**ACTIVITY: Detecting toxins**

**Activity idea**

In this activity, students explore the processes scientists used to analyse and identify the toxic substance responsible for dog deaths on Auckland beaches.

By the end of this activity, students should be able to:

* identify a given toxin using known chemical structures
* explain what a molecular formula is
* explain in simple terms how liquid chromatography-mass spectrometry (LC-MS) can be used to detect toxins
* show an understanding of the concept of LD50.

[Introduction/background notes](#kix.d8x384s3amf7)

[What you need](#kix.nhhpevd8s7jl)

[What to do](#kix.lvrxyodo4v59)

Student handout: [Learning about marine toxins](#kix.txv0mrfc25ee)

Student handout: [Information on five marine toxins](#kix.yl1ztnc6adot)

Student handout: [Liquid chromatography-mass spectrometry (LC-MS)](#kix.v889ecsmvfjp)

Student handout: [Lethal dose 50](#kix.ie7ofkr8dhtv)

**Introduction/background**

[Solving the dog death mystery](https://www.sciencelearn.org.nz/resources/359-solving-the-dog-death-mystery) follows the procedure, in a broad sense, of how the Cawthron scientists tracked down what killed several dogs on Auckland beaches in 2009 and how they identified the toxin involved. This activity takes a closer look at some of the key aspects of the detective story and how it was solved:

* The chemistry of toxins

The student handout features five marine toxins including the chemical make-up of the toxins via their formulae, structures and molecular mass.

* Liquid chromatography-mass spectrometry

Liquid chromatography-mass spectrometry is a method for separating out individual molecules within a mixture and then determining their molecular mass.

* Lethal dose

LD50 is a measurement in toxicology that indicates how poisonous something might be. It is the dose required to kill half of a specific animal population. Since LD50 is obtained through causing the death of animals, it may be a problem for some students. The video [The ethics of research animals](https://www.sciencelearn.org.nz/videos/1366-the-ethics-of-research-animals) introduces some ethical considerations associated with the use of animals in research.

**What you need**

* Access to the article [Solving the dog death mystery](https://www.sciencelearn.org.nz/resources/359-solving-the-dog-death-mystery) and videos within the article
* Access to the articles [Monitoring shellfish](https://www.sciencelearn.org.nz/resources/372-monitoring-shellfish), [Sea slugs and TTX](https://www.sciencelearn.org.nz/resources/360-sea-slugs-and-ttx), [Chemicals everywhere](https://www.sciencelearn.org.nz/resources/363-chemicals-everywhere) and [Measuring toxicity](https://www.sciencelearn.org.nz/resources/366-measuring-toxicity)
* Copies of the student handouts [Learning about marine toxins](#kix.txv0mrfc25ee), [Information on five marine toxins](#kix.yl1ztnc6adot), [Liquid chromatography-mass spectrometry (LC-MS)](#kix.v889ecsmvfjp) and [Lethal dose 50](#kix.ie7ofkr8dhtv).

**What to do**

1. Read the article [Solving the dog death mystery](https://www.sciencelearn.org.nz/resources/359-solving-the-dog-death-mystery) and watch the video [Finding the dog killer](https://www.sciencelearn.org.nz/videos/161-finding-the-dog-killer). Make a list of terms and ideas students find difficult to understand.
2. Find out about some marine toxins, using the articles [Monitoring shellfish](https://www.sciencelearn.org.nz/resources/372-monitoring-shellfish), [Sea slugs and TTX](https://www.sciencelearn.org.nz/resources/360-sea-slugs-and-ttx) and [Chemicals everywhere](https://www.sciencelearn.org.nz/resources/363-chemicals-everywhere). Use this information plus the [Information on five marine toxins](#kix.yl1ztnc6adot) to answer the questions in [Learning about marine toxins](#kix.txv0mrfc25ee) in pairs.
3. Learn about liquid chromatography-mass spectrometry. Watch the video [Identifying the toxin](https://www.sciencelearn.org.nz/videos/162-identifying-the-toxin), then work through [Liquid chromatography-mass spectrometry (LC-MS)](#kix.v889ecsmvfjp).
4. Learn about LD50. Read the article [Measuring toxicity](https://www.sciencelearn.org.nz/resources/366-measuring-toxicity), then read and discuss [Lethal dose 50](#kix.ie7ofkr8dhtv).
5. Watch the video [Finding the dog killer](https://www.sciencelearn.org.nz/videos/161-finding-the-dog-killer) a second time to gauge student understanding.

