Nano enabled RNAi based pesticides: modes of action, risks and regulation

Steve Suppan
Senior Policy Analyst
March 31, 2022
A Quick Overview

- Why nanotechnology enabled RNA interference pesticides?: 3 responses to herbicide resistant weeds
- Introduction to the GE ‘editing’ of RNA molecules
- Some risks of unintended mutations of interference RNA
- Nanomaterial coating for RNAi pesticide delivery: risks
- Why do RNAi pesticide developers dismiss these risks?
- Principles for risk assessing and regulating RNAi pesticides
- A state of play regulatory postscript on the GreenLight Bioscience RNAi pesticide
Two perspectives on new Modes of Action in pesticides


1. ‘Outsmart’ weed resistance by GE targeting of existing RNA pathways in weeds and insect pests

2. Opening Pandora’s Box of Technology Fixes: unleashing new forms of weed/pest resistance and Environmental Health and Safety (EHS) harms
CROP PRODUCTION

Breaking Pandora’s Box: Resistant Weed Future Looms Large for US Farmers

“In the next three to seven years, we’ll see the opening of the newest version of Pandora’s Box,” says Jason Bond, a weed scientist at Mississippi State University.

(Photo by Kenner Patton, Delta Research and Extension Center, MSU)

By CHRISS BENNETT August 31, 2021
Transgenic factor in herbicide resistance

https://www.intechopen.com/chapters/71135
Response 1 to weed resistance to glyphosate:
apply a more toxic pesticide

Iowa farmers make record number of pesticide misuse claims

Donelle Eller, deller@dmreg.com  Published 6:39 p.m. CT Sept. 11, 2017 | Updated 11:24 a.m. CT Sept. 12, 2017

Dicamba drift is damaging soybean crops at an unprecedented rate, experts say. Zach Boyden-Holmes/The Register
Response 2: nano-coat existing Active Ingredients (AIs)

B. Huang et al: *Nanomaterials* (11.2.2018)
doi: 10.3390/nano8020102

![Diagram](image)

**Figure 3**
Response mechanism diagram of thermosensitive polymer.
Nano-coating double strand (ds) RNA interference molecules resistance:

**ORIGINAL RESEARCH** article

**First sprayable dsRNA-based biopesticide product targets proteasome subunit beta type-5 in Colorado potato beetle (Leptinotarsa decemlineata)**

Provisionally accepted
The final, formatted version of the article will be published soon

Thais B. Rodrigues¹, Sambit K. Mishra¹, Rich Tuttle¹, David Garby¹, Ethann R. Barnes¹, Andrei Alyokhin², Krishnakumar Sridharan¹, Nicholas J. Skizim¹, Yu-Wen Tang¹, Brian Manley¹, Ronald Flannagan¹, Carole Cobb¹ and Kenneth E. Narva¹

¹GreenLight Biosciences (United States), United States
²University of Maine, United States
Idealized CRISPR-Cas9 DNA sequence editing: genomic ‘library’ data guides sequence cutting and messenger RNA repair.

Figure 1: Nuclease (DNA-scissors): CRISPR-Cas9 – Clustered Regularly Interspaced Short Palindromic Repeats
GE editing technique can cut and repair the DNA sequence inaccurately:
GE editing of RNA to interfere with protein expression vital for pest survival
Mode of Action in the AI of this new kind of pesticide

Figure 1: The Interfering RNA Mechanism

RNAi interference functions to regulate gene expression. Interfering RNAs block protein synthesis. Proteins are the instructions for carrying out many important processes inside an organism.

Double-stranded RNA (dsRNA), dubbed 'interfering RNAs', are processed into smaller, active fragments.
The interfering RNA and a target messenger RNA bind, as they share a sequence that is matching or similar to each other.

The messenger RNA is then cleaved and destroyed. No protein is produced, resulting in 'interference' of gene expression, also known as gene-silencing.
### Figure 2: Key agricultural RNAi products in development

<table>
<thead>
<tr>
<th>Developer</th>
<th>Product</th>
<th>Target</th>
<th>Stage of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASF</td>
<td>Spray</td>
<td>Plant fungal pathogen fusarium</td>
<td>Unclear</td>
</tr>
<tr>
<td>BAYER</td>
<td>Spray</td>
<td>Reverse glyphosate resistance in weeds</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td>Spray</td>
<td>Canola flea beetle</td>
<td>Early stages</td>
</tr>
<tr>
<td></td>
<td>Feed additive for honey bees</td>
<td>Varroa mite</td>
<td>Submitted for EPA registration in 2019</td>
</tr>
<tr>
<td>GreenLight</td>
<td>Spray</td>
<td>Colorado potato beetle</td>
<td>Expected to be submitted to EPA in 2020</td>
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<tr>
<td>syngenta</td>
<td>Spray</td>
<td>Colorado potato beetle</td>
<td>Expected commercialization within 7-10 years</td>
</tr>
<tr>
<td></td>
<td>Spray</td>
<td>Diamondback moth</td>
<td>Unclear</td>
</tr>
<tr>
<td>VIAQUA</td>
<td>Feed additive for shrimp</td>
<td>White Spot Shrimp Virus</td>
<td>Potential commercial launch in 2021</td>
</tr>
</tbody>
</table>
Risks associated with unintended mutations from GE editing of RNA molecules


