University of Guelph Research Guidelines

Agricultural Biotechnology – Plants with Novel Traits (PNTs)

Effective Date: March 2008

Applicable Legislation:

Administered by the Canadian Food Inspection Agency (CFIA):
- Feeds Act
- Fertilizers Act
- Health of Animals Act
- Plant Protection Act
- Seeds Act

Administered by Health Canada:
- Food and Drugs Act
- Pest Control Products Act

Administered by Environment Canada:
- Canadian Environmental Protection Act

Intent: to promote compliance with federally prescribed requirements for human and animal health protection, environmental protection, and safety performance concerning biotechnology-derived plants, novel feeds, and related novel agricultural products;

to reference appropriate plant biosafety levels and containment/confine ment guidelines for PNT research; and

to publicize University requirements and resources for due diligence and performance of best agricultural practices.

Scope: all University and contract PNT research involving field crop, horticultural and ornamental plants, turf grasses, fruit and forest trees; includes indoor research and development involving experimental PNTs, and outdoor confined research field trials with experimental PNTs.

When Plant Biosafety Level 2 containment is necessary for research that presents potential for human allergenic exposure, University Safety Policy 851.11.01 will also be relevant (see http://www.uoquelpah.ca/ehs/policies/11-01.pdf).
<table>
<thead>
<tr>
<th>Definitions:</th>
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</thead>
<tbody>
<tr>
<td><strong>accidental release</strong></td>
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<td><strong>biodiversity</strong></td>
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<td><strong>biotechnology</strong></td>
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<td><strong>CFIA</strong></td>
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<td><strong>confined release</strong></td>
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<td><strong>confined research field trial</strong></td>
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</table>
**ecosystem**
a dynamic complex of plant, animal and microorganism communities and their non-living environment interacting as a functional unit.

**environment**
components of the earth including the atmosphere, land, water, all organic and inorganic matter and living organisms, and all interacting natural and managed (e.g., agricultural) ecosystems.

**exotic**
on-non-native, refers to an organism that has been introduced into an area.

**Health Canada (HC)**
has regulatory responsibility for the health and safety assessment of novel human foods, drugs, cosmetics, medical devices and pest control products.

**microorganism**
any bacteria, mycoplasma, Chlamydia, rickettsia, protozoa, fungi, algae, viruses, parts of these microorganisms and any combination thereof.

**novel trait**
a new characteristic or attribute scientifically introduced to a plant, a food or food ingredient, see [http://www.inspection.gc.ca/english/sci/biotech/reg/novnoue.shtml](http://www.inspection.gc.ca/english/sci/biotech/reg/novnoue.shtml)

**NSNR** *New Substances Notification Regulations* pursuant to the *Canadian Environmental Protection Act*; regulate genetically engineered living organisms and microorganisms not regulated by other legislation (e.g., *Seeds Act*).

**organism**
any unicellular or multicellular biological entity capable of independent function and replication.

**plants**
includes but not limited to mosses, liverworts, macroscopic algae and vascular plants including crops, forest, weed and ornamental species.

**PMF**
plant molecular farming; the cultivation of plants for industrial, medicinal or scientifically useful bio-molecules (e.g., vaccines, antibodies, pharmaceuticals, industrial enzymes) rather than traditional uses as foods, feeds or fibres.
plant with novel trait: a plant with a characteristic not normally found in that species or a trait expressed outside the normal range of similar existing characteristics in that species. Novel traits are introduced via genetic modification techniques including conventional selective breeding, genetic engineering or mutagenesis. Most genetically engineered plants are PNTs but not all PNTs are created by genetic engineering. A livestock feed (including feeds from non-traditional sources and feeds used or approved in other countries) is also considered novel until listed in Schedule IV or V of the Feeds Regulations. Experimental PNTs (e.g., unapproved novel human or animal food crops, horticultural and marine plants, trees) are subject to regulatory controls by CFIA and extensive safety assessments before approval for unconfined release.

molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from the replication of these constructed molecules.

the means used to prevent movement of plant material, particularly pollen, from experimental containment or confinement. There are four principal methods to achieve reproductive isolation: geographic isolation, spatial separation, temporal isolation and physical isolation.

refers to each plant species/genetic modification combination documented for CFIA.

biological material that incorporates genetic material from completely unrelated organisms in order to exhibit or enhance desired characteristics.

any release of any genetic material, seeds or plant propagules of an experimental PNT into the environment without authorization by CFIA.

the unrestricted use of a CFIA-approved PNT in the environment, i.e., the PNT is no longer experimental.

Guidelines

1. Research and development and contract research involving plants with novel traits (PNTs), confined research field trials of experimental PNTs, and field work with PNTs approved for unconfined release, shall be conducted in compliance with federal and provincial requirements, and in an environmentally responsible manner pursuant to the University’s policy on environmental protection, http://www.uoquelp.ca/ehs/policies/01-01.pdf.

2. The Principal Investigator shall anticipate the following extraordinary issues that are common to all experimental PNT research whether contained indoors or confined outdoors:

- the novel traits of the agricultural product and the containment/confinement level required for biosafety;
- documentation needed for regulatory authorities;
- permissions to use growth facilities and/or field sites;
- physical security and biological containment;
- reproductive isolation for indoor experiments;
- field site confinement and reproductive isolation for outdoor experiments;
- presence of endangered species at the field trial site;
- orientation and training for all personnel growing PNTs;
- potential need for dedicated facilities and equipment;
- need for detection methods and quality control procedures for experimental PNTs;
- need for biosecurity and standard operating procedures;
- restrictions concerning harvested seeds and PNT biomass;
- monitoring, surveillance and stewardship commitments;
- prescribed record-keeping requirements;
- labeling, transportation, secure segregated storage, de-vitalization and destruction (by approved methods) of all experimental PNT biomass;
- management plans for herbicide-tolerant or insect-resistant experimental PNTs;
- annual compliance reporting obligations to CFIA;
- compliance with any third-party (technology) agreements;
- compliance obligations, commitments and costs related to prescribed post-harvest land-use restrictions and field site surveillance;
- contingency plans for accidental releases;
- communication strategies (e.g., for un-planned events);
- responsibility for costs to remedy any situation associated with incidents and accidental releases (as prescribed).

