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GROUP OF NATIONAL EXPERTS ON SAFETY IN BIOTECHNOLOGY

REPORT ON THE "SEMINAR ON SCIENTIFIC APPROACHES FOR THE ASSESSMENT OF RESEARCH TRIALS WITH GENETICALLY MODIFIED PLANTS"

6 and 7 April 1992,
Jouy-en-Josas

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 1993

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NOTE BY THE SECRETARIAT

1. In 1991, the fifth session of the Group of National Experts on Safety in Biotechnology (GNE) endorsed the proposal of the French Delegation to hold a scientific seminar to compare different national approaches for the review of genetically modified plants.

2. The seminar, entitled "Seminar on Scientific Approaches for the Assessment of Research Trials with Genetically Modified Plants", took place on 6-7 April 1992 in Jouy-en-Josas, France, hosted by the French authorities.

3. The sixth session of the GNE held on 17-18 June 1992 reviewed the documents presented at the seminar, which consisted of DSTI/STP/BS(92)4, Annexes I, II, III & IV and Addenda to Annexes I & III. The GNE endorsed the recommendation that the documents be derestricted, after being reviewed by the authors, due to the usefulness of the documents for future reference.

4. Consequently, the documents were reviewed by the authors and compiled into one document in OECD format.

5. The Committee for Scientific and Technological Policy (CSTP) approved, by written procedure, the derestriction of the report under responsibility of the Secretary-General on 29 June 1993.

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Summary Report

1. What follows is not a strict summary of the presentations, nor does it reflect the breadth and liveliness of the discussions that took place. It is essentially an interpretation of the proceedings. However, the three consensus statements presented at the end were written during the closing session of the seminar and accepted unanimously.

2. Participants from 14 countries and the CEC attended the seminar. They included representatives of the competent authorities, other scientific and administrative experts in the domain of transgenic plants and two industrial applicants. The purpose was to share and compare experiences with field tests in various countries, primarily in order to identify areas of disagreement and consensus in the scientific approaches used to assess releases and to encourage harmonization of approaches in Member countries.

3. The two days were organised along different axes. The first was under the general chairmanship of Mr. A. Kahn, with Mr. J. Beringer chairing the session on rapeseed and Ms. S. McCammon that on maize. It centred on comparison of the different manners of treating field tests of three plant species -- potato, rapeseed and maize -- each of which raises specific questions. The second day, presided by Mr. J. Schell, addressed more general issues, particularly questions concerning the molecular characterisation of transformed plants and the use of antibiotic and herbicide resistance genes as selectable markers. A number of questions raised during the first day will be reported here as part of the discussion of general points debated on the second.

1. Potato

4. Presentations concerning potato were made by Mr. P. Dale, Mr. P. de Haan, and Mr. M. Schechtman. They described how field tests are carried out in the United Kingdom, the Netherlands, and the United States, and emphasized methods of preventing dispersal. Conclusions were similar on many points, e.g. the potential for crosses with wild species in these countries is extremely low; crossing with other potato plants is a short-range phenomenon. The three countries approach the prevention of dispersal via seeds or residual tubers differently. In the United States, winter conditions in Idaho, where most tests are carried out, are rigorous enough to prevent regrowth from seeds and tubers. In the Netherlands and the United Kingdom, where the milder climate allows regrowth from overwintering tubers, other means of elimination are required, e.g. planting of monocots the following year, associated with treatment with dicot-specific herbicides.

5. Though potato presents specific problems because of the potential for dispersal by tubers, the general sense was that this is quite manageable, and that in any case, none of the genes introduced so far into potato would enhance

its weediness. Mr. Kahn presented a report transmitted to the seminar by New Zealand in which similar results on pollen dispersal and the lack of crossing with weedy species were described.

2. Rapeseed

6. Presentations on rapeseed were made by Ms. Y. Dattée, Mr. M. Renard, Ms. L. Duke, and Mr. P. Rüdelsheim.

7. The questions associated with rapeseed releases are quite different, in particular since shattering leads to release of large numbers of seeds and hence numerous volunteers in following years, and since rapeseed can be pollinated over longer distances by both wind and insects. Results of studies of dispersal via pollen were described by both Mr. Renard and Mr. Rüdelsheim.

8. Mr. Renard also presented novel results concerning the possibility of crosses between rapeseed and other crucifers. It is generally considered difficult to obtain crosses with the wild crucifers growing in Europe, viable seed production usually requiring embryo rescue and culture {in vitro}. However, Mr. Renard presented results showing good seed production in the field in crosses between rapeseed and {Brassica adpressa} and {Raphanus raphanistrum}. The ability to cross with rapeseed seems to be highly genotype specific, which may explain why these crosses were previously deemed impossible under field conditions. These results raise the question of whether interspecific and intergeneric crosses between other cultivated plants and wild relatives might also prove possible if more diverse genotypes were tested. The viability of the hybrids is currently under study.

9. Mr. Rüdelsheim also described the differences in emphasis in the requirements for field tests in France, Belgium and the United Kingdom. Overall requirements are similar in the three countries, but differ significantly in emphasis. The point of particular concern in France is molecular characterisation of the transformants. This question was discussed in greater detail the following day. In the United Kingdom, particular accent is placed on environmental considerations, and detailed evaluation -- of potential weediness, interactions with wild species, possibilities of dispersal, potential effects on human health, and potential social aspects -- is required. Belgian authorities are particularly concerned with monitoring and require detailed descriptions of protocols for detecting dispersal of the new genetic information. Stressing the difficulty and burden of completely and simultaneously satisfying the demands of all three countries, Mr. Rüdelsheim made a plea for greater uniformity of requirements and protocols.

3. Maize

10. Presentations concerning field tests of maize were made by Ms. S. McCammon, Mr. F. Quétier, and Mr. E. Chasseray. Ms. McCammon pointed out that there are no wild relatives of maize in the United States, and thus transfer of genetic material to wild species is not of environmental concern there. The case might be different in countries where wild relatives exist, or that are centres of origin for maize. However, the concerns should be analysed

in comparison to the parental line. Mr. Chasseray raised the important point that even under the best conditions, at least ten to 14 years would be required to go from gene development to commercial release of a transgenic plant variety, depending on the need for testing for plant variety registration. A plea for international harmonization as a means of avoiding insurmountable hurdles followed.

4. Discussion of general points

11. Ms. Casse-Delbart discussed the issue of field testing plants expressing genes of unknown functions, such as those of {Agrobacterium rhizogenes}. Current views on the assessment of the potential dangers associated with such genes are quite divergent. Mr. Beringer, Mr. Schell, and Ms. McCammon stated that the genes described did not appear to affect negatively the plants or their agronomic characteristics. Thus, there is no reason not to carry out initial field tests.

12. Mr. Kahn raised the question of whether {A. rhizogenes} is a plant pathogen. The French Commission is still reviewing applications for field tests and thinks that more information is necessary, but that tests could be carried out if no concerns are identified. The possibility of any genes conferring a selective advantage to the plants in initial limited tests would be minimal. Ms. McCammon said that the United States has not reviewed any field tests involving {A. rhizogenes} and that the only {Agrobacterium} species exempt from USDA's list of plant pathogens is {A. radiobacter}. Thus, any review of {A. rhizogenes} would be done on a case-by-case basis. This issue was thought to be an open question which deserves further consideration.

13. The degree of molecular characterisation necessary for assessing safety field tests of transformed plants was a major subject of discussion. Although all countries require molecular analysis of transformants, two fundamentally different points of view were expressed. The French authorities prefer extensive characterisation of transformants before the first field trials, including determination of the limits of the DNA transferred, whereas representatives of other countries are more willing to allow small-scale tests for establishing gene efficacy with transformants that are less well characterised, or that contain genes of unknown function, depending on the case.

14. The question put to the French participants was how one could justify, on scientific grounds, not allowing small-scale tests with genes having no obviously dangerous characteristics, if reproductive isolation of the plants is achieved. The response was that the French commission considers that its role goes beyond risk assessment in the strict sense, and that it also has a role to play in cautioning experimenters against transformants that, by their genetic complexity, may pose additional problems in large-scale tests, in which reproductive isolation would not necessarily be maintained. Several participants from other countries agreed that the presence of non-essential genes could pose problems for large-scale release and commercial use of transgenic plants. This was one of several occurrences of the issue of the uncertainties involved in making the transition from tightly controlled small-scale tests to less controlled large-scale tests, and from there to commercial release.

15. The question of scale (relating to size and numbers in a test) in increasing the potential for gene transfer was raised for the use of selectable marker genes, such as antibiotic (e.g. kanamycin) or herbicide resistance genes. The potential for horizontal transfer of kanamycin resistance, for instance to soil bacteria, has been a subject of contention for several years. Both Mr. J.E.N. Bergmans and Mr. J. Schell presented results of scientific studies aimed at clarifying these questions. Apparently, no horizontal transfer has been detected, even using sensitive PCR techniques. The observations that kanamycin resistant bacteria are ubiquitous, and that this antibiotic is not used clinically, except to a limited extent for veterinary purposes, are also reassuring.

16. Herbicide resistance genes can be used as selectable markers, but in fact their use as markers poses other questions that remain more difficult to resolve. The source of concern is that herbicides are important in agriculture, and that it is hard to predict how vertical transfer (to progeny) could affect certain herbicide uses, such as elimination of volunteers during crop rotation. Mr. J. Antognini pointed out that herbicide resistance genes would not categorically decrease or increase herbicide use; it would depend on the individual case.

17. There was general satisfaction with the research being carried out concerning both the potential for horizontal transfer of kanamycin resistance genes and related effects and also the dispersal of introduced genes via pollen to either weedy relatives or to individuals of the same species in nearby fields.

18. During the two days, several speakers expressed concern for potential risks associated with expression of viral genes in plants, and the effects that such genes could have on virus populations. It was agreed that the question merits scientific investigation.

5. Final remarks

19. There was a general consensus among the participants that the seminar had been useful and enriching, and it was agreed that further seminars of this sort could be worthwhile if new issues of general importance were raised. However, no clear agreement was reached on what other issues might be discussed.

20. At the close of the seminar, the participants wished to conclude with statements describing the significant points of consensus that had been reached. The following were accepted:

1) There is a general consensus that the evaluation of the potential risk associated with field tests of transgenic plants requires a sufficient description of the genetic modifications of the plant. However, there are different views as to what constitutes a sufficient description of the genetic modifications.

2) The presentations made and the ensuing discussions indicated that there is no experimental evidence supporting a deleterious effect from the use of kanamycin resistance genes in transgenic plants.

3) Issues and controversies regarding herbicide resistance genes centre on the use and management of herbicides, rather than on the gene modifications themselves.

I. POTATO

General Presentation

Dr. P.J. Dale
Cambridge Laboratory
John Innes Centre, United Kingdom

1. Introduction

21. Potato belongs to the species {Solanum tuberosum} ssp. {tuberosum}, and originated from the Peru/Bolivia region of South America. There are ca. 2000 {Solanum} species and ca. 150 are tuber-bearing. Potato is tetraploid with a chromosome number of 48. It originated either from one diploid {Solanum} species or from a hybrid between two or more diploid species. Most wild diploid species are self-incompatible. Potato is self-compatible, with most of its seeds produced from self-pollination, but it suffers inbreeding depression and loss of vigour with prolonged inbreeding.

Species•••- {Solanum tuberosum} ssp. {tuberosum}
Origin•••- Peru/Bolivia
ca. 2 000 {Solanum} species
ca. 150 species are tuber-bearing
Polyploid series••- 24, 36, 48, 60, 72 chromosomes
Potato•••- tetraploid with 48 chromosomes
Originated from diploid ancestor

Several possibilities:•- {S. brevicaule}
••••- {S. leptophyes}
••••- {S. canasense}• All hybridise freely
••••- {S. soukupü}
••••- {S. sparsipilum}
••••- {S. vernei}

Originated by• •- chromosome doubling of one diploid
••• • species, or
••• •- hybridization and chromosome doubling

Breeding system

• Most diploids••- self-incompatible
• {S. tuberosun}••- self-compatible
• •••- seeds frequently from self-pollination
• ••• (inbreeding depression)

2. Propagation and survival

22. Potato is able to propagate and survive by the production of true seeds and tubers. True seeds give a means of generating genetic variability, and tubers, of clonal propagation and genetic uniformity. The number of true seeds produced by a potato crop depends on the potato variety, weather conditions, soil conditions, insect activity, etc., and can be as high as 160 million per hectare. Seeds generally have a dormancy period of six to 24 months and there are reports of survival over a seven-year rotation to the next potato crop. The number of groundkeeper tubers is dependent on the efficiency of tuber harvest and can be as many as 100 000 to 300 000 per hectare.

23. Survival of true seeds and groundkeeper tubers is influenced primarily by their depth in the soil and the temperatures during the winter months. Seeds and tubers survive less well when covered by several cm of soil, and tubers are killed by 25 hours at -2 C or 5 hours at -10 C.

24. Weediness of potatoes in subsequent cropping is generally controlled by cultivations and herbicide application (e.g. Glyphosate, aminotriazole) and is generally not a problem. Growth from seeds or groundkeeper tubers can cause difficulties after mild winters or in milder climates. The survival of true seeds in soil for several years may create problems with maintaining genetic purity in seed tuber crops.

{2.1 Method of propagation}

True seeds• -•generate new variation by genetic recombination
Tubers•• -•genetic uniformity

Europe ca. 1579 Spanish
USA ca. 40 years later
200 years -> Long days.

{2.2 Survival in agricultural crops}

Survival by• -•true seeds
• •• -•tubers

{2.2.1 True seeds}

In practice, the number of seeds varies with:

-- variety (Desiree, Maris Piper)
-- season (cool, humid)
-- soil conditions
-- insect activity (bumble bees)

Number of seeds possible:

-- 200 per berry
-- 20 berries per plant
-- 40 000 plants per hectare
-- total 160 million seeds per hectare

Dormancy period:

-- depends on variety
-- frequently six months (breeding GA3)
-- can be two years

{2.2.2 Tubers}

Number:

-- depends on efficiency of harvest

-- 100-300 thousand per hectare

{2.3 Survival depends on:}

-- depth in soil and winter temperatures

{2.3.1 True seeds}

Data on survival at different soil depths

Depth (cm)	Per cent of berries producing seedlings	Mean no. seedlings
2.5	62	10
5.0	9	6
7.5	<1	-
10.0	0	-

{Source}: Lawson (1980).

{2.3.2 Tubers}

Temperatures to kill tubers

- 25 hours at -2 C
- 5 hours at -10 C

{Source}: Lutman (1977).

{2.4 Weediness under agricultural conditions}

Survival of true seeds/groundkeeper tubers depends on:

-- number present in soil
-- depth in soil
-- exposure to low temperatures
-- other

Controlled by:

-- cultivation
-- herbicides (glyphosate, aminotriazole)
-- competition from subsequent crop

Evidence of survival of true seeds:

-- survival over a seven year rotation

- ware tubers: four year rotation;
- seed tubers: seven to eight year rotation

-- problem of genetic purity of seed tubers

Weediness in natural habitats:

-- does not appear to be a problem

Presence of related species:

-- {S. nigrum}
-- {S. dulcamara}

3. Cross-pollination with related species

25. Potato is cross-compatible with its relatives in South America, but there is little or no evidence of sexual compatibility with commonly occurring {Solanum} species in temperate regions. There are a few reports of attempts to pollinate potato with common solanaceous species, e.g. tobacco, petunia, {Datura}, but successful hybridization is extremely unlikely. Experiments with the common weeds of potato {S.} {nigrum} and {S. dulcamara} have failed to give viable hybrid plants.

4. Genetic transformations and field releases

26. To date, the field releases with transgenic potatoes in Europe and the United States fall into four groups: 1) herbicide resistance (phosphinothricin acetyl transferase); 2) insect resistance (Bt); 3) virus resistance (X,Y, leaf roll); and 4) various marker genes (GUS, NPTII, etc).

{4.1 Genes inserted and the number of field trials (approximate number)}

Plant character	Europe (90)	USA (3/92)
Herbicide resistance		
PAT	13 (B,F,I,NL,UK)	
bromoxynil	1 (ID)	
2,4-D	1 (ID)	
Insect resistance		
Bt	5 (B,S)	7 (IL,WA,OR,WI,ME)
"insect resistance"	1 (UK)	
Virus resistance		
virus X	4 (IL,ID,WA)	
virus Y	4 (IL,ID,WA)	
leaf roll	7	
	(IL,ID,WA,FL,ME,MI,WI)	
Other disease resistance		
chitinase	1 (WI)	
Modified product		
carbohydrate enzyme	3 (ND,ID)	
Marker genes and miscellaneous		
Patatin - GUS	3 (UK)	
"marker"	2 (ID)	
"larval serum protein"	1 (ID,ME,MN)	
"metabolic enzyme"	4 (WI)	
"pea DRRG"	1 (WA)	
"chicken lysozyme"	1 (ID,ME,MN,ND)	
"cecropin"	2 (ID,ME,MN,ND)	
"stress alleviation enzyme"	1 (WI)	

{Note}: Abbreviations in brackets show names of countries in Europe or names of states in the United States.

{4.2 Risks and concerns}

Effect of introduced genes on weedinesss of potato (true seeds or groundkeeper tubers)

{Herbicide resistance} -- several herbicides used for control

••• -- could be problem if several different resistance genes were introduced

{Virus resistance}• -- may give slight selective advantage

••• -- unlikely to give significant advantage

{Insect resistance} -- may give slight selective advantage

••• -- unlikely to be significant in agricultural setting

{Other}

5. Risks and concerns identified

27. Of the transgenes inserted into potatoes to date, it is unlikely that any will confer a sufficient advantage to make true seeds or groundkeeper tubers a greater weed problem. If several herbicide resistance genes are introduced into potato, it could restrict the choice of herbicides for controlling growth in subsequent cropping. The incorporation of virus resistance would have the advantage of preventing/reducing the carry-over of the corresponding virus disease from one year to the next by survival of potato tubers over winter.

28. A comparison will be made of procedures adopted over five years of field trials with transgenic potatoes in the United Kingdom. Some procedures have been relaxed through more detailed consideration; others have changed little over that time. Recent data on the distance of pollination in field experiments and on sexual compatibility with other {Solanum} species can perhaps be used to redefine some of the requirements for field trials with transgenic potato and help to define a general approach to handling transgenic potato experiments and field trials with potential new varieties.

