Use of Stable Isotope Technologies to Accomplish *In-Situ* Biological Remediation of Explosives

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Introduction to Isotopic Carbon

6 12.011

C

Carbon

- Carbon comes in different weights
- ^{12}C and ^{13}C are most common isotopes
- Approximate 98.5: 1 ratio in naturally occurring compounds and by-products of naturally occurring compounds
- Not radioactive – working with STABLE isotopes

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Introduction to Isotopic Carbon

- Can distinguish between ^{12}C ,^{13}C
- Can artificially alter the ratio of ^{12}C : ^{13}C
- Thus, highly inflated ^{13}C levels act as isotopic “tag”

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Introduction to Stable-Isotope Probes

- In-situ sampling method
- Can confirm presence of indigenous, degrading population
- Utilizes isotopically-labeled target compounds
  - Can be any carbon-based compound
  - Ex: RDX

Bio-Trap, from Microbial Insights

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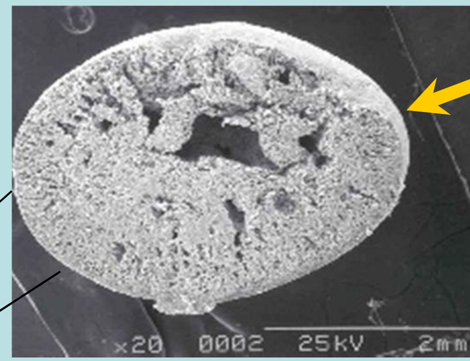
SIP: Six Simple Steps

- Step 1: “Baiting” the Beads - obtain ^{13}C labeled compound, sorb ^{13}C labeled compound onto beads

Bio-Trap Assembly



PAC + Nomex®

SEM of Bio-Sep® bead; 600 m^2 internal surface area / g

Porosity

- Significant surface area to sorb labeled target compound
- Encourages bacterial residence within beads

SIP: Six Simple Steps

Step 2: Construct in-situ sampler

Step 3: Deploy into MWs, suspended within the groundwater

Step 4: Incubate in-situ

- Timeframe will vary for different compounds under different site conditions
- Reflects actual site conditions versus laboratory microcosms

Step 5: Remove from MWs

Step 6: Analyze biomarkers and residual, labeled RDX

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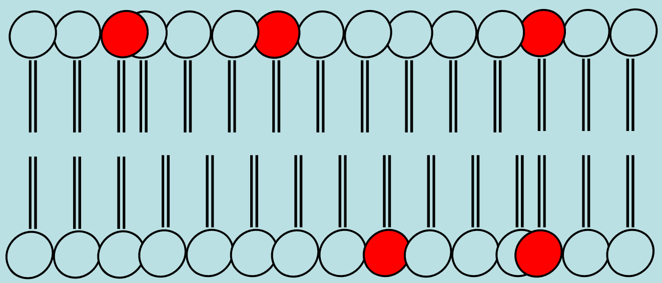
Biomarker of Choice - PLFAs

- High concentrations of phospholipids fatty acids (PLFAs) in microbial cell membranes
  - High concentration of carbon in PLFAs



microbial membrane

- ^{13}C incorporated into PLFAs
  - Result of incorporation of ^{13}C-label from RDX into biomass
  - Analyze using GC-IR-MS
  - Insight into indigenous microbial ecology and activity
  - Quantified via resulting ^{12}C : ^{13}C ratio