3. All experimental PNT research proposals and proposed contract research agreements (e.g., for indoor research and development work and for outdoor confined research field trials with experimental PNTs) should be reviewed by the Principal Investigator and the Director of Research Risk Management. PNT risk issues will be identified and the Plant Biosafety Level of containment should be determined before proposals are submitted to sponsors. (CFIA should also be consulted; see http://www.inspection.gc.ca/english/plaveg/bio/consult/tridec/doctype.shtml.)


5. Containment levels for laboratory research (e.g., for plant microorganisms, plant pathogens, media protection) shall be determined in accordance with Canada’s Laboratory Biosafety Guidelines, 3rd edition, 2004, http://www.phac-aspc.gc.ca/ols-bsl/lbg-ldmbl/.

6. Growth facility containment features and operational procedures (e.g., PNT inventories, seed logs, transit logs, waste logs) as summarized within this document describe the University’s performance standards for experimental PNT research.

7. The Principal Investigator, the Director of Research Risk Management, and the departmental growth facility coordinator should assess greenhouse space intended for PNT research, i.e., for containment features and physical security.
8. Experimental PNT research requiring Plant Biosafety Level 2 shall be documented for the University’s Biosafety Committee whenever potential exists for human allergenic response. See Safety Policy http://www.uoguelph.ca/ehs/policies/11-02.pdf.

9. The Vice-President Research may convene an ad hoc Biotechnology Review Committee to make recommendations about research proposals that involve issues such as transgenic plant research ethics, genetic provenance and third-party rights, material transfer agreements, utilization of genetic resources and derivatives, biodiversity, outstanding concerns about safety performance, containment or confinement, etc. This Biotechnology Review Committee shall include at least two members who are non-stakeholders.

10. The Director of Research Risk Management should be consulted about landowners’ permissions needed and/or granted for University–managed confined research field trials and for post-harvest field site monitoring. Property owners whose lands lie within the isolation distance from a confined research field trial must be notified. Neighbouring owners should be notified.

11. The Director of Research Risk Management will assist Principal Investigators with import permit applications for PNTs, confined research field trial applications, letters of authorization, standard operating procedures, inspections, audits and other CFIA compliance-related matters. See http://www.inspection.gc.ca/english/plaveg/bio/pbobve.shtml.

12. The Principal Investigator must provide mandatory orientations for research staff regarding CFIA-prescribed terms and conditions for experimental PNT research. Topics should include the nature of the PNT, containment required, transfer and confinement protocols related to seeding, cultivation, harvesting, seed collection, and equipment clean-up, post-harvest surveillance and record-keeping requirements, contingency plans for unauthorized releases, incident notification, and de-vitalization/destruction methods for all PNT biomass.

13. Confined research field trial site managers must retain trial protocols, field maps, seed logs, site activity and monitoring records, contingency plans and corrective action reports at or near the field site. PNT research personnel shall accommodate
CFIA and other inspectors to enable authorities to carry out their duties. The Principal Investigator and the Director of Research Risk Management must be notified about all external (e.g., CFIA) field site inspections.

14. Experimental PNT research laboratory records, confined research field trial site records, PNT biomass destruction records and other prescribed documentation are subject to internal and external audits. The Principal Investigator must notify the Director of Research Risk Management about all external (e.g., CFIA) laboratory and growth facility audits.

15. Accidental or unauthorized releases, breaches of containment and removals of PNTs must be reported immediately by the Principal Investigator to the Director of Research Risk Management, to his/her Department Chair and College Dean, and to the Vice-President Research. In accordance with CFIA requirements, the Vice-President Research shall notify the Canadian Food Inspection Agency (613-225-2342) and/or Environment Canada. Written corrective action reports may be required.

16. Experimental PNT research facilities (laboratories, growth chambers, greenhouse spaces) must be cleaned upon completion or termination of PNT research projects. All PNT biomass must be destroyed as prescribed by CFIA.

17. Research and development with PNTs intended for plant molecular farming (PMF) should be discussed with the Director of Research Risk Management. Containment standards, management protocols and disposal of all PMF biomass will be determined in accordance with directions from CFIA and additional guidelines herein.


19. Research trials with novel feeds will require unique standard operating procedures (e.g., security of the novel feed, management of animal manure and by-products, disposition of
all animals, record keeping). Contact the Director of Research Risk Management (extension 52048) for advice.

20. The Director of Research Risk Management is available to assist researchers with all regulatory compliance (e.g., CFIA, NSNR) obligations.

Additional Guidelines:


Before being approved, all biotechnology-derived products must undergo pre-market science-based safety assessments to investigate potential harms to the environment and to animal and human health. The novelty of the agricultural product, not its method of production (e.g., recombinant DNA technology, mutagenesis, selective breeding techniques), triggers the extent of the regulatory scrutiny. Researchers and product developers are responsible for submitting all data requested by federal authorities for their reviews.