{5.1 Conclusions}

{5.1.1 Distance of pollination}

Distance	Percentage of outcrossing
0 m	24
0-3 m	2
10 m	0.017
20 m	0

{5.1.2 Cross-pollination}

Solanaceous weeds

-- {S. nigrum}
-- {S. dulcamara}
No evidence of cross-pollination

{5.1.3 Genes inserted into potato}

Unlikely to make potato
-- more persistent - agriculture
-- more invasive - nature

{5.1.4 Are we moving to a standardized approach for potato?}

{5.2 Field releases in potatoes in the United Kingdom}

Procedure	1987a	1988a	1989a	1990a	1991b	1992c
	Pat-gus	Pat-gus	Pat-gus	Herb. tol.	Herb. tol.	
Transport of plants to site in secure containers	yes	yes	yes	yes	yes	yes
Disinfect equipment	yes	no	no	no	no	no
Nearest potato plot (m)	600	500	500	400	200	5
Control of aphids, viruses and blight	good practice	good practice	good practice	good practice (record)	good practice (record)	good practice (record)
Removal of flower buds	yes (1d)	yes (3d)	no	no	no	no
Removal of berries	n/a	n/a	yes (3d)	when mature	when mature	no
Removal of sola-naceous weeds	n/a	n/a	within field	within 100m	within 100m	no
Tubers dug by hand	yes	yes	yes	transg. only	transg.	machine
Tubers stored securely	yes	yes	yes	yes	yes	yes
Tubers destroyed by	burial (1.5m)	burial (1.5m)	burial (1.5m)	burial (1.5m)	steam-ing	burial (1m waste tip)
Fallow period	12 mth	12 mth	12 mth	12 mth	none	none
Site monitoring after trial	1+3 yrs	1+3 yrs	1+3 yrs PLUS (15m)	1+2 yrs PLUS	3 yrs PLUS	3 yrs
No potatoes on site for	3 yrs	3 yrs	3 yrs	3 yrs	3 yrs	4 yrs

a Institute of Plant Science Research
b Scottish Crops Research Institute c Nickersons

Note: no space suites
no special fencing
no hazard notices
moderately restricted access

6. References

{6.1 General}

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Risk Identification and Risk Management of Field Trials with Potatoes in The Netherlands

Prof. P. De Haan
Provisional Committee on Genetic Modification
The Netherlands

1. Summary

29. In the Netherlands, the competent authority for the endorsement of field trials with genetically modified organisms is the Minister of the Environment. The Provisional Committee on Genetic Modification (VCOGEM) advises the Minister on the risk and the risk management of the field trials (Figure 1).

30. The endorsement of the Minister for a field trial and the final endorsement for placing on the market are the first steps in the commercialisation of a GMO. Other agencies can, at a later stage, look at the GMO from a different point of view, for instance, whether the GMO is safe when used as food for animals or man.

31. This system has been operational for more than two years and it works satisfactorily.

32. In general, the Committee discusses two issues:

a) Does the GMO pose a risk to human health or to the environment?

- The items to be reviewed are the characteristics of the host, the nature and the function of the insert, and the characteristics of the site.

b) Can the trait (insert) be transferred to other organisms and can these organisms pose a risk to human health or to the environment?

- The item to be reviewed is the potential gene flow to wild relatives of the GMO. If no data is available on the gene flow, the contents of the Dutch State Herbarium are examined for the presence of hybrids and, if necessary, the gene flow is studied under experimental conditions in a greenhouse.

33. It is the aim of the Committee to give the applicants, as early as possible, an idea of the trial conditions and of the data needed for modifying risk management and moving from small-scale to large-scale field trials. For example, if the alkaloid content is determined and the Committee feels that the amount of alkaloids is acceptable, the Minister of the Environment can determine that, in the following year, the clipping of flowers will no longer be necessary.

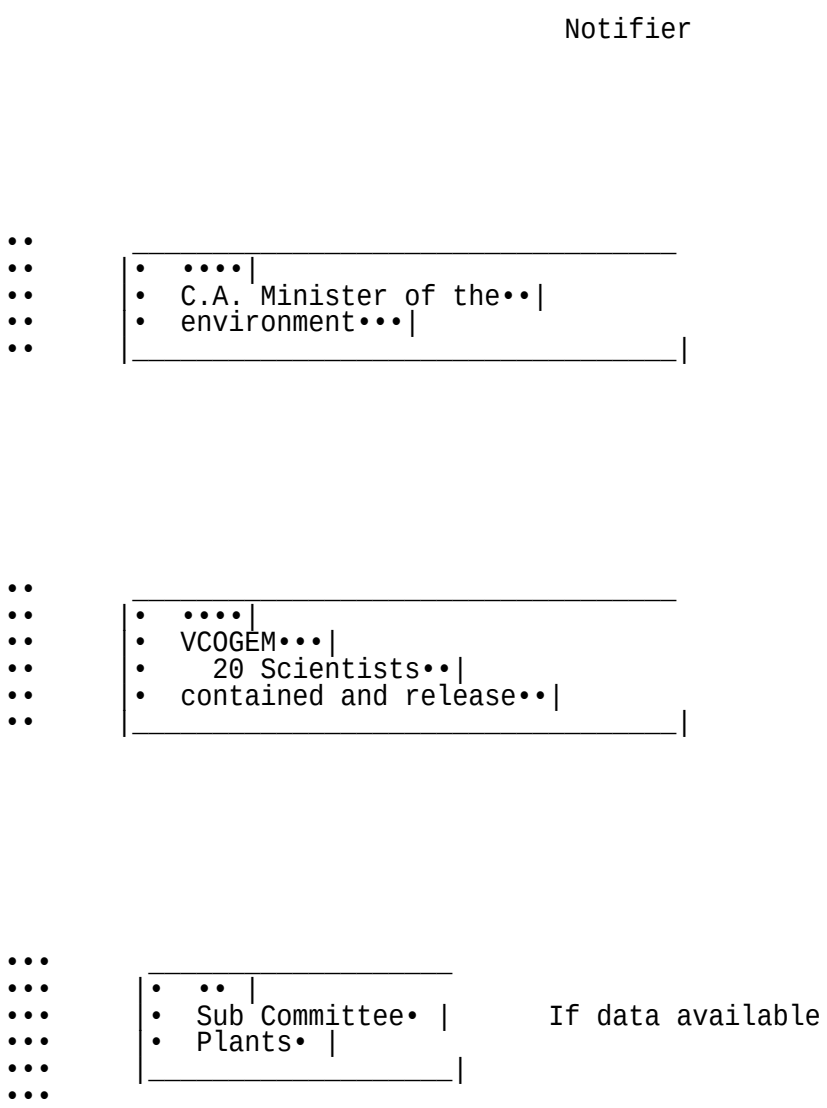
34. Table 1 summarises the present situation in the Netherlands. The first column contains the various traits inserted into {Solanum tuberosum}, the second summarises the safety considerations for the small-scale field trials, the third presents the trial conditions and the fourth indicates the considerations to be addressed for scale-up.

Table 1. Examples of risk management in the Netherlands

Trait * Insert	Safety Considerations	Trial Conditions	Up-Scale Considerations
Virus Resist. * PVX * PLRV	- Virus Rec. * 1STYR: R.I. * + Cage		- Virus Rec.
Herbicide Resist. * BASTA	- (Toxic. Cons.) * 1STYR: R.I.		- Toxic. Cons. - INCR. HB. USE
Insect Resist. * CRYI(A)/BT	- * 1STYR: R.I.		- Resistance Against BT
	- (Effect on non-target insects)		- Effect on non-target insects
Bacteria Resist. * Apeadicine IB * Cecropine B	- (Effect on beneficial soil M.O.) * 1STYR: R.I.		- Effect on beneficial soil M.O.
Fungi Resist. * Osmotine II	- (Effect on beneficial soil M.O.	* 1STYR: R.I.	- Effect on beneficial soil M.O.
Antibiotic Resist. * NPT II,HPT,CAT			
Non Select Mark. * GUS (-INT)			
Starch Composit. * A.S. cDNA	- (Frost Sens.) * 1STYR: R.I.		- Frost Sens.
Diminished Bruise * A.S. cDNA	- (Alkaloid) * 1STYR: R.I.		- Alkaloid
S.Brevidens Fus. * Erwina Resist.	- (Alkaloid) - Volunteers	* 1STYR: R.I. * Post Trial	- Alkaloid - Volunteers

Abbreviations:
- A.S. = antisense
- 1STYR = first year
- R.I. = reproductive isolation
- INCR = increased

Figure 1. Field trial endorsement system in the Netherlands



2. Risk assessment

{2.1 Two fundamental questions:}

1) Does the GME, because of the modification, pose a risk to human health and the environment?

2) Can the genetic material of the GMO be transferred to other organisms, which, as a result, can pose a risk to human health or the environment?

{2.2 General issue}

35. Does the intended release pose an unacceptable risk to human health or the environment?

{Poses a risk -> risk assessment}

Two elements:

1) Identification of a potential to cause harm (e.g. hazard identification)

2) Evaluation of that potential (likelihood, possibilities for management, etc.)

{2.3 Items to be reviewed}

1) Characteristics of the GMO, derived from relevant characteristics of:

- -- the host organism(s)
- -- the trait/insert(s)
- and from empirical data on the GMO from earlier stages.

2) The circumstances of the intended release.

3. Case study: Potatoes

AD 2) Gene transfer

- -- Indirect approach: Herbarium, field surveys of the PPS
- -- Direct approach: Reciprocal crossings, followed by techniques such as embryo rescue.

{3.1 Conclusion}

36. In the Netherlands, under natural circumstances, potatoes only cross-fertilize with potatoes.

{3.2 Examples of traits/inserts introduced}

Virus resist.

Herbicide resist.

Insect resist.

Bacteria resist.

Fungi resist.

Antibiotic resist.

Non select. markers

Change in starch composition

Diminished bruise sensit.

Erwina Resist. (Fus. Prod.)

US Evaluations of Field Tests of Transgenic Potatoes

Dr. Michael Schechtman
United States Department of Agriculture (USDA)
Animal and Plant Health Inspection Service (APHIS)
Biotechnology, Biologics, and Environmental Protection (BBEP)

1. General review

37. The USDA has been reviewing proposed field tests with transgenic potatoes for three years as of April 1992. About 30 field tests involving transgenic potatoes in the United States have been approved, of which six have been renewals of previous tests; one approval was for a three-year field test. About another ten are pending.

38. Field trials in the United States have included tests on potatoes carrying genes conferring:

-- insect resistance (carrying delta-endotoxin from {B. thuringiensis} ssp. {tenebrionis} against Colorado potato beetle: six tests);

-- metabolic changes (e.g. altered sugar/starch content, changes in bruising responses in potato: eight tests);

-- herbicide tolerance (bromoxynil resistance, 2,4-D resistance: two tests);

-- virus resistance (resistance to potato virus X, potato virus Y, potato leaf roll virus: ten tests);

-- bacterial or fungal disease resistance (e.g. carrying genes encoding cecropin, lysozyme, chitinase, disease response genes: five tests);

-- marker genes (kanamycin resistance or beta-glucuronidase: two tests).

39. Evaluations of the safety of the modified potatoes are based on environmental analyses involving careful review of the biology of potato as well as an examination of the modification introduced into the plant. They are designed to protect American agriculture against plant pests. Approval of an application means that the test has no significant potential for plant pest risk to the environment.

40. The reviews are carried out in the context of the considerable existing knowledge about potato as a crop. This biological information is essential for deciding what kinds of safeguards are vital, important, or irrelevant.

Whatever the modification made in the organism, however, field testing is being carried out with organisms which retain the fundamental biological properties of potato.

41. The features of potato biology that are relevant to applications for the field testing of transgenic potato are:

-- Potato is a solanaceous plant in the same family as tomato, tobacco and nightshade.

-- Potato can be propagated asexually, by planting pieces of seed tubers that contain "eyes", i.e. lateral buds.

-- Commercial potatoes destined for market are propagated exclusively asexually, i.e. clonally, through tuber propagation.

-- Potato can also produce fruit, i.e. berries.

-- In two separate studies, (the PROSAMO studies in Europe, and the studies on transgenic potato in New Zealand), evidence has recently been adduced that potato pollen, which can be insect-transmitted, moves only over very short distances. Beyond ten metres, no pollen movement was detectable in tens of thousands of plants screened.

-- Potato fruit generally arise by self-pollination, although interbreeding will occur if different potato cultivars are interplanted in the same field.

-- Potato seed is often used for breeding purposes and for elimination of viral infection, which can be a problem in clonally propagated potatoes.

-- Potato plants are noted for their sterility, both male and female. This causes difficulties for potato breeding. Most commercial cultivars (including Russet Burbank, which accounts for almost 40 per cent of US potato production) are sterile. For those cultivars that are male-fertile, high summer temperatures reduce pollen viability.

-- Survival of both potato fruit and potato tubers is temperature-dependent. In many of the locations where potatoes are grown in the United States, winter conditions are almost always sufficiently harsh that neither fruit nor tubers are known to overwinter. One needs, however, to account for both of these propagules in order to ensure containment.

-- The potato has the richest genetic resources of any cultivated plant, and these genetic resources are generally easily incorporated into fertile cultivars. The center of origin for the genetic variability of potato is in Andean South America. In the United States, the potential sexually compatible relatives of a transgenic potato would be other cultivated potato, and a few wild members of {Solanum} section {Tuberarium} that occur in the southwestern United States.

-- Potato has a series of ploidy levels, based on a haploid number of 12, ranging from diploid (2n=24) to hexaploid (6n=72), and including triploid, tetraploid, and pentaploid. Cultivated potatoes are autotetraploid (4n=48); many wild species are diploid, but may range up to hexaploid. Diploid potatoes usually will not cross with tetraploid potatoes, but bridging crosses, ploidy doubling, and somatic cell hybridisation can produce hybrids.

-- Although there is no evidence to suggest that such an event might happen, some experiments have been done to see whether there is any evidence for horizontal gene transfer to plant-associated bacteria. In the European Community, experiments have been performed to see whether genes could be transferred to {Erwinia} soft rot bacteria from potato. No evidence of gene transfer was found in these experiments.

42. In USDA reviews of proposed field trials involving genetically engineered potato, a series of factors are evaluated before it is certified that a given field test will not pose a risk to agriculture or a risk of plant pest introduction. These factors, and how applicants need to address them, will be discussed below. The USDA approach is to compare the engineered potato with its traditional counterpart in the environment into which it is to be introduced and to consider the agricultural practices that will be employed. It is not assumed that the engineered organism is dangerous, and must be proved "safe". Rather, the aim is to make the best, scientifically informed, decision about a proposed field trial.

43. When USDA safety evaluations are performed, a number of factors are considered relevant. They are discussed below.

2. Environmental consequences

44. In evaluating the environmental consequences of a particular field trial, potential impacts of the field tests on the human environment are addressed, in accordance with the USDA's environmental responsibilities under law (the National Environmental Policy Act -- NEPA).

{2.1 Effects on non-target organisms}

45. Are any potential effects on animals or plants, e.g. from a potato plant carrying a herbicide tolerance gene or a delta-endotoxin gene from {Bacillus } {thuringiensis}, limited to the field site itself? If there were any reason to think that a harmful metabolite or toxin would be transmitted out of the site or have very serious effects on-site, there would be considerable concern. By law, effects on organisms that are listed by the US government as threatened or endangered must be considered, and a field trial that will have significant adverse effects on any such organism cannot be allowed.

{2.2 Effects on agricultural practice}

46. Will the field trial itself have any effect on general agricultural practice involving potato? Will this test alone change agricultural practice? It is important to realise that the relevant question is not, "If the test is

successful, and the transgenic organism is commercialised and widely adopted by farmers, will there be any effect on agriculture?" Each test is a research endeavour. So far, no transgenic potato field test would have any noticeable effect on agricultural practice. At some later date, the effects of genetically engineered cross-protection on disease diagnosis in particular transgenic potato cultivars may change agricultural practice, but the changes are not yet relevant. Since many of the molecular probes for disease diagnosis being used or in development involve immunological reagents that detect viral coat proteins, new reagents, which would be applicable for plants that are virus-resistant but express a viral coat protein, would need to be developed.

{2.3 Effects on disease or pest susceptibility}

47. Will the field test affect the susceptibility of potato or any other neighbouring crop to disease or pests? Bearing in mind the same limitations as before, no application for a field test involving transgenic potato to date would have such effects. However, there are concerns regarding the possibility that novel viral types and/or novel vectors for plant viruses could be inadvertently introduced through the use of genetically engineered cross-protection in crop plants, and that, by any of several potential mechanisms, novel hybrid viruses (either hybrid by virtue of having hybrid DNA or by having the genome of one virus and the protein coat of another) or virus carried in new hosts could be created. This is a concern for luteovirus and potyvirus diseases of potato. At present, on the basis of the frequency with which such events could conceivably occur, the data regarding the field viability of hybrid viruses that have been created in the field, and the likelihood that such hybrid-creating events are not different from natural multiple infection events in the field, this has not been considered a significant factor for controlled field trials. However, applicants have been advised that it might be useful for them to develop field data addressing this point if they plan to commercialise such a variety.

{2.4 Effect on human health}

48. There must be no reason to believe that the conduct of the test will have any deleterious effect on human health. The use of toxic pesticides in the United States is regulated by the US Environmental Protection Agency, and any uses of added chemicals in the conduct of the field trial must be in accordance with EPA requirements.

3. Confinement of the transgenic organism

{3.1 Destruction of vegetative material, including tuber disposal}

49. At most field sites, vegetative plant parts above ground are either incorporated into the soil directly, or, more commonly, treated with herbicide for vine kill before harvest. The tuber material requires more consideration. Transgenic potato may be spread outside the confined field test if the tubers

are physically removed from the site. Therefore, all tubers must be accounted for. All those that are harvested must either be destroyed or stored in a secure facility. Destruction off-site may involve chemical treatment, autoclaving, or freezing. On site, tubers may be destroyed in the ground in the natural course of winter freezing and thawing, if there is a general consensus that the climate is sufficiently severe to guarantee tuber kill. In addition, the site will be monitored for the appearance of volunteers. It may be advantageous to spread the potatoes on the surface of the soil to facilitate their devitalisation, but only if there is no risk that the potatoes will be picked up and transported to another site by unauthorised individuals, or used as food. Excess tubers can also be destroyed by composting. At those sites where the weather is not sufficiently severe to kill tubers during the winter, the following regimen is allowed: rotating with corn during the following two winter seasons and leaving the field fallow but treating with herbicides during the summer seasons. In addition, the fields are monitored for two seasons for the appearance of volunteer potato plants; if volunteers arise, they are removed and destroyed as before.

{3.2 Pollen containment}

50. For varieties such as Russet Burbank, which are male-sterile, potato pollen is not produced and pollen containment is irrelevant. For varieties such as Lemhi Russet, which produce viable pollen, this pollen is not known to move beyond a few metres from test plants. Therefore, while no interplanting of transgenic potato and potato destined for commerce is allowable, field separations employed in normal agronomic practice, i.e. several metres to allow for the easy movement of farm equipment, are adequate to ensure pollen containment during a field trial. Most applicants surround their plots or subplots with border rows of nontransgenic potato to eliminate "edge effects" in their experiments.

{3.3 Potato berry destruction}

51. Because pollen dissemination by potato is so limited, berry production involving transgenic pollen will essentially be limited to the plants involved in the field trial itself, i.e. self-pollination or pollination of neighbouring plants. For those varieties that are male-sterile, no pollen will be available to produce viable fruit. Also, at most field sites in the United States, harsh winter weather precludes survival of potato fruit to the next growing season. At the field site itself, monitoring for, and destruction of, volunteer potato takes care of any concerns regarding potato berries. The regimen described for tuber accountability suffices here as well.

{3.4 Food use and entry into commerce}

52. None of the test potatoes are to be sold as food or are to otherwise enter into commerce while the test organism is under permit.

4. Oversight

{4.1 Site selection and security}

53. The integrity of the field site must be verified. Is the site sufficiently isolated so that unauthorised individuals are not likely to come on site? If not, will access be restricted to prevent unauthorised entry? Is care being taken to ensure that the test is not demarcated in the field in such a way as to attract vandals? (This has not proven a problem in the United States).

