Brain Reward Pathway and Addiction

Teacher Information

Summary

Students explore the case of a young man who may be addicted to Floratryp, a fictitious drug. They learn about the role of the brain reward pathway and dopamine in addiction. They compare the effects of sugar, cocaine, and Floratryp on brain dopamine levels and rat lever-pressing behavior.

Core Concepts

- Drug addiction is defined as “uncontrollable, compulsive drug seeking and use, even in the face of negative health and social consequences.”
- The brain reward pathway creates sensations of pleasure which cause organisms to repeat certain behaviors.
- Drugs of abuse cause increased levels of the neurotransmitter dopamine in the brain reward pathway.

Time Required

Three 40-minute class periods

Kit contains

- Tube of Dopamine Indicator
- Tubes of brain fluid samples (0, 30, and 60 min Floratryp),
- Droppers for Dopamine Indicator and brain fluid samples
- Color chart for dopamine concentration
- Dopamine 4 Test Cup Strip

Teacher Provides

- Safety goggles
- Paper towels for clean up

Teacher Information

- Floratryp is a fictitious drug.
- Before or after Part 2, consider showing animations from “The New Science of Addiction” website at: http://learn.genetics.utah.edu/content/addiction/

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.
Reusing *Brain Reward Pathway and Addiction* kits

Teachers will need to instruct students on how to handle clean-up and return of the re-usable kit materials. For example, teachers might provide the following information for students:

<table>
<thead>
<tr>
<th>Discard</th>
<th>Rinse with water</th>
<th>Return to kit bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Contents of Dopamine Test Cup Strip</td>
<td>• Dopamine Test Cup Strips</td>
<td>• All labeled droppers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Dopamine Test Cup Strips</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(rinsed)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• All labeled microtubes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Dopamine color chart</td>
</tr>
</tbody>
</table>

**Note:** It is **not** necessary to rinse or wash the droppers after use. Because the droppers are labeled, there is little chance for contamination. Washing the droppers may make the labels difficult to read. Simply ask students to squirt out any extra liquid from the droppers.

Consider laminating printed parts of the kits (such as colored graphics or instruction cards) that will be reused.

Refills for *Testing for Genetic Disorders that Can Cause Brain Damage* kits are available at www.sciencetakeout.com. The 10 Kit Refill Pack includes the following materials:

- Instructions and Quick Guide for refilling kit
- 4 graduated transfer pipets (for teacher use)
- 10 ml of Dopamine Indicator
- 10 ml of 0 min. – Floratryp
- 10 ml of 30 min. – Floratryp
- 10 ml of 60 min. – Floratryp
Read these instructions before using Science Take-Out kits

Parental or 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.

Safety Goggles and Gloves Strongly Recommended
We encourage students to adopt safe lab practices, and wear safety goggles and gloves when performing laboratory activities involving chemicals. Safety goggles and gloves are not provided in Science Take-Out kits. They may be purchased from a local hardware store or pharmacy.

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. Material Safety Data Sheets (MSDS) provide specific safety information regarding the chemical contents of the kits. MSDS information for each kit is provided in the accompanying teacher instructions.

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.

General Safety Precautions
1. Work in a clean, uncluttered area. Cover the work area to protect the work surface.
2. Read and follow all instructions carefully.
3. Pay particular attention to following the specific safety precautions included in the kit activity instructions.
4. Goggles and gloves should be worn while performing experiments using chemicals.
5. Do not use the contents of this kit for any other purpose beyond those described in the kit instructions.
6. Do not leave experiment parts or kits where they could be used inappropriately by others.
7. Never taste or ingest any chemicals provided in the kit – they may be toxic.
8. Do not eat, drink, apply make–up or contact lenses while performing experiments.
9. Wash your hands before and after performing experiments.
10. 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.
11. At the end of the experiment, return ALL kit components to the kit plastic bag. Dispose of the plastic bag and contents in your regular household trash.

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.
Brain Reward Pathway and Addiction

Teacher Answer Key

Part 1: Is Floratryp Addictive?