The regulatory agencies responsible for plant biotechnology in Canada are the Canadian Food Inspection Agency (CFIA), Health Canada (HC) and Environment Canada (EC). (See www.bioregulations.gc.ca.) CFIA serves as the lead agency for plant biosafety and involves HC and EC for any necessary concurrent approvals. As experimental agricultural products progress through the stages of research and development, confined research field trials, assessments for environmental, animal and human safety, variety registration, commercialization and marketing, the regulatory agencies apply stage-related controls.

The Canadian Food Inspection Agency (CFIA) is responsible for regulating plants with novel traits (PNTs), novel animal feeds and feed ingredients, fertilizers, and veterinary biologics. CFIA will assess the potential risks of environmental effects and risks to animal and human health, issue import permits for experimental PNTs, administer the confined research field trial process, conduct safety approval assessments, oversee variety registration, and will dictate the process...
for unconfined release of approved PNTs. (See http://www.inspection.gc.ca/english/plaveg/bio/pbobbve.shtml.)


Environment Canada (EC) administers the New Substances Notification Regulations (NSNR) for organisms, chemicals and polymers pursuant to the Canadian Environmental Protection Act (CEPA). New products of biotechnology that are not assessed by CFIA or HC under other legislation, are evaluated by EC for potential harm to human health or the environment prior to approval for commercialization. See http://www.ec.gc.ca/substances/nsb/eng/home_e.shtml.

**Research and Development**

A plant with a novel trait is a plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct stable population of cultivated species in Canada, and that have been intentionally selected, created or introduced into a population of that species through a specific genetic change. It is the responsibility of the plant breeder or plant developer to determine the range of selected trait of cultivated populations of the plant species in Canada and to determine if the product of his/her research is a PNT.

Research and development organisms (e.g., PNTs) grown in containment are currently exempt from the NSNR notification requirements if there is no release into the environment of the organism, the genetic material of the organism, or material from the organism involved in toxicity. (Proposed amendments to legislation will require prescribed notifications to EC about PNT research.)

PNTs are initially produced in research laboratories or tissue culture facilities and are propagated in growth chambers or greenhouses under controlled conditions with physical and biological isolations to protect the natural environment. The containment and operational...
procedures recommended herein are intended to help promote due
diligence and best agricultural practices for research involving plants
with novel traits.

Pursuant to the *Plant Protection Act*, the import into Canada of
experimental (unapproved) PNTs, seeds, plant-associated organisms,
and novel livestock feeds derived from plants (for research and
development) requires a permit from the Plant Health and Production
Division of CFIA. Potential harm to biodiversity is assessed.

**Directive D-96-13: Import Permit Requirements for Plants with
Novel Traits and their Products** gives guidance; it is available at
http://www.inspection.gc.ca/english/plaveg/protect/dir/d-96-
13e.shtml.

Further instructions and the application form are available at

For more information about import permit requirements, see
http://www.inspection.gc.ca/english/plaveg/internat/d-94-
14lste.shtml.

It is advisable to consult the Director of Research Risk Management
(ext. 52048) about import permits and/or phytosanitary certificates for
exotic plants, seeds, cuttings or bare roots. Please see

**Plant Biosafety**

Plant Biosafety Levels for indoor containment of experimental PNTs
and associated organisms (that may contain recombinant DNA) are
based upon recommendations in Appendix P of the National Institutes
of Health (NIH) *Guidelines on Recombinant DNA and Gene
Recommended containment standards for plant biosafety minimize the
likelihood of accidental releases and unanticipated deleterious effects
on organisms and ecosystems. Experimental PNTs that may not
survive outdoors are not exempt from CFIA regulations. In general:

**Plant Biosafety Level 1** provides a low level of containment for
PNTs, transgenic plants and associated organisms for which there is no
evidence that the modified organism would survive and spread
uncontrolled in the environment;
**Plant Biosafety Level 2** provides a moderate level of containment for PNTs, transgenic plants and associated organisms that, if released, could be viable in the surrounding environment with negligible or manageable biological impact;

**Plant Biosafety Level 3** provides an even higher level of containment for PNTs, transgenic plants, plant pathogens and other organisms that have recognized potential for detrimental impacts on organisms and ecosystems beyond the containment facility;

**Plant Biosafety Level 4** provides the highest level of containment for experiments on exotic readily transmissible infectious agents that are potentially serious pathogens of Canadian crops, and that are performed in the presence of their arthropod vectors.

**Risk Assessments for PNTs**

Criteria for risk assessment and the identification of appropriate containment for PNTs (which are flexible given species-specific information), should include:

- source of the novel trait (*i.e.*, whether pathogenic or infectious, whether derived from a fragment of DNA, or from a complete genome);
- recipient organism (*i.e.*, mode and ease of dissemination, invasiveness, whether a noxious weed or capable of interbreeding with noxious weeds; potential for outcrossing with related species);
- nature of novel trait (*i.e.*, toxicity, biological activity, stability);
- potential for release into the natural environment (*i.e.*, reproductive limitations, regulated gene expression);
- local environment (*i.e.*, nature of nearby crops, presence of sexually compatible wild or weedy species);
- likelihood of fitness and survival in the natural environment;
- possibilities for multiplication in the environment;
- potential for detrimental impact on managed or natural ecosystems and probability of harm (toxicities) to non-target species.

Other factors for consideration include:

- ease of detection of the novel trait (*i.e.*, phenotypic characteristics or laboratory detection; see
• complexity of procedures needed to manage the transfers of plant materials;
• scale of the experiment (i.e., number of plants cultivated).

