A Greenhouse Study to Model Potential Field Use of Genetically Modified Bacterial Symbionts for Chagas Disease Control

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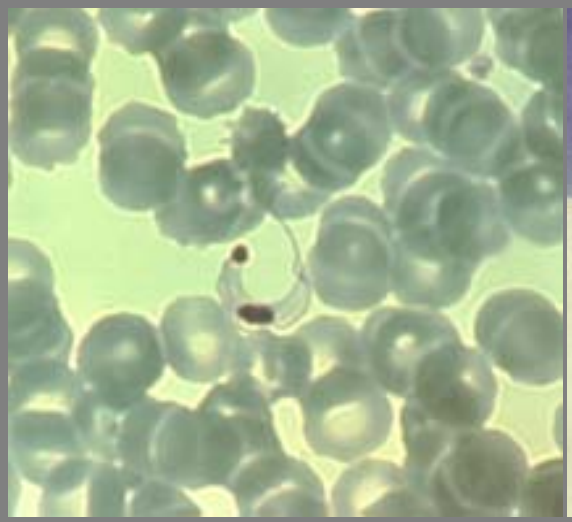

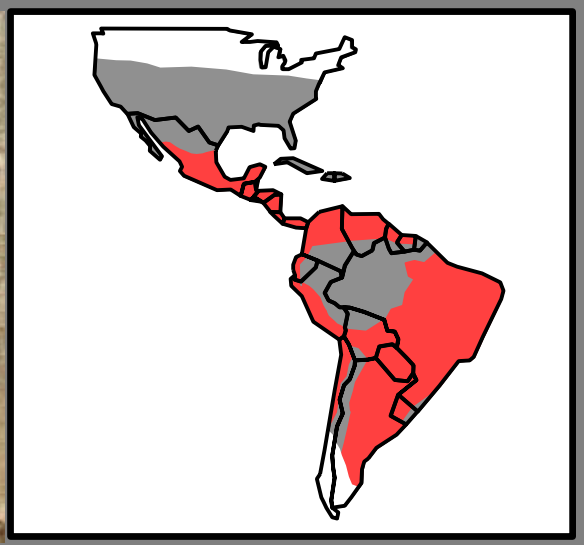

Chagas Disease

Impact: 16-18 million cases
Mortality: 50,000 deaths per year
At risk: 90 million in 21 countries
Agent: *Trypanosoma cruzi*
Vector: triatomine bugs
Distribution: The Americas
Control: Three multi-national control programs (Southern Cone, Andean Pact, and Central American)
Potential Obstacles: Incomplete insecticide coverage, reinfestation of treated homes, insecticide resistance, program sustainability

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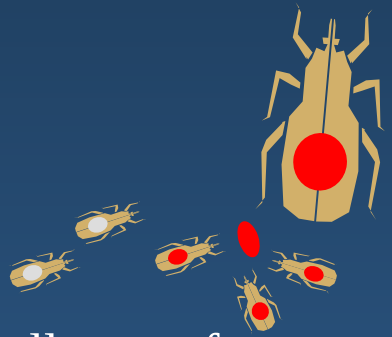
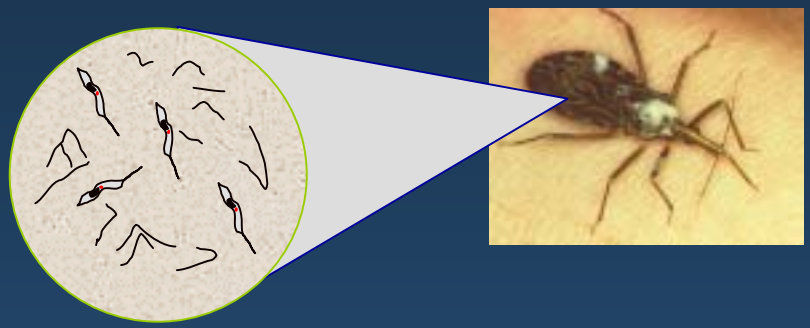
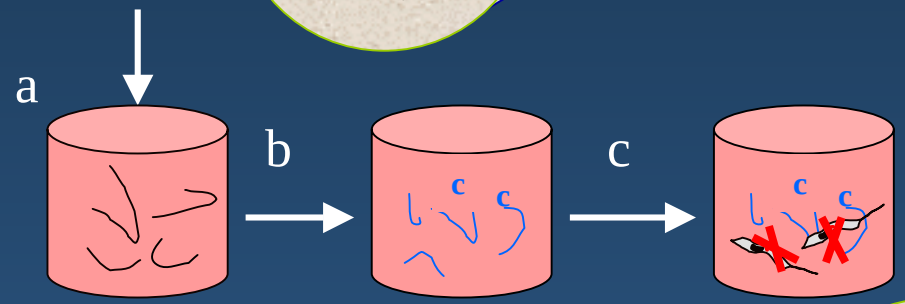
Vector-Symbiont-Intervention