Medical Report

Ray is feeling horrible. He is very depressed and nervous. He has a headache and nausea, and he is having trouble sleeping. Ray has experienced some of these symptoms in the past, but they disappear when he uses “Floratryp,” a mixture of over-the-counter and herbal medications.

Ray doesn’t think his symptoms are caused by Floratryp addiction. “Lots of people use Floratryp. It’s not illegal. It’s not addictive. What’s wrong with feeling good?” He likes using Floratryp because it makes him feel happy and confident. “I only take it when I’m feeling low or stressed out. A little Floratryp and I feel great.”

He has been using Floratryp for about a year. Over the past few months Ray’s use of Floratryp has increased from once a day to five or more times each day. Ray has not been able to use Floratryp for the past few days because he ran out of money. He was recently fired from his part-time job because he skipped work a lot – especially after late nights of doing Floratryp with friends.

"Drug addiction is defined as “uncontrollable, compulsive drug seeking and use, even in the face of negative health and social consequences.”

Scientists from the National Institute on Drug Abuse

1. Based on the information in the Medical Report, do you think that Floratryp is addictive even though it is not illegal? Support your answer by listing three pieces of evidence from the Medical Report.
Part 2: The Brain Reward Pathway

The reward pathway is a group of neurons (nerve cells) that conduct impulses (electrical signals) to specific brain regions that produce a sensation of pleasure. The arrows on diagrams below show the parts of the human brain involved in the reward pathway.

Parts of the Brain Involved in the Human Reward Pathway

The reward pathway causes animals to associate certain behaviors with a sensation of pleasure. When an animal carries out certain behaviors, such as eating or sexual activity, electrical signals travel through neurons in the reward pathway and cause a sensation of pleasure. This pleasure sensation is a reward that causes the animal to learn to repeat these behaviors again and again. Researchers theorize that this is how behaviors necessary to survival, like reproduction and eating, are learned. Research has also shown that drugs of abuse, such as cocaine, affect the brain reward region and can lead to addiction.

Scientists have identified dopamine as the neurotransmitter (chemical signal molecule) that carries information from one neuron to another in the brain reward pathway.
Base your answers to questions 1 through 6 on the information in the box on page 2.

1. What is the function of the reward pathway of the brain?

2. List two behaviors which cause impulses (electrical signals) to travel through the reward pathway in the brain.
   
   Eating and sexual

3. What causes animals to repeat behaviors that stimulate the brain reward region?

4. Addictive drugs have been shown to increase the activity in the brain reward pathway. What neurotransmitter (chemical signal) may be increased in the brain reward region during drug use?

5. Do you think that shopping, smoking, and gambling may activate the reward pathway and lead to addiction for some people? Explain your answer.

6. The parts of the rat brain involved in the reward pathway are shown in the diagram on the right. Some scientists who study the effects of addictive drugs on the brain use rats, instead of humans, in their experiments. What are two advantages of using rats instead of humans when conducting research on the effects of drugs on the brain?
Part 3: How does Floratryp affect dopamine levels in the brain reward region?

A scientist wants to determine if Floratryp is an addictive drug. If a drug is addictive, it will increase the dopamine levels in the brain reward region to levels that are higher than typical for normal rewarding substances. To determine if Floratryp is addictive, scientists can do animal experiments to determine how Floratryp affects dopamine levels in the brain reward region.

In this activity, you will conduct tests to compare the levels of dopamine in samples of brain fluid collected from the reward regions of three rats. Rat A was injected with sugar (a normal rewarding substance). Rat B was injected with cocaine (a known addictive drug). Rat C was injected with Floratryp that scientists think might be addictive.

The brain fluid samples were collected from the reward regions of the rat brains at three times: 0 minutes (immediately before the substance was given to the rat), 30 minutes after the substance was given to the rat, and 60 minutes after the substance was given to the rat.

The scientist has already tested the dopamine levels for brain fluid samples from Rats A and B. The data from the scientist’s research is provided in Data Table 1.