For experimental (unapproved) PNTs, control must be maintained over the entire life cycle of the novel plant wherever they are grown; i.e., in containment or confinement. When the likelihood of accidental releases of viable PNT material or (waste) biomass are minimized, the overall ecological risks of transgene escape are negated. Potential environmental risks (e.g., novel weed phenotypes) could be difficult to mitigate after-the-fact if accidental releases occurred.

**Recommended Containment Levels for Experimental PNTs**

Progressively stringent requirements for containment and biosafety are warranted as the risk potential of the experimental PNT increases:

<table>
<thead>
<tr>
<th>Risk Criteria</th>
<th>Description</th>
<th>Plant Biosafety Level</th>
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</thead>
<tbody>
<tr>
<td><strong>Weediness</strong></td>
<td>Not a noxious weed, cannot outcross with one</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Noxious weed or can inter-breed with weeds</td>
<td>2</td>
</tr>
<tr>
<td><strong>Dissemination</strong></td>
<td>Not easily disseminated</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Can outcross with other populations or related species</td>
<td>2</td>
</tr>
<tr>
<td><strong>Environmental Risk</strong></td>
<td>No detriment from PNT or associated DNA-modified common microorganism</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Release would have predictable minimal environmental impact</td>
<td>2</td>
</tr>
<tr>
<td><strong>Transgenic plant-associated microorganisms</strong></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Microorganisms are indigenous, or exotic but presenting no harm to natural ecosystems</td>
<td></td>
<td></td>
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<table>
<thead>
<tr>
<th><strong>Plant-associated transgenic insects</strong></th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insects that present no threat to ecosystems</td>
<td></td>
</tr>
</tbody>
</table>

| **Potential for detrimental impact on organisms or ecosystems beyond the containment facility** | 3 |

<table>
<thead>
<tr>
<th><strong>Transgenic Origin</strong></th>
<th>2</th>
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</thead>
<tbody>
<tr>
<td>Contains the complete genome of a non-exotic indigenous infectious agent or pathogen</td>
<td></td>
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</table>

| **Contains genome of an exotic infectious agent** | 3 |

| **Treated with an exotic infectious agent** | 3 |

| **Involves an exotic infectious agent with detriment to the environment** | 3 |

| **Genome of the infectious agent may be reconstituted in planta** | 3 |

| **Contains vertebrate toxin** | 3 |

| **Involves an exotic readily transmissible pathogen** | 4 |

<table>
<thead>
<tr>
<th><strong>DNA Used</strong></th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-coding DNA (not part of any organism or virus)</td>
<td></td>
</tr>
</tbody>
</table>

| **Segments are from a single non-chromosomal or viral source** | 1 |

| **From a particular organism propagated only in that organism** | 1 |
From species that exchange DNA by well-established physiological means (natural exchangers)

*In vitro* work with transiently transformed tissues

The highest Plant Biosafety Level associated with a risk criterion will determine the containment required for the experimental PNT.

**University Facilities for Experimental PNT Research**

The University of Guelph has laboratory and growth facilities suitable for Plant Biosafety Level 1 and Level 2 containment. Higher-level containment facilities do not exist at the University. Access to research spaces must be arranged via growth facility coordinators.

Experimental field crop and other PNTs that require Level 2 containment can be isolated in the Department of Plant Agriculture’s PNT Greenhouse at the Crop Science Building. The PNT Facility Coordinator may be contacted at extension 52476. The PA greenhouse at Bovey Building provides Plant Biosafety Level 1 containment. The Bovey greenhouse coordinator may be reached at extension 52788.

The College of Biological Sciences has PNT containment facilities in the New Science Complex that meet standards for Plant Biosafety Level 1 and 2. The greenhouse coordinator may be contacted at ext. 52685.

It may be desirable for security reasons to accommodate Level 2 greenhouse research, supervised by different principal investigators, in separate compartments.

Greenhouse research, supervised by different principal investigators at different Plant Biosafety Levels may be accommodated concurrently in the same compartment if the higher-level plant biosafety precautions are exercised by all personnel using this same space.

Confined field trial locations for research with PNTs, and contract research field trial locations for PNTs managed by University personnel (regardless of whether the University is the Applicant to CFIA or not), should be discussed with the Director of Research Risk Management, Office of VP Research, ext. 52048. Landowners’ consent, site security
and public relations are matters of potential concern. All confined research field trial sites must be approved by CFIA.

**Plant Biosafety Level 1 - Physical Containment Features**

*Laboratory Features*

As Plant Biosafety Level 1 involves little or no plant, pest or environmental risk, no special containment features are required beyond those features suitable for a well-designed, functional and secure microbiology laboratory. Noteworthy features include:

- locks on doors and on any openable windows;
- drain screens to collect pollen and detritus;
- proximity hand-washing facilities;
- separate spaces for hanging street clothing and lab coats;
- an autoclave available at or near the laboratory;
- screens with 30-mesh size or higher are recommended on all openings (window and ventilation openings) to preclude the transit of arthropod vectors.

*Growth Chamber and Greenhouse Features*

- locks on growth chamber access doors;
- locks on greenhouse access and compartment doors;
- self-closures on greenhouse access and compartment doors;
- drain screens to collect pollen and detritus;
- galvanized or stainless steel (easily cleanable) work surfaces;
- proximity hand-washing or hand sanitization facilities;
- separate spaces for hanging street clothing and lab coats;
- an autoclave available at or near the growth facility;
- screens with 30-mesh size or higher are recommended on all openings (window and ventilation openings) to preclude the transit of arthropod vectors.

