# LEGISLATION FOR GMO's IN NORTH AMERICA: *DESIGN OF CONTAINMENT FACILITIES*

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I stand before you with the utmost humility. It is truly an honor to have been invited to participate in a conference that brings together the world's experts in controlled environments for biological research. As a greenhouse and growth chamber manager of University facilities for most of my professional life, I've benefited from your discoveries and applications. My talk today will cover the regulatory aspects of using genetically modified organisms (GMOs) for plants and plant-related organisms in controlled environments. I will also touch on design aspects as they relate to this topic. Facility design of high level containment is well beyond the scope of a short presentation.

I remember a few short years ago chuckling with folks at Minnesota about a visiting scientist from China who was telling us he had introduced animal DNA into his cabbage plants in the greenhouse. I'd bet he probably did but he simply had trouble communicating his discovery to us! About that same time I saw a National Enquirer in the grocery store, an American tabloid famous for it's libel actions, with a headline about how scientists are creating plants that will have animal characteristics. It claimed that the plants would be pumping fuel into our vehicles before long. The next phase will be a corn plant pouring it's own ethanol into the vehicle's fuel tank! This points out the obvious need for containment.

I've distributed a new publication, *A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes* (Traynor, Adair, and Irwin, 2001), that covers points being made today. Is it the new bible on greenhouse containment? For this group, it's hardly the Book of Revelations and it's not the Ten Commandments though it may point out sins of omission.

Regulation of GMOs in North America is primarily targeted to field release, food, and feed applications. Mexico, for example, requires a phytosanitary certificate for field release and transport of non-maize related GMOs. Maize is protected due to it's Mexican center of origin status. If the path leading to a field trial involves controlled environments, the documentation must include these facilities but no mention is made of how material should be contained. "Biosecurity measures…to prevent …escape"[1] must be provided, too.

Canada has no regulations *per se* on controlled environment research but there is mention of greenhouses and growth chambers in the Health Canada Laboratory Biosafety Guidelines[2]. Four risk groups and four corresponding containment levels are described especially as it relates to "large scale production of microorganisms". The Canadian Food Inspection Agency regulates the importation, release, and feed use of transgenic plants as a subset of "plants with novel traits".

[1]Norma oficial Mexicana NOM-056-FITO-1995 http://www.sagarpa.gob.mx/Conasag/norma_fi.htm

[2] http://www.hc-sc.gc.ca/hpb/lcdc/biosafty/docs/index.html

The United States has several agencies that regulate GMOs. Under what is known as the Coordinated Framework for Regulation of Biotechnology, all agencies refer to the National Institutes of Health's Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) when working in controlled environments. The full text of the NIH Guidelines can be found at http://www4.od.nih.gov/oba/rac/guidelines/GUIDELINjan01rev.pdf I will spend the remainder of this talk referring to the NIH Guidelines since they offer the most direction in relation to controlled environments.

The NIH Guidelines, an outcome of the 1975 Gordon and Asilomar conferences, were issued in 1976 by the Office of Recombinant DNA Activities and adopted by all other Federal agencies in 1983. Appendix P for plants and plant-associated microorganisms or small animals was added in 1994 and describes performance standards for working in greenhouses and screenhouses. Growth chambers, tissue culture rooms, etc. are defined as laboratories by NIH and are covered in a separate appendix (Appendix G). Revisions to the document are as recent as January 2001 but none were made regarding plant research.

Regulation and oversight in the U.S. are handled by several agencies. Note that the NIH Guidelines are simply that, guidelines, whereas law directs certain aspects of handling GMOs. It should also be noted that not complying with NIH may endanger NIH funding which can be massive at many research institutions.

The US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) regulates the *introduction* of GMOs. Introduction is defined as the importation, interstate movement, or release to the environment of known pest organisms or even those organisms that may become pests. Additionally, they issue permits for movement of non-genetically engineered plant pests, noxious weeds, and exotic plants and plant products. The APHIS Form 2000 is used for determining requirements. APHIS has little comment on controlled environments though they do have a facility checklist that is used when inspections occur.

The US Environmental Protection Agency, under several laws, regulates the testing, tolerance, and labeling of "plant pesticides" i.e. transformed organisms that have pesticidal properties. Defining these plants and related organisms is still a debated issue.

The US Food and Drug Administration regulates any transformed food products. If no "substantial difference" is found between GMO and non-GMO food product, then the GMO product is considered equivalent to the non-GMO

At a research station or laboratory level, an Institutional Biosafety Committee (IBC) is the best vehicle for tracking and compliance for all GMO research. Higher level containment may require a biological safety officer (BSO). The principal investigator (PI) is the most likely person for ensuring compliance though all staff must be involved in the process. Table 1 (Traynor, Adair, and Irwin, 2001) summarizes the different agencies that can come into play when developing a GMO product.

