



Late Blight Resistant Potato expressing VNT1 Case Study

**Specialty Crop
Regulatory Assistance**

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Pesticide Laws

- **Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) → Registration**
- **Federal Food, Drug, and Cosmetic Act (FFDCA) → Food Safety and Tolerance Exemption**
- **Food Quality Protection Act (FQPA) → Food Safety and Tolerance Exemption**



code of federal regulations

40 CFR Part 174

**Contains detailed
information on PIPs**



PIP Products for Four Varieties of Potatoes w/ VNT1 for Late Blight Protection Registered by EPA

Varieties
X17 - Ranger Russet
W8 - Russet Burbank
Y9 - Atlantic
Z6 - Snowden

- These potato varieties were covered by separate PIP product registrations having the same VNT1 PIP active ingredient
- Final Registration Decision for the New Active Ingredient and Food Use of VNTI Protein and the Genetic Material Necessary for its Production in Potatoes

<https://www.regulations.gov/document/EPA-HQ-OPP-2016-0036-0013>



What are PIPs?

- **Plant-Incorporated Protectants (PIPs)** including pesticidal substance (e.g. VNT1 protein) and the genetic material necessary for its production in the plant (e.g. *Rpi-vnt1* gene)
- **Inert Ingredients** (e.g., NPTII selectable marker)



From 40 CFR Part 174:

- **Plant-incorporated protectant** means a pesticidal substance that is intended to be produced and used in a living plant, or in the produce thereof, and the genetic material necessary for production of such a pesticidal substance. It also includes any inert ingredient contained in the plant, or produce thereof.



From 40 CFR Part 174:

- **Inert ingredient** means any substance, such as a selectable marker, ...where the substance is used to confirm or ensure the presence of the active ingredient, and includes the genetic material necessary for the production of the substance, provided that genetic material is intentionally introduced into a living plant in addition to the active ingredient.



EPA's Oversight Focuses on PIPs

- EPA does not regulate non-PIP traits. For example, the gene for vacuolar invertase RNAi in Z6 potato is not a PIP:
- This gene is supposed to improve the quality and flavor of processed potato products while reducing the formation of acrylamide during high temperature cooking. This trait has no relation to pest mitigation.



Supporting Data

- Product Characterization
- Human Health/Toxicology
- Environmental Effects
 - Non-target organisms
 - Environmental fate
- Resistance Management

Specific requirements may be waived with sufficient justification.



Product Characterization

- Identification of transformation event and PIP ingredients
- Biology of the plant
- Spectrum of pesticidal activity
- Mode of action
- Transformation system
- Inheritance and Stability after Transformation
- Analytical detection method
- Characterization of inserted DNA
- Protein characterization and expression levels
- Test substance characterization and demonstration of equivalency with plant-produced substance



Product Characterization data submitted using Z6 as an example:

- Product Characterization of Event Z6
- Stability of Inserts in Vegetatively Propagated Z6.
- Event Detection Method for Z6
- Z6 Insert Characterization by Southern Blotting
- Evidence for the Absence of Plasmid Backbone DNA in Event Z6 (PCR)
- Sequence characterization of the inserts and insertion site in Z6
- Data Requirements and Waivers for Z6 (This volume contains VNT1 description, mode of action, arguments for its safety, and data waivers.)



Product Characterization data submitted using VNT1 potatoes as an example:

- Expression of VNT1 was undetectable by western blot, a method with the LOD of 500 ppb. The presence of mRNA was detectable using RT-PCR.
- VNT1 was also undetectable by Western blot for products X17, W8 and Y9.



Toxicology/Human Health

- Acute oral toxicity
- Allergenicity and toxicity bioinformatics analysis: for AI and for novel ORFs formed
- Stability to gastric proteases
- Heat stability
- Hypersensitivity incidents

Specific requirements may be waived with sufficient justification such as a history of safe exposure argument.