{4.2 General monitoring}

54. Will the site be regularly visited by responsible parties? Sites are visited at least once a week, and more frequently if possible. General monitoring examining the general agronomic properties of the test organisms, verifying that morphology and behaviour are as expected (including somaclonal variation, which is a real issue for potato), and checking for disease/weed infestations, nutrient status, growth, dryness, etc. As with all field trials approved, the field site is also to be monitored during the course of the following year for volunteers.

{4.3 Inspection}

55. Sites are visited by USDA inspectors to verify compliance with the terms of the permit near the start of the field trial, and may be visited more frequently. Inspectors are to be notified at least one week before the termination of the trial, and in some instances may be present at the termination of the field trial.

5. Summary of the results

56. The following is a summary of the results:

- -- All the tests were based on the use of disarmed {Agrobacterium} vectors. Details of the construct were examined to verify that they were indeed disarmed, and there has been no evidence of any problems with {Agrobacterium} infections at the test sites.
- -- There has been no unexpected survival of plants; also, volunteers did not appear at sites where winter weather was expected to kill tubers.
- -- There has been no unexpected movement of genetic material off test sites.
- -- No unexpected phenotypes have appeared in the transgenic potato, outside the range of what has been expected for the induced modifications plus somaclonal variation.

-- Somaclonal variation is known to be a problem for potatoes put through tissue culture, since tissue culture frequently induces aneuploidy and other anomalies in potato. For potatoes, about half the Russet Burbank marker gene transformants planted in the field have the quality and agronomic properties of the parental variety. {Several generations of testing with field-grown transgenic seed are } {necessary, however, to identify those clones that maintain the } {quality in the subsequent planting}. (This result has been observed using plants obtained via transformation of microtuber slices. Researchers have indicated that, using potato protoplast regenerants, one gets essentially no normal plants regardless of whether or not there has been DNA uptake.)

-- Levels of marker gene expression in Russet Burbank appear to be stably maintained through three seasons (two field seasons).

57. Field data continues to be produced as the tests proceed. All of the data are useful; most are predictable, but occasionally an unexpected bit emerges. The more data available, the easier it becomes to eliminate organisms about which the evidence is very reassuring, to continue to simplify requirements where this is scientifically justified, and to focus on organisms or field trials which raise issues that merit specific concern.

Monitoring "Escapes" from Field Trials of Transgenic Potatoes: A Basis for Assessing Environmental Risks

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1. Introduction

58. The "escape" of viable plant material carrying foreign genes is considered to be a potential problem in the initial field testing of transgenic plants. In crops such as potatoes, this may result from gene transfer via the dispersal of pollen to other potato crops or to related weedy species, and from volunteer plants from tubers left in the ground following the harvest of previous trials. Since 1988/89 nine regulatory approved field trials involving male fertile transgenic potatoes have been performed in New Zealand. A major component of these trials involved monitoring the sites for "escapes" of transgenic plant material. The results are summarised here in order to provide data on which informed decisions can be made about the environmental risks of field trials of transgenic plants.

2. Pollen dispersal from transgenic potatoes

59. Pollen dispersal was monitered by screening the progeny of wild-type (i.e. non transgenic) plants growing within and around the trials for the expression of the foreign genes. All flowers that developed on the wild-type potatoes before the transgenic plants started flowering were removed and destroyed. All berries that subsequently developed were hand picked upon maturity, seeds extracted and germinated seedlings screened for transgenic individuals. The most useful data originated from field trials of potatoes resistant to the herbicide chlorsulfuron (these plants were also transgenic for kanamycin resistance and •-glucuronidase activity). For these field trials, potato seedlings were screened {in vitro} for resistance to 50•g/1 chlorsulfuron, and their transgenic status was confirmed by assaying for •-glucuronidase activity.

60. In a 1988/89 trial of chlorsulfuron-resistant potatoes (see Table 1), the frequency of transgenic seedlings from wild-type potato plants growing within the trial was about one per cent, whereas up to 4.5m from the trial only five in 10 000 progeny were transgenic. Transgenic progeny were not recovered from wild-type plants growing 4.5 - 10m from the trial. In a 1989/90 trial of the same transgenic lines (see Table 2), only 5 in 10 000 of the progeny from wild-type plants within the trial were transgenic. From over 250 000 progeny

of wild-type plants growing around the trial only nine transgenic seedlings were recovered, all from rows 3.75m or 6.0m west of the trial. It is possible that these transgenic progeny originated from single berries in each row, thereby representing only 2 pollination events.

61. During field testing of other transgenic potatoes in 1988/89 and 1989/90, there was only limited seed set due to late planting dates which hindered flower and berry formation and/or somaclonal variation in some of the transgenic lines resulting in non-flowering phenotypes. In these trials the seedling progeny were screened for resistance to 50mg/1 kanamycin. Only minimal pollen dispersal onto wild-type plants within these trials was detected, with no transgenic progeny originating from wild-type plants surrounding the trials (Table 3).

3. Gene transfer to black nightshade

62. Seeds were harvested from black nightshade ({Solanum nigrum}) plants growing among transgenic chlorsulfuron-resistant potato lines in a field trial (these potato lines also expressed kanamycin resistance and •-glucuronidase activity). All black nightshade plants with berries growing in plots not sprayed with chlorsulfuron contributed to the seed sample. These seeds were germinated and screened for resistance to 100mg/1 kanamycin.

63. No evidence for gene transfer from potato to black nightshade was observed, despite the screening of nearly 54 000 seedlings (Table 4). Twenty-five putative kanamycin-resistant seedlings were identified with longer roots than usual on kanamycin selection medium. However, all of these proved to be negative for •-glucuronidase as assessed via histochemical staining and were therefore considered to be rare escapes through the seedling screen.

4. Appearance of volunteer potatoes

64. The field trial sites were continually monitored throughout subsequent growing seasons for the appearance of volunteer potato plants. Relevant data on the appearance of the volunteers is presented in Table 5. The 1988/89 field trials were harvested by mechanical lifter and hand picking.

65. In the 1989/90 field trials only the buffers were harvested in this manner, with the plants within the trials being hand dug and picked. The density of volunteers at both the trial sites for transgenic potatoes (2.9/m• and 1.3/m•) was low compared to figures commonly reported for potato volunteers.

66. The proportion of volunteers that were transgenic was substantially lower than the proportion of transgenic plants in the field trials. This may have resulted from the low tuber production in many of the transgenic lines, and/or the greater care being taken over the harvest of plots within the trial compared to the buffer rows. The substantial reduction in the proportion of transgenic volunteers following the 1989/90 trial would appear to be primarily a consequence of hand digging the plots within this trial compared to mechanical lifting of the buffer rows. The value of careful hand harvest to

reduce the appearance of transgenic volunteers the following season is also well illustrated by the ratio of transgenic volunteers to transgenic tubers harvested. Although some of this difference is no doubt related to the different sites and seasons being compared, the magnitude of the difference presumably reflects the importance of the harvesting approach.

5. Conclusion

67. The data collected from the monitoring of field trial sites of transgenic potatoes for "escapes" of transgenic plant material have clearly established negligible environmental risks from such preliminary field trials. The dispersal of pollen carrying foreign genes was limited to within 6m of the trials, and then only occurred at exceptionally low frequencies. Consequently, isolation distances between field trials of transgenic potatoes and other potato crops do not have to be great to mitigate gene transfer via pollen. Gene transfer to weedy species such as black nightshade was not detected, despite screening a large seed population originating from plants growing among the transgenic potatoes. Although volunteer transgenic potato plants do appear at trial sites in seasons following the initial field trial, they can be easily managed and eliminated.

Table 2: Dispersal of transgenic pollen from a field trial (22 m x 13.5 m) of chlorsulfuron-resistant potatoes (cultivar Iwa) in 1988/89

Distance from trial	Number of seedlings screened	Number of seedlings transgenic(1)	Proportion of seedlings transgenic (%)
Within trial	4 476	51	1.14
0.0 - 1.5 m	12 946	4	0.03
1.5 - 3.0 m	16 716	9	0.05
3.0 - 4.5 m	11 209	6	0.05
4.5 - 6.0 m	15 212	0	0.00
6.0 - 10.0 m	822	0	0.00
Total	61 381	70	0.11

{Note}:

1. 49 of these seedlings were tested for •-glucuronidase activity, and all were positive.

{Source}: Tynan {et al}. (1990), {Journal of Genetics and Breeding,} Vol. 44, pp. 303-305.

Table 3. Dispersal of transgenic pollen from a field trial (88 m x 24 m) of chlorsulfuron-resistant potatoes (cultivar Iwa) in 1989/90

Distance from trial		Number of seedlings screened(1)	Number of seedlings transgenic(2)	Proportion of seedlings transgenic (%)
Within trial		54 213	25	0.046
North	0.0 - 1.5 m	24 579	0	0
	1.5 - 3.0 m	27 267	0	0
	3.0 - 4.5 m	23 122	0	0
	4.5 - 6.0 m	12 905	0	0
	Total	87 873	0	0
East	0.0 - 1.5 m	11 543	0	0
	1.5 - 3.0 m	13 755	0	0
	3.0 - 4.5 m	10 231	0	0
	4.5 - 6.0 m	10 188	0	0
	6.0 - 7.5 m	4 306	0	0
	Total	50 023	0	0
South	0.0 - 1.5 m	16 744	0	0
	1.5 - 3.0 m	9 032	0	0
	Total	25 776	0	0
West	0.0 - 1.5 m	6 595	0	0
	1.5 - 3.0 m	5 902	0	0
	3.0 - 4.5 m	3 155	8	0.254
	4.5 - 6.0 m	12 461	0	0
	6.0 - 7.5 m	23 375	1	0.004
	7.5 - 9.0 m	11 556	0	0
	9.0 - 10.5 m	25 603	0	0
	Total	88 647	9	0.010
Grand total		306 532	34	0.011

{Notes}:

1. Based on a 1 000 seed weight of 1.132 g and 96 per cent seed germination.

2. All of these seedlings were •-glucuronidase positive. Thirty-four additional seedlings were putatively identified as chlorsulfuron-resistant, but were later considered to be escapes since they were found to be •-glucuronidase negative. The accuracy of the seedling screen was confirmed by assaying seedlings identified as chlorsulfuron-sensitive for •-glucuronidase activity. As expected, over 4 000 such seedlings (20-30 seedlings from each of 200 randomly chosen pottles) were all •-glucuronidase negative.

Table 4. Absence of pollen dispersal from other small-scale field trials of potato

Field trial	Position within or around trial	Number of progeny screened	Proportion of progeny transgenic
Kanamycin-resistant (1988-89)	Wild-type Rua plants within trial	22 940	0.06%
	Wild-type Iwa plants within trial	19 037	0.34%
	Wild-type Ilam Hardy plants within trial	34	0
	Wild-type plants around trial (up to 10 m)	3 629	0
Hygromycin-resistant (1988-89)	Wild-type plants within and around trial (up to 5 m)	0	0
Kanamycin-resistant (1989-90)	Wild-type Rua plants within trial	8 406	0
	Wild-type Iwa plants within trial	3 382	0
	Wild-type Ilam Hardy plants within trial	664	0
	Wild-type plants around trial (up to 9 m)(1)	0	0
Chlorsulfuron-resistant (1989-90)	Wild-type plants within and around trial (up to 9 m)(1)	0	0
Thaumatin producing (1989-90)	Wild type Iwa plants within trial	259	0
	Wild-type plants 5.25 m east of trial	233	0
	Wild-type plants around trial (up to 9 m)(1)	0	0

{Note}:

1. The wild-type plants around these trials were male sterile potatoes, incapable of self pollination. Consequently, seeds could only develop on these plants following dispersal of pollen from within the trials.

Table 5. Absence of gene transfer from transgenic potatoes (cultivar Iwa) to black nightshade ({Solanum nigrum}) plants.

Associated potato line	Number of black nightshade seeds collected(1)	Number of seedlings screened(2)	Number of seedlings transgenic(3)
SC11	35 139	18 272	0
SC12	30 686	15 957	0
SC14	37 861	19 688	0
Total	103 686	53 917	0

{Notes}:

1. Based on 1 000 seed weight = 0.918g

2. Based on 52 per cent germination of the seed sample

3. 25 putatively "kanamycin-resistant" seedlings were identified; all were •-glucuronidase negative.

Table 6. Appearance of volunteers in the season following field trials of transgenic potatoes

A. Data on the field trials

Season of field trial	1988-89	1989-90
Area of trials	1 248 m	1 098 m
Number of transgenic plants	2 368	810
Number of wild-type plants as controls or buffers	2 242	2 946
Proportion of transgenic plants in field trial	51%	22%
Number of transgenic tubers harvested	32 543	5 759

B. Data on the appearance of volunteers the following season

Number of volunteers	3 615(1)	1 428
Density of volunteers	2.9/m	1.3/m
Proportion of volunteers transgenic	31%(2)	3%(3)
Estimated number of transgenic volunteers	1 121	43
Ratio of transgenic volunteers to transgenic tubers harvested	1:29	1:134

{Notes}:

1. 24 volunteers appeared at this site during the second season following the field trial (1990-91).

2. Assessed by screening leaf segments from a sample of the volunteers (n=128) for kanamycin resistance in tissue culture.

3. Estimated on the basis of tuber colour, since the majority of wild-type plants (i.e. the buffers) had red tubers, whereas plants from within the trials had white tubers.

II. OILSEED RAPE

General Presentation

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1. Background information on winter and spring oilseed rape

{1.1 Deliberate release in France}

Year•••	88	89	90	91	T
Submissions••	1	4	8	7	20

{1.2 Main objectives of the experiments}

{1.2.1 Research}

-- Risk assessment: pollen dispersal.
-- Experimental design to detect {in situ} production of androgenetic plants (using Ka+ transformed male-sterile genotypes).

{1.2.2 Improvement of cultivars}

-- Resistance to herbicides.
-- Resistance to fungus.
-- Improvement of quality (amino-acid) including transformation into cattle cakes.

{1.2.3 Hybrid seeds production}

-- Test of male sterility and restorer genes
-- Test of F1 hybrids.

{1.3 Methods of transformation}

-- Electroporation of protoplasts (2)
-- Agrobacterium rhizogenes (1)
-- Agrobacterium tumefaciens (17)

2. The approach of the CGB [Commission du Génie Biomoléculaire (Biomolecular Engineering Commission, France)] on how to handle trials with genetically modified oil seed rape

{2.1 General points}

{2.1.1 Related to the proposed experiments}

-- all experiments must be carried out according to the good practice guidelines (NF x 42071 Dec. 88);

-- all experiments are carried out under the juridical responsibility of the proposer;

-- multilocal experiments can be authorised, but a new submission is required each year for advanced generations;

-- the transformed plants must be characterised at the molecular level (insertion sites, right and left borders, selectable markers, etc.);

-- the toxicity that may occur as an effect of the newly produced molecule or the degradation products of the applied molecule;

-- the location, the area, the exposure of the field trial must be described;

-- special care is advised for transport of seeds.

{2.1.2 Related to the release}

-- Isolation distance from other rape fields: 400 m - 500 m (according to technical rules for seed production).

-- Biological containment: (buffer crops) 6 m borders of the same, but untransformed, cultivar prevent dispersal of G.M. pollen.

-- Related (intercompatible) spontaneous species likely to be present in the vicinity of the trial are determined. They are eliminated (5 m surrounding the trial):

- . by hand;
- . by herbicide treatment;
- . bt farming monocot species and applying antidicot herbicide.

-- Harvest can be carried out three or four days before complete maturation to prevent seed shedding.

-- Threshers are carefully cleaned.

-- Appropriate waste disposal is advised according to the mass to be eliminated, the characteristics of the G.M. plants, and the state of knowledge.

-- Controlling the plot and its surroundings after the release can be carried out by:

- . increasing germination of the remaining seeds with superficial ploughing followed by herbicide treatment;
- . leaving the soil unploughed for one year and destroying fresh growths before flowering;
- . cultivating monocot species and using antidocot herbicide.

{2.2 Specific points}

Specific points depend on the introduced genes:

-- the research on expression of the gene coding chitinase for fungus resistance has been associated with a specific project on the impact of chitinase on pollinators (honey-bees);

-- after trials with herbicide-resistant plants, the plots can be used to detect crops developing resistance to the same herbicide.

- (The mechanism of the resistance must be understood at the cellular level but is generally not crop-specific.)

{2.3 How was this approach developed?}

-- rape seed biology and farming are well known;

-- rape seed has been chosen as model crop for BAP and BRIDGE programmes on deliberate release;

-- following the advice of the CGB, the proposers have carried out experiments to determine how to control plots, release, etc.

Applying for field trials with genetically modified oilseed rape: Enhancing the nutritional value of oilseed rape

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68. As a plant biotechnology company, Plant Genetic Systems has been involved in field testing genetically modified crops since 1986. In particular, the first trial with genetically modified oilseed rape was performed in 1988. Focusing on the development of value-added crops, traits under investigation include insect resistance, stress tolerance, enhanced nutritional value and a complete hybrid system (male sterility and restoration), possibly in combination with selectable markers such as kanamycin resistance, hygromycin resistance and/or phosphinotricin resistance.

69. In addition to the actual development of potential lines of interest, emphasis has been placed on several aspects of assessing the safety of introducing a genetically modified oilseed rape line into the environment (PGS is co-ordinator of a safety assessment project in the EEC programmes BAP and BRIDGE, a contribution to the PROSAMO initiative).

1. General situation of the project

70. The seed cake resulting from the extraction of oil from {Brassica napus} could heretofore be used in animal feeds only in limited quantities. This limitation was in part due to the presence of antinutritional factors (glucosinolates). Using traditional breeding methodologies, lines have been developed in which glucosinolate levels are significantly reduced. A second limitation for its use in monogastric animal feeds has been the amino acid composition of the protein in rapeseed meal. In particular, increases in the levels of methionine, lysine, and tryptophan would make rapeseed meal more competitive with the meal from other species such as soybean (see Table 7).

71. In this project, 2S albumin storage proteins are applied using different strategies to increase the level of specific amino acids. 2S albumins are small storage proteins consisting of two subunits linked by disulfide bridges. In {Brassica} they contribute about 24 per cent of total seed protein. The 2S albumin of {Bertholletia excelsa} (Brazil nut) is unusual, in that it contains 18 per cent methionine. In one approach, this gene was transferred to {Brassica} using regulatory signals from a 2S gene of a closely related species, {Arabidopsis thaliana}. This was done to avoid the problems associated with understanding differences in expression between the multitude (12-16) of {Brassica} 2S albumin genes; in {Arabidopsis} only four genes are present.

72. The structure of the 2S albumin is such that it will tolerate certain compositional changes. No lysine-rich 2S albumins have been described, and thus a second strategy was used, with modification of existing 2S albumins. The construction was designed so as to minimise possible disruption to the normal structure.

Table 7. Enhancing the nutritional value of oilseed rape

Goal:

-- Improve relative amino acid composition of oilseed rape cake (methionine, lysine).

Strategy:

-- Target storage proteins rich in AA to seed meal.
-- Improve AA composition of storage proteins.