**Plant Biosafety Level 1 – Recommended Operational Procedures**

In addition to good research practices (*e.g.*, planning, record keeping, performance, termination), recommended Biosafety Level 1 operational procedures are appropriate:

- confirm whether CFIA must be notified about the research;
• verify that potential use and storage locations for PNTs meet the aforementioned standards for plant biosafety containment;
• provide interactive orientations for staff and students that focus on managing containment for environmental protection;
• issue your departmental Greenhouse Manual to all staff;
• restrict greenhouse keys (or access cards) to a minimum number of authorized users;
• limit the number of locations where PNT biomass is developed, cultivated or stored; keep these locations locked when unattended and restrict third-party access as/when appropriate;
• maintain location-specific inventories of PNT materials;
• use distinctive colour labeling or identification codes to differentiate PNT and non-PNT materials;
• use ‘transit logs’ to record re-locations of PNTs;
• prohibit transfers of PNT materials to persons outside your research group, unless approved by the Principal Investigator;
• maintain chronological laboratory notes to document experimental PNT work and results;
• restrict PNT bench-top work to designated workstations;
• wear protective lab coat, gloves and eyewear as appropriate;
• ‘decontaminate’ and clean workstations each day;
• prevent the ‘escape’ of germplasm or novel product using physical containment, reproductive isolation from cross-fertile plants, separation distances, and appropriate procedural precautions;
• inspect plants for insect damage and implement an integrated pest management program in co-operation with the greenhouse facility manager;
• identify seed from PNTs in clearly marked containers with labels that identify the Principal Investigator’s name and date;
• segregate PNT seed from all other seed and store it in a locked laboratory or greenhouse cabinet; maintain ‘seed logs’;
• use closed non-breakable containers when transporting PNT seed, transgenic material and associated microorganisms outside of the containment area;
• contain waste PNT biomass for safe transport to the treatment area and ensure that exterior surfaces of waste containers are clean before removal from containment;
• destroy (autoclave or chemically devitalize) all PNT biomass when research is finished; record details (name, date, description of PNT biomass, method of treatment) and results of autoclave quality control indicator tests;
• prepare contingency plans for spills (i.e., employ effective chemical disinfectants, concentrations and contact times);
• prepare contingency plans for breaches of containment;
• decontaminate skin with an alcohol/ethanol hand sanitizer;
• report loss or theft of PNT material immediately to the Principal Investigator, the growth facility coordinator, the Department Chair and to the Director of Research Risk Management (ext. 52048).

Experiments involving organisms requiring no prescribed containment may be conducted concurrently with those requiring Plant Biosafety Level 1 containment if all work is conducted with Level 1 protocols.

**Plant Biosafety Level 2 – Physical Containment Features**

Further to the physical features for Level 1 containment, mandatory Plant Biosafety Level 2 security and containment features include:

**Laboratory Features**

• a Plant Biosafety Level 2 warning sign (re: potential environmental risk) should be posted at the laboratory entrance;
• easily cleanable work surfaces such as galvanized steel or stainless steel;
• a Class I or Class II biological safety cabinet for manipulations of agents with potential to create aerosols.

**Growth Chamber and Greenhouse Features**

• 24-hour environmental monitoring and alarm systems to signal mechanical malfunctions, loss of environmental conditions, and/or loss of containment;
• a Plant Biosafety Level 2 warning sign (re: potential environmental risk) posted on the growth chamber door or at the greenhouse compartment entrance;
• a Contingency Plan for Breach of Containment must be posted;
• galvanized or stainless steel (easily cleanable) work surfaces;
• screening (30-mesh size or higher for the size, shape and morphology of the arthropods to be excluded) is required on all ventilation openings;
• a sealed (impervious) concrete greenhouse floor is required.
Plant Biosafety Level 2 – Recommended Operational Procedures

In addition to the procedures recommended for Plant Biosafety Level 1 containment, the following procedures are appropriate for Level 2:

- seek Biosafety Committee review and approval whenever the work involves the potential for human allergenic response;
- use laboratory biological safety cabinets whenever manipulations produce aerosols;
- conduct pre-experiment inspections of the growth chamber and/or greenhouse compartment to confirm suitability for Plant Biosafety Level 2 containment; the departmental facility coordinator and the Director of Research Risk Management will assist;
- discuss all growth chamber or greenhouse uses of arthropods and other motile macroorganisms with the departmental facility coordinator to determine appropriate protocols and precautions;
- grow experimental plants and microbes at a time of year when susceptible plants are not growing;
- choose microorganisms that have an obligate association with the host plant;
- conduct experiments at a time of year when survival of any escaped organism (arthropod) is impossible;
- provide written SOPs for staff and students concerning regular facility inspections, reproductive isolation techniques, PNT biomass waste management, disinfection and cleaning of surfaces, spills and incident (loss) reporting;
- post a Contingency Plan for Breach of Containment at the growth chamber and/or greenhouse compartment door;
- decontaminate and clean workstations each day;
- avoid taking unnecessary personal effects into containment areas;
- wear personal protective clothing as directed by your supervisor;
- clean or contain personal protective equipment before removing from the containment area; decontaminate as instructed;
- autoclave or devitalize all detritus from screens prior to disposal;
- inspect shoes and clothing before leaving the containment area;
- sanitize the growth chamber or greenhouse after the completion of projects with experimental PNTs;
- conduct inspections after any event that may damage the containment structure;
- maintain chronological growth facility maintenance records.
When Plant Biosafety Level 2 and Level 1 (or lower) experiments are performed concurrently in the same growth facility, operational procedures for all experiments must conform to Level 2 containment standards.

To summarize, containment for plant biosafety is achieved via appropriate physical containment combined with plant-specific reproductive isolation techniques and good microbiological, horticultural, pest control and operational practices.

