Antibiotic-Resistant Bacteria and Recycled Water

Teacher Information

science take-out
just add students™

Summary

Are antibiotic resistance genes in recycled water a threat to human health?

- Test for the presence of antibiotic resistance genes in the water leaving a wastewater treatment plant.
- Interpret graphics that illustrate how bacteria can become resistant to multiple antibiotics.
- Explain how the presence of antibiotics in wastewater might increase the frequency of multi-drug resistant bacteria.

Core Concepts

- Infections with antibiotic-resistant bacteria are difficult and sometimes impossible to treat.
- Transfer of antibiotic resistance genes between different bacteria speeds up the evolution of bacteria that are resistant to multiple antibiotics.
- Antibiotics in wastewater may lead to natural selection of antibiotic-resistant bacteria.
- Antibiotic resistance genes found in recycled water may pose a risk to animal and human health.

Time Required

Two to three 40-minute class periods

Kit Contains

- **Instructions for Antibiotic Resistance Gene Test Kit**
- Well strip for antibiotic resistance gene tests
- Recycled wastewater (simulated) and dropper
- **Flowchart: Fairfield Wastewater Treatment System**
- **How Bacteria Acquire Antibiotic Resistance Genes**
- **Natural Selection of Antibiotic-Resistant Bacteria**
- **Testing Wastewater for Antibiotics** sheet
- Antibiotic Test Solution (simulated) and dropper

Teacher Provides

- Safety goggles
- Paper towels for clean-up

Warning: Choking Hazard

This Science Take-Out kit contains small parts. Do not allow children under the age of seven to have access to any kit components.

Teacher Suggestions

- Part 4 is designed promote thinking and discussion about recycled water and antibiotic-resistant bacteria in the context of community life. If possible, allow time for students to share and discuss their answers. It is particularly important to allow time for students to discuss their answers to question 6 in Part 4.

- Consider using the Science Take-Out kit **Antimicrobials: Is Keeping Clean Risky** (STO-151) to provide a model of the natural selection process.

- As a follow-up, students who want more information about antibiotic resistance genes in recycled water should find this overview paper written by an engineering student interesting. **Balancing Water Sustainability and Public Health Goals the Face of Growing concerns about Antibiotic Resistance** https://pubs.acs.org/doi/10.1021/es403883p

Teacher Resources

There is a growing body of evidence that recycled (reclaimed) water, and even air, contains antibiotics and antibiotic resistance genes. Risk assessments are needed to determine the level of threat to human health. Below is a list of some suggested resources for information.

- **CDC: Antibiotic/Antimicrobial Resistance** https://www.cdc.gov/drugresistance/index.html

- **Antibiotic-Resistance Genes in Waste Water** https://www.ncbi.nlm.nih.gov/pubmed/29033338

- **A Closer Look at Antibiotic Resistance Genes in the Air**
  https://phys.org/news/2018-10-closer-antibiotic-resistant-genes-air.html

- **Balancing Water Sustainability and Public Health Goals in the Face of Growing concerns about Antibiotic Resistance** https://pubs.acs.org/doi/10.1021/es403883p

- **EPA: Water Reuse and Recycling: Community and Environment Benefits**
  https://www.epa.gov/waterreuse

- **Rethinking wastewater risks and monitoring in light of the COVID-19 pandemic**
  https://www.nature.com/articles/s41893-020-00605-2

Reusing the Kit

Teachers will need to instruct students on how to handle cleanup and return of the reusable kit materials. For example, teachers might provide the following information for students:

Discard	Return to kit bag
• Used clear plastic well strip • Used **Testing Wastewater for Antibiotics** sheet	• **Instructions for Antibiotic Resistance Gene Test Kit** • Recycled Wastewater tube and dropper* • **Flowchart: Fairfield Wastewater Treatment System** • **How Bacteria Acquire Antibiotic Resistance Genes** • **Natural Selection of Antibiotic-Resistant Bacteria** • Antibiotic Test Solution tube and dropper

*** Note:** It is <u>not</u> necessary to rinse or wash the droppers after use. Washing the droppers may make the labels difficult to read. Simply ask students to squirt out any extra liquid from the droppers.