Tools:

{A. Functions}

-- Storage proteins
• 2S albumins - Brazil nut
• •• - Arabidopsis thaliana

-- Selectable marker: neo, hyg

{B. Expression systems}

-- Seed expression and storage
• 2S albumin Arabidopsis

-- Selectable markers
• pTR1/pTR2

{C. Vector system}

Plasmid / T-DNA borders
(remnant T-DNA sequences)

{D. Transformation system}

{Agrobacterium tumefaciens}

2. General outline of the trial programme

73. The aim of the field trial programme (see Table 8) was to evaluate the relative performance of the experimental lines under normal agronomical practices (in a comparison between the modified material, the unmodified basic material and a local check variety) and to yield sufficient material for subsequent detailed quality analysis.

74. Typically, a trial consisted of at least four repetitions of all variables (plots of 12 to 20 sq. m) (see Table 9). All basic operations (drilling, harvest) were performed using equipment applicable in traditional breeding. Additional containment at this experimental stage was based on a combination of netting of the trial site, border rows of control non-modified oilseed rape and spatial (>500 m) and/or temporal (spring versus winter varieties) isolation of any other {Brassica} crop. The standard observations applicable in oilseed rape breeding (germination; establishment; date of flowering; height; maturity; yield and post-trial quality analysis, such as glucosinolate content and composition; protein content; humidity; oil content; and fatty acid composition) were complemented by monitoring of the trial area during and after the actual trial [records of weed species, removal of (related) weeds, follow-up of volunteers].

Table 8: List of field trials included in agronomic evaluation of prototype nutritional value transformants

Year	Location	Tested gene isolated from
1989	Canada	{Bertholletia excelsa}
		{Arabidopsis thaliana}
	Sweden	{Bertholletia excelsa}
		{Arabidopsis thaliana}
1990	Belgium	{Bertholletia excelsa}
		{Arabidopsis thaliana}
	Canada	{Bertholletia excelsa}
	France	{Bertholletia excelsa}
	Sweden	{Arabidopsis thaliana}
	United Kingdom	{Bertholletia excelsa}
1991	Belgium	{Bertholletia excelsa}
	United Kingdom	{Bertholletia excelsa}

Table 9. Agronomic evaluation of oilseed rape with a modified seed storage protein

{Multi-site/Multi-year approach:}

Year	Country	# Transformed Lines
1989	Canada	2
	Sweden	2
1990	Belgium	3
	Canada	1
	France	1
	Sweden	1
	United Kingdom	1
1991	Belgium	1
	United Kingdom	1

{General design:}

-- Plots of 15-20 sq. m.
-- 4-6 repetitions/site
-- Open flowering

{Additional measures:}

-- Isolation from the other oilseed rape crop
-- Guard rows (2-6m.)
-- Bird netting
-- Harvest before full maturity
-- Monitoring
-- Authorisation by competent authority

{General design:}

{Conclusions:}

-- some delay in initial developmental stages due to production conditions of starting material;
-- evaluation of agronomic performance does not indicate any negative influence by the modification;
-- extensive quality analysis confirms that transformed line is true to type to the original line;
-- as predicted, no significant increase of specific amino acids was recorded;
-- no unexpected events have disturbed the trials and/or indicated hazardous situations due to the transgenic nature of the plants.

75. From a practical point of view, the entire design and implementation of the trial are quite similar to the procedures used in classical breeding: agronomy, isolation, observations, keeping track of the material both physically (during transport, at sowing, etc.) and by documentation (logbooks, fieldbooks, pedigrees, etc.). As many of the practices and experiments are applicable, the field person not only has to preserve the experimental material, but must also certify the absence of significant impacts from the trial and the material on the environment. Although most of the current procedures support bidirectional protection (trial -> environment and environment -> trial), this does impose a substantial increase of workload for all monitoring procedures (in and post-trial), and, due to the specific destruction methods needed, for the supplementary requirements on post-trial land use and the administrative paperwork involved (submission, safety report, etc.).

76. During scale-up to large-scale and commercial release, and on the basis of the increased safety record of the product, the stringent requirements need to be revised.

3. The regulatory procedure: applying for a field trial

77. All trials were performed upon receipt of approval by the competent national authorities. In all cases, submissions included the complete description of the lines to be tested (variety, genetic modification, identification of the lines) with a first product safety evaluation, the trial design and purpose, an environmental safety assessment with a description of the containment measures, the post-trial treatment and disposal of the material and monitoring intentions (the main components of the required data set of EEC directive 90/220). The information included in the applications was essentially the same for all countries, with differences merely arising from the use of specific data formats.

78. Indeed, the first hurdle the "trans-European" applicant was (and unfortunately still is) confronted with is the variety of "formats" used by the different member states, most of which undergo drastic changes over the years, so that keeping up with the latest version is not easy. Also, the legal framework of the different member states may require that an official document be in an official language. To meet with all these requirements, it is important that one is able to remain up-to-date and to dispose of excellent translation/word processing skills and facilities.

79. The procedure after the submission is again quite different from country to country: some require the presentation of the project at a commission meeting, others go through an administrative procedure and lengthy exchanges of mail. Further questions to the applicant were generally similar, but the specific emphasis depended on the commission or administration dealing with the submissions.

80. The following comparison concerns three "neighbouring" European countries, i.e. Belgium, France and the United Kingdom, and the experience of Plant Genetic Systems when presenting applications.

{3.1 Additional emphasis in France (Commission du Génie Biomoléculaire): } {molecular analysis}

81. A good definition of the experimental material is a starting point for the preparation of a field trial in France. This includes a complete description of all the stages leading to the obtainment of the final genetically modified line (vector construction, basic plant material, transformation procedure, regeneration, and selection) and a thorough molecular identification (number of inserts, segregation data, indications on the limitation of the insert). Depending on the stage of the experiment (comparing a first-time introduction with a full agronomic evaluation), the body of data supporting the molecular definition of the plants and their stability will be evaluated. All subsequent safety assessments are related to this basic knowledge of the newly created product (see Table 10A).

Table 10. Additional areas of emphasis in France, the United Kingdom and Belgium

A. Additional emphasis in France

{Molecular analysis}

-- Obtaining the GMO:

- -- vector construction;
- -- transformation;
- -- regeneration and selection.

-- Description of the GMO:

- -- copy number;
- -- insert identification and limitation;
- -- expression characteristics.

-- Stability of the GMO:

- -- phenotypical/genotypical;
- -- generations.

B. Additional emphasis in the United Kingdom

{Environmental interactions}

-- GMO related: how different is the "new" crop? (weediness).
-- Plant related: interactions with wild species.
-- Animals: direct and indirect influence (dispersal).
-- Humans: public health considerations and public-social aspects.

C. Additional emphasis in Belgium

{Monitoring capabilities}

-- Monitoring techniques:

- -- description;
- -- sensitivity.

-- Monitoring planning:

- -- frequencies;
- -- scope.

-- Monitoring results:

- -- in-trial;
- -- post-trial.

{3.2 Additional emphasis in the United Kingdom (Advisory Committee on } {Releases into the Environment):} { environmental interactions}

82. Environment-related questions are central to the evaluation by ACRE. These range from aspects of weediness of the genetically modified crop (dispersal, dormancy, etc.) to interactions with related wild species, interactions with animals and with the workers in the field. More broadly, this includes the people living in the neighbourhood of the trial and subsequent health and social concerns (see Table 10B).

{3.3 Additional emphasis by the Belgian authorities (Ministry of } {Agriculture): } {monitoring capabilities}

83. Detailed information is requested on the actual plans for monitoring gene and crop containment. What kind of methods will be used in and post-trial? What is the sensitivity of this method and how many plants can be screened? How will the post-trial land use be secure from excessive volunteers, and how will this be controlled? The monitoring of past trials (and of some of the safety assessment project) has indicated that the containment levels proposed do actually guarantee a reasonable limitation of the release area (see Table 10C).

{3.4 Conclusions}

84. The three different emphases indicate basic differences in approach. The Belgians stress the importance of controlling (by the applicant as well as by the authorities) what happens in reality and how well it fits with what was predicted. ACRE concentrates on identifying interactions and assessing the safety of the particular product in that respect. The CGBM looks at the basis of the new product itself -- the introduced genetic change -- as the essential element of the safety assessment.

85. For an applicant, it is interesting to be confronted at a very early stage with the diverse demands. The combination requires detailed analysis, safety research and good knowledge of the crop, which should form the basis for any development. But the same level of detail cannot be guaranteed for every individual regenerated transformant entering a field trial for a first evaluation. A gradual build-up of information, while collecting data and performing more detailed analysis, should make possible the safe development of agronomically acceptable products.

4. Small-scale field trials and beyond

86. Prototype lines continue to be used to study basic questions of stability of the storage proteins and nutritional aspects. In 1991, a first field production yielded approximately 600 kg. of genetically modified oilseed rape seed. This batch -- as well as a control batch -- was crushed in an experimental plant. A substantial amount of oil (for further detailed quality analysis) and cake was obtained and entered into animal nutrition trials. Each of the steps taken (transport, crushing, feeding trials, etc.) was notified to the competent authorities.

87. The field data and subsequent quality analysis of the first prototypes were the basis for fine-tuning subsequent developments. A first series of approximately 50 new lines were scheduled to be evaluated in the 1992 spring season, and the selected lines to be entered into agronomic multi-site evaluation in 1993.

III. MAIZE

Biology and Environmental Considerations

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88. The information in the following presentation outline is contained in the attached Environmental Assessment for a corn field test and list of permits issued for field testing by the Animal and Plant Health Inspection Servie, the United States Department of Agriculture.

1. US corn tests

21/214 tests as of 25 March, 1992

1988	1989	1990	1991	1992
1	1	2	15	2

Herbicide tolerance 10
Bt + Clavibacter 6
Markers 3
Value 2

8 states + Puerto Rico.

2. Taxonomy of corn

Family: {Graminae}
Genus: {Zea}

4 species:

{Zea mays} ssp. {mays} = corn - United States
{Mexicana} - teosinte
{Zea diploperennis}
{Zea luxurians}
{Zea perennis} - James Island

{2.1 Tripsacum}

7 species - 3 in the United States
n=9 vs. n=1
all cross with corn
1. extreme difficulty
2. extreme sterility

{2.2 Teosinte}

Annual, perennial
Mexico, Guatemala
Varying phenotypes
Corn x 2n, 4n = fertile F1

{2.3 Introgression}

-- flowering time
-- geographic separation
-- developmental morphology
-- timing
• reproductive structures
• dissemination
• dormancy

3. Morphology and reproduction

Monoecious annual
Similar to many grasses

1. pollen on staminate inflorescences
• eggs on pistillate
2. self- and cross-pollination can occur
• -- frequencies depend on physical factors and proximity
• -- sterility factors and growth rates of pollen tubes
3. pollen viable for 10-30 min.
4. inflorescences do not develop at the same time.

{3.1 Pollination - hybrid corn}

Inbred lines• - self-pollination
Hybrid seed• - cross-pollination

Breeder seed• - F8 to F10 - Sib mating
• •• Isolation blocks = 1/8 mile = 660 feet
Foundation seed • - Physical barriers
• •• - Border rows
• •• - No volunteers

Hybrid seed• - additional factors
• •• flowering dates, male sterility

{3.2 Weediness}

Volunteers in some fields and roadsides
• no establishment

4. Transformations

Direct transformation
- particle gun - embryonic callus
- protoplants - cocultivation
- application to corn silks

{4.1 Marker genes}

GUS genes
Hygromycin (antibiotic resistance)
Basta (herbicide resistance)

{4.2 Plant pest sequences}

CaMV 35S promotors
- Mannopine, Nopaline, Octopine promoters and terminators
- TMV enhancers - 5′ mRNA
- polyadenylation signals

-- cannot cause disease in themselves.

{4.3 Genes}

Herbicide tolerance
- Basta (PAT)
- Glufosinate
- Sulfonurea

Insect tolerance
- Wheat germ agglutinin
- Bt
- ({Clayibacter xyli})

Storage protein
Marker genes
- Basta, hygromycin, others

{4.4 Stability}

Mendelian inheritance of nuclear genes
Some cases several loci, only one expressed
May have to be confirmed after test
Instability -• gene amplification, unequal crossover,
• •• chromosomal disjunction
• •• transposon mediated
Deleterious to organism itself.

5. Environmental concerns

{5.1 Small-scale tests;} { initial tests}

{5.2 Gene escape - pollen}

- wind + 30 min.
- distance
- time
- physical - detasselling or bagging
- - border rows
- - shed pollen

{5.2.1 Grain movement}

- collect or destroy ears
- monitor for volunteers next year
- disk ears into soil
- disk and irrigate 3 times
- plant to non-corn
- leave fallow
- herbicide

5.2.1.1• Safeguards

- -- temporal safeguards could be used but would defeat purpose of test i.e. efficacy;
- -- physical or spatial safeguards good for small-scale only.

5.2.1.2• Familiarity

-- not novel plants, genes from novel sources.

5.2.1.3• Agricultural concerns.

{5.3 Environmental consequences}

Non-target organisms

-- Floral

- no compatible species
- no alternate means of transfer known

-- Faunal

- vertebrate or invertebrate

Endangered species

Beneficial insects - bees

-- Impact on agriculture

-- Alteration in susceptibility.

{5.4 Scale-up}

Affect on genetic resources
-- increased fitness of relative that causes community disruption
-- competition
-- pest density.

{5.5 Questions:}

1. Dissemination
2. Genes or sequences from plant pest
3. Selective advantage
4. Horizontal movement
5. Comparison to existing agriculture and organism

Environmental Assessment
and Finding of No Significant Impact (1)

Biotechnology Permits
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
US Department of Agriculture

1. Purpose and need

{1.1 Summary}

89. This Environmental Assessment (EA) presents scientific data and other information evaluated by the Animal and Plant Health Inspection Service (APHIS), US Department of Agriculture (USDA), prior to issuing a permit for the introduction of an article regulated under Title 7 Code of Federal Regulations Part 340 (7 CFR 340) [52 Federal Register (FR) 22892-22915, 16 June 1978].

90. A permit, number 91-295-01, was requested by The Holden's Foundation Seeds, Incorporated, Williamsburg, Iowa for a planned field test of genetically engineered corn plants to be carried out in Maui County, Hawaii. The corn plants have been modified to express a gene for herbicide tolerance. This EA is intended to provide documentation of the APHIS review and analysis of data in which it was determined that this limited field trial does not pose a risk of introduction or dissemination of a plant pest and does not present a significant impact on the quality of the human environment.

{Note}:

1. This document gives notice that the Department intends to issue a permit for release into the environment of a regulated article under regulations issued pursuant to the Federal Plant Pest Act and the Plant Quarantine Act [{N. de l'éd.} Permit Number 91-295-01: corn; herbicide tolerance gene, signed by Robert Melland, Administrator, Animal and Plant Health Inspection Service, 22 January 1992]. The permit is for a planned field test of genetically engineered corn plants to be conducted by The Holden's Foundations Seeds, Incorporated, in Maui County, Hawaii. The request for a permit has been thoroughly reviewed with a finding that there is no significant risk of introduction or dissemination of a plant pest from conducting this test as described by The Holden's Foundation Seeds, Incorporated. This document also contains an Environmental Assessment and Finding of No Significant Impact on the environment relative to the field testing of the genetically engineered corn plants.

{1.2 Finding of no significant impact}

91. APHIS has determined that this field trial, authorised by the issuance of permit number 91-295-01, will not pose a risk of the introduction or dissemination of a plant pest and does not present a significant impact on the quality of the human environment. This finding of no significant impact (FONSI) is based on the following factors:

1. Corn plants have been genetically engineered to contain a novel gene. In nature, genetic material contained in a chromosome of these plants is transferred to another sexually compatible plant by cross-pollination. In this field trial, the dissemination of the introduced genes by cross-pollination is not possible because all of the ears will be covered to prevent open pollination. In addition, border rows of a taller hybrid will surround the entire plot to bar the flow of any pollen outside of the test plot in the event that a bag is accidently removed. Other mitigation procedures are in place to prevent the survival of these genes after the test is terminated.

2. The introduced gene was derived from an organism that is not on the list of plant pests, and the introduced gene will not confer any plant pest characteristics on the recipient corn plants.

3. The introduced gene does not provide the transformed corn plants with any measurable selective advantage over nontransformed corn plants in their ability to be disseminated or to become established in the environment.

4. Select noncoding regulatory regions derived from plant pests have been incorporated into the plant DNA but do not confer any plant pest characteristics on the transformed corn plants.

5. Horizontal movement of genetic material after insertion into the plant genome (i.e. into chromosomal DNA) has not been demonstrated. No mechanism is known to exist in nature to horizontally move an inserted gene from a chromosome of a transformed plant to any other organism.

6. The field test plot will be small, with a maximum of 0.23 acre.

92. This EA and FONSI have been prepared in accordance with 1) the National Environmental Policy Act of 1969 (NEPA) [42 United States Code (U.S.C.) 4331 {et } {seq}.]; 2) Regulations of the Council on Environmental Quality for Implementing the Procedural Provisions of NEPA (Title 40 CFR Parts 1500-1509); 3) USDA Regulations for implementing NEPA (7 CFR Part lb); and 4) APHIS Guidelines for implementing NEPA (44 FR 50381-50384 and 44 FR 51272-51274).

{1.3 US Department of Agriculture regulations}

93. The request for a permit was submitted pursuant to regulations published in the Federal Register on 16 June 1987 (52 FR 22892-22915), that became effective on 16 July 1987. The regulations, "Introduction of Organisms and

Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There is Reason to Believe Are Plant Pests", will be codified in Title 7 of the Code of Federal Regulations in new Part 340. The regulations, which were promulgated pursuant to authority granted by the Federal Plant Pest Act, as amended (7 U.S.C. 150aa-150jj), and the Plant Quarantine Act, as amended (7 U.S.C. 151-164a, 166-167), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. Under Section 340.0 of the regulations, a person is required to obtain a permit prior to introducing a regulated article. A genetically engineered organism is deemed a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest. The genetically engineered corn plants in The Holden's Foundation Seeds submission are deemed "regulated articles" because they contain sequences of DNA that were derived from plant pests. The introduced gene was not derived from an organism on the list of plant pests. However, certain noncoding, regulatory sequences such as promoters and terminators were derived from organisms on the list of plant pests.

{1.4 Need for field testing of experimental products}

94. Limited field tests are needed so that information can be gathered for scientific evaluation of the efficacy of the genetic change. The plants have been tested in a greenhouse to obtain initial data relating to the genetic stability of the plants and to the efficacy of the changes. It is normal during the course of agronomic experiments to perform a planned field test following preliminary greenhouse evaluations. These tests help confirm the efficacy data, which can only be evaluated in an agricultural ecosystem using standard agronomic practices. Such limited field testing is necessary and is required to develop a potential agricultural product.

2. Background

95. This EA presents scientific data and other information evaluated by APHIS, USDA, prior to issuing a permit for the introduction of an article regulated under 7 CFR 340 (52 FR 22892-22915, 16 June 1987). This EA describes the information that was evaluated in determining whether to issue Holden's Foundation Seeds a permit for a planned field test of genetically engineered corn plants.