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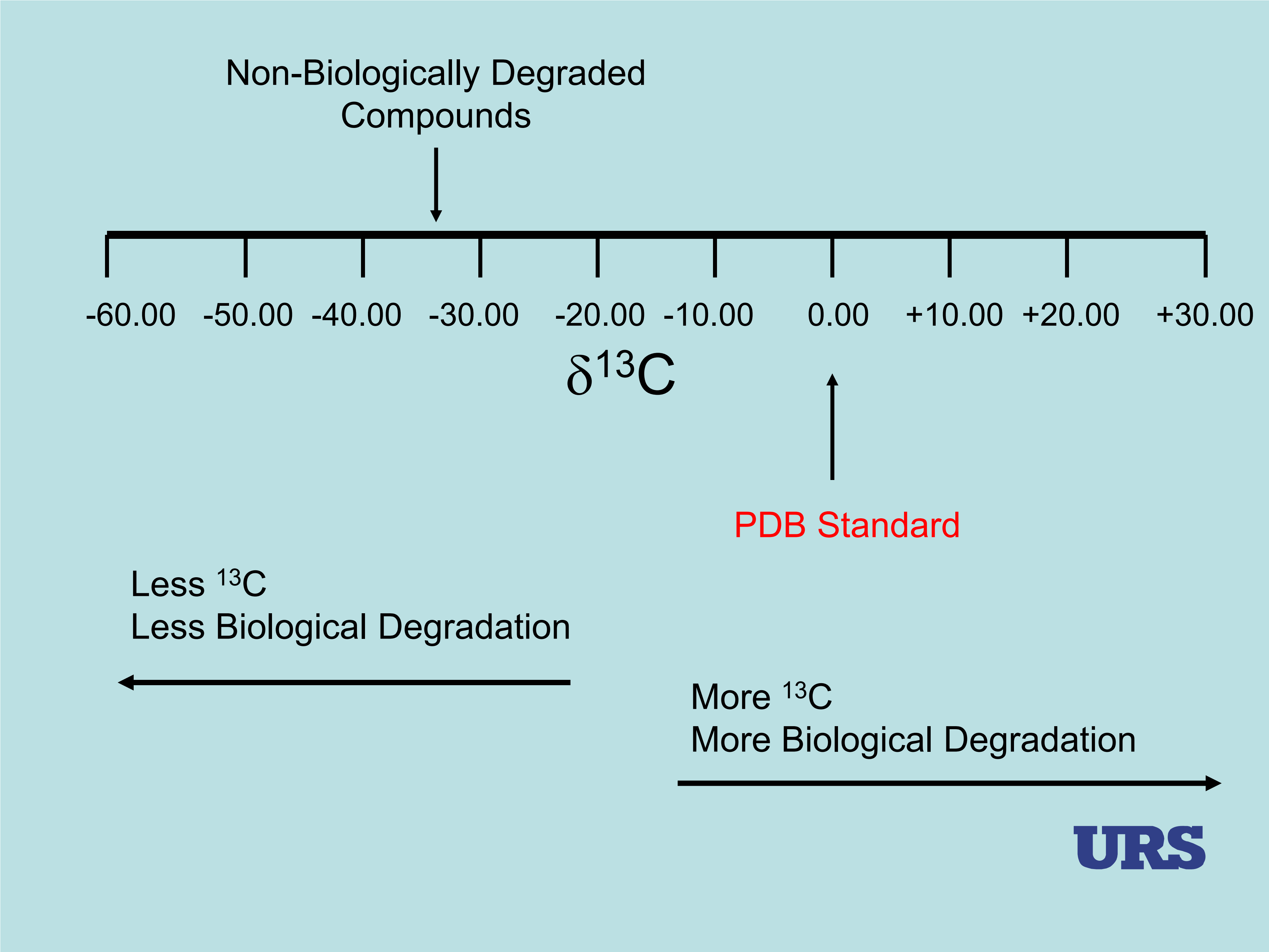
Non-Biologically Degraded Compounds
-60.00
-50.00
-40.00
-30.00
-20.00
-10.00
0.00
+10.00
+20.00
+30.00
$\delta^{13}C$
PDB Standard
Less ^{13}C
Less Biological Degradation
More ^{13}C
More Biological Degradation
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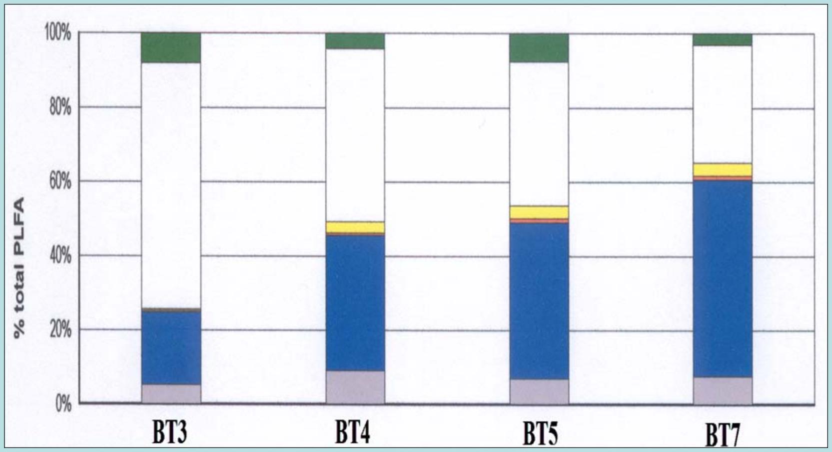
Quantitative Data from PLFA Analysis

