National Institute for Public Health
and the Environment
*Ministry of Health, Welfare and Sport*

# Educational material for addressing Safe-by-Design in biotechnology: *Cases and guidance*

## Case 3: Converging techniques

# Content of the case:

# 3.1 General information

## Biotechnology domain and focus

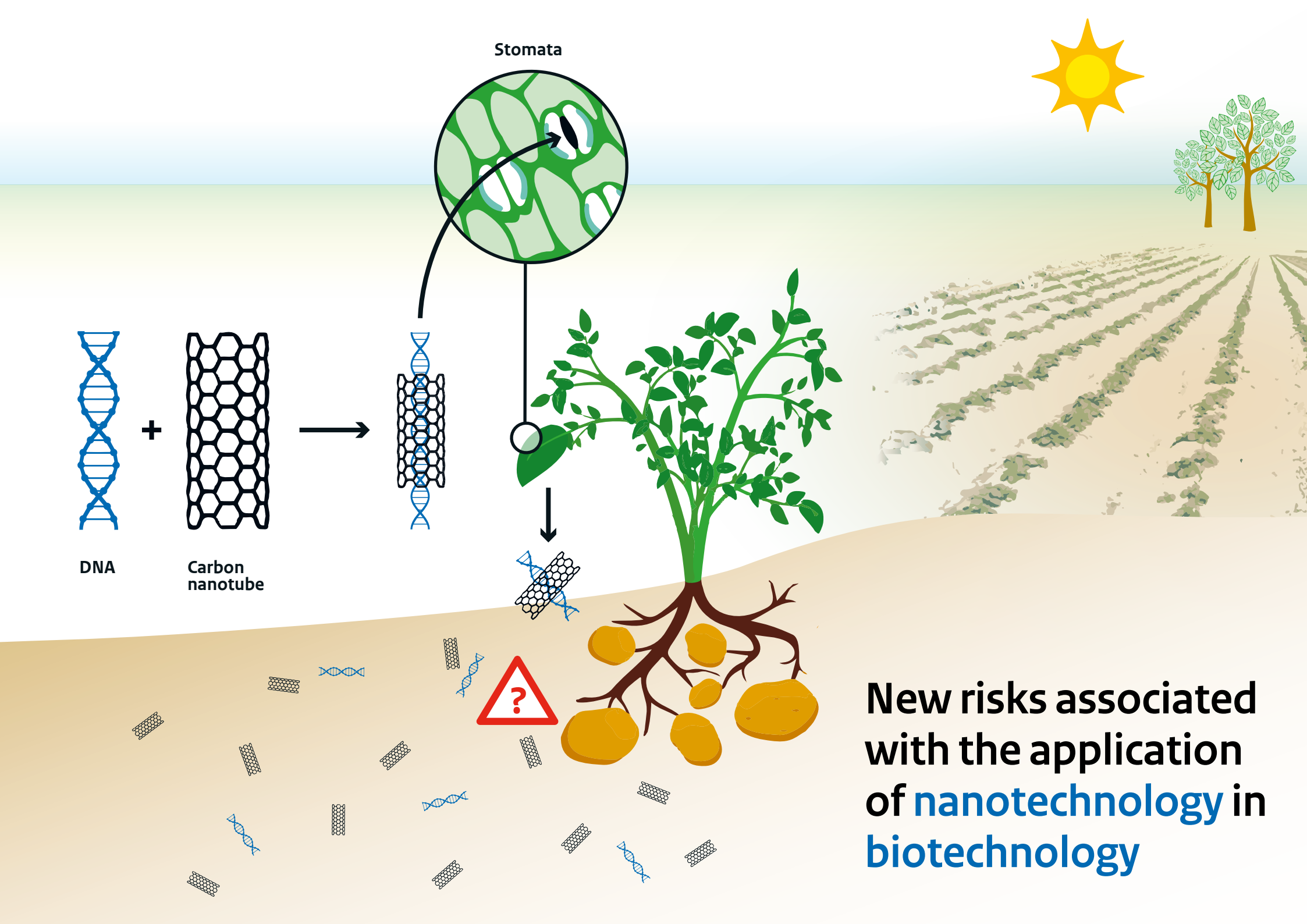
- plant biotechnology;
- multidisciplinary research;
- do-it-Yourself Biology.

## Learning objectives

- understand risks of biotechnology;
- understand risks of nanotechnology;
- evaluate risk of new technologies to society (Safe-by-Design)

## Case specific knowledge required

- knowledge of plant biotechnology;
- knowledge about the hazards of nanomaterials




# New risks associated with the application of nanotechnology in biotechnology

# 3.2 Case description

Scientists have developed a DNA delivery method based on the use of carbon nanotubes as carriers of the DNA to plant cells. The carbon nanotubes are coated with chitosan (a sugar). Because of an electrostatic interaction, the DNA is attached to these nanoparticles. The particles enter the plant leaf through the natural openings (leaf stomata) and pass through the membranes of the plant cell and chloroplast.
As the pH within the chloroplast is low, the DNA is released from the particles. The scientists have demonstrated the specific localisation of the DNA in the chloroplast and its absence in the nucleus. Moreover, the technique seems to be easy to use and applicable to different plant species, for example to rucola.
Its straightforward use enables application of the technique by DIY-biologists. The risks to humans and the environment caused by carbon nanotubes demands a safety evaluation.

