The Application of SPATT (Solid Phase Adsorption Toxin Tracking) for the pre-concentration of biotoxin and organic compounds from marine, estuary and freshwaters

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11th May 2022

Ireland's European Structural and Investment Funds Programmes 2014-2020

Co-funded by the Irish Government and the European Union

Tionól Réigiúnach an Deiscirt
Southern Regional Assembly

Llywodraeth Cymru
Welsh Government

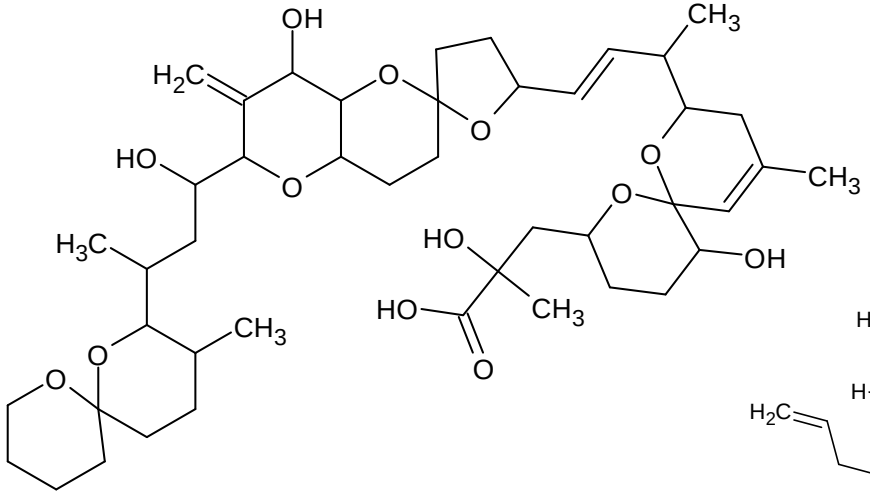
UNDEB EWROPEAIDD
EUROPEAN UNION

Cronfa Datblygu Rhanbarthol Ewrop
European Regional Development Fund

Marine biotoxins

1. Lipophilic:

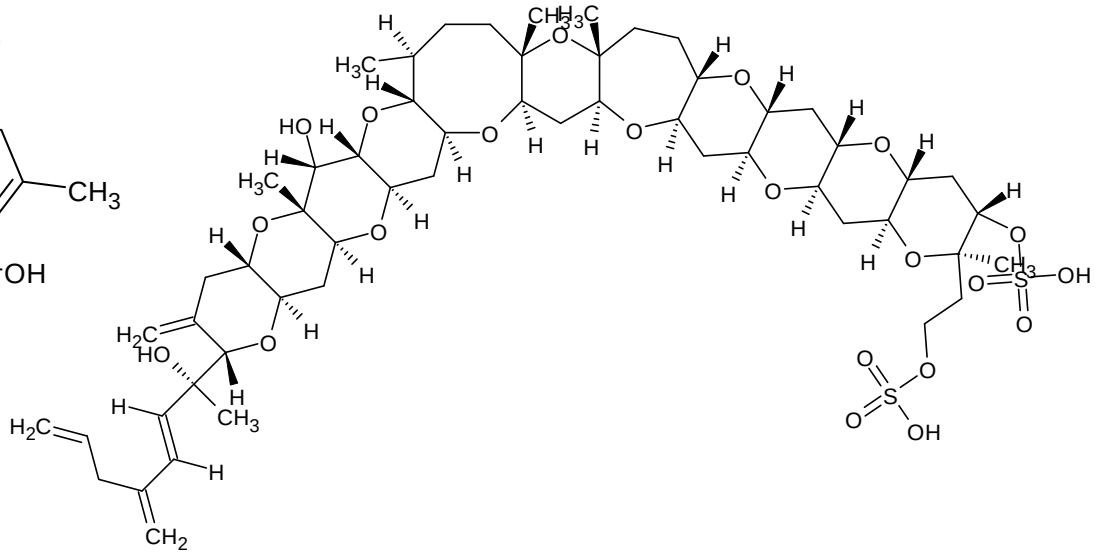
- Lipophilic biotoxins (lipid soluble) can be found individually or grouped as a biotoxin mixture in shellfish
- They are categorised into four groups:
  - (i) okadaic acid (OA) and its derivatives dinophysistoxins (DTXs),
  - (ii) pectenotoxins (PTXs),
  - (iii) the yessotoxins (YTXs),
  - (iv) the azaspiracids (AZAs).