Data Table 1: Concentration of Dopamine in Rat Brain Fluid Samples

<table>
<thead>
<tr>
<th>Substance Given to Rat</th>
<th>Time after being given substance (minutes)</th>
<th>Concentration of dopamine in rat brain (picograms/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat A - Sugar (a normal rewarding substance)</td>
<td>0 min.</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>30 min.</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>60 min.</td>
<td>10</td>
</tr>
<tr>
<td>Rat B - Cocaine (a known addictive drug)</td>
<td>0 min.</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>30 min.</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>60 min.</td>
<td>100</td>
</tr>
<tr>
<td>Rat C - Floratryp</td>
<td>0 min.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 min.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 min.</td>
<td></td>
</tr>
</tbody>
</table>
1. You will test the dopamine levels in the brain fluid samples from Rat C (the rat exposed to Floratryp).

2. Obtain a 4 Test Cup Strip from your kit. Leave the small cup on the left empty. Do not add anything to the left well (as shown on the diagram).

3. Add 1 drop of “Dopamine Indicator” to each of the small cups on the Dopamine Test Cup Strip.

4. Obtain three tubes of brain fluid samples (labeled 0 min. Floratryp, 30 min. Floratryp, and 60 min. Floratryp) from your lab kit. These tubes contain brain fluid samples collected at different time points in the experiment after the rat was injected with Floratryp.

5. Add 6 drops of the brain fluid sample (0 min. Floratryp, 30 min. Floratryp, and 60 min. Floratryp) to the appropriate cups on the Dopamine Test Strip.

6. Compare the color of the brain fluid samples with the “Color Chart for Dopamine Concentration” that is included in your lab kit. *It is easier to see the colors of the brain fluid samples in the test cups if you hold the cup strip up to the light.*

7. Record the estimated dopamine concentration in **Data Table 1: Concentration of Dopamine in Rat Brain Fluid Samples** on page 4.
8. What happens to the level of dopamine in the brain when a rat is given sugar (a normal rewarding substance)?

9. Compare the levels of dopamine in the rat given Floratryp with the levels of dopamine in the rat given sugar.

10. Compare the levels of dopamine in the rat given Floratryp with the levels of dopamine in the rat given cocaine.

11. Based on the results of this experiment, do you think that Floratryp is addictive? Explain why or why not.
Part 4: Does Floratrypt cause repetitive drug-seeking behavior?

To be considered addictive, a drug must activate the reward center and produce the repetitive drug-seeking behavior associated with addictive drugs. To determine if Floratrypt is addictive, scientists can do experiments to see whether it results in repetitive drug-seeking behavior.

In this activity you will analyze data collected by a scientist who investigated drug-seeking behavior in rats.

For this experiment, rats were placed in individual cages with a lever they could press that delivered a rewarding substance.

- When Rat A pressed a sugar lever, it received an injection of sugar solution.
- When Rat B pressed a cocaine lever, it received an injection of cocaine.
- When Rat C pressed a Floratrypt lever, it received an injection of Floratrypt.

Each day (for 6 days), the rats were placed in the appropriate cages and scientists observed and recorded the number of times the rats pressed the levers in 5 minutes.

The results of the experiment are presented in Data Table 3 on the next page.
Data Table 3: Effect of Sugar, Cocaine, and Floratryp on Rat Lever Pressing Behavior

<table>
<thead>
<tr>
<th>Lever</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat A Sugar (a natural reward)</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Rat B Cocaine (an addictive drug)</td>
<td>3</td>
<td>7</td>
<td>12</td>
<td>29</td>
<td>52</td>
<td>77</td>
</tr>
<tr>
<td>Rat C Floratryp</td>
<td>4</td>
<td>8</td>
<td>10</td>
<td>27</td>
<td>49</td>
<td>73</td>
</tr>
</tbody>
</table>

1. Summarize the data from the experiment by plotting the total number of times that each of the rats pressed the levers versus days.
   - Label the vertical axis and the horizontal axis.
   - Mark an appropriate scale on both of the axes. Use equal intervals.
   - Plot the data using the legend provided.