## Origin of case and background information

This case is based on a scientific paper[1] which describes the development of nanomaterial as a carrier of gene delivery to plant chloroplasts. The authors demonstrate the safety of the technique for application in food crops as the nanoparticles are lost during breeding cycles.
By using nanomaterial as a carrier instead of a biological vector such as Agrobacterium mediated transformation, permission for handling the genetically-modified Agrobacterium is not required. The resulting plant, however, is a GMO and requires permission. The safety, for humans and the environment, of the nanomaterial used for this technique of genetic modification is questionable. As only small amounts of nanomaterial are needed, a REACH registration (regulation for chemical substances) is not required.

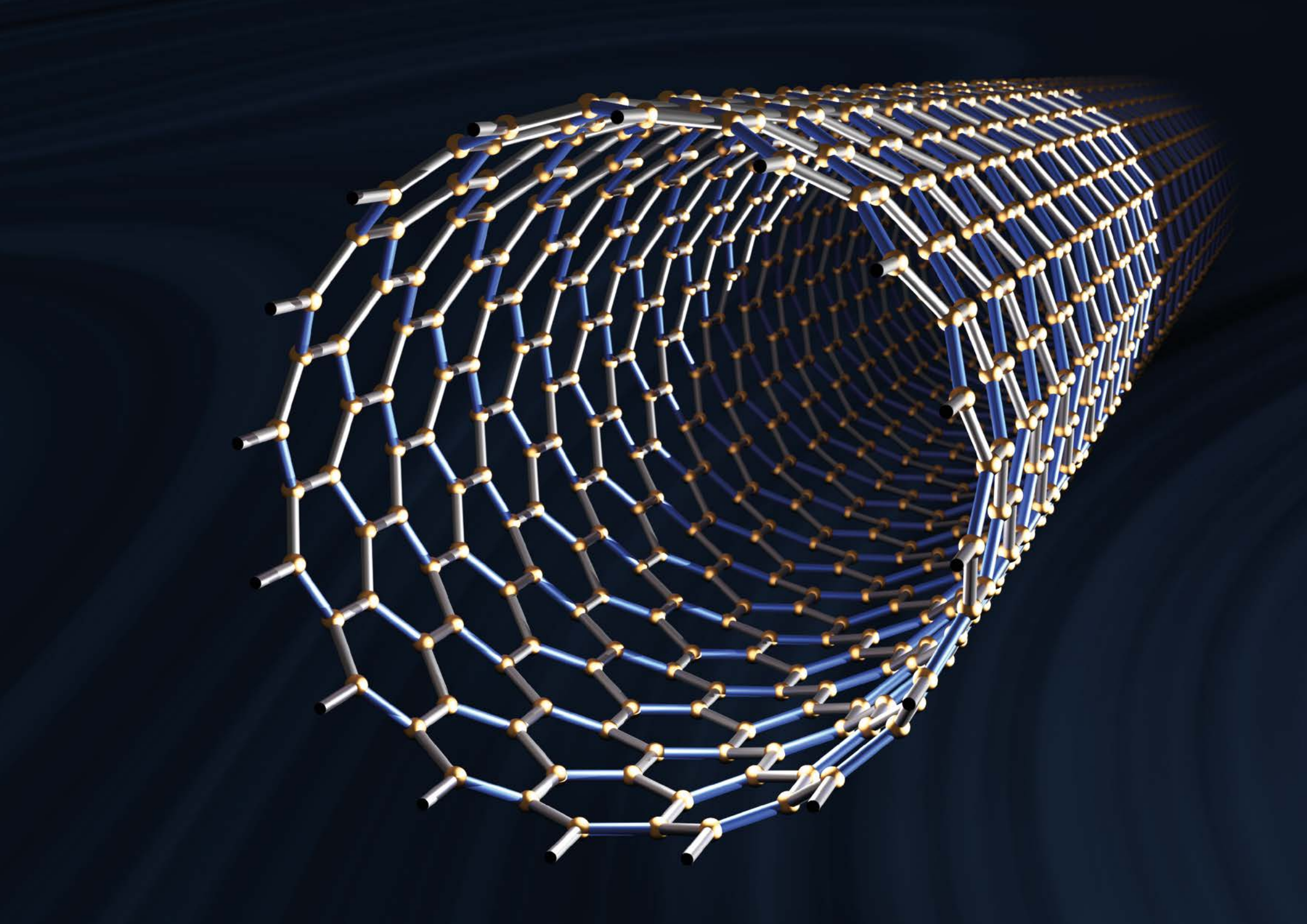
## Additional sources

General information on plant biotechnology:
https://www.nature.com/scitable/topicpage/genetically-modified-organisms-gmos-transgenic-crops-and-732/