OA, DTXs and PTXs

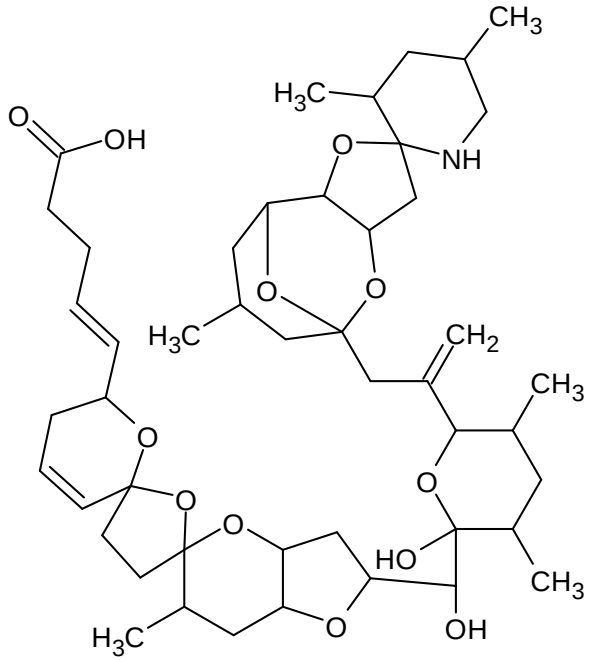
- Diarrhoeic Shellfish Poisoning (DSP) biotoxins
- Initially detected in the Netherlands
- Gastrointestinal incidents caused by consuming mussels.
- DSPs are **heat resistant**

Salas, et al. Toxins 2019, 11, 61



- YTX was first detected in the digestive gland of a bivalve scalene called *Patinopecten yessoensis* (Japanese scallop), in 1986 in Japan.
- In addition:
  - Europe, including Spain, Italy, Norway, Adriatic Sea, Russia; Chile; and New Zealand

Alfonso, et al. biochem pharmaco



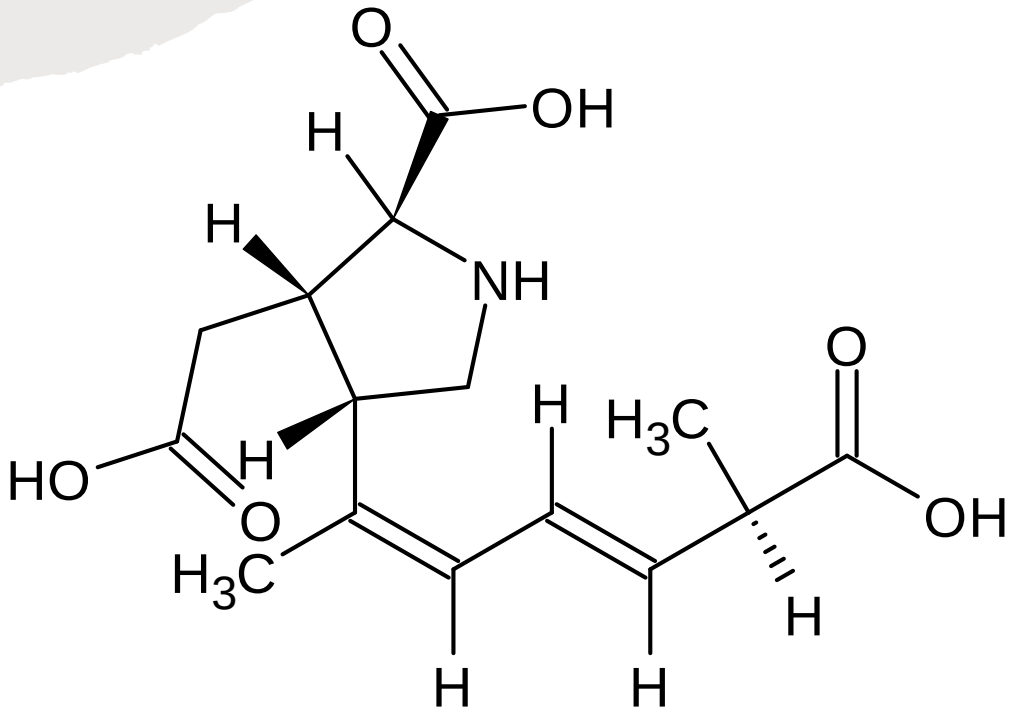
- AZA was first discovered after the ingestion of contaminated mussels originally from Ireland in 1995 caused human illness.
- AZAs toxins are **heat resistance**, **acid stable** and have a **long-term stability** under refrigeration.