**Legend:**
- Rat A – Sugar
- Rat B – Cocaine
- Rat C – Floratryp

**Effect of Sugar, Cocaine, and Floratryp on Rat Lever Pressing Behavior**
2. What is the independent variable in this experiment?

3. What is the dependent variable in this experiment?

4. Rat A and Rat B were controls in this experiment. They provided a basis of comparison.
   - Which rat provided a control (basis of comparison) to show the difference between a rat’s reaction to a normal rewarding stimulus and Floratryn?
   - Which rat provided a control (basis of comparison) to show the difference between a rat’s reaction to an addictive drug and Floratryn?

5. Why does the number of lever presses for sugar increase?

6. Compare the number of lever presses for Floratryn with the number of lever presses for sugar.

7. Compare the number of lever presses for Floratryn with the number of lever presses for cocaine.
8. Does the data from the rat lever pressing support the hypothesis that Floratryp is addictive? Explain why or why not.

9. State one way that the scientist’s experiment could be improved to make it more reliable.

10. Based on the information and data from this lab activity, discuss the pieces of evidence you would provide to lawmakers who are considering classifying Floratryp as an illegal drug.
MATERIAL SAFETY DATA SHEET

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name (as printed on the label): “Dopamine Indicator”

Product identity: 0.1% bromothymol blue solution

Distributor: Wards Natural Sciences, 5100 West Henrietta Road. PO Box 92912, West Henrietta, NY 14692-9102

Telephone number for information: (800) 962-2660

Medical emergency phone number (Chemtrec): (800) 424-9300

Date of this MSDS: 5/28/13

2. COMPOSITION/INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>CAS Numbers</th>
<th>% Weight/Volume</th>
<th>TLV Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromothymol blue sodium salt</td>
<td>34722-90-2</td>
<td>0.1%</td>
<td>None established</td>
</tr>
<tr>
<td>Water</td>
<td>7732-18-5</td>
<td>99.9%</td>
<td>None established</td>
</tr>
</tbody>
</table>

3. HAZARDS IDENTIFICATION – for all pH buffer products

**EMERGENCY OVERVIEW**

Do not ingest. Avoid skin and eye contact. Avoid exposure to vapor or mists.

Potential Health Effects

- **EYES:** May cause irritation.
- **SKIN:** May cause slight irritation.
- **INGESTION:** May cause gastrointestinal discomfort
- **INHALATION:** n/a

4. FIRST AID MEASURES

- **EYES:** Flush with water for at least 15 minutes, raising and lowering eyelids occasionally. Get medical attention if irritation persists.
- **SKIN:** Thoroughly wash exposed area for at least 15 minutes. Remove contaminated clothing. Launder contaminated clothing before reuse. Get medical attention if irritation persists.
- **INGESTION:** Do not induce vomiting. If swallowed, if conscious, give plenty of water immediately and call a physician or poison control center. Never give anything by mouth to an unconscious person.

5. FIRE FIGHTING MEASURES

- **NFPA Rating:** Health: 1 (slight) Fire: 0 Reactivity: 0
- **Extinguisher Media:** Any means suitable for extinguishing surrounding fire
- **Firefighting Procedures:** Firefighters should wear full protective equipment and NIOSH approved self-contained breathing apparatus.
- **Unusual Fire and Explosion Hazards:** None

6. SPILL OR LEAK PROCEDURES

Ventilate area of spill. Clean-up personnel should wear proper protective equipment and clothing. Mop up, or absorb material with suitable absorbent and containerize for disposal.

7. HANDLING AND STORAGE

Store in a cool dry place. Handle using safe laboratory practices.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Respiratory Protection: None required
Ventilation: Local Exhaust: Preferred
Protective Gloves: Natural rubber, Neoprene, PVC or equivalent.
Eye Protection: Splash proof chemical safety goggles should be worn.
Other Protective Clothing or Equipment: Lab coat, apron, eye wash, safety shower.