Refills for **Antibiotic-Resistant Bacteria and Recycled Water** kits are available at www.sciencetakeout.com. The **10 Kit Refill Pack** includes the following materials:

- 10 well strips for antibiotic resistance gene tests
- 15 mL of Recycled Wastewater (simulated)
- 10 **Testing Wastewater for Antibiotics** sheets
- 15 mL of Antibiotic Test Solution (simulated)
- 2 transfer pipets

Next Generation Science Standards (NGSS) Correlation

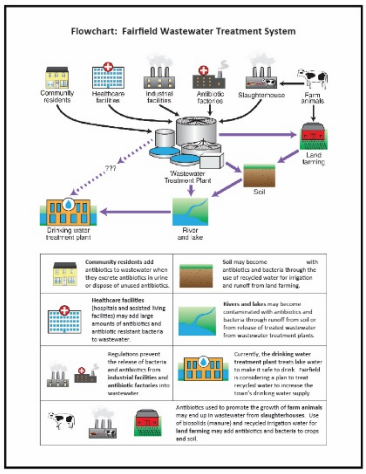
Working Towards Performance Expectations

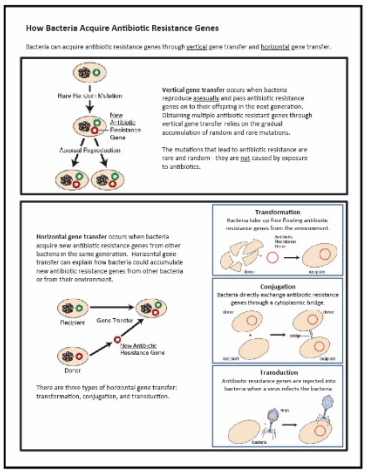
HS-LS4-4. Construct an explanation based on evidence for how natural selection leads to adaptation of populations. [Clarification Statement: Emphasis is on using data to provide evidence for how specific biotic and abiotic differences in ecosystems (such as ranges of seasonal temperature, long-term climate change, acidity, light, geographic barriers, or evolution of other organisms) contribute to a change in gene frequency over time, leading to adaptation of populations.]

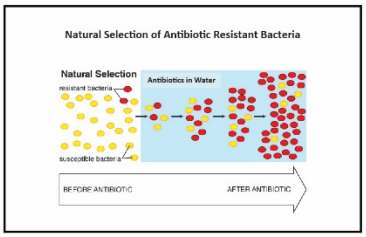
HS-LS4-2. Construct an explanation based on evidence that the process of evolution primarily results from four factors: (1) the potential for a species to increase in number, (2) the heritable genetic variation of individuals in a species due to mutation and sexual reproduction, (3) competition for limited resources, and (4) the proliferation of those organisms that are better able to survive and reproduce in the environment.

Science and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future. (HS-LS4-4) (HS-LS4-2)	Natural selection leads to adaptation, that is, to a population dominated by organisms that are anatomically, behaviorally, and physiologically well suited to survive and reproduce in a specific environment. That is, the differential survival and reproduction of organisms in a population that have an advantageous heritable trait leads to an increase in the proportion of individuals in future generations that have the trait and to a decrease in the proportion of individuals that do not. (HS-LS4-4) Natural selection occurs only if there is both (1) variation in the genetic information between organisms in a population and (2) variation in the expression of that genetic information — that is, trait variation — that leads to differences in performance among individuals (HS-LS4-2) Evolution is a consequence of the interaction of four factors: (1) the potential for a species to increase in number, (2) the genetic variation of individuals in a species due to mutation and sexual reproduction, (3) competition for an environment's limited supply of the resources that individuals need in order to survive and reproduce, and (4) the ensuing proliferation of those organisms that are better able to survive and reproduce in that environment. (HS-LS4-2)	Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects. (HS-LS4-4)