Furey, et al ,Toxicon 56 (2010) 173–190

2. Hydrophilic biotoxins: Domoic acid (DA)

- Domoic acid (DA) can bioaccumulate in shellfish and thus can be introduced into the human food chain.
- DA can contaminate different shellfish such as:
  - mussels,
  - crabs,
  - scallops, razor clams
- Amnesic shellfish poisoning (ASP)
- **Gastrointestinal** and **neurological** symptoms
- DA is primarily located in the digestive glands of the shellfish
- DA is **heat resistance** and is stable during cooking temperatures **but**

there is the possibility of concentration reduction due to the hydrophilicity



Jeffery, et al Food and Chem Toxicology 42 (2004)

Legal limit of toxins

operators must ensure that live bivalve molluscs placed on the market for human consumption **must not** contain marine biotoxins **in total quantities** (measured in the whole body or any part edible separately) that exceed the following limits. *

Toxin	Quantity (equivalents per kilogram)**
PSP (paralytic shellfish poison)	800 µg
OA	160 µg (0.16 mg)
YTX	1 mg
AZA	160 µg (0.16 mg)
DO	20 mg/kg

***The amount of toxins expressed as the amount of okadaic acid that gives the same toxic response followed intraperitoneal administration to mice. This applies similarly for the group of yessotoxins and azaspiracids, respectively*

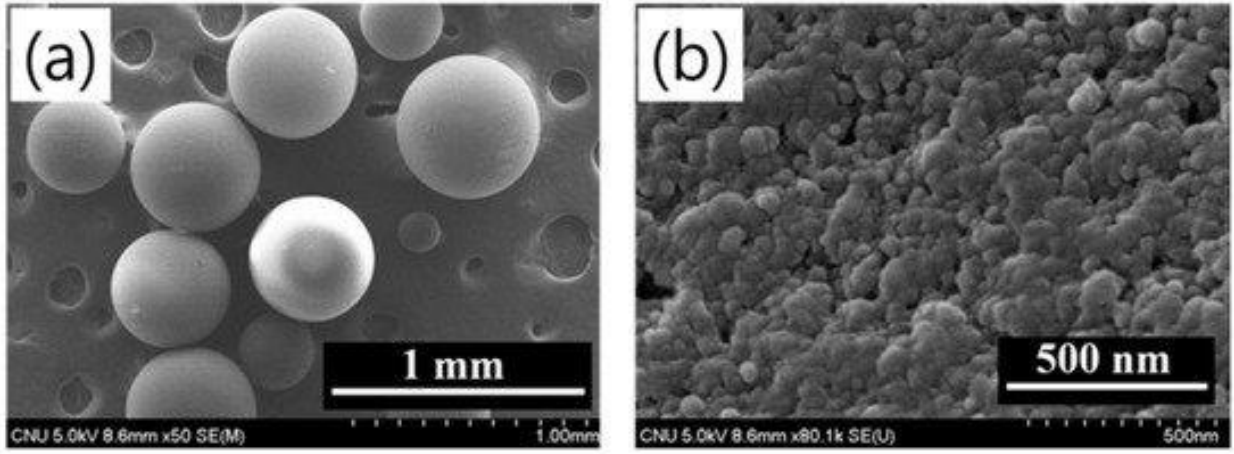
**** EFSA Journal 2010; 8(6):1627***

SPATT sorbents

- Synthetic sorbents are:
  - Spherical particles,
  - Large surface areas,
  - High porosities,
  - Three-dimensional crystalline lattices, including pores,
  - Water molecules are held loosely.

Synthetic sorbents :

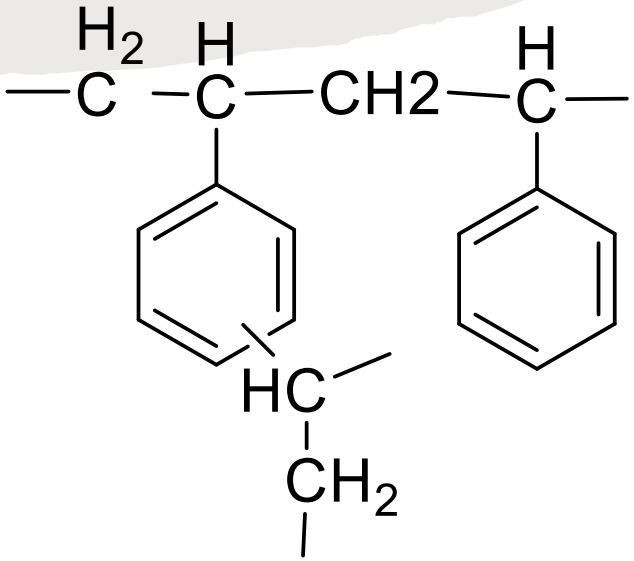
- remain stable in acidic and alkaline environments
- resistance to organic solvents.
- Analyte extraction is safe and reduces solvent usage.