96. The recipient organism is corn, {Zea mays}, which has been modified to contain a gene for herbicide tolerance. The gene for herbicide tolerance was derived from an organism that is not a plant pest. The resulting genetically engineered corn plants look and grow like unmodified plants, with the exception that they express one of the introduced genes. These corn plants do not display any growth abnormalities. The genes were physically incorporated into the plant chromosome so that they are transmitted to progeny in a manner consistent with the predictions of Mendelian inheritance.

97. In the sections that follow, the alternatives available to APHIS are first described. In subsequent sections, the field plot design, field test protocol, and other factors necessary to identify the aspects of the environment that would potentially be affected are presented. Then the biology of the genetically engineered recipient corn plants is examined in detail. In an attempt to identify the potential impacts to the environment from this field trial and to describe the ways in which the risk to the environment is limited either by the nature of the host plant or by specific safeguards that have been designed into the protocol. The final section concludes that no significant impact to the quality of the human environment will occur as a result of issuing the permit described in this EA.

3. Alternatives

98. The regulations in 7 CFR 340 set forth the conditions under which a permit is required and identify the responsibilities of APHIS in responding to a request for a permit. Under Section 340.3(b), APHIS has 120 days to process a permit for introduction that is deemed by the Agency to be complete. APHIS is faced with two alternative actions after a permit application is deemed to be complete. Section 340.3(e) provides:

99. "A permit shall be granted or denied. If a permit is denied, the applicant shall be promptly informed of the reasons why the permit was denied and given the opportunity to appeal the denial in accordance with the provisions of paragraph (g) of this section. If a permit is granted, the permit will specify the applicable conditions for introduction of the regulated article under this part."

100. These two alternatives are discussed below.

{3.1 Alternative 1: issue a permit for the introduction (release into the } {environment) of a regulated article}

101. One alternative is to issue a permit for the introduction of a regulated article. The permit would allow the applicant to conduct a limited field test as proposed in the permit request. To issue a permit for the introduction of a regulated article pursuant to 7 CFR 340, APHIS must find that there is no significant risk of introduction or dissemination of a plant pest due to the permitted activity under the specified conditions [7 CFR 340.3(e)]. APHIS may specify conditions in addition to those included in the submission with the request for a permit if those conditions are necessary to prevent dissemination of a potential plant pest.

{3.2 Alternative 2:} { deny a permit}

102. APHIS must deny the permit if the proposed field test would present a risk of introduction or dissemination of a plant pest that is new or not widely prevalent. If a permit is denied, the applicant must be fully informed by APHIS of the reasons for the denial. The applicant has a right to appeal the denial of a permit [7 CFR 340.3(g)].

4. Description of recipient plants and donor DNA

103. As stated in the preamble to APHIS's regulations in 7 CFR 340 (52 FR 22892-22915), an article is not regulated merely because of the process by which it was produced; however, certain genetically engineered organisms and products that present some potential for plant pest risk are regulated. APHIS has determined that it is important to evaluate a genetically engineered organism that has been engineered using a recognised plant pest as the recipient organism, or as the source of inserted genes (donor), or that use a vector or vector agent from a pest organism. In this experiment, the corn plants were genetically engineered using noncoding regulatory sequences that were derived from plant pests.

104. The sections that follow treat the biology of the recipient plant and the nature of the donor DNA sequences, focusing on the potential impacts to the environment inherent in each of these components. Specifically, the ways in which the risk to the environment is limited, either by the nature of the organisms or by specific safeguards that have been designed into the field test protocol, are described.

{4.1 Recipient-related impacts}

105. This section of the EA discusses the potential impacts to the environment of the introduction of genetically engineered corn. The biology of corn and plants related to corn are considered. Because the mechanism by which genes are moved from one flowering plant to another in nature is through cross-pollination of sexually compatible plants, the plants with which corn can cross-pollinate are described. The methods by which corn is commonly cultivated are examined to identify any unique risks that the transformed plants might express such pest characteristics as weediness. Other potential impacts from the corn plants are also analysed.

{4.1.1 Corn as a crop}

106. {Zea mays Linnaeus}, known as maize throughout most of the world, and as corn in the United States, is a large, annual, monoecious grass, that is grown for animal feed, silage, human grain, vegetable oil, sugar syrups, and other miscellaneous uses. It is the premier cash crop in the United States, and its cultivation, genetics, processing, financing, and distribution on a national and international scale is pervasive and complex.

107. World production in 1987/1988 was 439 million metric tonnes, of which the United States produced 179, China 76, Brazil 23, and France 12. Corn is grown commercially in almost all states of the United States (Jewell, 1989). US production in 1987 was 7 064 million bushels, of which the top state producers were Iowa (1 306), Illinois (1 201), Nebraska (812), Minnesota (635), and Indiana (632). Corn has the highest value of production of any US crop; 1987 value was $12.1 billion, compared to soybeans at 10.4, hay at 9.1, wheat at 5.4, and cotton at 5.0.

108. Corn has been cultivated since the earliest historic times from Peru to central North America. The region of origin is now presumed to be Mexico (Gould, 1968). Dispersal to the Old World is generally deemed to have occurred in the sixteenth and seventeenth centuries (Cobley and Steele, 1976); however, recent evidence indicates that dispersal to India may have occurred prior to the twelfth and thirteenth centuries by unknown means (Johannessen and Parker, 1989)

{4.1.2 Taxonomy of corn}

109. {Zea} is a genus of the family {Gramineae} ({Poaceae}), commonly known as the grass family. The genus consists of some four species; {Zea mays}, cultivated corn and teosinte; {Zea diploperennis} Iltis {et al}., diploperennial teosinte; {Zea luxurians} (Durieu et Asch.) Bird; and {Zea perennis} (Hitchc.) Reeves et Mangelsd., perennial teosinte. Various of the species have been assigned to the segregate genus {Euchlaena}, which is not currently recognised, or have been divided into numerous small species within the genus {Zea} (Terrell {et al}., 1986).

110. Of the four species of {Zea}, only {Zea mays} is common in the United States. It is known only from cultivation; it occasionally is spontaneous in abandoned fields or roadsides, but is incapable of sustained reproduction outside of cultivation (Gould, 1968). The other species are occasional university or experiment station research subjects. {Zea perennis} is reported as established from James Island, South Carolina (Hitchcock and Chase, 1951).

111. The closest generic relative to {Zea} is {Tripsacum}, a genus of seven species, three of which occur in the United States (Gould, 1968). {Tripsacum} differs from corn in many respects, including chromosome number (n=9), in contrast to {Zea} (n=10). All species of {Tripsacum} can cross with {Zea}, but only with difficulty and only with extreme sterility (Galinat, 1988).

112. Cultivated corn is presumed to have been transformed from teosinte, {Zea } {mays} subspecies {mexicana} (Schrader) Iltis, more than 8 000 years ago. During this transformation, cultivated corn gained several valuable agronomic traits, but lost the ability to survive in the wild. Teosinte, however, remains a successful wild grass in Mexico and Guatemala. Despite some confusion over proper taxonomic groupings of the non-cultivated members of {Zea}, wild members maintain a successful array of annual or perennial plants with visible chromosomal peculiarities and ploidy levels, and many adaptive macroscopic phenotypes. Cultivated corn and the wild members of diploid and tetraploid {Zea} can be crossed to produce fertile F1 hybrids. Nonetheless, in the wild, introgressive hybridisation does not occur because of differences in flowering time, geographic separation, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Galinat, 1988).

113. The second major transformation of cultivated corn occured in the United States in the twentieth century, and particularly since the 1930s. This transformation occurred through inbred lines for hybrid seed production and by other methods. Almost all corn grown in the United States now comes from hybrid seed that is obtained every planting season from private enterprises;

the older open-pollinated varieties are virtually unknown in commerce (Hallauer {et al}., 1988). This transformation has resulted in more uniform commercial plants with superior agronomic characteristics, and has contributed to the six-fold increase in per acre yields in the last 60 years.

{4.1.3 Morphology and reproduction of corn}

114. Corn is a tall, robust, monoecious annual, with overlapping sheaths and broad, conspicuously distichous blades; staminate spikelets in long spikelike racemes, which are numerous, forming large spreading terminal panicles (tassels); pistillate inflorescence in the axils of the leaves, the spikelets in 8-16 (30) rows, on a thickened, almost woody axis (cob), the whole enclosed in numerous large foliaceous bracts or spathes, the long styles (silk) protruding from the summit as a mass of silky threads; grains at maturity greatly exceeding the glumes (Hitchcock and Chase, 1951).

115. Pollination, fertilization, and caryopsis development of corn follows a fairly standard pattern for chasmogamous wind-pollinated grasses, with the following points of exception and note:

1. Pollen is produced entirely in the staminate inflorescences. Eggs are produced entirely in the pistillate inflorescences.

2. Self-pollination and fertilization and cross-pollination and fertilization are usually possible, and frequencies of each are usually determined by physical proximity and other physical influences on pollen transfer. A number of complicating factors, such as genetic sterility factors and differential growth rates of pollen tubes, may also influence the frequencies of self-fertilization versus cross-pollination.

3. Corn styles and corn pollen tubes are the longest known in the plant kingdom.

4. Shed pollen typically remains viable for 10 to 30 minutes, but may remain viable for much longer under refrigerated conditions (Coe {et al}., 1988).

5. The staminate and pistillate inflorescences do not develop at the same time. The pistillate inflorescence is precocious. However, there is the appearance of slight protandry, because the elongating styles are delayed for about seven days in emergence from the bracts of the pistillate inflorescence, while the development of the later-developing staminate inflorescence is fully visible.

6. The genetics of corn is better known than that of any other crop plant.

{4.1.4 Pollination of corn}

116. Studies of pollination of corn have mostly centered on the needs of hybrid seed production. This production involves the development and maintenance of inbred lines and the subsequent crosses to produce commercial seed. In the former, self-pollination is mandatory. In the latter, cross-pollination is mandatory. Mechanisms have been developed to ensure each kind of pollination.

117. Breeder seed is usually derived from self-pollinated seed at the F8 to F10 generation of inbreeding (Wych, 1988). A high degree of self-pollination is ensured by planting well isolated blocks that virtually guarantee natural random sib mating. Minimum isolation distances for foundation seed are one-eighth mile (660 feet) from the nearest contaminating source. Other safeguards, such as physical barriers or unharvested border rows, can further reduce the possiblity of contamination. Fields that have not been recently planted in corn are preferred. This is to minimise the appearance of volunteer corn from a previous season.

118. Hybrid seed production fields also require isolation similar to that for foundation seed. Isolation distance may be modified by such factors as high winds, additional border rows, size of field, natural barriers, and differential flowering dates. Flowering dates are often adjusted by differential planting dates, planting depth, or fertilizing. The two different parents are planted in a regular pattern of rows, such as four pistillate to one staminate (4:1), or 4:2, or 6:2, or a variety of other combinations. Detasseling or use of cytoplasmic male sterility prevents pistillate plants from shedding viable pollen, and thus ensures cross-pollination.

{4.1.5 Cultivation of corn}

119. Corn is grown in the United States as rowcrops of monocultures of uniform plants from hybrid seed. Agronomic practices have developed a high degree of sophistication in the use of tillage, pesticides, planting, fertilizer, harvesting, distribution, and all other agronomic aspects.

{4.1.6 Weediness of corn}

120. Corn appears as a volunteer in some fields and roadsides, but it never has been able to establish itself outside of cultivation (Gould, 1968). Some of the other species of {Zea} are successful wild plants, but have no pronounced weedy tendencies (Galinat, 1988).

{4.1.7 Modes of gene escape in corn}

121. Genes of corn may escape from the test plot in two ways. The first is by pollen transfer. The second is by movement of the grains.

122. If viable pollen of the transgenic plants can be transferred by wind to any receptive corn stigma within the 30 minute period of pollen viability, an escape of genetic material could take place. This potential transfer becomes

more unlikely as distance increases from the transgenic plants, and from a practical standpoint becomes completely unlikely at distances much beyond the foundation seed isolation distance of 660 feet. Temporal isolation would further reduce the likelihood of effective pollination and fertilization. In addition, any physical impediment to this movement, such as effective detasseling or bagging, would completely eliminate the possibility of gene escape by way of pollen.

123. To prevent grain from remaining in the field or otherwise escaping, all ears would have to be collected or otherwise destroyed. To ensure that no grain escaped harvest, the field would have to be monitored for volunteer corn plants in the following season.

{4.2 Donor-related impacts}

124. In this section of the EA, the potential impacts to the environment of the introduced gene and the associated regulatory sequences are discussed. The use of corn plants that express the introduced gene is examined to identify any unique risks to the environment that might be posed by the controlled introduction of the corn plants.

{4.2.1 Phosphinothricin acetyl transferase}

125. The transgenic corn plants have been genetically engineered to contain a gene that encodes the enzyme phosphinothricin acetyl transferase (PAT). The PAT gene was isolated from {Streptomyces viridochromogenes}. Because the {Streptomyces} gene had a high G:C content, which is atypical for plants, a modified PAT gene containing codons more typically used in plants was synthesised {in vitro}. The biology of the PAT gene and the mechanism by which herbicide tolerance is conferred on the genetically engineered soybean plants are described below.

126. Members of the genus {Streptomyces} are gram-positive sporulating soil bacteria. These organisms synthesise numerous unique compounds, secondary metabolites, that often possess antibacterial, antitumor, or antiparasitic activity (Demain {et al}., 1983). One such compound, the antibiotic bialaphos, is produced by several species of {Streptomyces}. The PAT enzyme functions in both the biosynthesis of bialaphos and the prevention of self-toxicity in bacteria that produce the antibiotic.

127. Bialaphos is a tripeptide consisting of two L-alanine molecules and an analogue of L-glutamic acid called phosphinothricin (Pt) (Kondo {et al}., 1973; Ogawa {et al}., 1973). Phosphinothricin is the active component of two commercial herbicides, Herbiace(R) (Meiji Seika Ltd.) and Basta(R) (Hoechst AG). Herbiace(R) is bialaphos that is commercially produced using {S. hygroscopicus}. Basta(R) is the ammonium salt of phosphinothricin and is chemically synthesised. Phosphinothricin is a potent inhibitor of glutamine synthetase in both bacteria and plants (Bayer {et al}., 1972). Glutamine synthetase plays a central role in nitrogen metabolism of higher plants. In higher plants, glutamine synthetase is the major enzyme responsible for the first step in the assimilation of ammonia produced by nitrate reduction or

nitrogen fixation in the roots as well as for the reassimilation of ammonia released by photorespiration in the leaves (Miflin and Lea, 1980; Keys {et al}., 1978). Ammonia, although a plant nutrient and a metabolite, is toxic in excess, affecting chloroplast morphology and a wide range of metabolic functions (Givan, 1979; Heath and Leech, 1978; Krogmann {et al}., 1959; Platt {et al}., 1977). Glutamine synthetase is the only enzyme in plants that can detoxify ammonia released by nitrate reduction, amino acid degradation and photorespiration (Miflin and Lea, 1976). Inhibition of glutamine synthetase by phosphinothricin causes rapid accumulation of ammonia which leads to death of the plant cell (Tachibana {et al}., 1986).

128. Acetylation of the phosphinothricin free amino group, as catalysed by the PAT enzyme, disables the compound's inhibitory activity towards glutamine synthetase, thereby attenuating its herbicidal activity. Therefore, plants that produce the PAT enzyme should be resistant to the phosphinothricin class of herbicides. To this end, the PAT gene has been introduced into tobacco, tomato, and potato plants (de Block {et al}., 1987; Leemans {et al}., 1987). The transgenic tobacco, tomato, and potato plants were completely resistant to high doses of the commercial formulations of bialaphos (de Block {et al}., 1987). In the field trial proposed by The Holden's Foundation Seeds, the PAT gene has been introduced into corn plants for resistance to the phosphinothricin class of herbicides.

{4.3 Vector and vector agent-related impacts}

129. In this section of the EA, the potential impacts to the environment from the vector system that was used to transfer the genes into plant cells are discussed. The term "vector" refers to the actual DNA molecule that carries the genes into the plant cells and facilitates their incorporation into plant DNA. In this submission, the novel DNA was introduced into the corn plants by direct gene transfer into protoplasts.

{4.3.1 Plant transformation}

130. Transgenic plants were produced by cocultivation of the plasmid DNA containing the gene of interest with protoplasts of corn cells (Negrutiu {et al}., 1987). The plasmid DNA used, pDH 51, was derived from the common cloning plasmid, pUC18 (Pietrzak {et al}., 1986). Protoplasts were derived from a synthetic corn line, HE/89, specifically developed for its superior capacity to regenerate. Southern blotting methods indicated that the initial transformants contained multiple copies of the engineered gene. Mendelian patterns of inheritance indicated that only one copy was expressed. The initial transformants were then crossed into elite inbred lines. Southern analysis demonstrated that fewer copies of the PAT gene were present in the progeny, indicating that nonfunctional copies were lost via recombination. Progeny were then backcrossed into the elite lines. Progeny from both the crosses and partial backcrosses will be used as donors of the PAT gene in the proposed experiment. The elite lines used for these crosses are proprietary lines developed by The Holden's Foundation Seeds and the inbred line, B73.

{4.3.2 Regulatory sequences}

131. Some regulatory sequences used in these experiments, including promoters and transcription termination and polyadenylation sequences, were derived from known plant pests. Although these sequences were derived from plant pests, they are well-characterised, noncoding sequences that are incapable of causing plant disease.

132. A key step in the design of a plant expression vector is the identification and characterisation of strong promoters (Tempe and Goldmann, 1982; Fraley {et al}., 1983; Sanders {et al}., 1987). Promoters are regions on a DNA molecule to which RNA polymerase binds and initiates transcription. Promoter DNA sequences are located upstream from functional gene sequences and are not transcribed into mRNA. A strong promoter results in increased mRNA synthesis which results in increased protein production. Promoters derived from plant pests can be divided into two classes: those derived from the Ti plasmid or those from cauliflower mosaic virus (CaMV). Those derived from the Ti plasmid include promoters from the mannopine synthase gene (Velten {et al}., 1984; Willmitzer et al., 1983), the octopine synthase gene (Koncz {et al}., 1983), and the nopaline synthase gene (Herrera-Estrella {et al}., 1983). These promoters have several advantages: they are several of the most highly transcribed Ti plasmid genes (Willmitzer {et al}., 1983; Velten {et al}., 1984), and they are constitutively expressed (Bevan {et al}., 1983). Promoters derived from the cauliflower mosaic virus are the promoters for the two major RNA transcripts, designated 35S and 19S (based on their sedimentation coefficients), that are produced during the replication of DNA-containing CaMV (Hull and Covey, 1983). The 35S CaMV promoter sequences have been characterised by Nagy {et al}. (1985), Odell {et al}. (1985), and Nagata {et al}. (1987), and the 19S promoter by Balazs {et al}. (1985).

133. Another step in the design of a plant expression vector is the identification of transcription termination and polyadenylation signal sequences. Most eukaryotic mRNAs possess a heterogenous length of polymerised adenosine monophosphate residues (called poly A) at their 3'-termini. The length of residues may vary from 20 to 200 bp. The poly A sequences are not coded for by the DNA but are added post-transcriptionally (Mainwaring {et al}., 1982).