Kit Contents Quick Guide

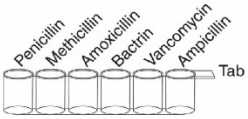




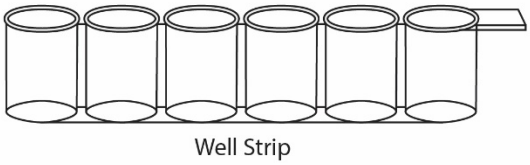


Instructions for
Antibiotic Resistance Gene Test Kit

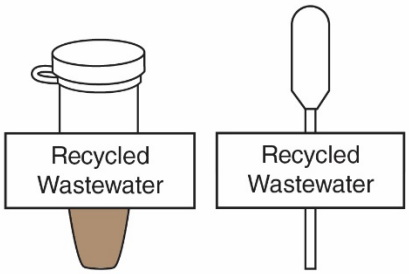
1. Each well on the clear plastic well strip is used to test for a gene that makes bacteria resistant to the antibiotic listed above the well.
2. Arrange the well strip as shown on the diagram below. Be certain that the tab on well strip is on the right side.

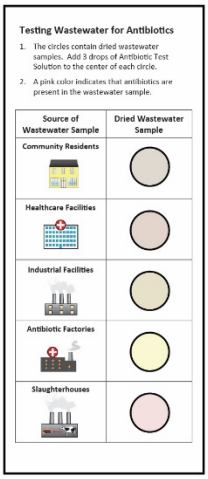


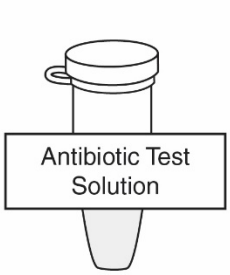
3. Use the dropper to put 2 drops of **Recycled Wastewater** sample in each of the wells.
4. If the well turns a pink color, it indicates that the recycled wastewater sample contains resistance genes for that antibiotic.

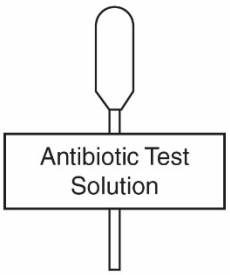


Well Strip









Read these instructions before using Science Take-Out kits

Adult Supervision Required

This kit should be used only under the supervision of an adult who is committed to ensuring that the safety precautions below, and in the specific laboratory activity, are followed.

Chemicals Used in Science Take-Out Kits

Every effort has been made to reduce the use of hazardous chemicals in Science Take-Out kits. Most kits contain common household chemicals or chemicals that pose little or no risk. Safety Data Sheets (SDS) provide specific safety information regarding the chemical contents of the kits. SDS information for each kit is provided in the accompanying teacher instructions. We encourage students to adopt safe laboratory practices when using chemicals.

Warning: Choking and Chemical Hazard

Science Take-Out kits contain small parts that could pose a choking hazard and chemicals that could be hazardous if ingested. Do not allow children under the age of seven to have access to any kit components.

No blood or body fluids from humans or animals are used in Science Take-Out kits. Chemical mixtures are substituted as simulations of these substances.

General Safety Precautions

1. Never taste, smell, or ingest any chemicals provided in the kit - they may be hazardous.
2. Chemicals used in Science Take-Out experiments may stain or damage skin, clothing or work surfaces. If spills occur, wash the area immediately and thoroughly.
3. Report any chemical spills or contact with chemicals to your teacher.
4. Work in a clean, uncluttered area. Cover the work area to protect the work surface.
5. Read and follow all instructions carefully.
6. Pay particular attention to following the specific safety precautions provided by your teacher or included in the kit activity instructions.
7. Do not use the contents of this kit for any other purpose beyond those described in the kit instructions.
8. Do not leave experiment parts or kits where they could be used inappropriately by others.
9. Do not eat, drink, or apply make-up or contact lenses while performing experiments.
10. Wash your hands before and after performing experiments.