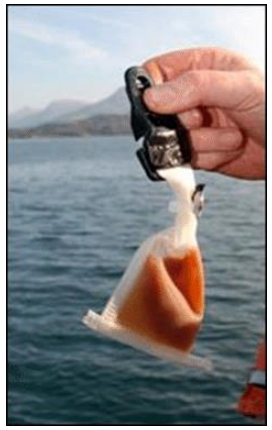


DIAION HP20- SEM image*

Jongho Kim et al, water research 2018

1. Aromatic adsorbents
a. DIAION HP20

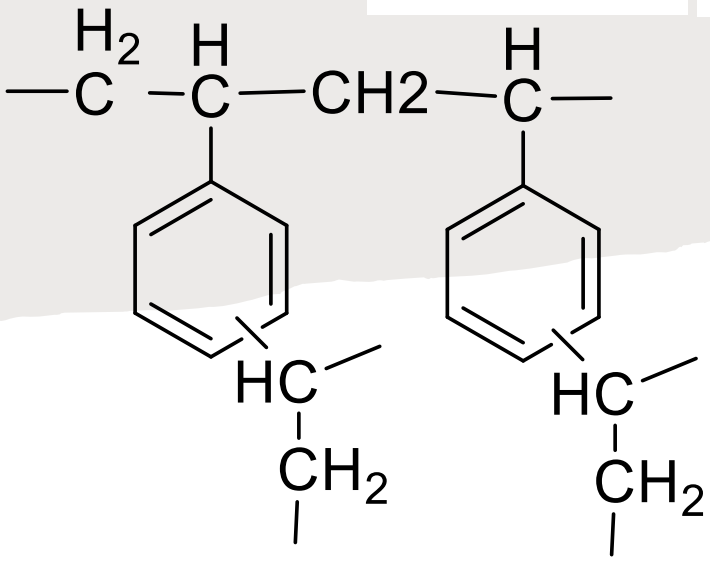




- DIAION HP20 is the most popular
- Non-polar
- Particle density of 1.01 g/mL,
- Specific surface area of 600 m^2/g and
- large pore size (200-300 Angstrom), (which enables adsorption of large natural products and organic compounds with MW >1000 such as peptides, proteins and phenols)
- Is able to adsorb **many lipophilic** marine toxins and **some hydrophilic** toxins

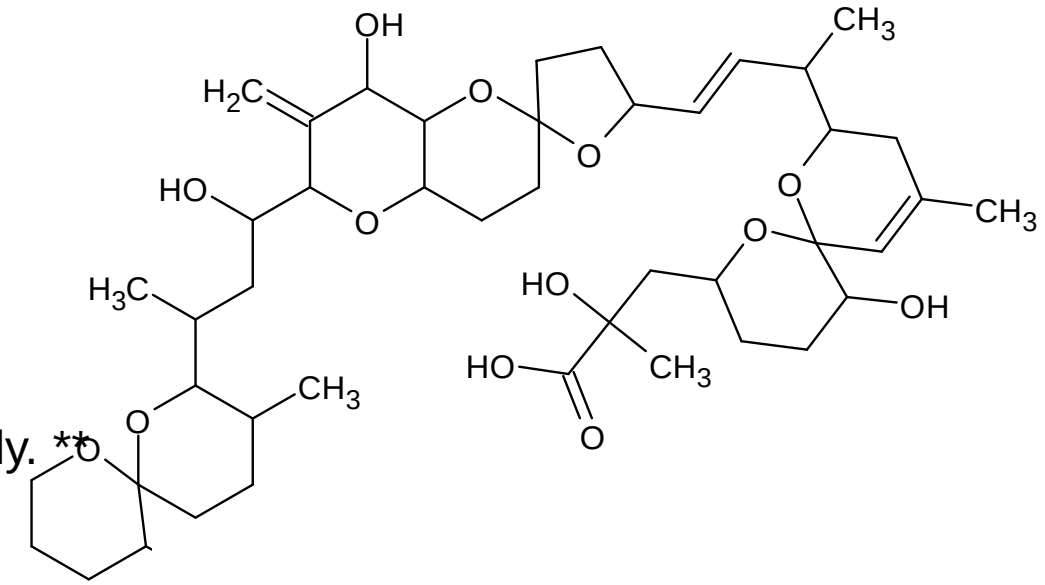
Source: www.google.com

Aromatic adsorbents
b. SEPABEADS SP700



- Effective lipophilic shellfish toxin synthetic sorbents
- Pore size distribution 90 Angstrom
- These provide a greater surface area (1100 m^2/g)
- with similar particle density (1.02 g/mL)

For lipophobic biotoxins, the efficiency order was reported as: **HP20 > SP700 > SP207 > SP207SS** *



The maximal capacity of :