Bioinformatics:

- Allergenicity bioinformatics analysis:
<http://www.allergenonline.org>
- Toxicity bioinformatics analysis:
 - Toxinpred
<https://webs.iiitd.edu.in/raghava/toxinpred/index.html>
 - T3DB <http://www.t3db.ca/>
 - NCBI database with custom filters applied
 - Custom databases



Product Characterization

- In addition to the sequences encoding PIPs and inert ingredients, EPA has since 1994 required companies to submit information on noncoding, non-expressed nucleotide sequences to better address the potential for novel open reading frames and new proteins.
- In particular, the applicant needs to perform bioinformatics analysis of the insertion site to confirm that no novel ORFs with allergenic or toxic properties have been formed.



Toxicology/Human Health data for VNT1-expressing potatoes:

History of Safe Use Arguments:

“Dietary Exposure to Rpi-vnt1 -A Bioinformatics Review of Genes Homologous to Rpi-vnt1 in Edible Plants: Potato Late Blight Resistance Gene”

- Proteins highly similar to VNT1 are already present in the human diet as constituents of tomato.
- Similar R-proteins are present in many crops naturally



Toxicology/Human Health data for VNT1-expressing potatoes:

- A study “Bioinformatics Assessment of Allergenicity and Toxicity for VNT1” was submitted.
- The VNT1 protein is produced at levels in potato that are not detectable by conventional techniques.
- Therefore, oral toxicity, heat stability, and stability to gastric proteases studies were waived.



Environmental Effects: Non-Target Organisms

- Wild mammal – oral toxicity
- Avian – oral toxicity
- Avian – dietary toxicity
- Freshwater fish – toxicity
- Freshwater invertebrate – toxicity
- Estuarine and marine animal – toxicity
- Honeybee toxicity
- Arthropod – toxicity
- Non-arthropod invertebrate – toxicity
- Plant studies
- Soil microbial community toxicity test



Environmental Effects: Environmental Fate

- Degradation rate
- Field persistence
- Evaluation of environmental impacts of gene flow between sexually compatible plants
- Evaluation of potential weediness
- Evaluation of potential horizontal gene transfer



For VNT1-expressing potatoes, environmental effects data waived

- VNT1 protein is expressed at non-detectable levels
- History of dietary exposure to non-target organisms:
 - *Rpi-vnt1* gene has a 90% homology with the Tomato mosaic virus ToMV Tm-2 resistance genes
 - Similar R-proteins are present in many crops naturally



For VNT1-expressing potatoes, environmental effects data waived

- **There is no significant risk of gene capture** by wild or weedy potato relatives due to the inability of wild potatoes and cultivated potatoes to cross fertilize. Cultivated potatoes are extremely difficult to breed because they are tetraploid, highly heterozygous, and subject to inbreeding depression. The W8 Russet Burbank and conventional Russet Burbank variety are sterile and cannot be bred. In a rare instance where outcrossing could occur, only true seeds would be produced, which are unlikely to survive. Furthermore, farmers are encouraged via the fungal resistance management plan to destroy any volunteer W8, X17, Y9 and Z6 potatoes to limit any potential for escape.²²



Resistance Management

- Knowledge of pest biology and ecology;
- Appropriate refuge;
- Resistance monitoring and a remedial action plan should resistance occur;
- Employment of integrated pest management (IPM);
- Communication and education strategies on use of the product;
- Development of alternative modes of action.



Resistance Management in the case of VNT1-expressing potatoes:

- As part of the applications for registration of potato varieties expressing the VNT1 protein, Simplot submitted a resistance management strategy.
- Simplot was required to submit a copy of the **IPM guide** to EPA, as well as an **annual report** each year documenting the activities conducted under the IPM stewardship program, and the company's **investigations of unexpected damage cases**.



Resistance Management in the case of VNT1- expressing potatoes:

- Use of certified seed and sanitized seed cutting equipment
- Scouting for infected volunteer plants
- Monitoring for late blight lesions in VNT1 potatoes
- Use of preventative fungicides in conjunction with VNT1 to reduce selection pressure for resistance
- Cull pile destruction



Questions?

For more information see the Final Registration Decision for the New Active Ingredient and Food Use of VNT1 Protein and the Genetic Material Necessary for its Production in Potatoes:

<https://www.regulations.gov/document/EP-A-HQ-OPP-2016-0036-0013>