**Containment Standards for Plant Pests**


**Screening**

Screens can keep pests and pollinators out of the growth cabinet, growth room or greenhouse. The mesh-size refers to the number of threads per linear inch (e.g., a 30-mesh screen has 30 threads per inch). Mesh size will greatly affect airflow, cooling efficiency, humidity and light transmission. Thread diameter and mesh material will affect insect exclusion properties. The following mesh sizes will generally restrict certain insect species:

<table>
<thead>
<tr>
<th>Adult Insect</th>
<th>Insect Screen Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>leafminers</td>
<td>40-mesh</td>
</tr>
<tr>
<td>silverleaf whiteflies</td>
<td>52-mesh</td>
</tr>
<tr>
<td>melon aphids</td>
<td>78-mesh</td>
</tr>
<tr>
<td>flower thrips</td>
<td>132-mesh</td>
</tr>
</tbody>
</table>

Consult the growth facility supervisor in your department about the screening provided at the facility you plan to use.

**Signs and Labeling**

No special warning signs are required for Plant Biosafety Level 1 experiments. For Level 2 experiments, the Principal Investigator should post a “Caution – Experiment in Progress” sign on the growth cabinet and/or at the entrance to his/her growth room or greenhouse compartment. The sign (provided by the departmental facility coordinator) should indicate the researcher’s name, Plant Biosafety Level 2, the plant (PNT) species, the potential environmental risk, whether any viable microorganisms or motile macroorganisms are being used, specific precautionary requirements, and should include 24-hour emergency contact information. Any risk to human health must be identified with a separate Biohazard Warning sign indicating the nature of the risk. Pursuant to CFIA recommendations, a Contingency Plan for Breach of Containment must be posted on your growth chamber and at the growth room or greenhouse compartment door.

**Orientation and Training**

The Principal Investigator must provide all personnel involved in experimental PNT research with project-specific pre-commencement orientation and training. Orientation topics should include:

- explanations about the novelties of PNTs and transgenic plants;
- species at risk in the environment and at the field trial location;
- necessity for laboratory containment or field confinement;
- foreseeable possibilities for breaches of security, containment or confinement, and appropriate contingency plans;
- procedures for safe transfers and cleaning dedicated equipment;
- procedures for labeling, storing and destroying PNT biomass;
- monitoring, surveillance and record-keeping requirements;
- compliance obligations prescribed by CFIA, Health Canada or Environment Canada;
- potential consequences of any non-compliance.
Biological Containment - Reproductive Isolation

CFIA-approved strategies for reproductive isolation of experimental PNTs in growth facilities or confined research field trials include, as appropriate:

- geographic isolation;
- spatial isolation;
- temporal isolation of flowering plants;
- physical isolation:
  - isolation cages;
  - bags over flowering parts to prevent dispersal of pollen or seeds;
  - physical removal of flowers prior to pollen maturity;
  - harvesting of plants before flowering;
  - guard rows (pollen traps).

Plant-specific reproductive isolation techniques are used to prevent the movement of plant material, particularly pollen, from the containment or confinement area into the natural environment. (See [http://www.inspection.gc.ca/english/plaveg/bio/dir/dir0007e.shtml](http://www.inspection.gc.ca/english/plaveg/bio/dir/dir0007e.shtml); [http://www.inspection.gc.ca/english/plaveg/bio/dir/dir0007e.shtml#3.4](http://www.inspection.gc.ca/english/plaveg/bio/dir/dir0007e.shtml#3.4).)

Confined and Unconfined Releases of PNTs

For experimental transgenic plants, the main ecological concerns are escape and establishment in the ecosystem, outcrossing with related species to produce more competitive species, and toxicities to non-target species.

CFIA regulates the confined and unconfined environmental releases of PNTs and their uses as ingredients in livestock feeds. Each type of release is subject to environmental safety assessments and, if approved, to applicable restrictions. The process to research, develop, test and assess the safety of a new novel product may take from seven to ten years. For more information about data needed, see [http://www.inspection.gc.ca/english/plaveg/bio/dir/dir9408e.shtml](http://www.inspection.gc.ca/english/plaveg/bio/dir/dir9408e.shtml).
Confined Research Field Trials

Confined research field trials, which are permitted only upon approval by CFIA, enable evaluations of experimental PNTs in the natural environment under confined and strictly controlled conditions. The conditions for field confinement are based on prescribed performance standards for reproductive isolation (to prevent the transfer of pollen), field site monitoring, the management of seed and harvested material, and post-harvest land use restrictions. CFIA audits records and inspects research laboratories and field sites to enforce all terms and conditions for authorized confined research field trials. General information about confined research field trials is available at http://www.inspection.gc.ca/english/sci/biotech/gen/pntvcne.shtml.

Performance standards (i.e., plant-specific terms and conditions) for confined research field trials (pursuant to the Seeds Regulations, Part V) and the Confined Research Field Trial Application Form are found with CFIA Directive 2000-07: Conducting Confined Research Field Trials of Plants with Novel Traits in Canada, available at http://www.inspection.gc.ca/english/plaveg/bio/dir/dir0007e.shtml. Application deadlines are March 15th for spring planting and June 15th for fall planting. Confined research field trials are normally limited to one hectare per site and to a maximum of five sites per province. CFIA regards field trial locations as confidential business information. Web-based information concerning various aspects of field trial work will assist with the Application process. For example, the CFIA prescribes crop-specific isolation distances for PNTs in confined trials, post-harvest land use restrictions and frequencies for field site monitoring and surveillance. This information may be found at http://www.inspection.gc.ca/english/plaveg/bio/isole.shtml.