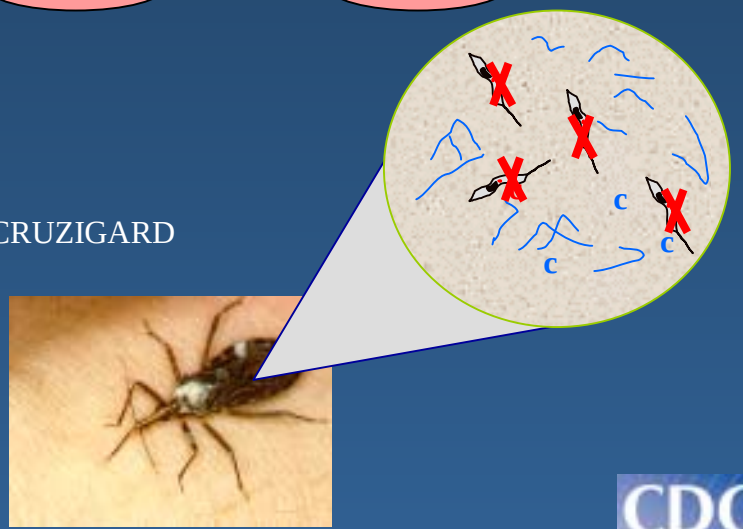
1.Triatomines harbor bacterial symbionts essential for survival and reproduction; symbionts passed by coprophagy.







2. Genetically transform symbionts to express an anti-trypanosomal agent



3. Bugs would feed on artificial feces (CRUZIGARD) containing genetically modified symbionts, resulting in diminished Chagas transmission (Paratransgenic vector).

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A Theoretical Strategy for Controlling Chagas Disease Transmission Using Genetically Modified Symbionts

- Apply genetically modified bacterial formulation to new homes or to insecticide-treated homes.
- Insects infest or reinfest homes.
- Triatomine nymphs ingest modified bacteria.
- Genetically modified symbionts are amplified and dispersed by newly-infected insects.

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Greenhouse Study of Transgenic Insects : Containment

- Two sets of barriers, both secured to the floor
- Contains a “clean” area for decontamination

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Greenhouse Study of Transgenic Insects : Release and Maintenance

- 36 fed and mated *R. prolixus* females released into the hut (5/1/01)
- Bugs given bloodmeal through condom to 37°C
- Temperature and humidity monitored constantly

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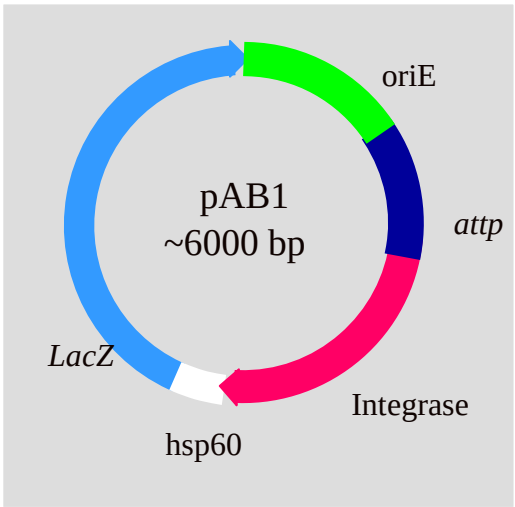
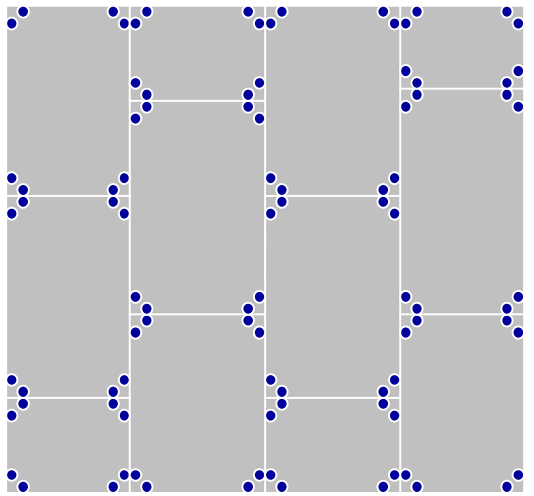
Composition and Placement of CRUZIGUARD