- Total population size
- What groups, how many
  - Community profile
  - Groups, not individual species
- Confirmation of compound biodegradation



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Quantitative Data from PLFA Analysis

- Which community members are responsible for degradation
- Information about possible environmental stress
    - Nutritional deficits or toxic compounds
- Information about <u>current</u> microbial population
- In-situ conditions

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Quantitative Analyses From Residual Isotopic Compound

- Degradation Rate
  - Under in-situ conditions, not laboratory conditions
- Compound Mineralization to CO_2
  - What bacteria are doing with carbon, where microbial energy is being directed

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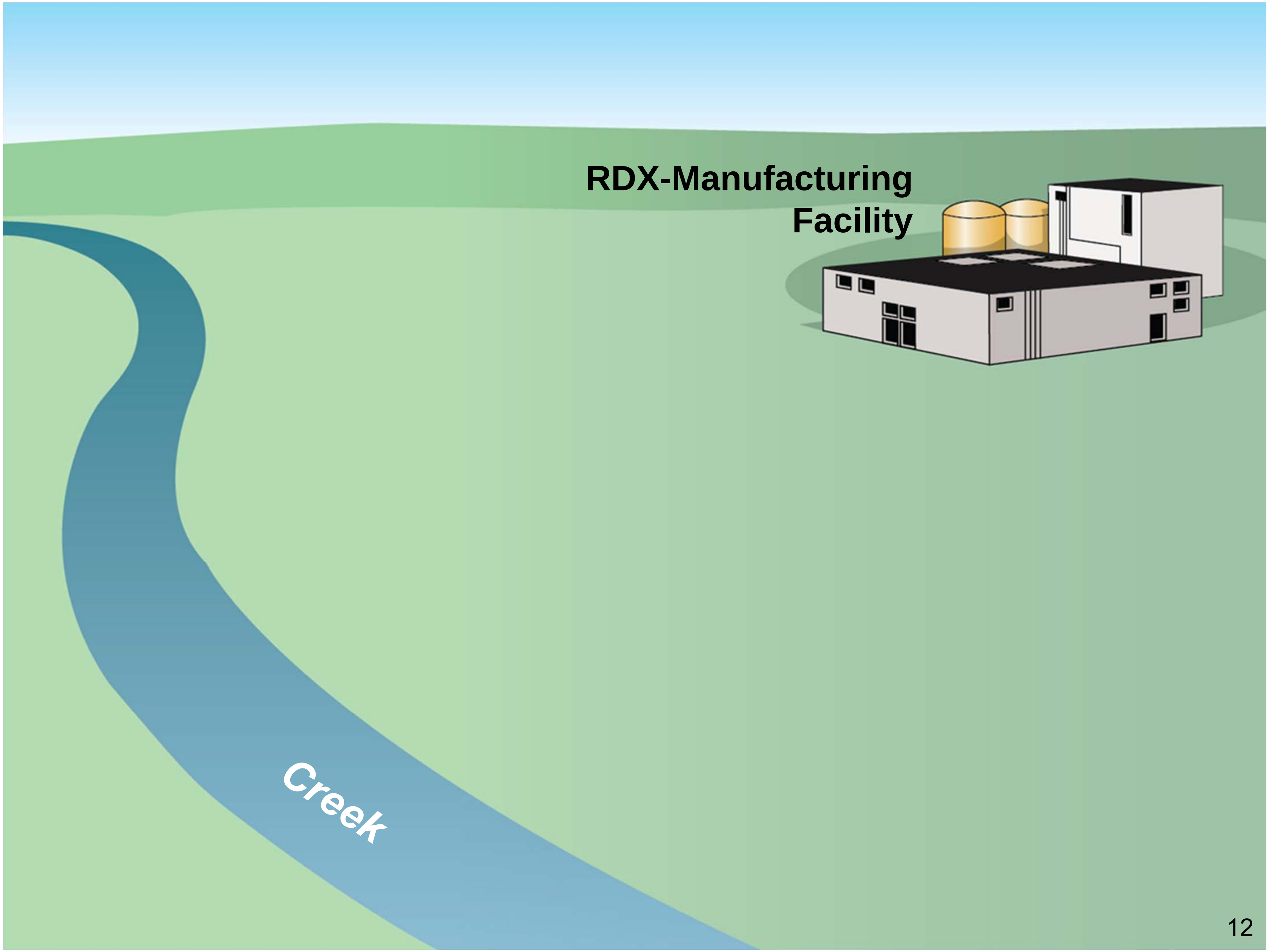
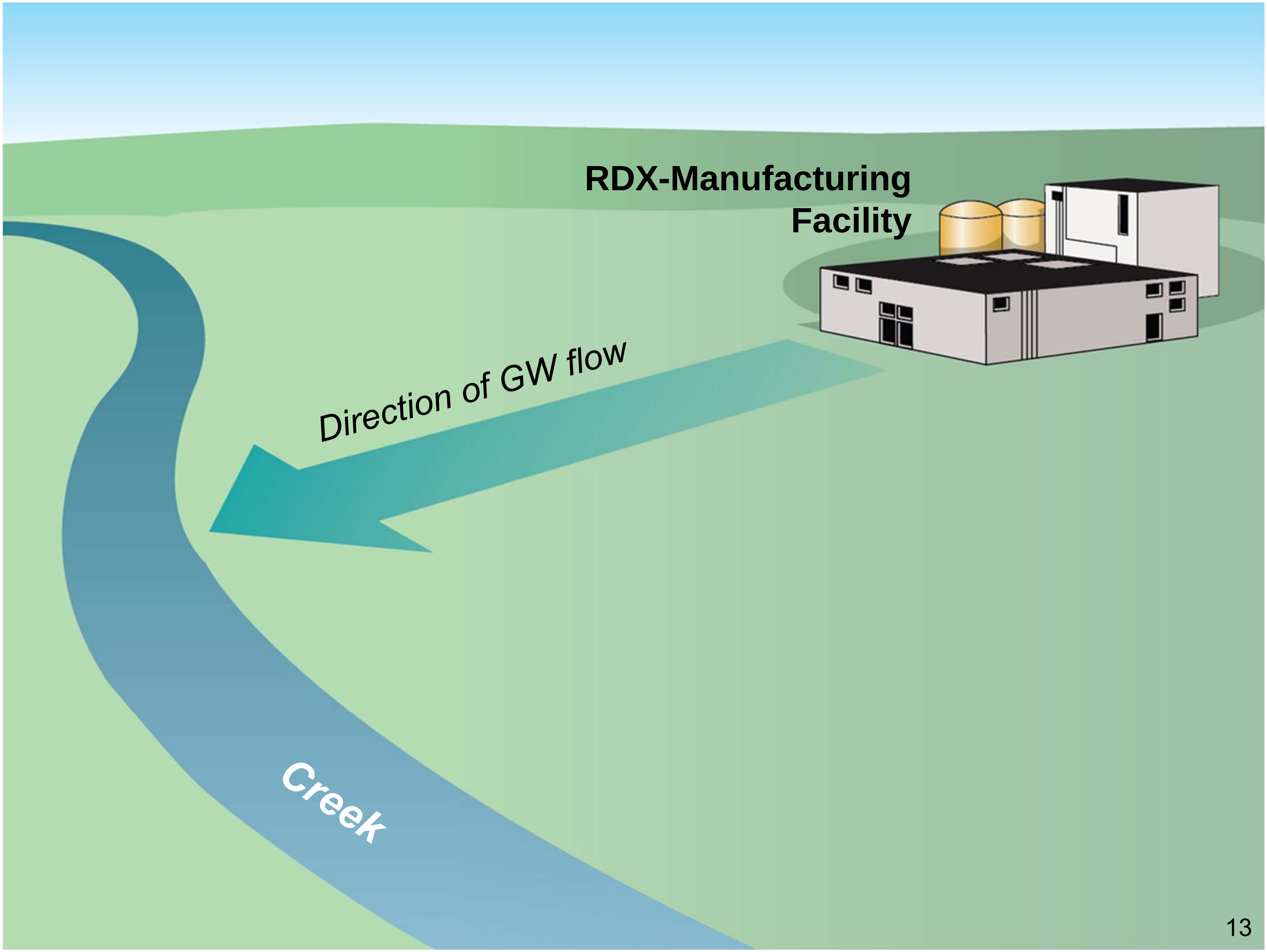
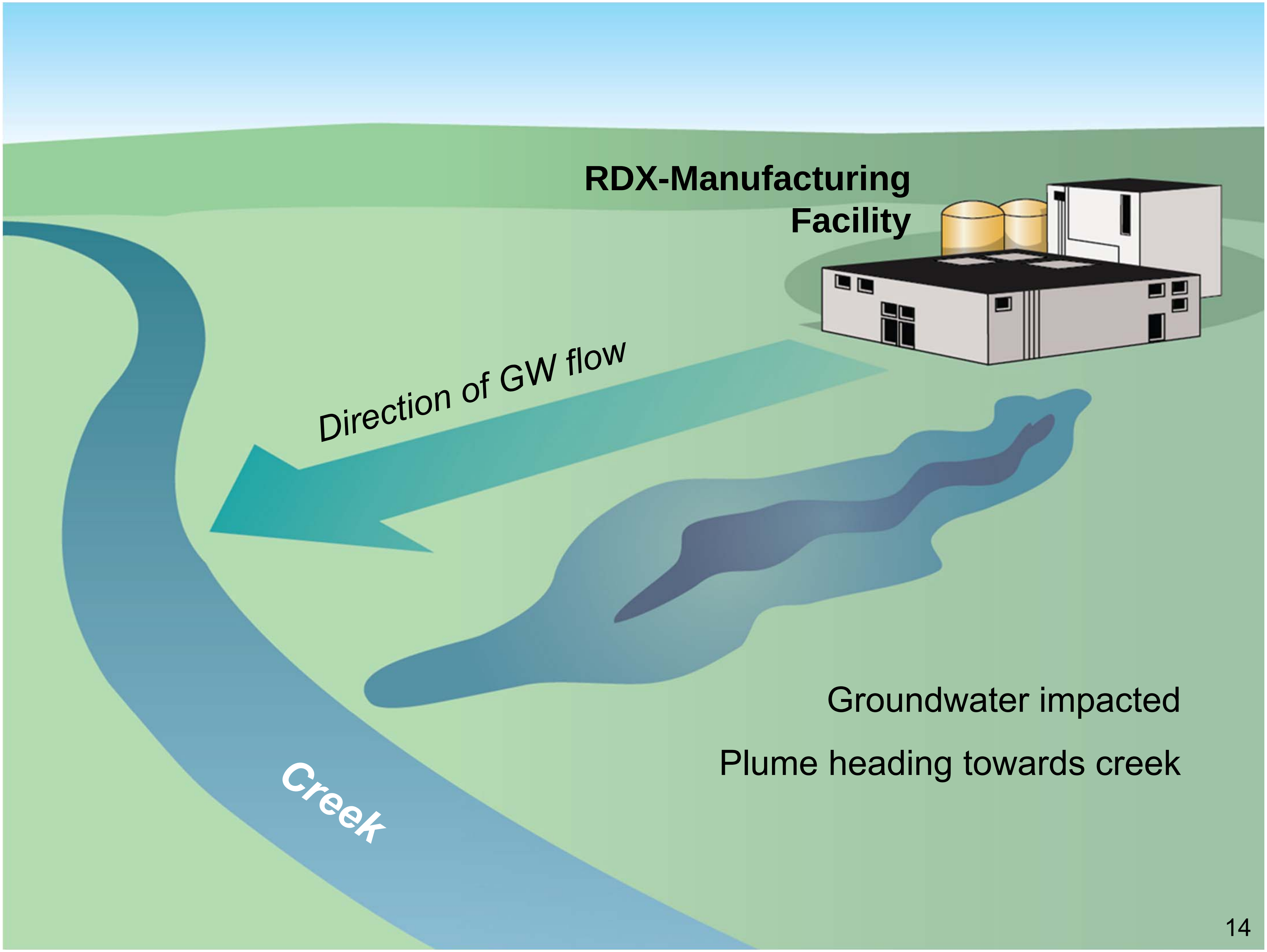