If the trial requires the use of an unregistered pesticide or the unregistered use of a registered pesticide, a research permit must be
obtained from the Pest Management Regulatory Agency of Health Canada (http://www.pmra-arla.gc.ca/english/appregis/appregis-e.html). Warning signs at all four corners of a trial site must indicate pesticide use each time the site is chemically treated.

CFIA plant biosafety evaluators and/or feed safety evaluators conduct initial environmental safety assessments for all research trial applications. If approved, confined research field trials are permitted under highly controlled conditions, which are described at http://www.inspection.gc.ca/english/plaveg/bio/dt/term/terme.shtml.

Unless explicitly exempted, all seeds and plant material harvested from confined field trials must be destroyed via CFIA-approved methods (e.g., incineration, autoclaving, deep burial).

Annual summaries of all confined research field trials approved by CFIA, and the terms and conditions, are found at http://www.inspection.gc.ca/english/plaveg/bio/confine.shtml#sum.

Principal Investigators should discuss confined field trial due diligence planning and on-going compliance obligations (e.g., post harvest surveillance) with the Director of Research Risk Management.

Unconfined Releases

CFIA environmental safety assessments are prerequisite for approvals of unconfined releases of PNTs and for approvals of novel livestock feeds derived from plants. Data from confined research field trials must address five criteria for environmental safety: altered weediness potential, potential for outcrossing, altered plant pest potential, impact on non-target organisms, and impact on biodiversity. Other data to be evaluated concerns impacts on human and animal health.

CFIA encourages the use of data checklists for safety assessment applications; see http://www.inspection.gc.ca/english/plaveg/bio/usda/usda04e.shtml.

Approval for unconfined release (pursuant to the Seeds Regulations, Part V) is an essential step towards the registration and commercialization of experimental novel plants. For guidance on the approval process for PNTs, see CFIA Directive 94-08: Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits, which is available at
As the CFIA approval process is subject to on-going development, applicants are advised to consult the Plant Biosafety Office early.

CFIA biology documents provide reference information on stable species with no novel traits. They are helpful for completing applications for unconfined releases of related PNTs; see http://www.inspection.gc.ca/english/plaveg/bio/dir/biodoce.shtml.


Concerns about outcrossing of novel traits to wild species are discussed at http://www.inspection.gc.ca/english/sci/biotech/enviro/transfe.shtml.

Environmental safety assessments are evaluated on a case-by-case basis. CFIA charges fees-for-services. See http://www.inspection.gc.ca/english/plaveg/bio/feepaie.shtml.

After completing their safety assessment process for environmental and biodiversity impacts (which can take 6 to 12 months), CFIA posts notices about every PNT approved for unconfined release. These notices are accessible via http://active.inspection.gc.ca/eng/plaveg/bio/pntvcne.asp

Novel product-specific decision documents are posted at http://www.inspection.gc.ca/english/plaveg/bio/dde.shtml. Any new post-approval information about an approved PNT or novel livestock feed derived from plants must be communicated to CFIA. The decision document will be re-evaluated. Post-approval monitoring and inspection programs may be imposed by CFIA.

Information about variety registration is found at http://www.inspection.gc.ca/english/plaveg/variet/vartoce.shtml.
Novel Foods

Pursuant to the *Food and Drugs Act* and the *Novel Food Regulations*, Health Canada is responsible for assessing novel human foods that have no previous history of safe use. HC is also responsible for nutritional, allergenicity and environmental assessments of novel foods. For more information, see [www.novelfoods.gc.ca](http://www.novelfoods.gc.ca), [http://www.hc-sc.gc.ca/fn-an/consultation/init/consultation_appendix-annexe1_e.html](http://www.hc-sc.gc.ca/fn-an/consultation/init/consultation_appendix-annexe1_e.html) and [http://www.hc-sc.gc.ca/fn-an/consultation/init/consultation_appendix-annexe3_e.html](http://www.hc-sc.gc.ca/fn-an/consultation/init/consultation_appendix-annexe3_e.html).


A listing of all HC-approved novel food products is found at [http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index_e.html](http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index_e.html).


Novel Feeds


Research trials with novel feeds involve extraordinary strategies for operations management (e.g., security of the novel feed, management of animal manure and by-products, disposition of all animals, record keeping). These matters should be discussed with the Animal Facility Manager and the Director of Research Risk Management (extension 52048).

**PNTs for Plant Molecular Farming**


Since PNTs for PMF produce pharmaceutical (or industrial) bio-compounds which may potentially be active in humans, livestock animals or non-target organisms, CFIA is developing risk assessment criteria exclusively for PMF safety assessments. CFIA may also prescribe additional standards for containment and confinement. CFIA inspectors must witness the destruction of all PMF biomass. No PNTs for PMF have been approved to date for commercial purposes.

**Ornamental Plants with Novel Traits**

Ornamental plants that express novel traits also come under the CFIA regulatory framework for environmental protection (due to physical or genetic invasiveness, weediness, etc.). Generally, environmental concerns are no different from those associated with exotic ornamentals. For more information, see [http://www.inspection.gc.ca/english/plaveg/bio/consult/ornamen/2005/ornamene.shtml](http://www.inspection.gc.ca/english/plaveg/bio/consult/ornamen/2005/ornamene.shtml).

University guidelines for physical containment and operational procedures for PNTs are also appropriate for ornamental PNTs.
New Substances

The Canadian Environmental Protection Act (CEPA 1999) is the key authority to ensure that all new substances, including living organisms, introduced into Canada are assessed for their potential to harm human health, the environment and biodiversity. The toxicity of a new substance is under regulatory scrutiny; i.e., capacities to cause injury to humans, animals, plants or to microorganisms; see http://www.ec.gc.ca/CEPAREgistry/gene_info/fact_01.cfm.