Background information to Agrobacterium mediated plant transformation:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6501860/

Toxicity and safety issues of carbon nanotubes:
https://www.sciencedirect.com/science/article/pii/B9780323482219000078
https://www.cleanproduction.org/static/ee_images/uploads/resources/ECN_casestudy_0325.pdf

[1] https://www.nature.com/articles/s41565-019-0375-4



# 3.3 The group discussion

## Theme 1: Safety concerns regarding biotechnology and nanotechnology

**Background information for discussion leader**
To genetically modify plants, *Agrobacterium* is the most commonly-used vector for DNA transfer to the plant nucleus. As this bacterial vector is a GMO, handling this vector is subject to GMO regulation. Using the carbon nanotubes as carriers for the DNA may be regarded as an advantage over *Agrobacterium*, as handling the nanoparticles is not subject to GMO regulation.
In relation to the carbon nanotubes, it is helpful to read the references in the additional information before discussing the safety concerns of nanomaterials with students in biotechnology. Many nanomaterials are persistent, which means they may accumulate in the environment and could have various toxic effects.
The chitosan-coated carbon nanotubes specifically target the plant chloroplasts. This can be seen as an advantage, as chloroplasts are maternally inherited in most plants. The absence of chloroplasts in pollen reduces the spread of the genetic modification in field grown plants. Bear in mind that plants originating from both transformation by *Agrobacterium* and the nanoparticles are GMO and subject to GMO regulation.
The authors argue in their publication that, if this method is used for the genetic modification of commercially-grown crops, the presence of nanoparticles in such a crop is highly unlikely. To develop a commercial crop, the breeding programme encompasses many plant generations, which causes the nanoparticles from the first generation to be lost.

**Suggestions for the group discussion**
The following issues are suggested for discussion:

- The DNA attached to the carbon nanotubes is a nanomaterial and is not a GMO like Agrobacterium. Discuss the properties of both carriers and their specific safety issues.
  - With regard to their use in the lab;
  - With regard to the genetic modification of a plant.
- Would you consider the nanotubes to be intrinsically safer than *Agrobacterium*?
  - With regard to its use in the lab;
  - With regard to the genetic modification of a plant;
  - With regard to commercial use of a crop.

By delivering the DNA coated nanoparticles to the plant cells, the resulting plant is regarded as a GMO.

- What safety issues are at stake when cultivating these genetically modified (GM) plants? And what kind of measures have to be in place for this cultivation?
  - With regard to the first generation plants that are exposed to the nanoparticles;
  - With regard to the GM plants grown as a commercial crop.

## Theme 2: Expanding the safety perspectives

**Background information (for discussion leader)**
Standard plant genetic modification techniques making use of a genetically modified (GM) bacterium (*Agrobacterium*) are more complicated and ask for technical experience and equipment. For the DNA delivery technique described in this case, the carbon nanotubes are easily obtainable and the protocol for preparing the particles and the application to the plant is doable. Moreover, delivery of the nanoparticles to plant leaves results in a quick effect, e.g. fluorescence of the leaves. This technique is likely to draw the attention of Do-it-Yourself biologists, as the 'Glowing Plant' project did a few years ago.

In contrast to the strict GMO regulation, carbon nanotubes are already on the market even though their hazardous properties are still under investigation.

**Suggestions for the group discussion**
The following issues are suggested for discussion:

- This technique could potentially be used by Do-it-yourself Biologists as the protocol is easy to apply and the material is readily obtainable. Discuss the different aspects of such a development:
  - Is safety to humans and the environment still guaranteed?
  - If something goes wrong (i.e. harm to human health or the environment), who is responsible?
  - Is the scientist, who developed the technique, responsible for its safe use by others, by non-experts?
- Permission for the use of chemicals (regulated by REACH) and for genetic modification (GMO regulation) are set up in different ways. A GMO can only be constructed and handled if a risk assessment has been carried out beforehand and the resulting risks are negligible. For nanomaterials, which are used in small quantities, REACH is not applicable, yet research on the cytotoxicity and immunological effects of carbon nanomaterials is still ongoing and harmful effects have already been shown to be associated with the materials.
  - Discuss this discrepancy. Is safe use possible while unregulated?

# 3.4 Wrap up

This case illustrates the occurrence of new risks when nanotechnology finds its way into biotechnology. Different knowledge fields have to be in place for safe use. Innovation and the simplification of techniques can lead to unforeseen risks related to the lack of regulation, or their use by unauthorised persons (here non-experts like DIY-biologists).

By taking into account the learning objectives (see Section 'knowing') you may summarise the collective view on the safety aspects of this technique and the additional issues that were discussed in Theme 2.

## Options for enriching the learning experience

- Gain insight into possible stakeholder perspectives. For example by performing a role play in which each student acts as a different stakeholder.

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