Antibiotic-Resistant Bacteria and Recycled Water
Teacher Answer Key

Part 1: Recycled water

Yesterday, Jason and his friends were sprayed with water from Fairfield High School's irrigation system. This worried Jason because he noticed signs throughout the town of Fairfield that said the town conserved water by using recycled water for irrigation. Recycled water is treated wastewater from a sewage treatment plant. It is <u>not</u> potable (safe for drinking or use in food) because it may contain bacteria that make people and animals sick. The town uses recycled water for irrigation because it had experienced several years of drought conditions.

1. How is recycled water different from potable water that comes from a water treatment plant?

2. Explain why using recycled water could be a good thing.

3. Explain why using recycled water could be a problem.

Jason's friend, Ben, is in the hospital with a serious skin infection. Ben was treated with several different antibiotics but these antibiotics have not worked to kill the bacteria that are causing the skin infection.

Antibiotic-resistant bacteria are not killed by some (or sometimes many) types of antibiotics. Antibiotic-resistant bacteria are dangerous because they can cause diseases that are difficult, and sometimes impossible, to cure.

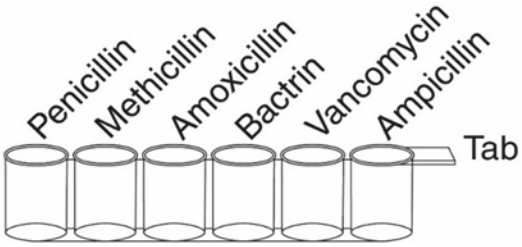
4. Explain why it is appropriate to conclude that Ben's skin infection is caused by antibiotic-resistant bacteria.

Some community members claim that the use of recycled water is responsible for the increase in Fairfield residents who have been sickened by antibiotic-resistant bacteria. To provide evidence to support their claim, scientists need to test the recycled water to see if it contains antibiotic-resistant bacteria.

The scientists explained that it is a time-consuming and expensive process to grow bacteria from the recycled water and then to test the bacteria for antibiotic resistance. It is faster and less expensive to do DNA tests to see if the recycled water contains **antibiotic resistance genes** (ARGs) that would make bacteria resistant to a variety of antibiotics.

5. Use the materials and instructions in the **Antibiotic Resistance Gene Test Kit** to determine whether antibiotic resistance genes are present in the recycled water sample from Fairfield's wastewater (sewage) treatment plant.

6. Mark each well in the diagram below with an "X" if the sample contains antibiotic resistance genes for that type of antibiotic.



7. Does the recycled water sample contain antibiotic resistance genes (genes that would make bacteria resistant to antibiotics)? If so, list these antibiotic resistance genes.

8. Multidrug-resistant bacteria ("superbugs") are bacteria that contain antibiotic resistance genes for more than two types of antibiotics. Why are diseases caused by multi-drug resistant bacteria difficult to treat or cure?

Part 2: Evolution of antibiotic-resistant bacteria

Jason wondered why people were worried that the evolution of "superbugs" would occur when bacteria are exposed to the antibiotics in wastewater. Jason knew that the wastes entering a wastewater treatment plant contain:

- Weak concentrations of many different kinds of antibiotics. These antibiotics enter the wastewater treatment plant when they are flushed down drains in homes or health care facilities.
- Many different kinds of bacteria. Some of these bacteria are **susceptible** (not resistant) to antibiotics. Other bacteria have one or more genes that make them resistant to antibiotics.

Base your answer to questions 1 through 6 on the **Natural Selection of Antibiotic Resistant Bacteria** diagram sheet in your lab kit.

1. Based on the diagram, what happens to the proportion (percentage) of antibiotic-resistant bacteria in a wastewater treatment plant over time?

2. Based on the diagram, what happens to the proportion (percentage) of antibiotic susceptible (not resistant) bacteria in a wastewater treatment plant over time?