**Table 1. Multiple Regulatory authorities oversee certain GMOs.**

| New Trait/Organism | Regulatory Review Conducted By: | Reviewed For: |
|---|---|---|
| Viral Resistance in food crop | USDA<br>EPA<br>FDA | Safe to grow<br>Safe for the environment<br>Safe to eat |
| Herbicide Tolerance in food crop | USDA<br>EPA<br>FDA | Safe to grow<br>New use of companion herbicide<br>Safe to eat |
| Herbicide Tolerance in ornamental crop | USDA<br>EPA | Safe to grow<br>New use of companion herbicide |
| Modified Oil Content in food crop | USDA<br>FDA | Safe to grow<br>Safe to eat |
| Modified Flower Color in ornamental crop | USDA | Safe to grow |
| Modified Pollutant Degrading soil bacteria | EPA | Safe for the environment |

NIH identifies 5 biosafety levels (Exempt, BL1P-BL4-P) which are simply a method of categorizing the risk so general measures can be employed. Table 2 (Traynor, et. al., 2001) below offers criteria for assigning particular biosafety levels. Additional measures may be required depending on the experiment. The Health Canada Laboratory Biosafety Guidelines are similar in that they also list biosafety levels and performance standards.

Experiments exempt from the NIH Guidelines:

- Pose no risk to the environment
- DNA from a particular host organism is propagated only in that same organism
- Transfer of DNA between two different species if they are known to exchange DNA by well established physiological means i.e. "natural exchangers".

No risk to the environment does not necessarily exempt the experiment from APHIS or other regulatory approvals. Note also that a list of "natural exchangers" (e.g. *Agrobacterium tumefaciens* on a documented host) is periodically revised and included in Appendices A-I through A-VI of the NIH Guidelines.

BL1-P (Biosafety Level 1 for Plants) is designed to provide a moderate level of containment This level of containment is appropriate for plants that are not noxious weeds and cannot interbreed with

noxious weeds in the surrounding area. For example, an experiment using potatoes transformed with genes from potato virus pathogens or genes with known anti-viral function would likely be classified as BL1-P.

BL1-P also applies to DNA-modified common microorganisms that do not have the ability to spread rapidly and are not known to have any negative affects on either natural or managed ecosystems, such as *Rhizobium* and *Agrobacterium*. Examples include *Rhizopus* strains engineered with altered enzyme genes, or the construction of *Agrobacterium* plasmid vectors containing genetic material for potato virus protein. BL1-P is also used for non-exempt GMO research that still requires containment.

BL2-P is used when there is a recognized possibility of survival, dissemination, or transmission but the consequence is predictably minimal. For example if GMOs under BL2-P were inadvertently released outside a greenhouse, they could survive and be transmitted to the surrounding environment but would have minimal negative impact. In this case, the genetically modified plants might be invasive weeds or be capable of interbreeding with weeds or they may contain the entire genome of an infectious agent or pathogen. For example, work to transform wheat to provide resistance to viral diseases would require a BL2-P containment facility *if* the work were done in a wheat-growing state in the Midwest. BL2-P is also used for DNA-modified insects or small animals as long as they pose *no* threat to managed or natural ecosystems.

The designing *goal* of BL3-P containment is to prevent the accidental release of plant pathogens or genetic materials that have a recognized potential for serious detrimental impact on managed or natural ecosystems. This includes:

- genetically engineered plants that must be allowed to shed pollen; exotic plant pathogens; exotic weeds; and potential biological control agents that must be evaluated for host range.
- the expression of genes from a quarantined pathogen in a non-quarantined pathogen.
- plant pathogens, such as the fungi that produce aflatoxin, that may have serious health consequences to humans or animals.
- microbial pathogens of insects or small mammals associated with plants if the DNA-modified material poses a serious risk to the environment.

BL4-P creates a highly restrictive environment due to a recognized potential to cause significant harm to environment or investigators. BL4-P would be recommended for experiments on certain exotic, readily transmissible infectious agents that are potentially serious pathogens of major US crops, such as soybean rust fungus, maize streak, or other viruses, *and* that are performed in the presence of their arthropod vector. It would also be used if producing vertebrate toxins in plants in order to protect the human investigators from contamination.