134. Both the promoter and transcriptional termination sequences used to express the PAT gene were derived from CaMV.

135. Although the promoters and the transcription termination and polyadenylation sequences are derived from known plant pests, they cannot cause plant disease by themselves or in conjunction with the coding sequences. The promoters and terminators are well-characterised noncoding DNA sequences with the sole function in these corn plants of regulating expression of the PAT gene. Recipient corn plants will acquire no plant pest characteristics when they are modified by the introduction of the PAT gene and its associated regulatory sequences.

5. Affected environment

136. The field trial will take place in Maui Counti, Hawaii. Site monitoring of the field trial and agronomic management practices that create a nonpropagative environment are expected to provide the necessary degree of both biological and physical containment. These factors are described at greater length below.

{5.1 Field plot design}

137. The field tests will be conducted in an isolated nursery owned by Hawaiian Research, Ltd. The purpose of the field trial is to transfer the PAT gene into elite inbred lines.

138. The field plot will contain 170 15-foot rows with 20 plants per row. The plot will have five rows grouped together. One of the five will contain the transgenic corn, the pollen source for the elite inbred lines that will be planted in the other four rows. The experimental plot will be surrounded on all four sides by a 5-6 border rows of hybrid corn. The entire test area occupies approximately 0.20 of an acre. Prior to silk emergence, all ears will be covered with glassine bags. Pollen from the transgenic plants will be collected and used to pollinate receptive silks in the rows containing the elite inbred lines. After pollination, ears will be covered with a paper bag. Pollinated ears will be hand-harvested. In order to select against segregants for the PAT gene, plants will be treated at the three-leaf stage by brushing the leaves with a 0.2 per cent solution of Ignite(TM). Plants with necrosis on the leaves will be labeled as PAT and will be destroyed mechanically. In no case will the herbicide be applied to the soil. The Ignite(TM)-treated plants will occupy 0.05 of an acre.

139. No other maize will be grown within 800 feet, a distance that exceeds the standard isolation distance of 660 feet required for foundation seed production. Ears will be covered with glassine bags. Only if a bag is accidently removed will there be an opportunity for open pollination. For additional security, border rows of a taller hybrid corn will form a perimeter around the entire plot to provide a barrier to pollen movement.

140. Pollinations will be controlled; however, in the event that glassine bags are blown off and leave these ears receptive to open pollination, the resulting ears will be mowed and disked into the soil at the time of harvest.

{5.2 Field observation and monitoring}

141. An APHIS representative will visit the plot at the initiation of the experiment or shortly thereafter to verify that the test is being conducted as proposed in the request for the permit. Further monitoring inspections will be performed by a Regional Biotechnologist for Plant Protection and Quarantine, APHIS.

{5.3 Security of the field test plot}

142. The field trial will take place at the Hawaiian Research, Ltd. nursery on the island of Molaki, Hawaii. The plot will be surrounded by fallow ground containing tropical pasture grasses such as buffalo grass, guinea grass, and green tannic grass. Holden's Foundation Seeds will take adequate precautions to provide for the physical security of the field test plot. A Holden's Foundation Seed employee stationed near the site will be responsible for the field trial.

{5.4 Final disposition of the experimental corn plants}

143. When the plants are mature, hand-pollinated ears will be harvested and put into cloth harvest bags for drying and storage. After shelling, kernels will be stored in locked cabinets in Hawaii.

144. After harvest, plant material will be mowed and disked into the soil. The area will be irrigated, and any plants that germinate two to three weeks afterwards will be disked. Past experience has indicated that three irrigation/disking cycles are sufficient to eliminate volunteer corn. However, the plot will be monitored closely after the third cycle and if volunteers are detected, additional rounds of irrigation/disking will be performed until no volunteers emerge.

6. Environmental consequences

145. The risk associated with the introduction of genetically engineered organisms is the same in kind as those associated with the introduction into the environment of unmodified organisms and organisms modified by other genetic techniques. Also, assessing the risks of introducing a genetically engineered organism into the environment should be based on the nature of the organism and the environment into which it is to be introduced. These statements are supported in a report by a group of distinguished scientists convened by the Council of the National Academy of Sciences to review the key issues in introducing genetically engineered organisms into the environment. The report states, "For the determination of ecological risk, the biological properties of the (genetically) engineered organism are paramount" (National Academy of Sciences, 1987).

{6.1 Impact on non-target organisms}

146. This section of the EA includes a discussion of impacts on non-target organisms in the environment, with particular attention to those that might be threatened or endangered.

{6.1.1 Native floral communities}

147. As described in section 5.1, unrelated plant species cannot be pollinated and successfully fertilized by corn pollen. No species interfertile with corn have been observed in the areas adjacent to the field test plot. No

means of transmitting the inserted gene(s) to other plant species have been identified in nature. APHIS concludes that it is highly unlikely that the genetically engineered corn plants will introduce any of the experimental genes into the gene pool of any local native floral community.

{6.1.2 Native faunal communities}

148. No factor unique to this field test trial has been identified that would have an effect on any vertebrate species. Since there is no identifiable direct effect of this field test trial on any wild vertebrate or invertebrate fauna, there is no apparent risk to any threatened or endangered faunal species.

{6.2 Impact on existing agricultural uses}

149. The field test is being conducted for the purpose of preliminary testing of the modified corn plants. No impact on existing agricultural uses are expected.

{6.2.1 Alteration in susceptibility to plant pathogens or palatability to } {insects}

150. There has been no intentional change in these plants to alter their susceptibility to plant pathogens or palatability to herbivorous insect pests, and there is no reason to believe that these characteristics are different in the transformed corn plants. The only physiological changes in the transformed plants is presumed to be the expression of the gene for herbicide tolerance, and this change is not expected to have any effect on plant disease organisms or insects.

151. Bacterial, fungal, or viral diseases can be limiting factors in corn production. There is no reason to believe that the genetic changes in the corn plants will alter the degree of susceptibility or resistance to bacterial, fungal, or viral pathogens. However, if there were any changes in susceptibility, the effects would be confined to the corn plants in this field plot.

152. Some insect pests can also be limiting for corn production. Any unusual change in insect population levels would be ecologically significant if the experimental corn plants were susceptible to a particular insect pest and were propagated extensively in the environment. This will not be the case in this experiment, and any environmental impact should be limited to the test plot.

{6.3 Impact on the immediate physical environment}

153. Due to the nature of the transformed and control corn plants and the safeguards built into this field test, no corn plant will survive upon termination of this experiment to cause an effect on the physical environment.

{6.4 Impact on human health}

154. None of the genetically engineered corn will be available for human consumption. No potential impact on people living in the area of the field test, or any other human population, can be identified.

7. Conclusions

155. APHIS concludes that no significant risk of introducing or disseminating a plant pest, and no significant impact to the quality of the human environment will result from issuing the permit described in this EA. The factors that were evaluated in reaching this conclusion can be summarised as follows:

1. Corn plants have been genetically engineered to contain a novel gene. In nature, genetic material contained in a chromosome of these plants is transferred to another sexually compatible plant by cross-pollination. In this field trial, the dissemination of the introduced genes by cross-pollination is not possible because all of the ears will be covered to prevent open pollination. In addition, border rows of a taller hybrid will surround the entire plot to bar the flow of any pollen outside of the test plot in the event that a bag is accidently removed. Other mitigation procedures are in place to prevent the survival of these genes after the test is terminated.

2. The introduced gene was derived from an organism that is not on the list of plant pests, and the introduced gene will not confer any plant pest characteristics on the recipient corn plants.

3. The introduced gene does not provide the transformed corn plants with any measurable selective advantage over non-transformed corn plants in their ability to be disseminated or to become established in the environment.

4. Select noncoding regulatory regions derived from plant pests have been incorporated into the plant DNA but do not confer any plant pest characteristics on the transformed corn plants.

5. Horizontal movement of genetic material after insertion into the plant genome (i.e. into chromosomal DNA) has not been demonstrated. No mechanism is known to exist in nature to move an inserted gene horizontally from a chromosome of a transformed plant to any other organism.

6. The field test plot will be small, with a maximum of 0.23 acre.

156. The test has been designed with safety factors to minimise the possibility of adverse ecological effects. At the conclusion of the experiment, all transgenic plant seed will be harvested by hand. After harvest, all transgenic and nontransgenic plant material at the test site will be incorporated into the soil. Any volunteers will be eliminated by disking. Should unanticipated effects arise, the isolation of the test site and manner of conducting the test indicate that the effects can be readily contained and would have no permanent effect on the environment.

157. This proposed field test should not have a significant effect on the environment. APHIS has determined that this limited field test will not pose a risk of introducing or disseminating a plant pest into the environment. Therefore, APHIS concludes that the proper choice of alternatives is that described in Alternative 1: the issuance of a permit for introduction of a regulated article, pursuant to the authority under 7 CFR 340, for a limited field trial of genetically engineered corn plants, to be conducted as described in the detailed proposal submitted by The Holden's Foundation Seeds.

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Reviewing a Field Trial Request for Maize

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1. External referee

The referee is:

-- chosen freely by the applicant among a list provided by the committee;
-- really independent, confidential.

Usually two half-days are necessary to analyse one project.

158. The external referee has two principal roles. The first is to check that all the information necessary to reach a decision is fully described. If any parameter is not sufficiently defined, the referee asks the applicant, directly, to complete the point before the session.

159. The second is to give his own statement on the absence -- or presence -- of risk, based on the biological parameters and on the different precautions to be taken. This statement is given to the committee in the absence of the applicant. Usually, the various points are individually reviewed and commented on; either a parameter is considered as satisfactory, or the attention of the committee is drawn to a lack, an insufficiency or to a supplementary precaution which should be required.

2. Points to be examined

-- the DNA sequence(s) to be introduced in the plant genome;
-- the transformation process (for some aspects);
-- the biological characteristics of the plant in relation to possible dissemination;
-- the molecular characterisation of the various plants proposed for the test;
-- the material precautions proposed by the applicant.

160. The following discussion concerns a field trial of maize, transgenic for hygromycin and GUS. The tests involved concern the expression of the hygromycin gene and biometric parameters not related to the foreign genes.

{2.1 The DNA sequence(s) to be introduced in the plant genome}

{Genes - the regulatory sequences}

Two different plasmids

-- amp (bacterial), encoding the •-lactamase with its own promoter;
-- lac i (bacterial), encoding the lactose operon repressor;
-- either the hpt gene (bacterial) encoding the hygromycin-phosphotransferase, with the 35S CaMV promoter and terminator;
-- or the GUS gene (bacterial) encoding the • glucuronidase, with the 35S CaMV promoter and terminator, and the Adh 1 intron (maize);
-- ori (bacterial).

{2.2 The transformation process}

161. The nature of the process is not important {per se}, but it is crucial to be sure that no other sequences than the ones described above have been used during the transformation. For instance, the question of the possible use of carrier DNA is systematically raised.

162. The transformation entailed biolistic transformation of micro-calli of maize, followed by plant regeneration under hygromycin selection pressure. A mixture of the two plasmids was used.

{2.3 The biological characteristics of the plants}

163. The biological characteristics examined are:

-- genus, species and line, variety/cultivar;
-- mode of reproduction;
-- known distance for natural pollen dissemination (wind, insects etc.);
-- list of the different plants which could be cross-pollinated;
-- distance to the nearest field of pollinatable plants.

164. {Zea mays} has male or female flowers (monoecious), physically separated on the plant. A male flower produces 25 million pollen grains, which can be disseminated by the wind, with a known limit of 400 m (the diameter of the pollen is 100 •m, among the largest). Pollen is not disseminated by insects. The minimal distance imposed for the certification of seed purity is usually 400 m. Male inflorescences can be manually castrated.

{2.4 The molecular characterisation of the various plants proposed for the } {assay}

165. The level of description of the plants usually needs to be improved; exact flow diagrams of the genealogies are systematically requested.

166. The Southern hybridisations are analysed in detail.

-- Plant DNA is usually analysed by two or more enzymes.
-- The probes should be completely described and need to encompass each sequence to be transferred and, if possible, all the plasmid used. So that the exact number of copies and their intactness can be known theoretically, quantitative reconstruction to complement junction determinations is encouraged. The relative position of the various copies can be approached through molecular analysis (which is difficult) or genetics.
-- Insertion into a single site (a very large Xho band is lit up) five to six copies more or less intact.

{2.5 Material precautions}

-- seed transportation to the field;
-- mechanical or manual sowing;
-- nylon net (bird protection) recommended to the committee;
-- 180 plants (3 lines 5m each, 80cm alleys) with a 6m wild maize border surrounding the plot (see Figure 1);
-- manual castration;
-- there is no wild plant species which can be crossed by {Zea mays} in France (Teosintes and tripsacum only exist in America);
-- cobs will be manually harvested before maturity and incinerated;
-- supplementary precaution proposed to the committee; since technicians will enter the experimental parcel frequently to make biometric observations, care must be taken that their clothes do not act as pollen vectors.

167. The various parts of the plants which will be cut for enzymatic determination will be frozen in liquid nitrogen and transported by car to the laboratory.

168. As to the soil treatment, the usual recommendation is that the parcel receive a total herbicide and be kept uncultured for a period. Alternatively, another culture can be allowed, with the obligation to collect all volunteer maize plants; this makes it possible to evaluate the efficiency of the various precautions taken to avoid dissemination.

Figure 2. Plan of the plot in the Field Assay Station

1. Line 0274, 8 peripheral rows, 3 x 2 central rows.
2. Line 0274 regenerated, 3 x 2 central rows.
3. Line 0274 transformed, 3 x 2 central rows.

80 cm-wide spaces between rows.

Transgenic maize trials

Dr. Emmanuel Chasseray
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Switzerland

1. Worldwide status of transgenic maize

169. The first open field trials of genetically modified plants were held in 1986 in the United States. Since then, over 370 trial applications have been approved in 21 countries, over half of them in the United States and France (see Figure 3).

170. The trials have concerned 26 species (see Figure 4). Maize alone obtained 18 field trial licences, the first held in 1990 in the United States.

Figure 3. Approved field tests of transgenic plants, 1986-91

Figure 4. Crop species having been transformed and field tested 1986-91

2. Development of transgenic plants at Ciba-Geigy

171. An initial open field trial on transgenic tobacco plants was conducted in 1986. Since then, several trials have been performed with the model plant. During the latest trials, plants in isolated plots were allowed to bloom.

172. Maize modified by a marker gene was obtained at Ciba-Geigy laboratories at the end of 1989. It underwent open field trials in 1991 in France and in the United States. The first trials using maize modified by an encoding gene for a delta-endotoxin of {Bacillus thuringiensis} for the purpose of protecting the plant against European corn borer ({Ostrinia nubilalis}) are scheduled in the United States and in France in 1992 (see Figure 5).

Figure 5. Transgenic plants: research field trials at Ciba-Geigy

3. Nature of transgenes, transformation methods

173. The 1991 trials concerned maize modified by the encoding gene for hygromycin phosphotransferase (Hpt) used both as selective gene and as marker gene.

174. The transformants were obtained by the bio-balistics technique (BIOLISTICS(R)) whereby the DNA of two whole circular plasmids, including a prokaryote selective gene (used for preparing plasmids) and selective and marker genes controlled by means of eukaryote sequences (CaMV), was introduced into plant cells by projecting microparticles coated with the DNA. Detailed analysis of the transformants using the Southern technique showed that at a single site there was integration of extensively rearranged genetic material from the two plasmids, with a functional and expressed Hpt gene and a functional and non-expressed Amp gene (see Figure 4).

{3.1 Transformation of maize with a marker gene coding for} { hygromycin } {phosphotransferase}

This gene is a selectable marker of transformed tissues.

This marker gene serves as a tool for the:

-- development and refinement of the techniques of transformation;
-- development and refinement of the methods of culture of transgenic tissues;
-- development and refinement of promoters which will be used later with agronomic genes;
-- assessment of the expression of the transgene in plants grown in the field.

Figure 6. Genes inserted: cotransformation of two plasmids

4. Open field trial protocols

{4.1 Field trials:} { objectives}

175. The modified maize was field-tested in 1991 in France, the United States (two plantings) and Argentina in order to investigate the expression stability of the transgene Hpt under natural agroclimatic conditions. The same material is to be tested in Italy and Switzerland in 1992, subject to authorisation by the competent authorities.

176. Ciba-Geigy's trial application files are drawn up in accordance with the recommendations issued by the Committees where applicable. The United States model was followed for the Argentina file and the French model for the Switzerland and Italy files.

{4.1.1 Field trials:} { marker gene}

4.1.1.1• Common objectives to all countries

-- to establish CG procedures for testing genes of agronomic values;
-- to build up experience on the behaviour of the transgenic maize (multilocation): phenotypic behaviour and plant fertility;
-- to test the expression of the transgene;
-- to test the inheritance of the transgene.

4.1.1.2• Objectives specific to countries

US • to refine technique to assess expression of the marker gene;

FRANCE • to build up co-operation with INRA (expertise on insect damage to maize);

ARGENTINA to prepare for counter-season capabilities;

SWITZERLAND to implement breeding with transgenic maize on site of biotech research.

{4.1.2 Field trials - regulatory issues}

4.1.2.1• Regulatory bodies

COUNTRY• r-DNA Committee•••••Guidelines

US• USDA••••••APHIS
• Animal and Plant Health Inspection Services

FRANCE• CGB••••••CGB
• Commission du Génie Biomoléculaire

ARGENTINA CONABIA••••••APHIS
• Comision National de Biotecnologia Agropecuaria
• (visit of the Commission to the site required)

SWITZERLAND SKBS••••••CGB/SKBS
• Biosafety Evaluation Committee

ITALY• Agriculture Ministry Committee•••CGB
• Ministry of Health decision

4.1.2.2• Prevention of gene dissemination

	At planting ••	Pollen• ••	Seeds• ••	Plant• Material•	Final Disposition
US	check• planter• ••	spatial/• temporal• isolation	harvested• ••	ploughed• under•	herbicide + soybean
F	protection against birds	emasculation ••	burned• ••	burned• ••	herbicide + no crop
A	check• planter• ••	spatial/• temporal• isolation	harvested• ••	ploughed• under•	herbicide + soybean
CH	protection against birds	emasculation ••	destroyed• ••	autoclaved• ••	herbicide + no crop
I	protection against birds	emasculation ••	destroyed• ••	destroyed• ••	herbicide + soybean

(n.b. all measures proposed by Ciba-Geigy and accepted by r-DNA Committee)

{4.2 Field trials:} { risk assessment}

177. In all cases, suitable measures for preventing transgene dissemination (in particular via pollen) were proposed and accepted as adequate by the evaluation committees. Spatial and temporal isolation was ensured in the United States and Argentinian trials, where the plants were allowed to bloom. It was decided to conduct the French trial in a maize field and to castrate the plants to prevent dissemination via pollen. It should be noted that the evaluation of potential risks in the case of maize should specifically take into account the fact that this species is naturally contained and there is no possibility of transgene dissemination to wild species.

178. Ciba-Geigy will continue to take utmost care to ensure that the evaluation of potential risks associated with the trials conforms to scientific criteria assessed case by case and that the evaluation authorities clearly define these criteria for their applicants.

{4.2.1 Issues related to risk assessment}

179. Ciba-Geigy encourages the regulatory authorities to use:

-- a case by case identification of the potential risk(s) associated with the release of the product, based on its biological characteristics;

-- if a risk is identified, to develop in co-operation the scientific studies required to assess the level of risk.