3. What is present in wastewater that acts as a selecting agent to cause the changes you described in questions 1 and 2?

4. Describe the characteristics of bacteria that would be best able to survive and reproduce in the wastewater.

5. Why is the presence of antibiotics in wastewater a problem?

6. What could be done to reduce the presence of antibiotics in wastewater?

7. Explain how evolution by natural selection may result in antibiotic-resistant bacteria that are resistant to a variety of antibiotics.

Part 3: How do bacteria obtain multiple antibiotic resistance genes?

Jason was interested in learning how bacteria in the wastewater or recycled water could obtain genes that made them resistant to multiple antibiotics. Bacteria that have multiple antibiotic resistance genes are more likely to survive and reproduce than bacteria with few or no antibiotic resistance genes.

Jason did a bit of research and discovered the article in your kit entitled **How Bacteria Acquire Antibiotic Resistance Genes**. Use the information in the article to answer questions 1 through 6.

1. Does exposure to an antibiotic cause the mutations that make bacteria resistant to that antibiotic? Explain your answer.

2. Explain why vertical gene transfer is slower than horizontal gene transfer.

3. State one way that horizontal gene transfer is similar to vertical gene transfer.

4. State one way that horizontal gene transfer and vertical gene transfer are different.

5. Which type of horizontal gene transfer might be modeled using a syringe? What would the syringe represent?

6. Which type of horizontal gene transfer might be modeled using a piece of straw? What would the piece of straw represent?

Part 4: Wastewater and recycled water in a community

Jason's friend, Ben, was still in the hospital with a serious skin infection caused by antibiotic-resistant bacteria. None of the antibiotics that doctors had tried was stopping the spread of the infection. The doctors thought the infection started when antibiotic-resistant bacteria entered a cut on Ben's leg that he got during football practice.

Jason wondered if the bacteria that caused Ben's infection came from the recycled water sprayed on school property. Jason decided he should learn more about wastewater and recycled water in Fairfield. He discovered that the town of Fairfield has increased the use of recycled water (reclaimed wastewater) over the past 10 years.

The town of Fairfield built a network of purple pipes to deliver recycled water. Purple is a color reserved for pipes that carry non-potable water (water that is not used for drinking). The purple pipe system delivers recycled water to parks, farms, school yards and factories in Fairfield.

Reclaimed wastewater is also discharged directly into a river that is a source of water for Fairfield's water treatment plant, which turns it into potable (drinkable) water for the community. Fairfield has also begun using biosolids (dried wastes) from the wastewater treatment plant to make fertilizer. Some people are concerned that reclaimed wastewater and biosolid fertilizer are spreading antibiotic resistance genes in the community.

Use the information above and the **Flowchart: Fairfield Wastewater Treatment System** in your kit to answer questions 1 through 6.

1. Predict <u>two</u> places on the flowchart where you would be likely to find high concentrations of bacteria that could enter the wastewater treatment plant. <u>Support your predictions.</u>

2. Predict <u>two</u> places on the flowchart where you would be likely to find antibiotics that could enter the wastewater treatment plant. <u>Support your predictions.</u>

3. Test your predictions for question 2 above using the instructions and materials for **Testing Wastewater for Antibiotics**. Which wastewater samples contained antibiotics?

4. Antibiotics reduce the survival and reproduction of susceptible (non-resistant) bacteria and allow antibiotic-resistant bacteria to survive and reproduce. Identify one possible place on the flowchart where the presence of antibiotics would favor the evolution of antibiotic-resistant bacteria. Support your choice.

5. Beyond recycled water from community irrigation systems, there are many other places where people are likely to be exposed to the recycled water from a wastewater treatment plant. Identify two places on the flowchart where people are likely to be exposed to antibiotic-resistant bacteria or antibiotic resistance genes from wastewater. Support your choices.

The area around Fairfield has been experiencing droughts that seem to be getting worse. The reservoirs that provide water to the town contain less water each year. Town officials have declared that water needs to be recycled in order to provide adequate water for the community. Jason wondered what he or his community could do to reduce the risks associated with the use of recycled water.