9. PHYSICAL AND CHEMICAL PROPERTIES

Melting Point: <2°C  Boiling Point: >98°C
Vapor Pressure: Ca 50 @ 20°C  Vapor Density: ~ same as water
Specific Gravity (H₂O=1): ~1
Percent Volatile by Volume: information not available
Evaporation Rate: ~ same as water
Solubility in Water: soluble
Appearance and Odor: Green liquid

10. STABILITY AND REACTIVITY

Stability: Stable
Materials to Avoid: none known
Hazardous Decomposition Products: none
Reactive under what conditions: none known

11. TOXICOLOGICAL INFORMATION

<table>
<thead>
<tr>
<th>Toxicity (rat) LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute oral toxicity = information not available</td>
</tr>
<tr>
<td>Acute toxicity from vapor = information not available</td>
</tr>
</tbody>
</table>

Effects of Overexposure:
Acute: Irritation of eyes/skin
Chronic: Irritation of eyes/skin
Target Organs: Eyes, skin.
Primary Route(s) of Entry: Ingestion

12. ECOLOGICAL INFORMATION No data available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods: Dispose in accordance with all applicable Federal, State and Local regulations. Always contact a permitted waste disposer (TSD) to assure compliance.

14. TRANSPORTATION INFORMATION No data available

15. REGULATORY INFORMATION No data available

16. ADDITIONAL INFORMATION

The information provided in this Material Safety Data Sheet represents data from the manufacturer and/or vendor and is accurate to the best of our knowledge. By providing this information, Science Take-Out LLC makes no guarantee or warranty, expressed or implied, concerning the safe use, storage, handling, precautions, and/or disposal of the products covered or the accuracy of the information contained in this fact sheet. It is the responsibility of the user to comply with local, state, and federal laws and regulations concerning the safe use, storage, handling, precautions, and/or disposal of products covered in this fact sheet.
MATERIAL SAFETY DATA SHEET

1. PRODUCT AND COMPANY IDENTIFICATION

<table>
<thead>
<tr>
<th>Label on Tube</th>
<th>Contents of Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min Floratryp</td>
<td>Buffer pH 5</td>
</tr>
<tr>
<td>30 min Floratryp</td>
<td>Buffer pH 7</td>
</tr>
<tr>
<td>60 min Floratryp</td>
<td>Buffer pH 9</td>
</tr>
</tbody>
</table>

Distributor: Wards Natural Sciences, 5100 West Henrietta Road. PO Box 92912, West Henrietta, NY 14692-9102

Telephone number for information: (800) 962-2660
Medical emergency phone number (Chemtrec): (800) 424-9300

Date of this MSDS: 5/28/13

2. COMPOSITION/INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Ingredients</th>
<th>CAS Numbers</th>
<th>% Weight/Volume (balance is water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5 buffer</td>
<td>Potassium biphthalate Sodium phosphate dibasic</td>
<td>877-24-7 7558-79-4</td>
<td>0.38% 0.08</td>
</tr>
<tr>
<td>pH 7 buffer</td>
<td>Potassium phosphate monobasic Sodium phosphate dibasic</td>
<td>7778-77-0 7558-79-4</td>
<td>0.15% 0.30%</td>
</tr>
<tr>
<td>pH 9 buffer</td>
<td>Sodium carbonate Sodium bicarbonate</td>
<td>497-19-8 144-55-8</td>
<td>0.10% 0.35%</td>
</tr>
</tbody>
</table>

For all the ingredients
NIOSH ILDH: none estab.

3. HAZARDS IDENTIFICATION – for all pH buffer products

EMERGENCY OVERVIEW
Do not ingest. Avoid skin and eye contact. Avoid exposure to vapor or mists.

Potential Health Effects
EYES: May cause irritation.
SKIN: May cause irritation.
INHALATION: n/a
INGESTION: May cause gastrointestinal discomfort and mouth burns.