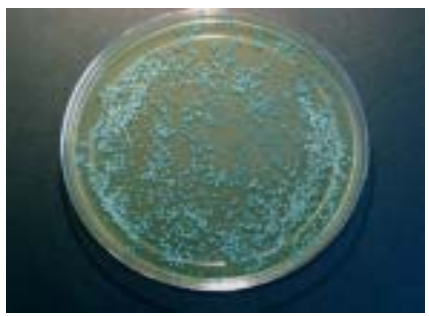


R. rhodnii were transformed with an integrative plasmid that contained the *LacZ* marker gene

- Hut treated with CRUZIGUARD (5/31/01)
- Assay of CRUZIGUARD (6/29/01)
- Reapplied CRUZIGUARD (7/10/01)
- Stability of CRUZIGUARD assessed on weekly basis
- Analysis of F1 progeny initiated (7/16/01 to 2/01/02)



Midgut Assays of *R. prolixus* from Greenhouse Study



- *R. prolixus* bugs removed at varied intervals from greenhouse hut
- External surface of bugs washed in 10% bleach and 70% ethanol
- Abdomen ground in 1 ml of PBS (0.01M, pH7.2)
- Serial dilutions of midgut contents plated on BHI-Xgal plates
- 5-7 days after plating, counted CFU's

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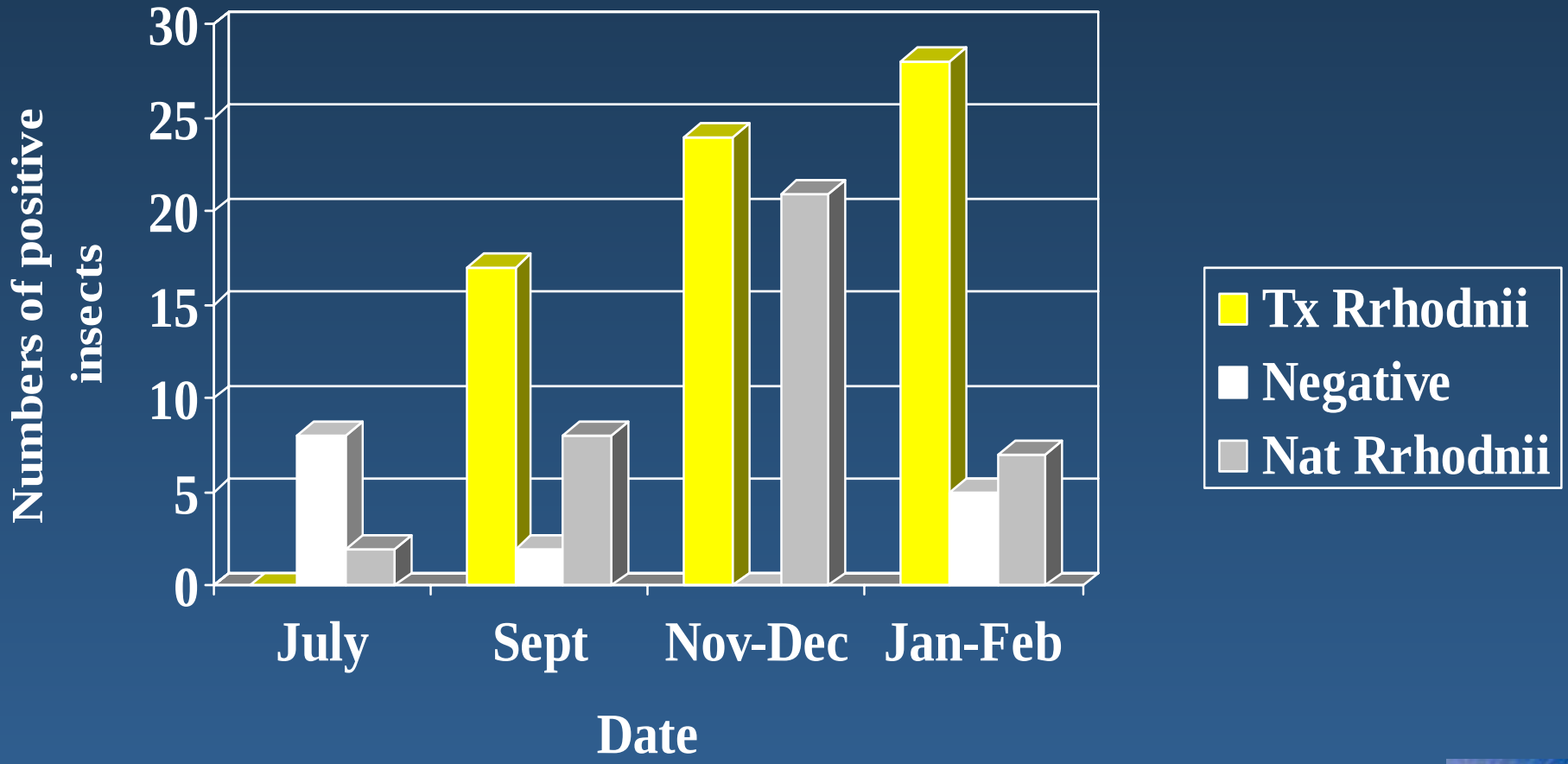
Greenhouse Study Summary