6. Imagine that you lived in Fairfield. Identify two important things that you would be willing to do to reduce the need for recycled water or to reduce the risk from antibiotic-resistant bacteria in recycled water. Explain how these actions would reduce the risks from antibiotic-resistant bacteria.

Section 1 Chemical Product and Company Information

Science Take-Out
80 Office Park Way
Pittsford, NY 14534
(585)764-5400

CHEMTREC 24 Hour Emergency
Phone Number (800) 424-9300
For laboratory use only. Not for drug, food or household use

.

Product	Buffer Solution pH10
Synonyms	“Recycled Wastewater” (simulated); “Antibiotic Test Solution” (simulated)

Section 2 Hazards Identification

This substance or mixture has not been classified at this time according to the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals.

Signal word: WARNING
Pictograms: None required
Target organs: None known

GHS Classification:
Skin irritation (Category 3)
Eye irritation (Category 2B)

GHS Label information: **Hazard statement(s):**
H316: Causes mild skin irritation.
H320: Causes eye irritation.

Precautionary statement(s):

P264: Wash hands thoroughly after handling.

P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P332+P313: If skin irritation occurs: Get medical attention.

P337+P313: If eye irritation persists: Get medical attention.

Ca Prop 65 - This product does not contain any chemicals known to the State of California to cause cancer, birth defects, or any other reproductive harm.

Section 3 Composition / Information on Ingredients

Chemical Name	CAS #	%	EINECS
Water	7732-18-5	99.08%	231-791-2
Potassium chloride	7447-40-7	0.40%	231-211-8
Boric acid	10043-35-3	0.33%	233-139-2
Sodium hydroxide	1310-73-2	0.19%	215-185-5

Section 4 First Aid Measures

INGESTION: Call physician or Poison Control Center immediately. Induce vomiting only if advised by appropriate medical personnel. Never give anything by mouth to an unconscious person.

INHALATION: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

EYE CONTACT: Check for and remove contact lenses. Flush thoroughly with water for at least 15 minutes, lifting upper and lower eyelids occasionally. Get immediate medical attention.

SKIN ABSORPTION: Remove contaminated clothing. Flush thoroughly with mild soap and water. If irritation occurs, get medical attention.

Section 5 Fire Fighting Measures

Suitable Extinguishing Media: Use any media suitable for extinguishing supporting fire.

Protective Actions for Fire-fighters: In fire conditions, wear a NIOSH/MSHA-approved self-contained breathing apparatus and full protective gear. Use water spray to keep fire-exposed containers cool.

Specific Hazards: During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion.

Section 6 Accidental Release Measures

Personal Precautions: Evacuate personnel to safe area. Use proper personal protective equipment as indicated in Section 8. Provide adequate ventilation.

Environmental Precautions: Avoid runoff into storm sewers and ditches which lead to waterways.

Containment and Cleanup: Absorb with inert dry material, sweep or vacuum up and place in a suitable container for proper disposal. Wash spill area with soap and water.

Section 7 Handling and Storage

Precautions for Safe Handling: Read label on container before using. Do not wear contact lenses when working with chemicals. Keep out of reach of children. Avoid contact with eyes, skin and clothing. Do not inhale vapors, spray or mist. Use with adequate ventilation. Avoid ingestion. Wash thoroughly after handling. Remove and wash clothing before reuse.

Conditions for Safe Storage: Store in a cool, well-ventilated area away from incompatible substances.

Section 8 Exposure controls / Personal Protection

Exposure Limits:

Chemical Name	ACGIH (TLV)	OSHA (PEL)	NIOSH (REL)
Potassium chloride	None established	None established	None established

Engineering controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower and fire extinguishing material. Personnel should wear safety glasses, goggles, or faceshield, lab coat or apron, appropriate protective gloves. Use adequate ventilation to keep airborne concentrations low.

Respiratory protection: None should be needed in normal laboratory handling at room temperatures. If misty conditions prevail, work in fume hood or wear a NIOSH/MSHA approved respirator.