**Table 2. Criteria for assigning biosafety levels.**

| Criteria | Transgenic Plants | Transgenic Microbes | | Transgenic Insects/Animals/ Assoc. Microbes |
|---|---|---|---|---|
| | | Exotic | Non-Exotic | |
| Not a noxious weed or cannot outcross with one | BL1-P | | | |
| Not easily disseminated | | | BL1-P | |
| No detriment to environment | | BL2-P or BL1-P + | BL1-P | BL2-P or BL1-P + |
| Noxious weed or can interbreed with weeds | BL2-P or BL1-P + | | | |
| Contains complete genome of non-EIA | BL2-P or BL1-P + | | | |
| Contains genome of EIA | BL3-P or BL2-P + | | | |
| Treated with an EIA | BL3-P or BL2-P + | | | |
| Detriment to environment | | | BL2-P or BL1-P+ | BL3-P or BL2-P + |
| EIA with detriment to environment | BL3-P or BL2-P + | | | |
| May reconstitute genome of infectious agent *in planta* | BL3-P or BL2-P + | | | |
| Contains Vertebrate Toxin | BL3-P | BL3-P | BL3-P | |

*EIA – Exotic Infectious Agent

Meeting the biosafety standards means, in a word, containment. The objectives of containment are to:

- Avoid unintentional transmission
- Minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility
- Avoid the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop
- Avoid the unintentional introduction and establishment of an organism in a new ecosystem

Containment can be achieved through physical, biological, or combined methods.

Biological containment generally can pick up where physical facility limitations stop. The key to achieving environmental protection and preventing the dissemination of propagules lies in understanding the biological systems involved.

BL1 or 2-P calls for the basic facilities, equipment, and protocols that one would find in most research greenhouses. Protocols must, though, be understood and followed by all.
Generally, plant-*related* organisms such as insects and microbes increase the standards. If insect quarantine is the goal, regardless of the presence of rDNA material, then it is advisable to read the APHIS/PPQ draft, *Containment Guidelines For Nonindigenous, Phytophagous Arthropods And Their Parasitoids and Predators.* Microbes, depending on their potential for causing disease, may need to be kept in higher containment.
Please bear in mind, though, that BL3-P and BL4-P facilities are very expensive to not only build but also to operate. A retrofit growth chamber or growth room may offer suitable containment at a fraction of the cost of larger facilities. It can also be cost effective to design research that meets the experimental objective without creating a need for containment.

Physical containment is achieved by employing various methods of glazing, screening, caulking and sealing, caging, creating negative air pressure, facility siting, and air filtration. Table 4 (Traynor, et. al., 2001) provides an example of the type of technical specifications needed to determine a physical containment choice. Additional details of physical containment will be touched upon later.

**Table 4. Mesh sizes* for insect containment[3]**

| Adult insect | Screen hole size | | |
|---|---|---|---|
| | mesh* | microns | inches[2] |
| Leafminers | 40 | 640 | 0.025 |
| Silverleaf Whiteflies | 52 | 460 | 0.018 |
| Melon Aphids | 78 | 340 | 0.013 |
| Flower Thrips | 132 | 190 | 0.0075 |

*The number of threads per linear inch defines the mesh size of the screen; e.g., a 30-mesh screen has 30 threads per inch.

Biological containment methods include reproductive, spatial, and temporal isolation. Examples include:

- For whole plants -Covering or removing flower and seed heads to prevent pollen and seed dispersal.

[3] Adapted from "Greenhouse Screening for Insect Control." Rutgers Cooperative Extension. http://www.wvu.edu/~agexten/hortcult/greenhou/fs640.htm

- For microorganisms-Inoculating plants with methods that limit reproduction of microorganisms.
- For insects and other small animals-Conducting experiments at a time of year when survival of escaping organisms is impossible.

Biological and technical knowledge plus creativity and common sense are indispensable in designing biological containment.

Combining physical and biological containment is formally recognized by NIH e.g. "BL1P+ biological containment". This allows you to use a lower level physical space with the addition of biological methods and can give a fairly wide latitude to the types of experiments that can be conducted in conventional facilities.

Any containment strategy is doomed to failure if personnel don't understand or refuse to adhere to the procedures for handling transgenic material. It is critical to provide the appropriate procedures i.e. proper biosafety level and standards so that staff comply without excessive burdens. I'll give a rough sketch of some of the management practices related to various biosafety levels.

- Facility access logically becomes more limited or restricted as the biosafety level increases.
- Apparel is nothing special until you get to BL3-P and higher.
- Entry and exit logging in is only required at BL4-P.
- Signage is required at BL2 and higher.
- Emergency exits for personal safety are always needed even if containment is potentially compromised.
- The universal biosafety symbol should not be displayed unless it is truly applicable.
- Identifying GMO from non-GMO material with tags is suggested yet all material in a contained space is treated at highest biosafety level assigned to that space.
- It is recommended that seed be locked away except when potting. Spill-proof containers should be used in the potting areas.
- Transport of BL2 and higher GMOs requires unbreakable containers or even secondary containers in some instances.
- Termination of GMOs requires that they be biologically inactivated. Work surfaces, too, must be decontaminated. At higher levels, runoff and all materials are to be autoclaved.
- An established pest control program is required at all levels.
- Security is required at higher levels but has become very important due to political concerns. It is recommended that one use the most you can afford since the more barriers established, the better chance you have of success. Political vandalism is officially labeled domestic terrorism by the US Federal Bureau of Investigation.
- Records are required at BL2 and up. They are also recommend as a research protocol.
- A manual is required at all levels. Emergency and contingency plans must be included at BL2-P and higher.