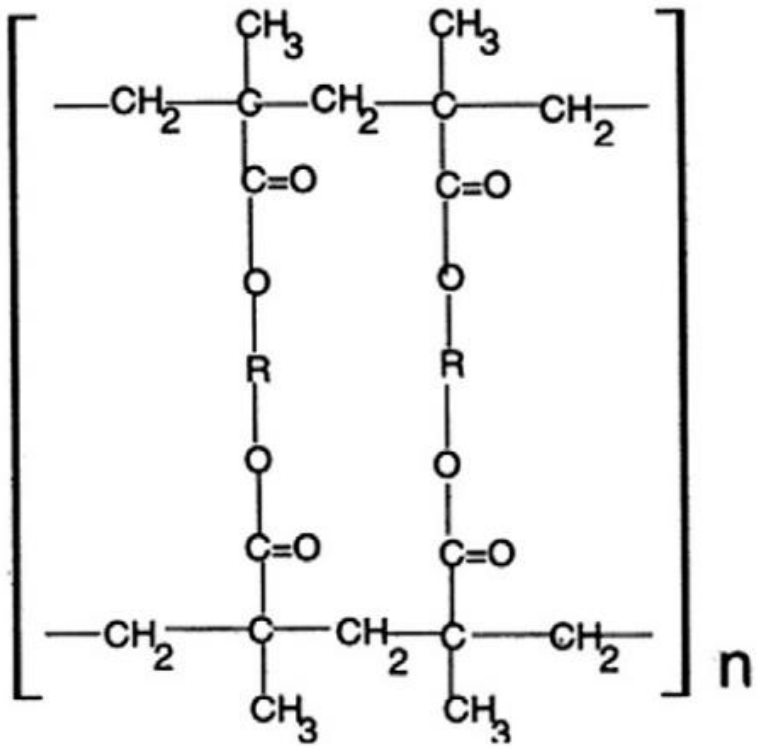
OA and DTX1 by **HP20** is 1639 and 2934 g/g, and

OA and DTX1 by **SP700** resin is 1088 and 1872g/g, respectively. **

**** Lane, et al. Limnol. Oceanogr.: Methods 8, 2010***
*****Aifeng Li, et al. J of Chrom A, 1218 (2011) ; Fux , et al. Harmful Algae 7 (2008)***

2. Acrylic ester resin Amberlite XAD-7HP

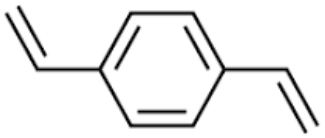
- Moderately polar and hydrophilic
- suitable for use in pharmaceutical applications, compounds up to 60,000 MW
- Surface area 450 m^2/g
- Pore size 90 Å



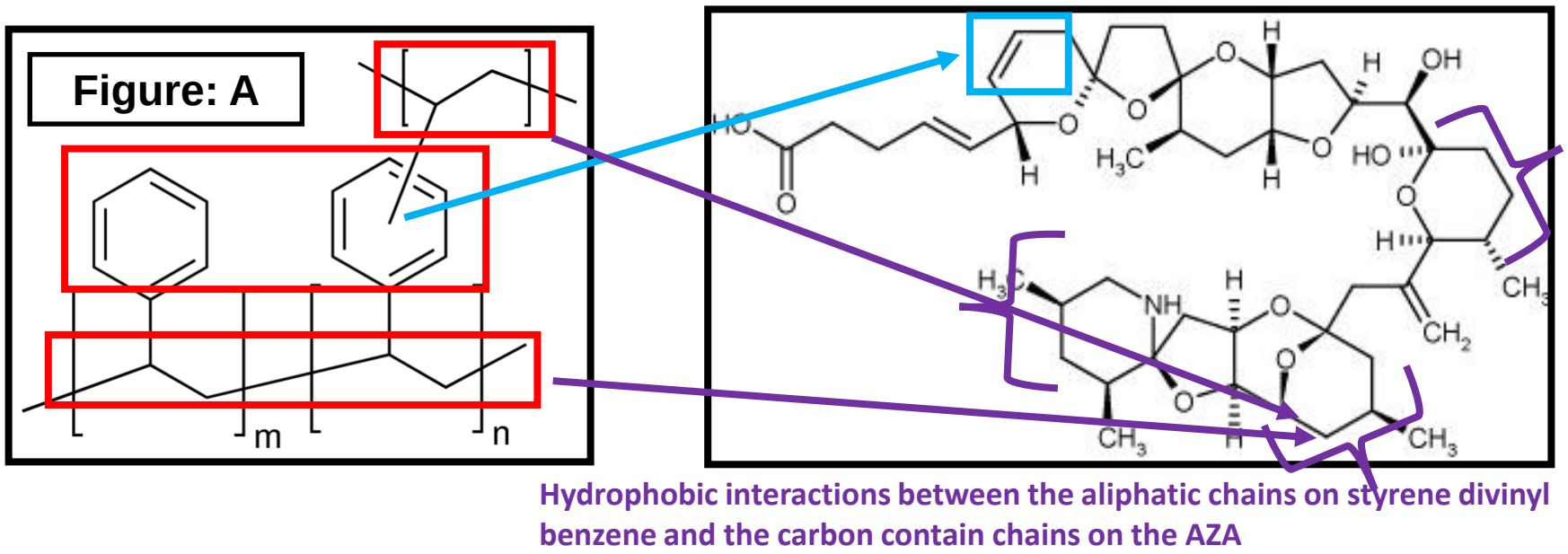
SPATT Technology Adsorbent Phase – HP20

Styrene-DVB Copolymer is the material used as the adsorbent phase in SPATT Technology (See Figure: A below). Particle size is approximately 250-850 µm.

DVB stands for Divinylbenzene



The structure of this compound (Styrene-DVB Copolymer) and the reasoning as to how attraction of the toxin AZA to the adsorbent phase (theorised) is shown below.
REMEMBER - Like attracts like!



Light blue Arrow (pi-pi interactions) and Purple Arrow (aliphatic carbon chain interactions) between HP20 stationary phase and Azaspiracid

The role of SPATT Technology

Solid **P**hase **A**dsorption **T**oxin **T**racking (SPATT) technology was first introduced in 2004 in New Zealand (MacKenzie, et al., 2004)

SPATT involves suspending small bags containing adsorption substrates (such as HP20) in a body of water which accumulate toxins.

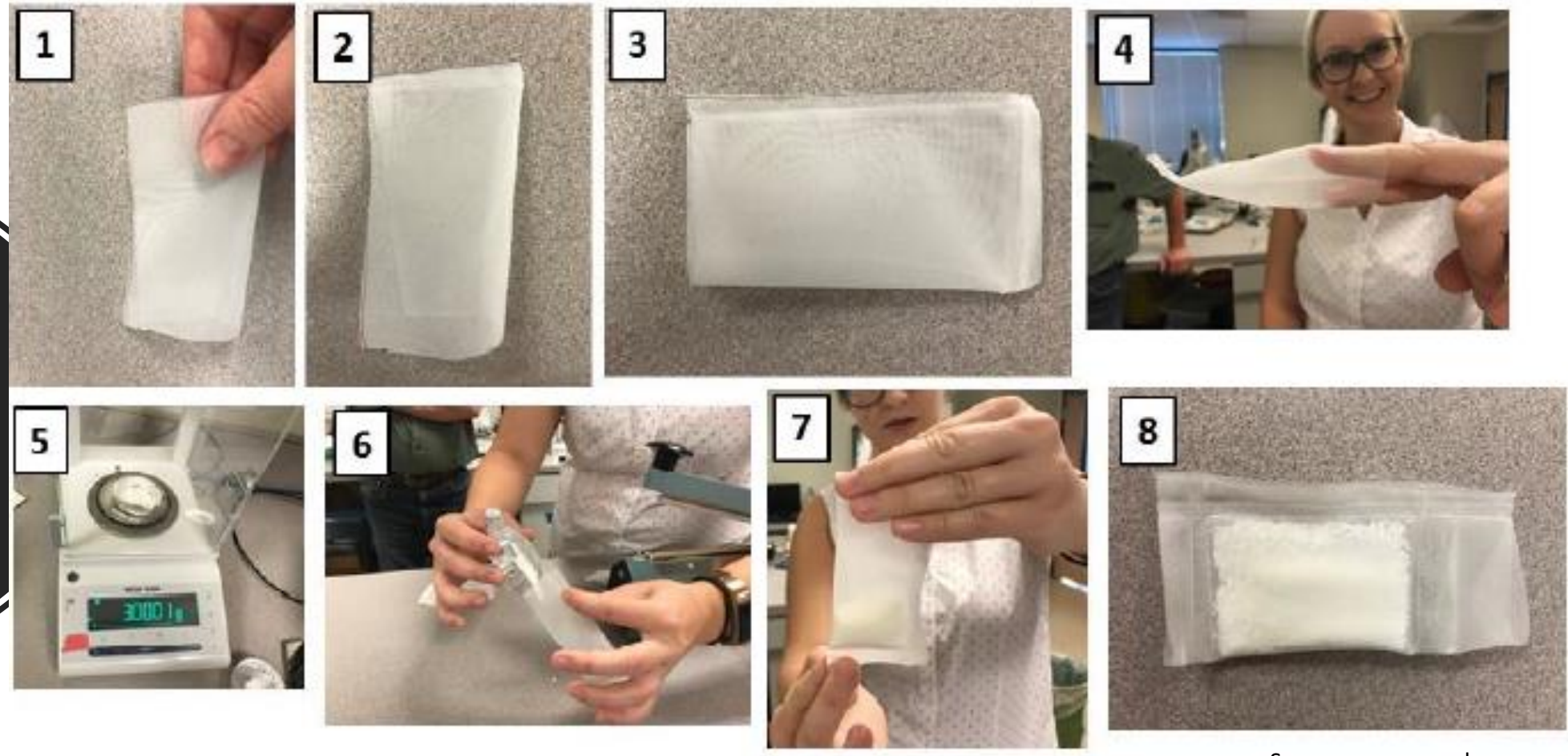
These toxins can then be extracted from the adsorbate phase and quantified thus providing information on toxins over an extended period!

Source: www.google.com

- low cost, easily deployed and recovered and it allow for continuous measurement.

- SPATT technique only measures dissolved toxins and not total toxins and is currently NOT commercially available as a pre-made unit, however it is easy to make in the laboratory.
- 7-day exposure

SPATT preparation- Tea bag design

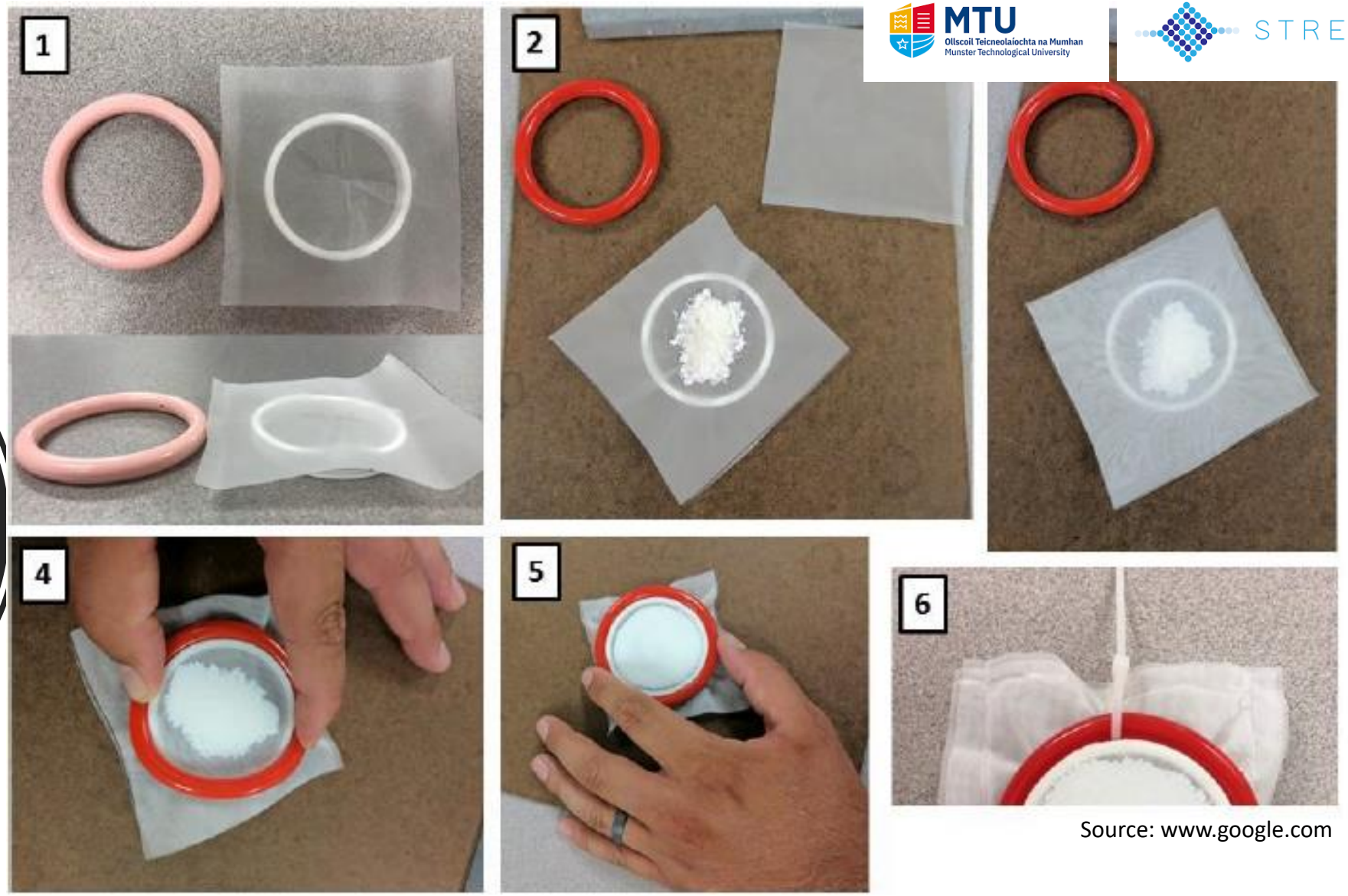


Source: www.google.com

Bag's dimension : 4.5" X 4.5"

Made from 95 µm polyester mesh

SPATT preparation using embroidery ring



Source: www.google.com

To form a thin layer of resin Rundberget *et al*.*, placed the resin between two layers of nylon mesh that was clamped tightly into a frame.

**T. Rundberget, et al, Toxicon 50 (2007) 960–970*

Resin activation and deployment preparation

1. Soak SPATT bag in MeOH for 24 hours.

2. After 24 hours, rinse off MeOH with ultrapure water:

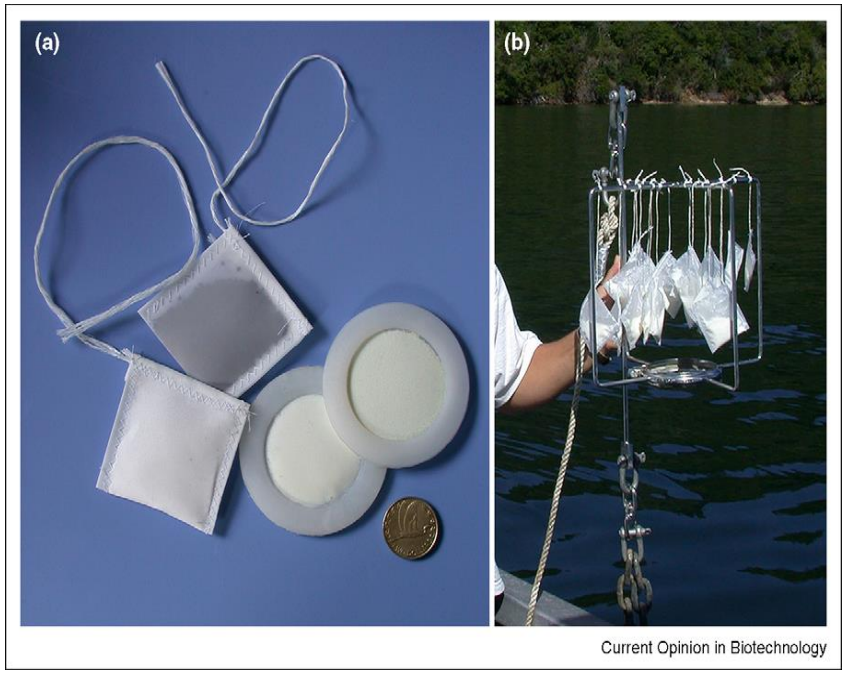
- a. Fill a 500ml beaker with ultrapure water and dip the bags in the water, gently agitate to ensure all the resin contacts the water
- b. Water temperature will increase upon the methanol will react with the water
- c. Pour the water out, refill the beaker, and repeat the previous step to water temperature remains steady while the bag is in the beaker.

3. Place the SPATT bags in a ziplock bag while covering with water to ensure the resin does not dry out.

4. Store in a refrigerator (4-6 °C) until deployment (resin is stable for months in this temperature).

5. Attach SPATT bags to a structure for deployment

- SPATT bags can be suspended in the water column*
- Example of the SPATT bags, in which bags were attached to a large aluminium alloy mounting tube by 4 mm diameter screws. **
- The aluminium tube was deployed in the river while SPATT bags were securing with clamping screws.



Source: www.google.com

**** Mackenzi, et al, Toxicon 44 (2004) 901–918***
*****A. Woods, at al, Chemosphere 82 (2011) 888–894***

Retrieving SPATT Samplers from the deployment site

Rinse	Upon collection rinse the bags using field water.
Store	Store SPATT bags into a labeled ziplock bag (Note: bags do not need to be in water).
Freeze	Immediately place the bags in ice container at < -4 °C until extraction of toxins in the lab.

Toxin extraction

- The extraction requires different eluents depending on which toxins are of the most interest

SPATT resin	Elute	References
DIAION HP20, SEPABEADS SP825L, SP850 and SEPABEADS SP700	50% MeOH	[McCarthy,et al 015]
DIAION HP20, SP 207, HP2MG	MeOH	[[Kudela2010]
DIAION HP20	MeOH	[Lane2010] [Peacock2018]
	Ammonium acetate in 50% MeOH,	
	Ammonium acetate in 50% MeOH	
DIAION HP20	MeOH	[Kudela2011]

Application of SPATT to Marine and Freshwater toxins

SPATT resins	Toxins detected	Year of study	Country	Elute	Adsorbent quantity	Analyte %Recovery	Ref
DIAION HP - 20 (Bags)	PTX, PTX2 SA, PTX11, PT11 SA, OA, OA-ester & YTX.	2004	New Zealand	MeOH Ace MeOH>Ace	3 g dry weight	Ave= 62%	L. Mackenzie 2004
DIAION HP-20 (Large scale pumping)	OA DTX-2 PTX-2 PTX-2SA	2007	Norway	MeOH	0.5 kg/Column	DTX-2: 4.5 mg 73% OA: 2.1 mg 78%	Rundberget2007
SEPABEADS SP700 (Bags)	OA, PTX2, AZA, YTX	2010	New Zealand	MeOH	-	OA 61% PTX2 22% AZA 41% YTX 47%	Mackenzie2010
HP20, SP700	domoic acid and saxitoxin	2010	US	MeOH	3 g	SP700: 69-72% HP20: 99%	Lane2010

Review publication (ready for submission)

A Review of *In-situ* methods - Solid Phase Adsorption Toxin Tracking (SPATT) and Polar Organic Chemical Integrative Sampler (POCIS) for the collection and concentration of marine biotoxins and pharmaceuticals in environmental waters

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