Section 9 Physical and Chemical Properties

Appearance: Clear, colorless liquid.
Odor: No odor.
Odor threshold: Data not available.
pH: 10.0
Melting/Freezing point: Approx. 0°C (32°F) (water)
Boiling point: Approx. 100°C (212°F) (water)
Flash point: Data not available

Evaporation rate (Water = 1): <1
Flammability (solid/gas): Data not available.
Explosion limits: Lower/Upper: Data not available
Vapor pressure (mm Hg): 14 (water)
Vapor density (Air = 1): 0.7 (water)
Relative density (Specific gravity): Approx. 1.0 (water)
Solubility(ies): Complete in water.

Partition coefficient: Data not available
Auto-ignition temp.: Data not available
Decomposition temp.: Data not available
Viscosity: Data not available.
Molecular formula: Mixture
Molecular weight: Mixture

Section 10 Stability and Reactivity

Chemical stability: Stable
Hazardous polymerization: Will not occur.

Conditions to avoid: Excessive temperatures which cause evaporation.
Incompatibilities with other materials: Acids, alkalies, and air will change the buffer's ability.
Hazardous decomposition products: Boron oxide and chlorine gas.

Section 11 Toxicological Information

Acute toxicity: Data not available
Skin corrosion/irritation: Data not available
Serious eye damage/irritation: Data not available
Respiratory or skin sensitization: Data not available
Germ cell mutagenicity: Data not available
Carcinogenity: Data not available

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity: Data not available
STOT-single exposure: Data not available
Aspiration hazard: Data not available
STOT-repeated exposure: Data not available

Potential health effects:
Inhalation: May be harmful if inhaled.
Ingestion: May be harmful if swallowed.
Skin: May cause mild irritation.
Eyes: May cause mild irritation.

Signs and symptoms of exposure: To the best of our knowledge the chemical, physical and toxicological properties have not been thoroughly investigated. Specific data is not available. Exercise appropriate procedures to minimize potential hazards.
Additional information: RTECS #: Data not available

Section 12 Ecological Information

Toxicity to fish: No data available
Toxicity to daphnia and other aquatic invertebrates: No data available
Toxicity to algae: No data available
Persistence and degradability: No data available
Bioaccumulative potential: No data available
Mobility in soil: No data available
PBT and vPvB assessment: No data available
Other adverse effects: An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Section 13 Disposal Considerations

These disposal guidelines are intended for the disposal of catalog-size quantities only. Federal regulations may apply to empty container. State and/or local regulations may be different. Dispose of in accordance with all local, state and federal regulations or contract with a licensed chemical disposal agency.

Section 14 Transport Information

UN/NA number: Not applicable
Shipping name: Not Regulated
Hazard class: Not applicable
Packing group: Not applicable
Reportable Quantity: No
Marine pollutant: No
Exceptions: Not applicable
2012 ERG Guide # Not applicable

Section 15 Regulatory Information

A chemical is considered to be listed if the CAS number for the anhydrous form is on the Inventory list.

Component	TSCA	CERLCA (RQ)	RCRA code	DSL	NDSL	WHMIS Classification
Potassium Chloride	Listed	Not Listed	Not Listed	Listed	Not Listed	Uncontrolled Product
Sodium hydroxide	Listed	1,000 lbs (454 kg)	D002	Listed	Not Listed	E

Section 16 Additional Information

The information contained herein is furnished without warranty of any kind. Employers should use this information only as a supplement to other information gathered by them and must make independent determinations of suitability and completeness of information from all sources to assure proper use of these materials and the safety and health of employees.
NTP: National Toxicology Program, IARC: International Agency for Research on Cancer, OSHA: Occupational Safety and Health Administration, STOT: Specific Target Organ Toxicity, SE: Single Exposure, RE: Repeated Exposure, ERG: Emergency Response Guidebook.

Revision Date: October 12, 2020 Supercedes: