

CASE STUDY 3: Application for Field Trials with Genetically Modified Bananas Containing a Vaccine against Hepatitis B

1. Brief Description of Proposed Trial Release

The major epitope protein of the Hepatitis B virus has been cloned into banana to enable an effective vaccine delivery system to children and adults in developing countries. It is necessary to compare the suitability of eight banana clones to your country's climatic conditions. These trials will examine two parameters relevant to potential adoption and use: (1) growth rates of newly propagated and five-year-old plants, and (2) level of gene expression in the fruit.

2. Objective

2.1 What is the aim of the proposed trial release of the genetically modified organism (GMO)? What are the benefits of this approach compared with other possible methods, especially those not involving planned release?

The current vaccine for Hepatitis B is the same cloned viral protein, but delivery is in a pharmacological package designed for injection. Storage and hygiene of injected vaccines have been problematic in rural areas of developing countries where refrigeration and supplies are not readily available. In addition, injections cause significant trauma to children and adults. Packaging the vaccine protein in an edible fruit is a convenient, cost-effective, and humane way of ensuring that adequate doses are delivered to all people in high-risk areas.

2.2 If the trial release is successful, do you intend to propose a general release of the GMO?

Yes

If so:

2.2.1 When do you propose that the general release would take place?

Once medical clearance is obtained—approximately three years.

2.2.2 Where do you propose that the general release would take place?

Worldwide in banana growing areas.

2.2.3 By whom do you propose that the GMO would be released?

An international health organization.

2.3 Do you intend to market the GMO as a product in this country?

Yes.

3. Nature of Organism and Novel Genetic Material

3.1 What is the species of GMO to be released?

Banana plants (*Musa* spp.).

3.2 Do the unmodified form(s) have any adverse effect on:

3.2.1 Humans, animals, or plants?

No.

3.2.2 Agricultural production?

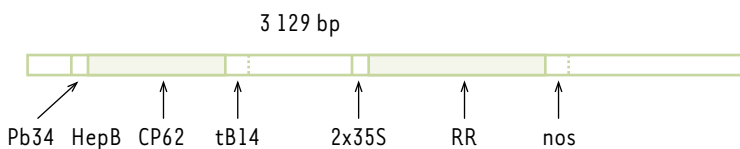
No.

3.2.3 Any other aspect of the environment?

No.

3.3 Furnish a description of the genetic, and resultant phenotypic, modifications of the GMO. This should include the origin of the inserted DNA, the procedure used to induce the genetic modification, and

the extent to which it has been characterized.



Linear DNA fragment inserted into banana

KEY

- Pb34 = fruit specific promoter from tomato
- HepB CP62 = major epitope coat protein from Hepatitis B virus strain 4589
- tB14 = terminator from tomato polygalacturonase gene
- 2x35S = two copies of the CaMV 35S promoter
- RR = glyphosate herbicide tolerance gene
- nos = nopaline synthase terminator from *Agrobacterium*

The Hepatitis B vaccine protein was cloned from the virus and inserted into a plasmid containing a tissue-specific promoter that only expresses the protein banana fruit. The plasmid also contains an herbicide-resistance marker gene. The plasmid was shot into banana tissue using a helium gun. Plantlets were regenerated from transformed cells of eight banana varieties. Stable transformants were selected over ten generations of plants regenerated in tissue culture.

3.4 What is the frequency of reversion, i.e., loss of genetic modification?

None.

3.5 How do you verify that you have the desired GMO?

Hybridization and herbicide tolerance.

3.6 What methods will you use to test for batch-to-batch consistency?

Elisa testing.

3.7 What is the expected survival time of the GMO under conditions likely to be found in the proposed release area(s) and surrounding environment(s)?

The plants will survive for three to ten years in cultivated fields.

3.8 Describe normal dispersal mechanisms of the species and characterize the capability of the GMO to disperse from the release area.

Bananas are cultivated vegetatively. New stock is obtained through tissue culture to optimize virus disease control in the crop.

3.9 If, at any stage in the future, biosafety regulators need to ascertain whether the GMO in a field is the same as that specified here, what means are available?

Hybridization and herbicide tolerance.

3.10 Provide a protocol and materials to enable detection of foreign gene(s) in surrounding microbial, plant, or animal life.

Protocols attached.

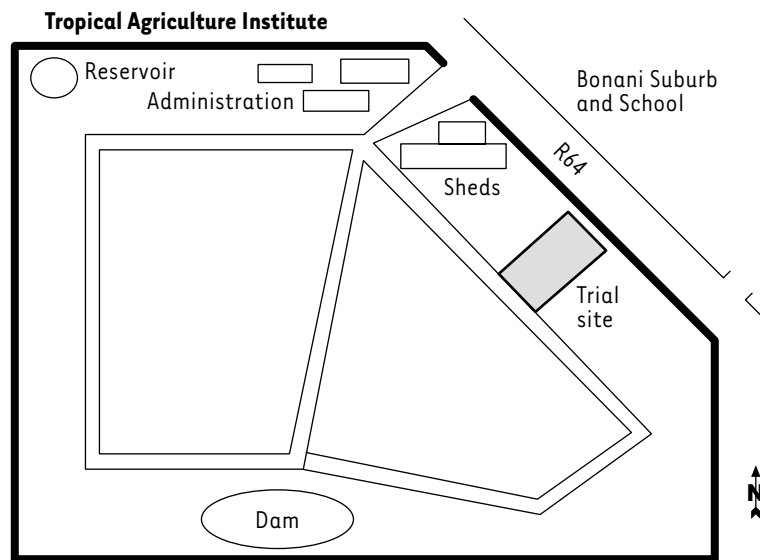
4. Trial Release: General

4.1 Full details are required about the manner in which the trial release of the GMO is to be undertaken.

The bananas will be planted at the Tropical Agriculture Institute's research facility, which is surrounded by a fence to minimize pilfering. Access to the trial site will require a key and will be by authorized personnel only. A sign on the fence will have a skull and crossbones and say, "Test bananas, not for human consumption."

The trial site is within the grounds of the institute, on a southwest slope. Water drains from the site to the dam. The institute is surrounded by subtropical vegetation to the north, west, south and by a suburb on the east. The R64 road separates the institute from the suburb. The site will have no bordering banana plantings. About 42 clones of each of the 8 transformed varieties will be planted. Plants that die or fail to grow will be replaced by seedlings from the lab stocks. The trial site will be fenced with a 2-meter-high mesh fence and locked with an access gate. Fruit from the plants will be collected and moved to the laboratory for further testing.

The trial will run for three years and will be supervised at all times by trained personnel. A flood



would, at the very worst, wash the plants down to the dam, but this is highly unlikely because no such floods have been recorded in the area.

After the trial the plants will be removed and burnt.

4.2 What potential hazardous or deleterious effects resulting from the trial release of the GMO can be postulated?

The vaccine bananas are not toxic even when consumed at a high rate.

4.3 Have similar releases of similar GMOs been made previously, either within or outside the country?

No.

4.4 Have similar requests or applications for the release of this particular GMO been made previously?

No.

4.5 Is there any evidence that the inserted genetic trait is transferable to other organisms in the release site and surrounding environment?

The banana flowers are mostly sterile, but will be bagged to prevent pollen release into the environment.

4.6 What data are available to suggest that the introduced genetic trait has no deleterious effect in the long term upon the species into which it has been introduced or allied species or any other organisms or the environment in general?

The Hepatitis B epitope protein is being used already as a vaccine, and its lack of toxicity was established before it was approved for medical use.

4.7 Is the GMO intended to modify the characteristics or abundance of other species?

No.