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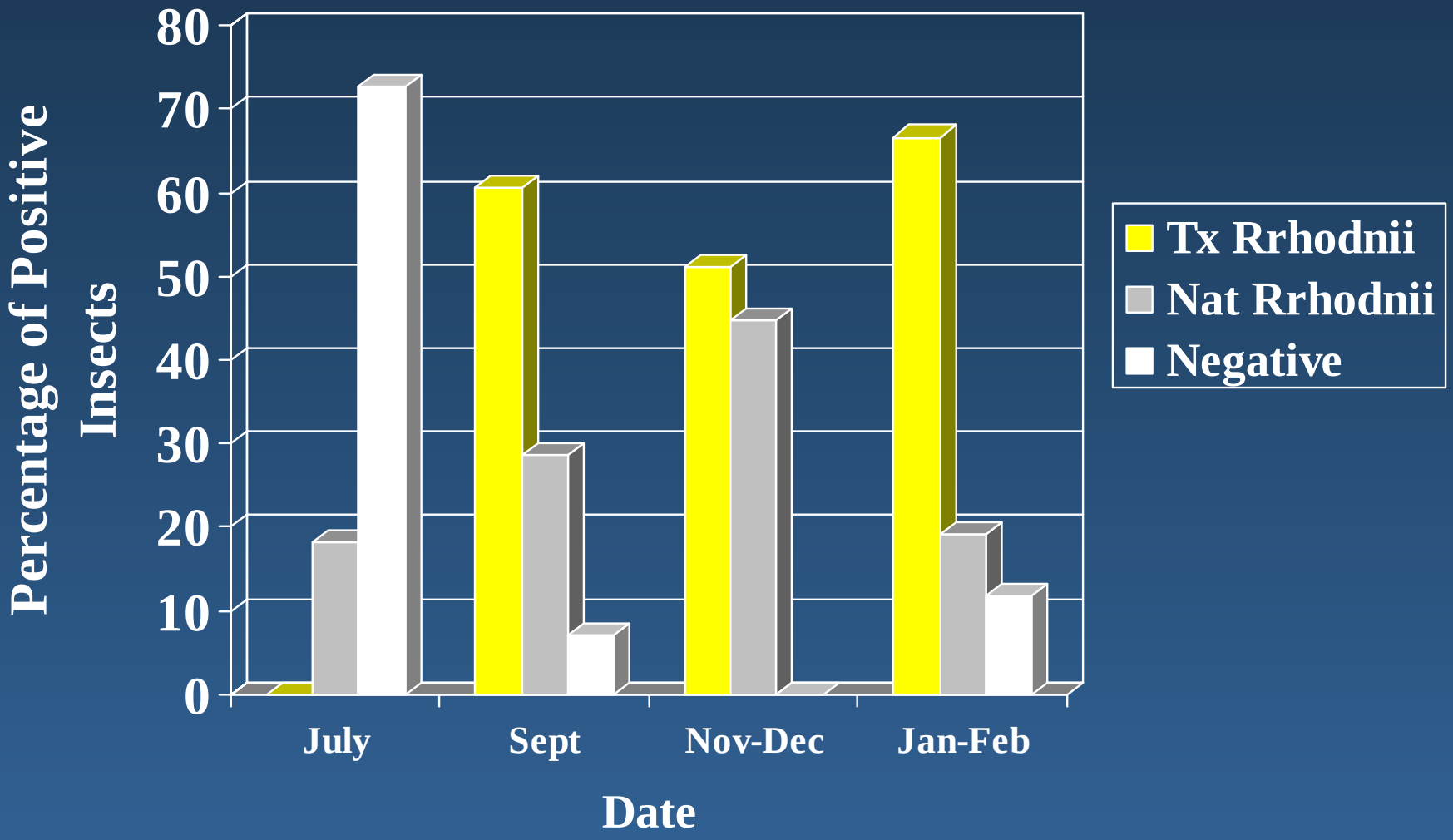
Greenhouse Study Summary