5. Marketing of transgenic plants and evaluation of potential risks

180. The objectivity of risk evaluation criteria will acquire considerable importance in view of the fact that commercial applications are being envisaged and regulations are being prepared or introduced in most OECD countries.

181. In particular, it is important to define the nature of the product to be subject to control.

182. Two scenarios (see Figure 7) are discussed:

-- transfer of the same gene independently in several varieties;

-- transformation of a variety followed by transfer of the transgene to other varieties through sexual crossing.

183. The scenarios will cover a time-span of eight to 12 years between cloning of the gene and obtention of the marketable variety. Authorisation for the free dissemination of this variety will be essential if the new variety is to be included in agronomic evaluation trials for several years prior to commercial release and then to be propagated. Particularly in countries where official registration is compulsory, the material will be tested under the responsibility of official evaluation bodies rather than under that of the obtainer, and this will give some idea of when transgenic seed will be placed at the disposal of farmers.

184. The competent authorities are therefore urged to prepare regulations as soon as possible that will be:

-- clear and based on scientific criteria;
-- relatively stable over time so as not to waste several years of work;
-- harmonized at international level.

Figure 7. Two marketing scenarios

{5.1 Issues related to the commercialisation of the product}

{5.1.1 How will it be regulated?}

-- For various varieties transformed with the same transgene (multiple transformation events)
-- For a transgene backcrossed in various varieties (unique transformation event).

{5.1.2 Situation:}

-- eight to 12 years from gene design to the product, entering registration process;
-- products already in the pipeline.

Table 11. New variety development from biotechnology innovation

	Number of Years
1. Biotechnology	
Gene development, transformation, regeneration	3 - 5
Greenhouse testing	1 - 2
Small plot trials	1 - 2
2. Plant breeding	
Development of varieties	
(local adaptation and evaluation)	5
{Clearance for free dissemination and unrestricted growing conditions}	
3. Agronomy	
Registration (Plant Variety Protection)	2 - 3

	(10 - 14 years)
4. Marketing	

{5.1.3 Therefore:}

1. Clear and scientifically based guidelines.
2. Consistent but flexible guidelines.
3. Harmonization between countries.

Figure 8. Maize Hybrid Prisma to be sold in 12 countries

IV. GENERAL QUESTIONS

Information requested for the description of a genetic construction, the problematic sequences in genetic insertion and the concept of minimal construction

Prof. A. Kahn
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France

1. Field tests of transgenic plants in France

2. Objective of the field tests of transgenic plants (CGBM 1987-91)

	(per cent)
Herbicide resistance	48
Virus resistance	13
Pest resistance (insect and fungus)	11
Risk assessment	11
Cultural properties (stress resistance)	5.5
Nutritional improvement	5.5
Male sterility	7

3. Activity of the CGBM 1987-91 (outside transgenic plants)

Animal vaccines•••• 8

Biopesticides•••• 2

Rhizobium•••• 2

Yeasts••••• 2

Recombinant products (amino acid, chymosin) 3

Transgenic animals••• 1

4. "Philosophy" of the French CGBM

-- A good evaluation requires a good description.
-- Biohazard is expected to be reduced if genetic modifications are limited as much as possible to what is strictly necessary to the desired effect.
-- The goal of progress in biotechnology is not only to do more quickly, but also more safely.

5. A good description of transgenic plants

{5.1 What does the construct really include?}

-- Genes, promoters (wild or modified), enhancers, terminators.
-- Sequences of unknown functions: Origin? (homologous recombination)
-- ORI

-> Maps and analysed sequences.

{5.2 What is really integrated?}

-- In a Ti system, respect of the left and right boundaries?
-- If these boundaries have not been respected (>10-20 per cent for LB), what is the extent of integrated Ti plasmid?
-- Does this aberrantly integrated fragment include an ORI?

-> PCR, Southern blots, "flanking" probes.

{5.3 How many integration sites do the plants contain?}

-- Genetic segregation (active copies)
-- Southern blot analysis
-- Physical segregation [what is/are the active copy(ies)?]

-> The risk of unpredictable insertional mutations is proportional to the number of integration sites.

{5.4 How many copies do the plants contain in a given integration site (rough } {estimate)}

-- Restriction with enzymes cutting once in the insert
-- Appreciation from comparison with quantitative markers

-> Stability of long repeats?

{5.5 ...is impossible when using poly-dispersed carrier DNA (electroporation, } {biolistic)}

- Insertional mutagenesis and positive effects of DNA fragments randomly integrated in multiple, undetermined sites.

{5.6 ...is often difficult with new methods aimed at transferring DNA into } {monocots (electoporation, biolistic), even when using homogenous DNA}

-- Rearrangements, multiple integration sites

-> Multiple generations selected for the desired phenotype are expected to progressively eliminate copies in inactive sites and to allow segregation of active sites, therefore to "clean" undesirable extra DNA fragments from the plants.

6. Functional characterisation of a transgene

-- The transcripts (observed vs. expected)
- Northern blot, cDNA PCR

-> hybrid transcripts originating from or terminating in flanking plant DNA, tissue and developmental specificity.

-- The proteins
- Western blot

-> amounts, MW, location

-- Does a virus capsid protein form virus-like particles?

-> Packaging of foreign infectious genomes?

7. Interference between function of a transgene, external agents and ecosystems

-- A herbicide resistance gene can degrade the herbicide: toxicity for humans and environment of the degradation products?
-- Is a pest resistance gene safe for domestic insects (honey-bees)?

8. Limited genetic modifications

Unpredictable effects could result from extensive genetic modifications

-- Procaryotic ORI and recombination between plant DNA and soil microorganisms.

9. Open questions

-- In the future, management by the farmers of different plants resistant to many different herbicides
-- Occurrence of natural resistance to biopesticides (e.g. Bt toxin)
-- In virus-resistant plants, occurrence of new pathogenic agents.

10. The ideal transgenic fragment in a commercialised plant

-- Short
-- Defined
-- Stable
-- Limited to what is indispensable to the desired effect.

Transformation by means of {Agrobacterium Rhizogenes}

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1. Introduction

185. {Agrobacterium tumefaciens} causes crown gall in sensitive plants, whereas {A. rhizogenes }causes hairy root syndrome. Both bacteria induce the proliferation of plant cells through a similar mechanism. They possess plasmids, referred to as Ti (tumor inducing) and Ri (root inducing) plasmids respectively, part of which (T-DNA) is transferred to the nuclear genome of the plant cell in which it is incorporated and its genes are subsequently expressed.

2. The mechanism of T-DNA transfer

186. The mechanism of T-DNA transfer from Ti or Ri plasmids to the nucleus of sensitive plant cells is basically identical. It may be summarised as follows:

-- {Agrobacterium} fastens to the plant cells under the control of its chromosome genes.

-- Phenol compounds produced by wounded plant cells induce the expression of virulence genes ({vir}) through a two-component regulation system (captor/regulator). The {virA} product acts as a membrane captor of these phenol compounds and then modulates the activity of the {virG} product by transforming the inactive protein into a positive regulation protein required for inducing the {vir} regulon. The {virG}-activated product recognises a common sequence upstream of all {vir} genes and acts on them as a transcriptional activator.

-- The function of the virulence genes induced in this way allows the transfer, from the plasmid to the plant cell nucleus, of one DNA region (T region), whose borders are defined through imperfect direct repeats of a specific sequence of 25 pairs of bases.

187. The {virB}-coded proteins are membrane proteins similar to those encoded by conjugative plasmids in bacteria that constitute the pili through which DNA is transferred during conjugation.

188. The role of {virC}, which is still unclear, might be to recognise the overdrive, i.e. the sequence close to the right-hand border of the T-region, thus increasing transfer efficiency by facilitating access to the right-hand border for {virD} products.

189. The {virD} operon encodes for a specific endonuclease that acts on a strand of the border sequence for the T-region and is responsible for forming the T-strand, a single strand linear DNA through which genetic information would be transferred.

190. A {virE} product capable of linking specifically to the single strand of DNA would facilitate the movement, protection and/or integration of T-DNA.

191. It is still not known how T-DNA is transferred to the plant cell nucleus and how covalent integration occurs, apparently haphazardly, within the nuclear genome.

192. The size of the T-DNA is determined only by the distance separating the two borders on the plasmid, and it is possible, without modifying transfer efficiency, both to delete the T-region and to add any genes to be introduced in the plants, provided that the 24bp repetitions are maintained when forming the borders. Since the virulence functions operate in {trans} fashion relative to the border repeats of the region to be transferred, region {vir} and the T-region need not occur on the same molecule. Thus, in a binary system, these functions are carried by two independent compatible replicons.

3. Virulence functions and disarming of plasmids

193. The virulence functions of the two types of plasmid are equivalent and interchangeable: the T-DNA of Ti may be mobilised by the virulence functions of a Ri plasmid and vice versa. The main difference between Ti and Ri plasmids lies in the nature of the genes forming the T-DNA, i.e. the genes located between the borders on the plasmid and which will be expressed only after they have been inserted in the host chromosome.

194. The T-DNA from pTi encodes, among other things, enzymes responsible for auxin and cytokinin synthesis. Excess production of these growth substances in transformed plant cells make them tumoral. Expression of these genes is incompatible with the regeneration of whole plants. Consequently, in order to obtain a transgenic plant, disarmed Ti plasmids must be used, or in other words, plasmids with a T-region from which oncogene functions have been removed. For dissemination trials, disarmed plasmids from which practically all genes have been removed in the T-region are generally used.

195. The T-DNA of pRi has been the subject of far less advanced studies than that of pTi. Some strains of {A. rhizogenes} show a pRi with one single T-region, while others have both a TL and a TR region.

196. The TR region of agropine pRi is a vector of the genes {aux1} and {aux2} responsible for auxin synthesis, equivalent to those of the T-DNA of pTi. It also carries the three genes involved in agropine synthesis and a highly homologous ORF to the {rolB} gene of the TL-DNA. TR-DNA alone allows root growth, although the roots do not proliferate on a medium without hormones once separated from the infected plant.

197. Agropine pRi TL-DNA is similar to the single T-DNA of mannopine pRi and causes hairy-root disease. The functions of its genes are far from being understood. Only the functions are currently identified of supposed for this T-DNA which bears at least ORF 18:

- -- ORF 1 corresponds to agrokinopine synthase;
- -- the ORF 10 ({rolA}) product increases sensitivity to auxins;
- -- {rolB} and {rolC} (ORF 11 and 12) encode an indole glucoside hydrolase and a cytokinin glucoside hydrolase, respectively;
- -- super-expression of ORF 13 causes types of deformation attributed to the synthesis of a morphogenic substance in plants;
- -- homologies are found between ORF 6 and a region of the {Rhizobium} genome, between ORF 8 and {aux1} and between ORF 13a and regulatory proteins.

198. Homology has also been found between ORF 11 and 12 and the genome of certain members of the Nicotiana family, and between the TR-DNA of agropine pRi and the apple genome. These observations are interpreted as traces of former transformation events. In the case of {N.} {Glauca}, it has been shown that the genes equivalent to {rolB} and {rolC}, which are not expressed in this species, are, on the other hand, expressed in hybrids {N.} {glauca x N.} {langsdorffi}, which have long been known to form tumours at injury sites. Transcription of these genes in hybrids is suppressed by adding exogenous auxin.

199. From the root hairs induced by {A. rhizogenes}, whole plants can be obtained. The presence of pRi T-DNA is therefore compatible with regeneration. Consequently, although the plants obtained exhibit a specific phenotype (crimped leaves, shorter internodes and greater root mass etc.), there is no need to disarm the T-DNA of pRi before using it as a plant transformation vector.

4. Practical applications and possible approaches

200. Because of the onset of root hairs and the specific phenotype of plants transformed using {A.} {rhizogenes}, the T-DNA of pRi is a convenient transformation marker which can therefore replace conventional markers of resistance to antibiotics or herbicides.

201. One of the practical applications of this system is the use of a binary system with a wild pRi as assistant plasmid and a second plasmid with a T region bearing only the gene of interest. Since double transformation is a relatively frequent occurrence, many of the transformed cells bear both T-DNAs, that of pRi and that of interest. Consequently, most of the transformed plants regenerated from the roots obtained show a specific phenotype and also contain the gene required. Because they arise from two independent T regions, the two T-DNA are not usually linked on the host chromosome. As a result of segregation of the two T-DNA during meiosis, among the progeny of the plants there will be transgenic plants containing the gene required alone, the T-DNA from pRi having been discarded. This is therefore a suitable method for obtaining transgenic plants without any superfluous sequences. In order to obtain ultimately one single integrated gene, this system of double transformation followed by segregation might of course also be envisaged between any selection marker and the gene required, but so far, only double transformation of T-DNA from pRi plus minimal T-DNA seems to have been used for this purpose.

202. Because maintenance of the T-DNA from pRi is not incompatible with regeneration, transgenic plants containing both the new genes required and also those of T-DNA from pRi may obviously also be obtained. The problem therefore arises whether dissemination of such plants should be authorised. There are two possible approaches to this problem:

-- In line with our past practice, dissemination is only acceptable for organisms which contain as new sequences solely genes whose function can be described and is known to be without risk to the environment. Obviously, it would be premature to envisage disseminating plants containing so many genes with as yet undetermined roles. This appears to be the more sensible approach.

-- On the other hand, it could be argued that the T-DNA of pRi is of "natural" origin since it is not the product of an {in vitro} operation, and in some plants there are traces of former transformation events, so that its dissemination could be regarded as harmless or not yet falling within the scope of our committees.

203. It would seem appropriate to discuss the basis for both ways of considering the question.

Acceptability of the use of antibiotic resistance genes as marker genes in transgenic plants

Dr. Hans Bergmans
Provisional Committee on Genetic Modification
The Netherlands

1. Marker genes

204. Marker genes are used in the development of transgenic plants, either as a selectable trait during transformation and selection of the transgenic organisms, or as a phenotypic trait indicating the expression of this transgene and the other transgenes linked to it.

205. Antibiotic resistance, notably kanamycin resistance and hygromycin resistance, are commonly used as selectable traits, while the {•-glucuronidase} gene (GUS), usually in the modified form carrying a eukaryotic intron (GUS-INT), is most often used as phenotypic reporter gene.

206. The kanamycin resistance gene commonly used, the NPT-II (neomycin phosphotransferase) gene derived from transposon Tn5, is an aminoglycoside-modifying enzyme belonging to the {aphA3} class of genes, that inactivate kanamycin and neomycin, but not the more recently developed aminoglycosides, e.g. amikacin and gentamycin.

207. Because the widespread use of the NPT-II gene as marker gene, the Dutch Ministry of the Environment commissioned a project for the evaluation of the possible risks of kanamycin resistance in transgenic plants. During the preparation of this report the Calgene report on the food safety of kanamycin resistance became available, in which basically the same conclusions were reached.

2. Identified risks

-- Selective advantage of kanamycin-resistant plants. Kanamycin resistance might enhance weediness of a plant, if environmental concentrations of kanamycin are high enough.

-- Transfer of the NPT-II gene to other organisms; effects in the receiving organism. Gene flow can occur to wild relatives. More hypothetical gene flow could occur to micro-organisms by horizontal gene transfer, either in the environment or in the gut. Uptake of the gene into gut epithelial cells could even be envisaged.

-- Toxicity of the gene product. This issue arises because transgenic plants carrying the NPT-II gene may, in many cases, be crops meant for human consumption.

{2.1 Selective advantage:} { concentration of neomycin/kanamycin in soil}

208. Neomycin and kanamycin are in extensive veterinary use; the drugs have only limited use in human medicine because of undesirable side effects. In the Netherlands, the yearly use of the antibiotics amounts to an estimated 30 000 kg in veterinary use, and approximately 100 kg in human medical use. From these data an estimate can be made of the concentration of the drugs in soil, based on the following assumptions: the total amount of the antibiotics used is spread (as part of the manure) on 75 per cent of the arable land and mixes in with 5cm top soil, at soil humidity of 0.2 ml/g. The final concentration then amounts to 0.13 •g/ml. The actual concentration will, however, be appreciably lower, due to inactivation of the drugs by adhesion to soil particles.

209. The conclusion is that the final concentration reached in soil is too low by at least two orders of magnitude to be of any effect on plant growth. Selective advantage of plants carrying the NPT-II gene is highly unlikely.

{2.2 Gene transfer}

210. The same reasoning leads to the conclusion that transfer of the NPT-II gene to other plants would also not lead to any selective advantage of these plants.

211. Reasonable calculations of the effect of DNA carrying the NPT-II gene on soil micro-organisms are not possible, as the parameters involved, notably the transformability of soil micro-organisms, are not sufficiently known.

212. However, calculations that have been made give extremely low values (10-12 KmR transformants per gram soil), while naturally occurring KmR soil micro-organisms are much more abundant (103-105 KmR organisms per gram, although KmR due to aminoglycoside modifying enzymes may occur less frequently).

213. Transfer of the NPT-II gene to gut microflora after ingestion of food containing the NPT-II gene is equally difficult to estimate. Calculations predict KmR transformants to be formed at a frequency of ten per year. Again, KmR micro-organisms are present in the normal gut flora at much higher frequencies.

214. Uptake of the DNA by gut epithelial cells will occur, as recent experiments have shown that (probably) any ingested gene will be taken up by gut epithelium; this has never been perceived as a risk.

{2.3 Toxicity of the gene product}

215. As bacteria containing the NPT-II gene occur regularly in the gut microflora, the NPT-II gene product has a long history of exposure in humans, without apparent toxic effects. Some caution may be needed, as it should be kept in mind that the concentration of the gene product in food may be much higher, and that the gene product may be post-translationally modified (e.g. by glycosilation) while the bacterial gene product is not. There is however no {a }{priori }reason to suspect that the gene product will have any toxic effects on humans.

216. On the basis of this argument, the Dutch Committee on Genetically Modified Organisms has advised the Ministry of the Environment that no adverse effects are to be expected from deliberate release of transgenic plants carrying the {aphA3} gene as selectable marker.

217. The risk evaluation of the {aphA3} gene may be used as a pilot study for the risk evaluation of other marker genes, e.g. the hygromycin phosphotransferase gene that is used in the development of transgenic plants that are naturally resistant to kanamycin.

Potential for Use of Herbicide Resistant Crop Plants

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1. Introduction

218. Many people are still using the term Herbicide Tolerant Crop (HTC) to refer to crops that were made tolerant by nature. The term Herbicide Resistant Crops is now used for crops made resistant by man.

219. In the United States, herbicides, by volume, comprise 65-70 per cent of pesticide use in US agriculture, so that if pesticide use is to be reduced significantly, herbicide use must be reduced. A number of economic and technical factors affect a farmer's decision to use a particular herbicide at a particular time at a given rate to control a specific weed.

220. Policy-makers want to formulate policies and enact laws that affect herbicide use in some socially desirable way. They attempt to understand the complexity of weed management decisions. Unfortunately, policy-makers generally do not attend weed science meetings, and weed scientists generally are not represented in the policy-making process. As a result, when policy is set or laws are passed, they may be based on simplified assumptions that ignore the complexities of weed management, and may be counter-productive or totally ineffective.