**Studen****t handout: Learning about marine toxins**

1. What is okadaic acid? How are people poisoned by okadaic acid? What is the origin of okadaic acid? What type of poisoning does it cause (in relation to how it affects people)? What are the symptoms of the poison?



1. What is tetrodotoxin (TTX)? How might people be poisoned by TTX? What type of toxin is it? What are the symptoms of the poison?
2. What are the molecular formulae for okadaic acid and tetrodotoxin? What do the letters C, H, O, and N stand for? Check the periodic table in [Chemicals everywhere](https://www.sciencelearn.org.nz/resources/363-chemicals-everywhere). Explain the numbers that follow each element.
3. Here are some chemical structures for some marine toxins. These structures show how the molecules in the toxin are made up or formed. Use the student handout [Information on five marine toxins](#kix.yl1ztnc6adot) to identify the chemical structures of these toxins.

|  |  |
| --- | --- |
| Picture 4 | saxitoxin |
| Picture 3 | Picture 1 |
| Picture 2 |

**Student handout: Information on five marine toxins**

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| --- | --- | --- | --- |
| **Toxin**  | **Molecular formula and structure** | **Molecular mass (daltons)** | **LD50****(µg/kg in mice)** |
| **Brevetoxin** Neurotoxic shellfish poisoning (NSP) | C52H74NO17SNaPicture 1 | 1039 | 200 |
| **Okadaic acid**Diarrhetic shellfish poisoning (DSP)  | C44H68O13Picture 2 | 805 | 200 |
| **Tetrodotoxin****(TTX)**Neurotoxin  | C11H17N3O8Picture 3 | 319.28 | 10 |
| **Saxitoxin**Paralytic shellfish poisoning (PSP) | C10H15N7O4Picture 2 | 294 | 10 |
| **Domoic acid**Amnesic shellfish poisoning (ASP) | C15H21NO6Picture 4 | 311.14 | 3600 |

**Student handout: Liquid chromatography-mass spectrometry (LC-MS)**



Liquid chromatography-mass spectrometry is a sophisticated laboratory technique.

Very briefly, when samples are loaded into the instrument, the liquid chromatography (LC) part separates the molecular components present.

These then enter the mass spectrometer (MS) where the molecular mass of each component is determined. The amount of a given component (pure substance) present can also be determined.

The unit for this measurement of molecular mass is given in daltons. One dalton is approximately equal to the mass of one proton or one neutron. Scientists have already worked out and recorded the molecular mass for most molecules. This means that, when they are given a molecular mass through LC-MS, they can look up that mass to find out what the molecule is.

Using the student handout [Information on five marine toxins](#kix.yl1ztnc6adot),answer the following questions:

1. Which of the five toxins has the heaviest molecule?
2. Which has the lightest?
3. What is the molecular mass of tetrodotoxin?
4. What is the molecular mass of okadaic acid?
5. What was the name of the other toxin detected?
6. Molecular mass is measured in daltons. This was named after John Dalton. Find out who John Dalton was.

**Student handout: Lethal dose 50**

Lethal dose 50(LD50) is a very useful measurement in toxicology. It is the dose required to kill half of a specific animal population. It tells us how poisonous a substance is. In the example given in [Measuring toxicity](https://www.sciencelearn.org.nz/resources/366-measuring-toxicity), salt has an oral (means it was fed to an animal) LD50 of 3 gm/kg in rats. Paracetamol has an oral LD50 of 1.944 gm/kg in rats. This means paracetamol is more poisonous than salt because a smaller amount of paracetamol will get the same result in rat deaths.

The LD50 of the toxins in the student handout [Information on five marine toxins](#kix.yl1ztnc6adot) was calculated using mice and is measured in micrograms (µg). A microgram is just one-millionth of a gram. A small paper clip might weigh a gram, so a microgram is one-millionth of that. It is not something you can see with your eyes. A kilogram is a billion micrograms, so one part per billion of solid measure is equal to a µg/kg.

Discuss these questions together:

1. What is the LD50 of okadaic acid?
2. What is the LD50 of tetrodotoxin?
3. Which is more toxic? Explain why
4. Which of the five toxins is/are the most deadly? Explain why.