As mentioned above, APHIS has a facility inspection checklist, reproduced in Traynor, Adair, and Irwin, 2001, that is useful in meeting many of the above points.

I'll now discuss some aspects related to the design of containment facilities by going through Table 7 (Traynor, et. al., 2001) below. This table summarizes design features as specified in the NIH Guidelines.

Typical research greenhouses would generally be compliant for BL1-P and BL2-P but the higher levels require significant accoutrements. Retrofitting is quite possible to meet the lower containment levels yet would be difficult if not impossible at the higher levels.

Success is most likely if an expert design team is created that includes researchers, facility managers, engineers, architects, and regulators. Engineers and architects with prior experience with containment facilities is highly recommended. Contact with regulators should be at the *beginning* of the project.

In summary, one can see that regulation for controlled environments is sparse. To date, the NIH Guidelines protect researchers and the environment with a minimum amount of bureaucracy. Therefore researchers, in concert with an IBC, are charged with a great deal of responsibility in determining needed containment. And containment is driven by the biology. Biosafety levels provided by NIH and others are useful as a broad categorization yet specific measures undertaken may vary widely under the same BSL. Facility design and choice of equipment is often no different than standard research facility concepts. High level containment, though, requires experience, knowledge, and a healthy construction and maintenance budget. Good management is the critical link in maintaining containment.

So we try. Good managers, good earth stewards, responsible folks that we are will only help us use what may prove to be the pivotal technology of our lifetime.

**Table 7. Enhanced features of containment greenhouses**

| | Conventional | BL1-P | BL2-P | BL3-P | BL4-P |
|---|---|---|---|---|---|
| Structure | Framing may be aluminum, steel, wood, or pipe | | | Rigid, wind resistant frame preferred; internal walls, ceilings, and floors resistant to liquids and chemicals | Reinforced, rigid frame required; walls, floors, and ceilings form sealed internal shell, resistant to liquids and chemicals; see Appendix P for others |
| Entry | Hinged or sliding entry doors | | Locks on entry doors | Double set of self-closing, locking doors | Double set of self-closing, locking doors with air-lock; shower and changing rooms |
| Glazing | Standard greenhouse glass or plastic material | | | Laminated, strengthened, sealed | Double-paned, laminated, strengthened, sealed |
| Screening | If used, standard 30 mesh fly screen | Recommended | 30-mesh or higher required | Not permitted | Not permitted |
| Ventilation | Roof or side vents, fans, cooling pads, fog system, or a combination these | | | Separate negative pressure system; air supply fans with back-flow damper; exhaust air HEPA filtered | Air-conditioned and HEPA filtered, closely monitored negative pressure, no roof or side vent allowed |
| Benching | Any material; solid or porous bottoms | | | Seamless water and chemical resistant bench tops | Seamless water and chemical resistant bench tops |
| Floors | Gravel (most common), soil, or concrete throughout | Impervious walkways recommended | Impervious material; collection of runoff water may be required | Impervious material; for microbes, runoff water collection and decontamination | Sealed floors as part of internal shell; runoff collection and decontamination |
| Drains | Discharge into groundwater or sanitary/storm sewer | | | Provision for collection and decontamination of runoff | Runoff collection required, sewer vents filtered |
| Other | Automatic control and utility systems meet basic operating requirements | | Autoclave available | Autoclave within facility; hand washing with hands free on/off; filtered vacuum lines; disinfectant traps for liquid lines | Double-door autoclave; self-contained vacuum system; in-line filters and back-flow protection for all liquid/gas services |

## References


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2. Health Canada Laboratory Biosafety Guidelines, http://www.hc-sc.gc.ca/hpb/lcdc/biosafty/docs/index.html

3. Kahn, R. P. and S. B. Mathur. 1999. *Containment Facilities and Safeguards: For Exotic Plant Pathogens and Pests.* St. Paul, MN. APS Press.

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5. National Institutes of Health, *Guidelines for Research Involving Recombinant DNA Molecules*. http://www4.od.nih.gov/oba/rac/guidelines/GUIDELINjan01rev.pdf

6. Traynor, P.L., D. Adair, and R. Irwin. 2001. *A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes.* Information Systems for Biotechnology, Blacksburg, VA.. http://www.isb.vt.edu