4. FIRST AID MEASURES – for all pH buffer products

EYES - Flush with water for at least 15 minutes, raising and lowering eyelids occasionally. Get medical attention if irritation persists.
SKIN - Thoroughly wash exposed area for at least 15 minutes. Remove contaminated clothing. Launder contaminated clothing before reuse. Get medical attention if irritation persists.
INGESTION - Do not induce vomiting. If swallowed, if conscious, give plenty of water immediately and call a physician or poison control center. Never give anything by mouth to an unconscious person.

5. FIRE FIGHTING MEASURES – for all pH buffer products

NFPA Rating:
Health: 1
Fire: 0
Reactivity: 0
Extinguisher Media: Any means suitable for extinguishing surrounding fire
Special Firefighting Procedures: Firefighters should wear full protective equipment and NIOSH approved self-contained breathing apparatus. Unusual Fire and Explosion Hazards: No data available

6. SPILL OR LEAK PROCEDURES – for all pH buffer products
Ventilate area of spill. Clean-up personnel should wear proper protective equipment and clothing. Absorb material with suitable absorbent and containerize for disposal.

7. HANDLING AND STORAGE – for all pH buffer products
Store in a cool dry place. This Material is not considered hazardous. Handle using safe laboratory practices.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION – for all pH buffer products
Respiratory Protection: n/a
Ventilation: 
Local Exhaust: Preferred
Mechanical(General): Acceptable
Special: No
Other: No

Protective Gloves: Natural rubber, Neoprene, PVC or equivalent.
Eye Protection: Splash proof chemical safety goggles should be worn.
Other Protective Clothing or Equipment: Lab coat, apron, eye wash, safety shower.

9. PHYSICAL AND CHEMICAL PROPERTIES – for all pH buffers
Melting Point: ~0°C
Boiling Point: ~100°C
Vapor Pressure: information not available
Vapor Density: information not available
Specific Gravity (H₂O=1): ~1
Percent Volatile by Volume: >99
Evaporation Rate: information not available
Solubility in Water: soluble
Appearance and Odor: Clear colorless liquid

10. STABILITY AND REACTIVITY – for all pH buffer products
Stability: Stable
Materials to Avoid: strong acids and bases
Hazardous Decomposition Products: none known
Hazardous Polymerization: will not occur

11. TOXICOLOGICAL INFORMATION

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Toxicity (oral-rat) LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium biphthalate</td>
<td>3200 mg/kg</td>
</tr>
<tr>
<td>Sodium phosphate dibasic</td>
<td>17 g/kg</td>
</tr>
<tr>
<td>Potassium phosphate monobasic</td>
<td>7100 mg/kg</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>4090 mg/kg</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>4220 mg/kg</td>
</tr>
</tbody>
</table>

Effects of Overexposure (for all pH buffers):
Acute: Essentially non-hazardous. Possible irritation of eyes/skin/stomach
Chronic: None known.
Conditions aggravated/Target organs: none known
Primary Route(s) of Entry: Ingestion or skin contact.

12. ECOLOGICAL INFORMATION – for all pH buffer products
No ecological data available
13. DISPOSAL CONSIDERATIONS – for all pH buffer products
Waste Disposal Methods: Dispose in accordance with all applicable Federal, State and Local regulations. Always contact a permitted waste disposer (TSD) to assure compliance.

14. TRANSPORTATION INFORMATION
D.O.T. SHIPPING NAME: Not regulated

15. REGULATORY INFORMATION – for all pH buffer products
EPA regulations: RCRA Hazardous waste number (40 CFR 261.33) – not listed
RCRS Hazardous waste classification (40 CFR 261) – not classified
SARA Toxic Chemical (40 CFR 372.65) – not listed
SARA EHS (Extremely Hazardous Substance (40 CFR 355) – not listed
OSHA regulations: Air Contaminant (29 CFR 1910.1000) – not listed

16. ADDITIONAL INFORMATION
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