RDX-Manufacturing Facility
Creek


RDX-Manufacturing
Facility
Direction of GW flow
Creek



RDX-Manufacturing Facility
Direction of GW flow
Creek
Groundwater impacted
Plume heading towards creek

Questions to Answer

- Is there an indigenous RDX degrading population?
- Can RDX-degrading population be further stimulated to increase degradation efficiency?
- Can enhanced bioremediation reduce the time to site closure and lifespan costs of the site?

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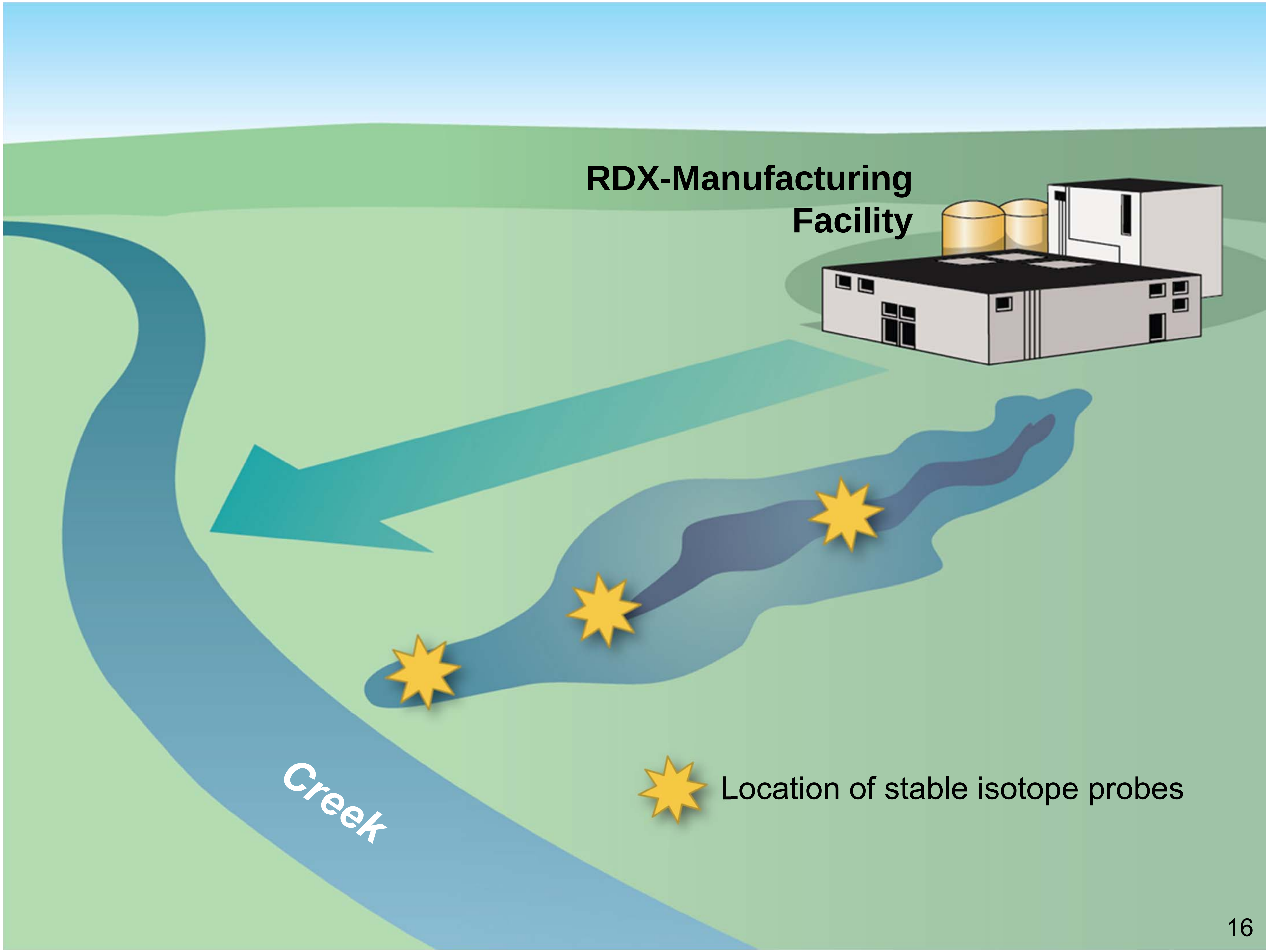
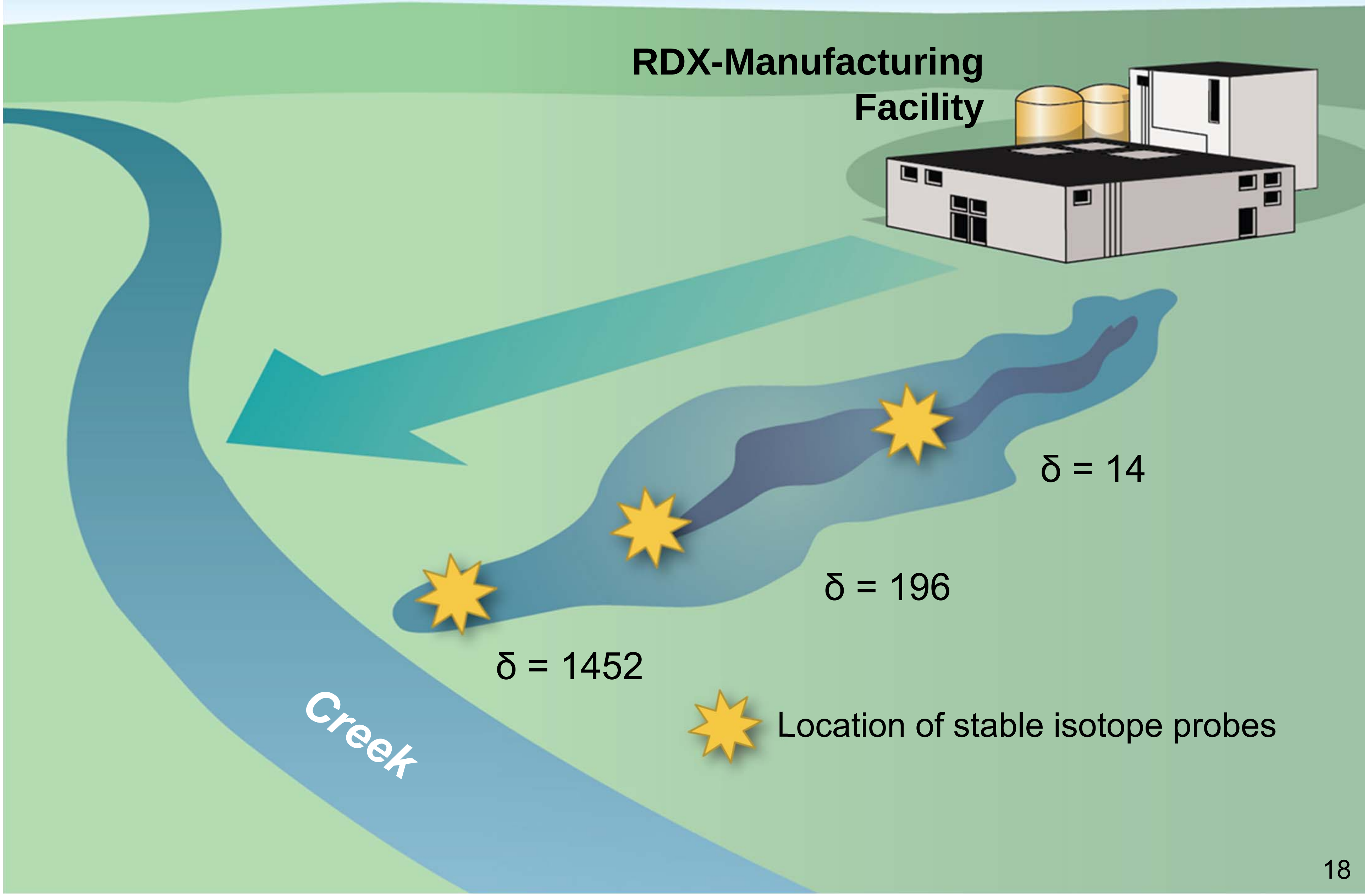
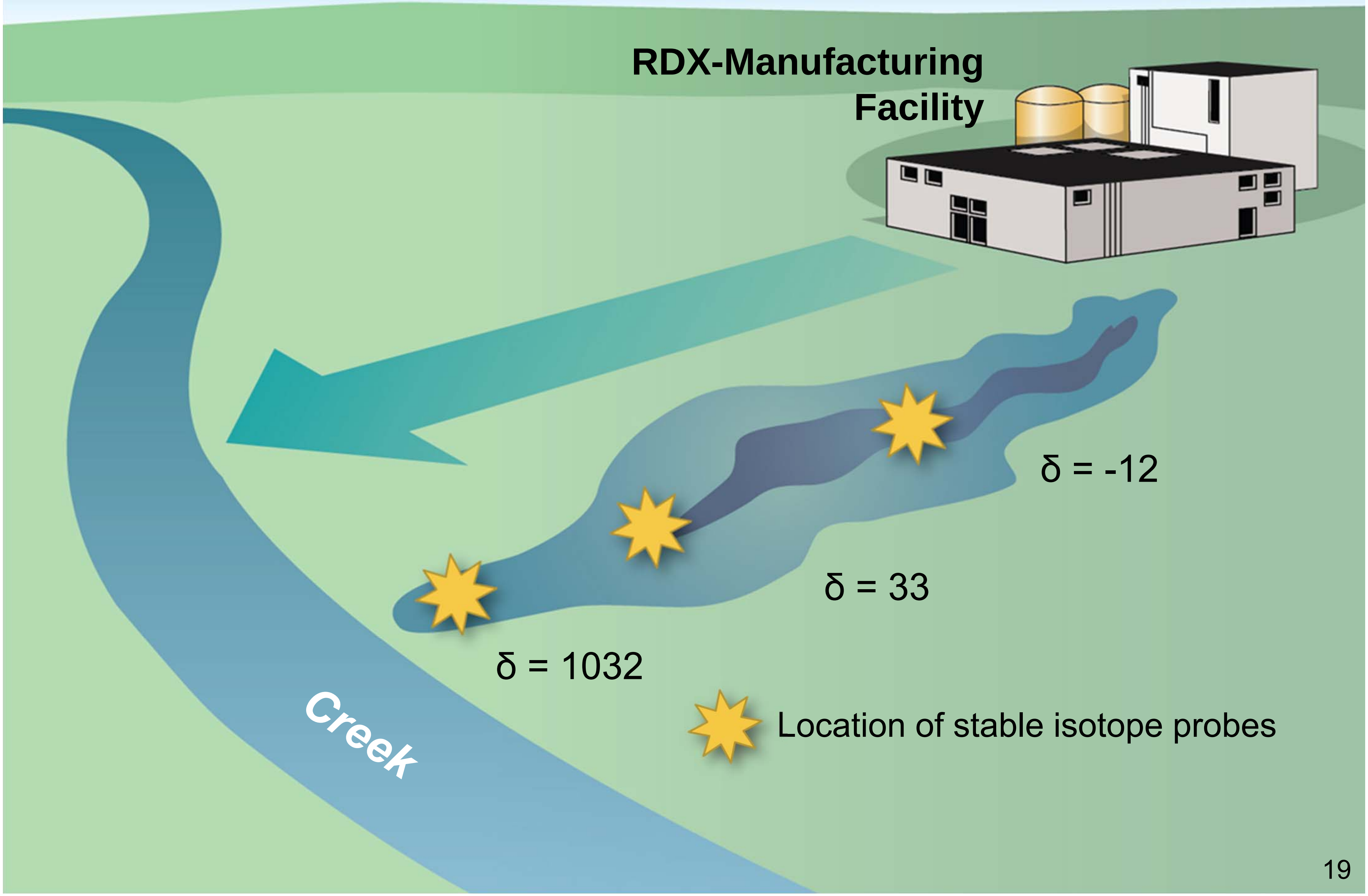

RDX-Manufacturing Facility
Creek
Location of stable isotope probes

Results

^{13}C Incorporation into Biomass



^{13}C Incorporation into Carbon Dioxide



Stable Isotope Probe Results

- Presence of substantial, robust RDX-degrading population confirmed
  - Significant incorporation of isotopic label into biomass, CO_2
  - Higher incorporation at plume periphery than in plume center
- Population was very active
  - Signs of nutritional deficits both in plume center and periphery
  - Confirmed by site geochemistry

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Benefits to this Project

- Detailed profile of microbial community members in RDX plume
  - Appropriate, customized site amendments developed to address nutritional deficiencies
  - Resulted in more rapid contaminant degradation
    - 85% increase RDX degradation rates
  - Allowed site to approach site closure more rapidly
    - Estimated 2.5 – 3 years saved
    - Substantial savings over lifespan of project

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Summary

- Stable isotope tools can assist with determining best remediation design
- Can have a significant impact on effectiveness of remediation system
  - Can help discern most effective MNA / bioremediation strategy
  - Determine how to most efficiently implement remediation enhancements

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Summary

- Can determine if an MNA / bioremediation strategy is working to its full potential

- Monitor remediation strategy

- Determine new closure timeline and associated, reduced costs

- Easily coupled to other isotopic / molecular technologies to increase resulting information

    - Stable Isotope Ratios
    - Quantitative Polymerase Chain Reactions (qPCR)
    - Denaturing Gradient Gel Electrophoresis (DGGE)

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Summary

- Can help demonstrate MNA / bioremediation to regulators
  - State level: CA, MD, and other states
  - Federal level: Multiple EPA regions
  - Can convince regulators to sometimes allow less expensive remediation methods by proving effectiveness
- Can reduce lifespan of remediation project by years, reducing lifespan costs
  - Reducing years of O&M, reporting costs

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Importance of Stable Isotope Technologies

- Can be used on wide range of contaminants
  - RDX
  - TNT
  - CL-20
  - Wide range of nitroaromatics

- Use is accepted by scientific community
  - Backed by years of peer-reviewed research
  - Widely used for other industrial /chem wastes

- International acceptance of their use

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Questions ???

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