221. Legislation is proposed in the United States that would ban using public funds for herbicide-resistant plant development research. However, development research would not prohibit basic research to develop the tools required to develop herbicide-resistant crops. The following discussion examines some of the issues surrounding the development of herbicide-resistant crops, in an attempt to clarify some important questions.

222. Farmers control weeds by mechanical seed bed preparation, by in-season cultivation or by herbicides. In the United States, 29 per cent of the crop acres are on conservation tillage, while 13 per cent of 60 million acres of soybeans and 11 per cent of 70 million acres of corn are in No-till, i.e. there is no mechanical soil disturbance for seed bed preparation or weed control.

{Note}:

1. This presentation was prepared on very short notice, and would not have been possible without a great deal of material supplied by Dr. Gianessi of Resources for the Future.

223. Although a wide variety of herbicides is available, not all herbicides can be used on all crops. Atrazine can be used safely for corn but damages soybeans. Imazaquin is selective for soybeans but damages corn. By contrast, neither corn nor soybean plants can be directly sprayed with glyphosate, since both are susceptible to it.

224. Traditional methods have been used to determine crops with natural tolerance to herbicides. In some cases, plant breeders have bred individual cultivars that have resistance to a particular herbicide. Until recently, such an exchange of genes was confined to cultivars within the same species.

225. Modern biotechnology techniques have made it possible to expand the gene pool for the development of plant cultivars that are resistant to certain herbicides. Researchers at Monsanto, for example, have developed a soybean plant that is resistant to glyphosate sprays by placing a single gene from another plant species (from a petunia plant) into a soybean plant. Thus, a new plant has been created that could not have been created without modern biotechnology.

2. Development of herbicide-resistant crops

226. The main question is whether resistant plants developed in this way will increase or decrease the use of herbicides. Four crops -- soybeans, lettuce, cotton and potatoes -- have been the subject of scientific papers at recent weed science meetings. They have been discussed in conjunction with two pesticides -- Monsanto’s glyphosate and Rhône Poulenc’s bromoxynil.

{a) Soybean}

227. About 95 per cent of the United States’ 60 million acres of soybeans are treated with herbicides, with an average use rate of about 1.5 lbs of active ingredient/acre. The herbicide cost/acre is approximately $15. The soybean herbicide market is very large and competitive with 26 active ingredients presently registered, and new herbicides continue to be introduced.

228. Growers may use residual soil applied herbicides (pre-plant or pre-emergence) to treat an anticipated problem, or foliar applications (post-emergence) to treat an actual problem.

229. Large acreages of soybeans are treated with pre-emergence, pre-plant herbicides, but soybean growers have been switching to post-emergence herbicides to control both grass and broadleaf weeds. This makes possible the adoption of conservation tillage or No-till production systems.

230. As mentioned previously, Monsanto has developed a glyphosate-resistant soybean. Glyphosate is a non-selective, foliar-applied herbicide; it will therefore control both grass and broadleaf weeds as well as any crop plant. It is strictly a post-emergence herbicide with insignificant residual soil activity. The current price of glyphosate is about $10.00/lb of active ingredient.

231. The four major weeds of soybeans in the Midwest -- foxtail, cocklebur, pigweed and velvetleaf -- can be controlled with 1.0 lb/acre of glyphosate, compared to the total of 1.5 lbs of herbicide presently being used. If a pound of glyphosate were used to control both the grass and the broadleaf weeds, the current cost would be $10/acre, compared to the present cost of $15/acre. If some 57 million acres of soybeans receive 1.5 lbs of herbicide per year and if, with the availability of glyphosate-tolerant cultivars, 20 per cent of these acres received glyphosate this would mean a reduction of 5.7 million lbs of herbicides/year and herbicide savings to the grower of $57 million/year.

232. It is important to remember that glyphosate-resistant soybeans do not require a glyphosate application to grow normally. The gene transfer has simply made it possible to apply glyphosate directly on soybean plants. It is worth asking whether the availability of the glyphosate-resistant soybean cultivar could increase the number of growers who would attempt to go without herbicides or go to No-till and use herbicides when needed.

233. The National Academy of Sciences report on alternative agriculture in 1989 included descriptions of a number of soybean farmers who had eliminated or attempted to eliminate their use of herbicides. One grower who plants on ridges plans to control weeds with cultivation. But if he is unable to cultivate at the proper time because of wet fields, he simple applies post-emergence herbicides. Another farmer tried to grow soybeans without herbicides in 1986, but could not cultivate because the fields were too wet. As a result, there were severe weed problems and his crop was not profitable. Another farmer, who was also unable to cultivate because of heavy rains and wet fields, applied a post-emergence herbicide by airplane to control grass weeds and followed with another post-emergence herbicide by airplane to control broadleaves.

234. The point is that it is very risky for farmers to give up the use of herbicides completely. It may well be that if growers had cheaper broad spectrum post-emergence options, more would try to grow soybeans without herbicides. If the system without herbicides did not work and if they had planted a glyphosate-resistant cultivar, they could use glyphosate and kill weeds economically without harming the soybeans. Providing more low-cost post-emergence options may persuade farmers to take the risk of trying to grow soybeans with cultivation and without herbicides. It would also encourage many to go to No-till in order to control erosion and improve the soil.

{b) Lettuce}

235. For lettuce, a recent estimate of annual herbicide sales by crop showed about $1 billion of sales for soybeans compared to sales of only $5 million for lettuce. As a result, it is highly unlikely that the herbicide industry will invest millions of dollars to develop a herbicide-resistant lettuce plant. In fact, there are no new herbicides presently being developed for lettuce -- the market is too small.

236. However, research is underway at the University of Florida to develop lettuce cultivars with resistance to glyphosate. The reason for the interest is that, in Florida, lettuce is grown on peat soils, while in other states, such as California, it is grown on mineral soils. The herbicides that are registered for use for lettuce are applied to the soil and are ineffective in the highly organic peat soils.

237. In 1979, Florida lettuce growers used 58 000 lbs of CDEC. However, the manufacturer withdrew the registration in the early 1980s. For ten years Florida lettuce growers have not had an herbicide that would control weeds without crop damage. Dr. Joan Dusky, a Florida Weed Scientist, pointed out in 1982 that without CDEC, lettuce growers would have to use scarce, expensive manual labour or mechanical cultivation to control weeds. They have been successful in controlling weeds using nonchemical means. About 10 000 acres of lettuce in Florida are hoed by hand for weed control purposes.

238. The cost of handweeding represents a substantial portion of the operating cost of growing lettuce in Florida. Handweeding costs about $200/acre according to the latest cost of production budgets for Florida lettuce. This represents about 13 per cent of the total operating costs for growing lettuce. If glyphosate were available to control weeds in lettuce, 1-2 lbs/acre would control the weeds adequately at a cost of $10-20/acre. Lettuce growers would save $180-$190/acre in production costs (or $1.8-$1.9 million/yr in total weed control savings). Herbicide use in Florida lettuce would go up by 10 000-20 000 lbs/year.

{c) Cotton}

239. With respect to cotton, research has shown that for cotton to achieve maximum yields, it needs to be grown in a weed-free environment for six to seven weeks after emergence. As cotton grows very slowly in comparison to many weeds, if weed control is not practised in a timely fashion, cotton can be easily overwhelmed by weeds.

240. In many areas, weed control in cotton involves: one pre-plant broadcast application of herbicide, one pre-emergence banded application, and three to five directed post-emergence banded applications. This weed management system has evolved because cotton is not completely tolerant to any post-emergence over-the-top herbicide. Even with this system cotton injury quite often results. If crop/weed height differences are not established, an over-the-top application of MSMA must be used, even though cotton is injured.

241. Yield losses due to weeds in Mississippi declined from 6 to 8 per cent to about 2 per cent in the mid-1980s; however, cotton yield losses due to weeds have climbed back to about 6 per cent in 1990. The reason for the increase is that certain weed species, such as morning glories, are not well controlled by the currently available herbicides. In an effort to achieve satisfactory control, growers have been applying more herbicides in the past ten years. In 1979, they sprayed 3 lbs. of herbicide per acre. In 1990 they sprayed 5 lbs.

242. Bromoxynil, which is a post-emergence herbicide, offers a potential solution to this problem. It controls broadleaf weed species, such as morning glories, has no residual soil activity, and disappears rapidly from the soil. Currently, bromoxynil is used in small grains, corn, garlic, onions and rice, which are naturally tolerant to bromoxynil. Bromoxynil is effective at rates of 1/4 to 3/4 lb of active ingredient/acre and costs about $20 a pound.

243. Genetic engineers at Calgene have managed to transfer a gene from a soil micro-organism into cotton plants that confers resistance to bromoxynil. Experiments were conducted in a number of states in 1991 and the initial findings were consistent: the gene-altered cotton plants showed a high level of resistance to bromoxynil -- up to 15 lbs/acre with no damage to cotton. Bromoxynil has also provided a high degree of control for the troublesome weeds, particularly morning glories, for which control has been in excess of 99 per cent.

244. Dr. Harold Coble at North Carolina State University is a weed scientist working with the bromoxynil-resistant cotton. He estimates that bromoxynil could be used twice at 0.38 lbs/acre and provide effective weed control and that this use would replace several current applications and reduce total lbs of herbicide use in cotton by 3.74 to 5.24 lbs/acre. If just 10 per cent of the 12 million US cotton acres used bromoxynil instead of the present herbicide system, it would result in 4.5 to 6.5 million lbs reduction in herbicide use.

{d) Potato}

245. There is a wide difference between cotton and potatoes in terms of US herbicide sales, with cotton at $200 million and potatoes at $25 million. As for lettuce, it is unlikely that chemical companies are going to develop herbicides or herbicide-resistant potato cultivars -- the market is too small.

246. Idaho is the largest potato-growing state with 390 000 acres of potatoes valued at about $700 million. In 1979 in Idaho EPTC was used on 14 per cent of the acres at 2.7 lbs/acre for a total of 131 000 lbs. Metribuzin was used on 60 per cent of the acres at the rate of 0.6 lbs per acre for a total of 125 000 lbs. Since 1979, heavy reliance on metribuzin on the same potato acres year after year has led to the development of some resistant weed species. One weed that is becoming a significant problem in Idaho potatoes is known as hairy nightshade.

247. In 1990, EPTC was being used on 47 per cent of the acreage and metribuzin was being used on 86 per cent. Herbicides have increased by 550 000 lbs per year since 1979, as growers have used more of them, particularly EPTC, to control some of these troublesome weed species. However, as Dr. Eberlein at the University of Idaho and her colleagues have pointed out, EPTC controls early germinating nightshades only. Nightshades that escape control with the early application cannot be adequately controlled with currently available herbicides. So metribuzin is not working as well as it used to, and EPTC fills in only to a certain extent. When the weeds are not controlled, the potato fields can have weeds towering over potatoes; this not only represents a yield loss, it also makes harvesting difficult.

Therefore, Dr. Eberlein conducted experiments to determine whether potato plants could be genetically engineered to have resistance for bromoxynil. She has been successful in inserting the bromoxynil-tolerant gene into several potato cultivars. Plants have been grown in greenhouses and in field trials and they show excellent resistance to bromoxynil. She estimates that if bromoxynil were available for use in Idaho potatoes, the total amount of herbicide application could be reduced between 40 and 85 per cent.

3. General considerations

{a) Loss of weed control technology}

248. More generally, there is at present a loss of weed control technologies. Herbicide registrations are being dropped by chemical companies. Rather than incur the costs of re-registering herbicides with low volumes, industry is choosing to re-register large volume uses and new compounds. Weed resistance problems are emerging as a result of continued use of particular herbicides. As a result, some older herbicides do not work as well as they did. Because of concerns regarding soil erosion, there are also restrictions on the amount of cultivation that farmers can use to control weeds.

249. There are several options for replacing these lost weed control technologies. Research could be targeted towards developing non-chemical means of weed control, such as biological control and use of cover crops. New herbicides will also be investigated, particularly by chemical companies. The development of herbicide-resistant crops is another strategy that, if successful, could increase weed control options.

250. With regard to the funding of herbicide-resistant crop research, it seems clear that incentives exist for large acreage crops but not necessarily for small acreage crops for which herbicide sales are small.

251. There are four crops -- corn, cotton, soybeans and small grains -- that account for about 70 per cent of all herbicide sales. Each of these crops represents an average of $587 million in herbicide sales. By contrast, there are 80 other crops that account for an average of only about $11 million each in herbicide sales. The development of new herbicides and herbicide-resistant cultivars by the commercial sector will, by economic necessity, be largely targeted to the four high volume crops.

252. Therefore, it appears that the development of Herbicide Resistant Crops involving minor uses of herbicides will be left to some area of the public sector or to the seed companies. One must remember, however, that the herbicide still has to be registered, and this requires a commitment from the registrant of the herbicide.

{b) Uncertainties in developing herbicide-resistant crops}

253. There are also uncertainties involved in the development of herbicide-resistant crops. Very little is known about the yields and agronomic characteristics of gene-altered plants. If the plants do not yield as well or are less fit as a result of the gene transfer, it is unlikely that there will be commercial development.

254. Another uncertainty revolves around the minor crop issue. Even if university researchers are successful in developing an herbicide-resistant plant, there are no guarantees that a chemical company will pursue the registration. It is not only a question of cost but also a question of whether or not the residue level in the raw agricultural commodity is such that registration is possible, i.e. whether there is sufficient allowable daily intake available to register additional uses.

255. With regard to the adoption of herbicide-resistant crops by farmers, there are uncertainties as well. There will be new herbicides developed for the major markets that will compete with the herbicides used with resistant crops. Obviously, shifts in herbicide prices affect what growers choose to use. Further, there is the issue of the cost of seed. Just because the gene-altered plants are introduced does not mean they are going to capture the market.

{c) Potential risks and benefits}

256. As to potential risks, a new plant introduction certainly presents some ecological risks, and there may be others due to residues in the raw agricultural commodity. Further potential risk are: increased use of herbicides, increased grower dependence on herbicides, and high cost of seed.

257. On the other side of the coin, the potential benefits include:

-- reduced cost of weed control;
-- reduced lbs of herbicide use;
-- use of more environmental- and human-compatible herbicides, i.e. more lbs of herbicide may be better than fewer, in some cases;
-- increased No-till to reduce soil erosion and consumption of fossil fuels;
-- reduced residues in soil, water, air and food;
-- ability to rotate herbicides and crops to avoid development of herbicide-tolerant weeds.

4. Conclusion

258. Weed control strategies are complex and vary from crop to crop and from location to location. It is really not possible to generalise about the impact of the development of herbicide-resistant crops. It will be necessary to make an assessment on a crop by crop basis, and in the final analysis both of the

following views may be correct, depending upon the crop and the particular situation of weed species, soil, etc.: "Herbicide-tolerant crops will increase the use of certain herbicides and perpetuate agriculture's dependence on herbicides", (Biotechnology's Bitter Harvest, March 1990). "There will be an opportunity to reduce the number and quantity of herbicides applied, and to enhance the use of No-till or minimum tillage systems" (Herbicide Resistant Crops - CAST - May 1991).

259. In the United States the total Weed Science expenditure of public funds is $31 777 000, of which only $1 917 000 (6.0 per cent) is devoted to genetic engineering aimed at development of herbicide-resistant crops. Of this amount 62 per cent or $1 194 000 supports research by the universities with $176 000 (9.2 per cent) being spent in this area by the USDA Agricultural Research Service.

ANNEX 1

PROGRAMME

5 APRIL 1992

18H00 Opening speeches by:

- Mr. Axel Kahn, Chairman of the Biomolecular Engineering Committee (France)
- Mr. Robert Ducluzeau, General Director of the Research Centre of Jouy-en-Josas

18H45: • Cocktail

6 APRIL 1992

09H00: • Welcome address: Mr. Axel Kahn, (France)

09H15: • Session on "{potato}"

• Chair: Mr. Axel Kahn (France)

• -- General presentation
• Mr. Phil Dale, Centre for Plant Science Research, Norwich, (Norfolk, United Kingdom)

• -- Case review: Mr. Peter de Haan, Provisional Committee on Genetic Modification (The Netherlands)

• -- Case review: Mr. Michael Schechtman, Animal and Plant Health Inspection Service (APHIS)/Department of Agriculture (United States)

12H00: • Lunch

13H15: • Session on "{rape} {seed}"

• Chair: Mr. John Beringer, Chairman of the Advisory Committee on Releases into the Environment (UK)

• -- General presentation

• Ms. Yvette Dattée, Research Director and member of the Biomolecular Engineering Committee (France)

• Mr. Michel Renard, Researcher

• -- Case review: Ms. L. Duke, Chief, Variety Registration Office, Food Production, Inspection Branch, Animal & Plant Health Directorate (Canada)

• -- Presentation by an applicant: Mr. Patrick Rüdelsheim, Director for Field Operations, Plant Genetic Systems (Belgium)

15H45: • Coffee Break

16H15: • Session on "{corn/maize}"

• Chair: Ms. Sally McCammon, Animal and Plant Health Inspection Service (APHIS)/Department of Agriculture (US)

• -- General presentation by a competent authority:

• Ms. Sally McCammon (United States)•••

• -- Case review: Mr. Francis Quetier, University of Orsay (France)

• -- Presentation by an applicant: Mr. E. Chasseray, Regulatory Co-ordinator, Seeds Division, Ciba-Geigy (Switzerland)

Conclusions: • Mr. Axel Kahn (France)

19H30: • Dinner offered by the French authorities at Château de Grignon (Salon Bessières), National Institute of Agronomy, Thiverval, Grignon.

• [A bus will take participants directly from the Seminar centre to the Château de Grignon].

7 APRIL 1992

08H30: • Analysis of general questions that emerged during the previous sessions

• • Chair: Mr. Jeff Schell, Director, Max Planck Institute for Breeding Research, Section for Fundamental Genetics of Plant Breeding (Germany)

• • -- Information requested for the description of a genetic construction, the problematic sequences in a genetic insertion and the concept of minimal construction: Mr. Axel Kahn (France)

• • -- Complementary interventions by different countries:
• • . The Netherlands: Mr. J.E.N. Bergmans;
• • . United States: Ms. Sally McCammon

10H00: • Coffee break

10H30: • -- Transformation with {Agrobacterium rhizogenes}:
• • Ms. Francine Casse-Delbart, Research Director, member of the French Biomolecular Engineering Committee (France)

• • -- Complementary interventions by different countries
• •

12H00: • Lunch•••• •••

13H00: -- Justification and restriction of the use of marker genes conferring antibiotic resistance:
• • Mr. J.E.N. Bergmans, Secretary of the Provisional Committee of Genetic Modification (The Netherlands)

• • -- Complementary interventions by different countries:
• • . United Kingdom: Mr. Beringer;
• • . United States: Mr. M. Schechtman
•

14H30: • -- Justification and restriction of the use of herbicide resistance genes: "Potential Use of Herbicide Resistant Crop Plants":
• • Mr. Joe Antognini, National Programme Leader, Weed Science Agriculture Research Service, Department of Agriculture (United States)

• • -- Complementary interventions by different countries:
• • . The Netherlands: Mr. Bergmans

16H00: • Coffee break

16H30: • Final conclusions, Mr. Jeff Schell

17H30: • End of the meeting

ANNEX 2

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