- Undesirable agronomic traits: e.g., lower germination percentage, inappropriate plant height, lower plant density
- Changes in genetic composition produce plant allergenicity, augmented toxicity, nutrient deficiencies (R. Steinbrecher, 2015)
- Undesirable heritable traits resulting from large sequence alterations that change plant structure
- Reductions in white blood cell count due to undesirable immune stimulatory impacts from chronic inhaling of RNAi
Government consensus building about risk analysis of RNAi based pesticides

OECD Conference on RNA interference (RNAi) based pesticides

10-12 April 2019, OECD, Paris
GLB and Bayer to OECD governments: ‘history of safe consumption of RNA’ and ‘very low risk’ from inhaling RNAi (nano-scale inhalation risks are ‘silenced’)

Review article

Safety Considerations for Humans and Other Vertebrates Regarding Agricultural Uses of Externally Applied RNA Molecules

Thais B. Rodrigues1 and Jay S. Petrick2

1GreenLight Biosciences, Inc., Medford, NC, United States
2Bayer Crop Science, Chesterfield, MO, United States
Properties and risks of the nanomaterials (NMs) coating the RNAi Active Ingredient

- RNAi is unstable and easily destroyed, e.g., by UV rays: requires protective nano-coating to be an AI
- NM coatings, e.g., nano-clays, are inert and of very low risk
- Nano-scaling enables RNAi penetration and motility in plant cells: impossible for macro-scale pesticide droplets
- NMs deliver RNAi to any adjacent plant or animal with an RNA sequence like that of the target pest
- The lungs cannot expel a large fraction of nano-scale RNAi

Chronic inhalation exposure to nano-RNAi pesticides, e.g., in farmworkers, may result in immunity effects.
Nano-bio inhalation risks are known but their fate and transit for specific NMs is not well understood.
Why would GLB and Bayer dismiss or downplay nano-bio inhalation risks?

- EPA assesses risks only of the AI, not the nano-pesticide delivery system or the formulated pesticide
- EPA classifies company studies and data as Confidential Business Information (CBI), so no full public review of company safety claims
- The industry needs new AIs to replace the AIs that are pest resistant and that are going off patent
- Industry: farmworkers should wear “the appropriate Personal Protective Equipment”
- Billions of dollars are at stake
Principles for regulating RNAi pesticides

• Risk assess the entire RNAi pesticide product, not just the AI
• Don’t base GE pesticide regulation on irrelevant comparisons with RNA as it occurs in nature and humans
• Don’t classify company studies and data relevant to environmental and human health as CBI
• Study the nano-bio interactions and mutations in non-target, as well as targeted, plants and animals
• Don’t forbid risk assessment of the process of production in the regulation of the pesticide product
GLB permit terms for April 2022-April 2023 field trials: 200 acres in 11 potato growing states

Summary
This notice announces EPA's receipt of application 94614-EUP-R from GreenLight Biosciences, Inc. requesting an experimental use permit (EUP) for Leiprona (CAS No. 2433753-68-3). The Agency has determined that the permit may be of regional or national significance. Therefore, because of the potential significance, EPA is seeking comments on this application.

Dates
Comments must be received on or before July 21, 2021.
GreenLight Bioscience Press Release:
Before receiving EPA field trial permit
https://greenlightbiosciences.com/in-the-pipeline-colorado-potato-beetle/

We have conducted more than 100 field trials over four years to develop a solution that is effective at just 9.9 g/hectare, equivalent to a spoonful of sugar spread on a football field. Consumption of the dsRNA causes the Colorado potato beetle to stop eating and expire from its own toxins.

The application has been designed to be highly specific to affect the target pest. Our testing has shown that our Colorado potato beetle product is safe for honeybees, butterflies, and several other nontarget insects and mammals at use rates 100 times higher than our recommended rate. It degrades in water and soil within three days to benign, natural nucleotides.
Needs and Challenges in Personal Protective Equipment (PPE) Use for Underserved User Populations

A Notice by the Centers for Disease Control and Prevention on 06/24/2021

AGENCY:
National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control and Prevention (CDC), Department of Health and Human Services (HHS).

ACTION:
Request for information.

SUMMARY:
NIOSH requests information on the Needs and Challenges in Personal Protective Equipment (PPE) Use for Underserved User Populations.
Agricultural Worker Protection Standard (WPS)

Esta página web está disponible en español

EPA's Agricultural Worker Protection Standard (WPS) aims to reduce pesticide poisonings and injuries among agricultural workers and pesticide handlers. The WPS offers occupational protections to over 2 million agricultural workers and pesticide handlers who work at over 600,000 agricultural establishments. In 2015, EPA revised the WPS to decrease pesticide exposure incidents among farmworkers and their family members. Fewer incidents means a healthier workforce and fewer lost wages, medical bills and absences from work and school.

Pesticide safety training materials with the expanded content required by the 2015 WPS must be used to train workers and handlers. EPA-approved training materials for national use are available on the WPS Materials webpage.

For complete information about the WPS rule requirements, refer to the final WPS rule.
NEW STUDY REVEALS DRAMATIC RISE IN GLOBAL PESTICIDE POISONINGS

Worldwide poisonings up from 25 million in 1990 to 385 million today

44% of farmers and farmworkers are poisoned by pesticides globally every year
Conclusions

• RNAi nano pesticide delivery systems and AI pose human and environmental health risks
• EPA will allow these risks if they authorize commercial use of RNAi pesticides based on evaluation only of the AI, and not the formulated pesticide as used
• To reduce the risks of immunity suppression in farmworkers applying RNAi pesticides, NIOSH must develop PPE adequate to prevent inhalation of nano-scale pesticide particles
• EPA must require use of such PPE by farmworkers and their employers under its Agricultural Worker Protection Standard