monitoring plan

SUMMARY

- substantial increase in information included in applications
  → competent authorities of EU MS have access to DMS at EFSA
- new guidance documents (e.g. equivalence test)
  → Increase in quality of data
- tasks often divided among national authorities
  (e.g. environmental risk assessment, food & feed safety assessment)
  → need for coordination and cooperation
- different expertise and stakeholders available here today & the most relevant information was presented for the case study ‘ *application for food & feed use of GM OSR HCN92 in Belarus*’

→ Let us start the exercise!