Environment Canada is responsible for enforcing the New Substances Notification Regulations (NSNR) pursuant to CEPA whenever other Acts (e.g., Feeds Act, Seeds Act) do not address proposed uses of the imported or novel organism (product). NSNR (Organisms) apply to certain PNTs. Proposed amendments to these regulations will likely remove research and development notification exemptions for new substance organisms by the summer of 2008.

General information about the new substances program is available at http://www.ec.gc.ca/substances/nsb/eng/home_e.shtml.

Persons wanting to conduct research with, import, manufacture (i.e., produce, develop or grow) or sell any new (plant) substance are required to notify the appropriate federal authority (i.e., Environment Canada, Health Canada, or the Canadian Food Inspection Agency) well in advance of the planned activity. Any organisms that do not appear on Canada’s Domestic Substances Lists (DSL) may be subject to NSNR; see http://www.ec.gc.ca/substances/nsb/eng/lists_e.shtml.

New substances subject to the notification requirements include organisms such as whole plants that are not indigenous to Canada and genetically modified (e.g., transgenic) exotic plants. Persons who import or manufacture microorganisms for use in an experimental field study must notify Environment Canada in accordance with Schedule 3 of NSNR (Organisms).

Since regulatory requirements concerning biotechnology research are evolving, University personnel are encouraged to contact the Director of Research Risk Management (ext. 52048) for advice and assistance with NSNR compliance matters. See http://www.uoguelph.ca/research/policies/Adobe/New%20Substances%20Notifications%20-%20Guidelines.pdf.
Plant Biosecurity

Biosecurity procedures for farm and greenhouse visits help to prevent the spread of animal and plant diseases. University personnel who visit commercial or private grow operations should respect local biosecurity procedures and/or follow recommended procedures. For more information, see references such as http://www.gov.mb.ca/agriculture/livestock/poultry/bba13s02.html.

For information about international phytosanitary protocols, see https://www.ippc.int/IPP/En/default.jsp.

Code of Practice for Biotechnology in Canada

Health Canada is currently leading a consultative process to develop a stewardship framework for the ethical advancement of biotechnology in Canada. Stakeholder consultants from the academic and industrial sectors have recommended that a Canadian Code of Practice for Biotechnology should address:

- ethical standards for performance;
- Canadian and international legislation governing biotechnology and biotechnology development;
- educational topics such the science of biotechnology, its applications and emerging technologies.

More information about the status of this federal initiative is found at: http://www.ioq.ca/view_project_section.asp?area=4#proj_186.

An issue-by-issue approach to biotechnology stewardship and due diligence is prudent. BiotechCanada is a source of information on ethical standards and related guidelines. See www.biotech.ca/.

Plant Gene Resources of Canada

Agriculture and Agri-food Canada supports conservation of and access to the country’s plant genetic resources. It sponsors Plant Gene Resources of Canada and its Canadian Plant Germplasm System to preserve plant diversity in national ‘gene banks’. Access to the collections by domestic and foreign breeders and research investigators is managed; the exchange of germplasm with other nations is controlled in accordance with international treaties. For further details, see http://pgrc3.agr.gc.ca/about-propos_e.html.
Convention on Biological Diversity and the Cartagena Protocol on Biosafety

The *Convention on Biological Diversity* is an international treaty adopted in 1992 under which the signatories agreed to develop national strategies for the conservation and sustainable use of biological diversity. The Convention has three goals; to promote conservation of biodiversity, the sustainable use of components or biodiversity, and the fair and equitable sharing of the benefits arising from the utilization of genetic resources. This United Nations Convention promotes sustainable development to achieve environmental conservation in a world climate of economic development. The CBD home page is [www.biodiv.org](http://www.biodiv.org).

Parties to the Convention recognized that modern biotechnology has the potential to contribute advancements towards these goals. Many countries have regulations to control the handling, use and disposal of living modified organisms (LMOs), but there were no international agreements to control transborder commerce involving LMOs. The *Cartagena Protocol on Biosafety*, pursuant to the Convention, was adopted in 2000 to establish an advanced informed agreement (AIA) procedure that expedites transborder movements of LMOs. A Biosafety Clearing House ([http://www.biodiv.org/chm/default.aspx](http://www.biodiv.org/chm/default.aspx)) was created to facilitate the exchange of information on living modified organisms and to assist countries to implement the Protocol.

Canada is a signatory to the Convention and supports the objectives of the Biosafety Protocol. Further guidance from CFIA concerning the import and export of plant material is available at [http://www.inspection.gc.ca/english/plaveg/internat/internate.shtml](http://www.inspection.gc.ca/english/plaveg/internat/internate.shtml).
References

For further information about agricultural biotechnology, see:

BiotecCanada
http://www.biotech.ca/

Government of Canada – Bioportal:
http://www.bioportal.gc.ca

Government of Canada – BioRegulations:

Biotechnology at the Canadian Food Inspection Agency:

Canadian Food Inspection Agency – Plant Biosafety Office:

International Agreements

National Agricultural Biotechnology Council:
http://nabc.cals.cornell.edu/?CFID=22651020&CFTOKEN=88563647

The Biosafety Clearing-House
www.bch.gc.ca

United States Department of Agriculture

Council for Biotechnology Information
http://whybiotech.com/index.asp

http://www.isb.vt.edu/greenhouse/green_man.intro.cfm