4.8 What experimental results or information exist to show the probable consequences (positive and negative) of the release of such a modified organism, including impacts on:

4.8.1 Human, animal, or plant health?

The protein will increase resistance to Hepatitis B in consumers able to invoke an immune response.

4.8.2 Agricultural production?

None.

4.8.3 Target and nontarget organisms in the area?

None.

4.8.4 The general ecology, environmental quality, and pollution in the area?

None.

4.8.5 The genetic resources (e.g., susceptibility of economically important species to herbicides, pesticides, etc.)?

None.

4.9 Will the trial release have any unlikely but possible impacts?

No.

4.10 What will be the consequences if the organism remains in the environment beyond the planned period?

None.

4.11 Has a trial release been carried out in the country of origin of the GMO?

No.

4.12 Provide a draft copy of a press release informing the public of the trial or general release of the GMO.

Attached.

5. Trial Release: Vaccines

5.1 For human clinical trials, what arrangements are proposed to dispose of waste containing any vaccine organisms?

Clinical trials are not part of this application. They are already under way in Europe.

5.2 Will the subjects carry live vaccine organisms at the end of the trial? If so:

5.2.1 Will they be likely to disseminate the live vaccine organisms to the general population?

N/A

5.3 On the basis of data obtained in contained experiments (please supply), what effects are expected when the vaccine organism interacts with target and nontarget species in the test area and surrounding environment?

N/A

5.4 What is the existing evidence regarding level and duration of immunity produced in the target species?

These data are being collected.

5.5 What challenge or other tests using virulent field strains are to be carried out on vaccinated animals?

N/A

5.6 Is there likelihood that the host vaccine organism would be used in other human or animal vaccines?

Yes.

5.7 Would the use of this vaccine preclude the future use of the host vaccine organism for immunization purposes?

No.

6. Crop or Pasture Plants

6.1 Will the plants in this experiment be allowed to set seed?

No.

6.2 Is vegetative propagation planned?

No.

6.3 What desirable effects are expected to result from the use of the modified plant (e.g., increased production, improved quality of product, new product, disease, insect or herbicide resistance, etc.)?

Improved vaccine delivery system for tropical and subtropical developing countries.

6.4 What undesirable effects may result from the release (e.g., reduced fertility, increased disease prevalence, production losses, etc.)?

None.

6.5 Are any of the likely gains directly linked to losses in other characteristics of the species?

No.

6.6 Are any members of the genus of modified plants known to be weeds?

No.

6.7 Can the genetic trait be transmitted by means other than by normal reproduction?

No.

6.8 Does the imparted characteristic have the potential to add or subtract substances from the soil (e.g., nitrogen)?

No.

6.9 Has the modified plant been shown to be nontoxic to animals and humans?

Yes.

6.10 Could any toxic products concentrate in the natural or human food chain?

No.

6.11 With regard to the pollination characteristics of the species, do wild populations of the species, or related species with which it can interbreed, exist in the vicinity of the field trial or agricultural site?

No.

6.12 With regard to the pollination characteristics of the plant, is it likely that the novel genetic material will enter a pre-existing gene pool? Provide information on the pollinators specific to the crop and the measures to be taken to prevent pollen spread to unmodified plants.

No. The flowers are mostly sterile, but will be bagged at the onset of flower development and kept bagged until harvest.

6.13 Should the imparted characteristic (e.g., insect, herbicide, or disease resistance) "escape" into a wild population, would it have the potential to affect the distribution and abundance of that population?

No.

6.14 Would there be any consequent problems with respect to:

6.14.1 Agriculture?

No.

6.14.2 The environment?

No.

6.14.3 Disease control?

The GM crop is designed to combat viral disease in humans.

6.15 If there is any possibility of 6.12 and/or 6.13 occurring, has any attempt been made to minimize the risk (e.g., by imparting male sterility)?

No.

6.16 Could the imparted characteristic (either in the cultivated population or in a wild population) provoke a genetic response in populations of other species (e.g., increase the resistance of an insect population to an insecticide)?

No.