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Summary and Future Studies

- In this study, we demonstrated:
  - Over 60 % of bugs acquired GM bacteria
  - An improved method for applying CRUZIGUARD
- In future iterations, we will:
  - Determine minimum concentration and density of GM bacteria for treating hut
  - Test other formulations of GM bacteria with added attractants that may enhance uptake
- Associated laboratory studies:
  - Assess *in vivo* competition between GM and wild-type bacteria
  - Develop improved anti-trypanosomal DNA constructs

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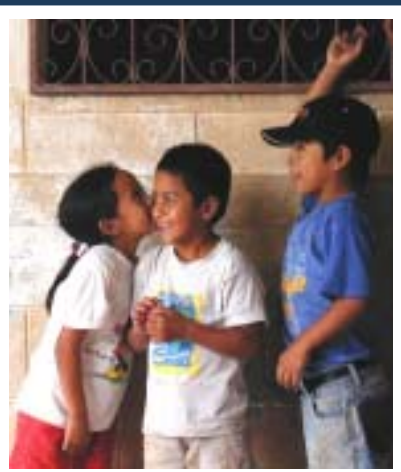
Transgenic Control of Insect Vectors



- Public health concerns
  - Human safety (direct and indirect)
- Environmental and ecological concerns
  - Effects on non-target organisms and horizontal transfer of genes must be evaluated
- Political concerns
  - Effective interaction at local, national, and international levels
- Public perception
  - Effective/proactive communication
  - Whether scientifically valid or not public opinion can determine the future of entire programs

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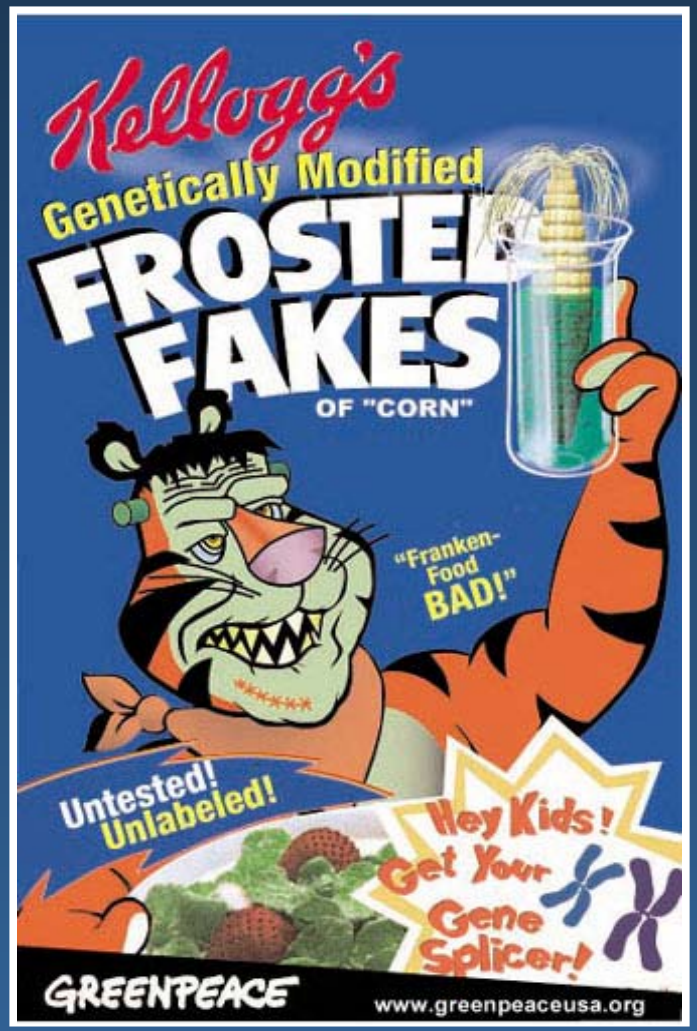
Collaborators



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