Medicinal Food and Marine Toxins Analysis

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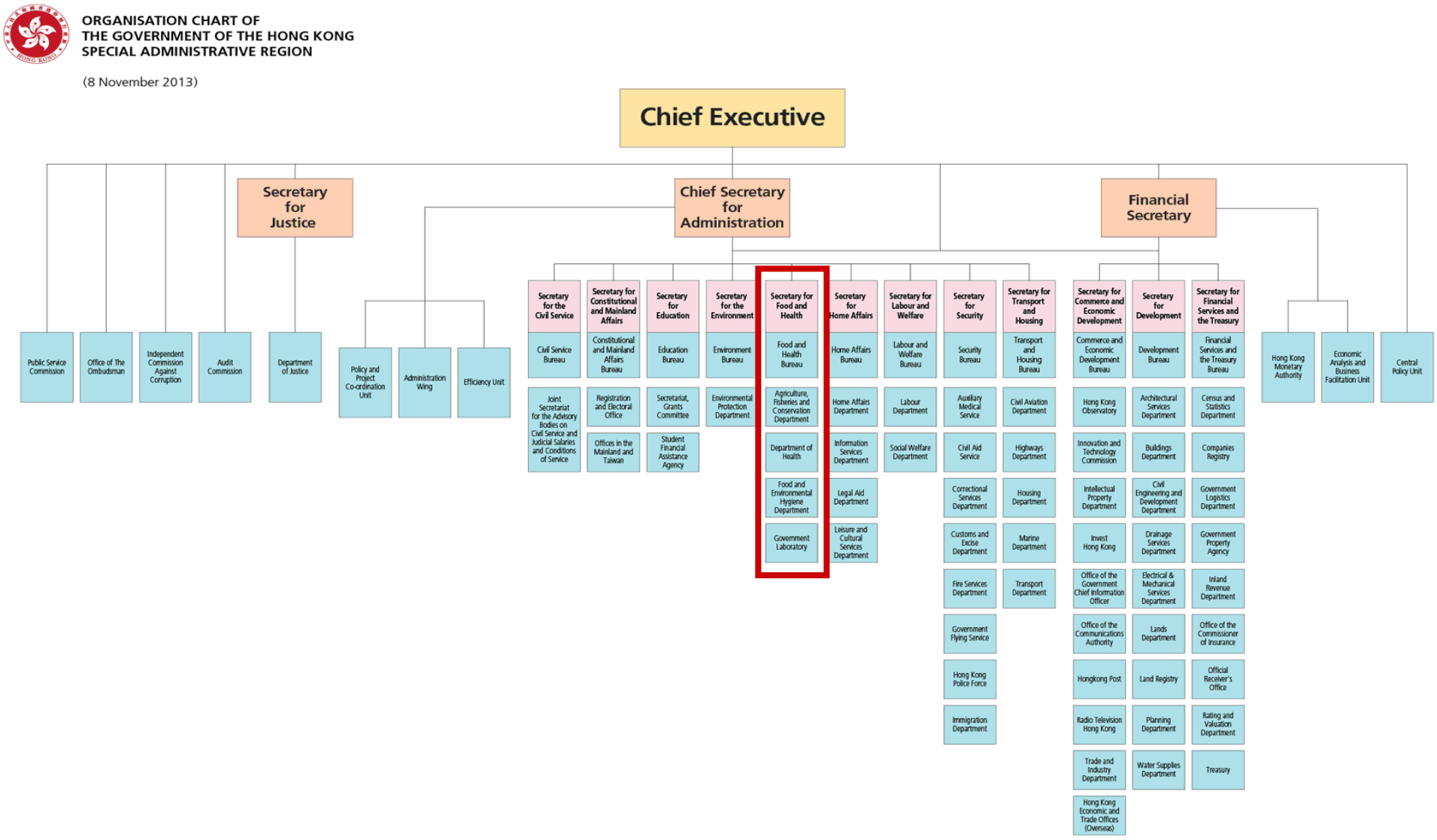
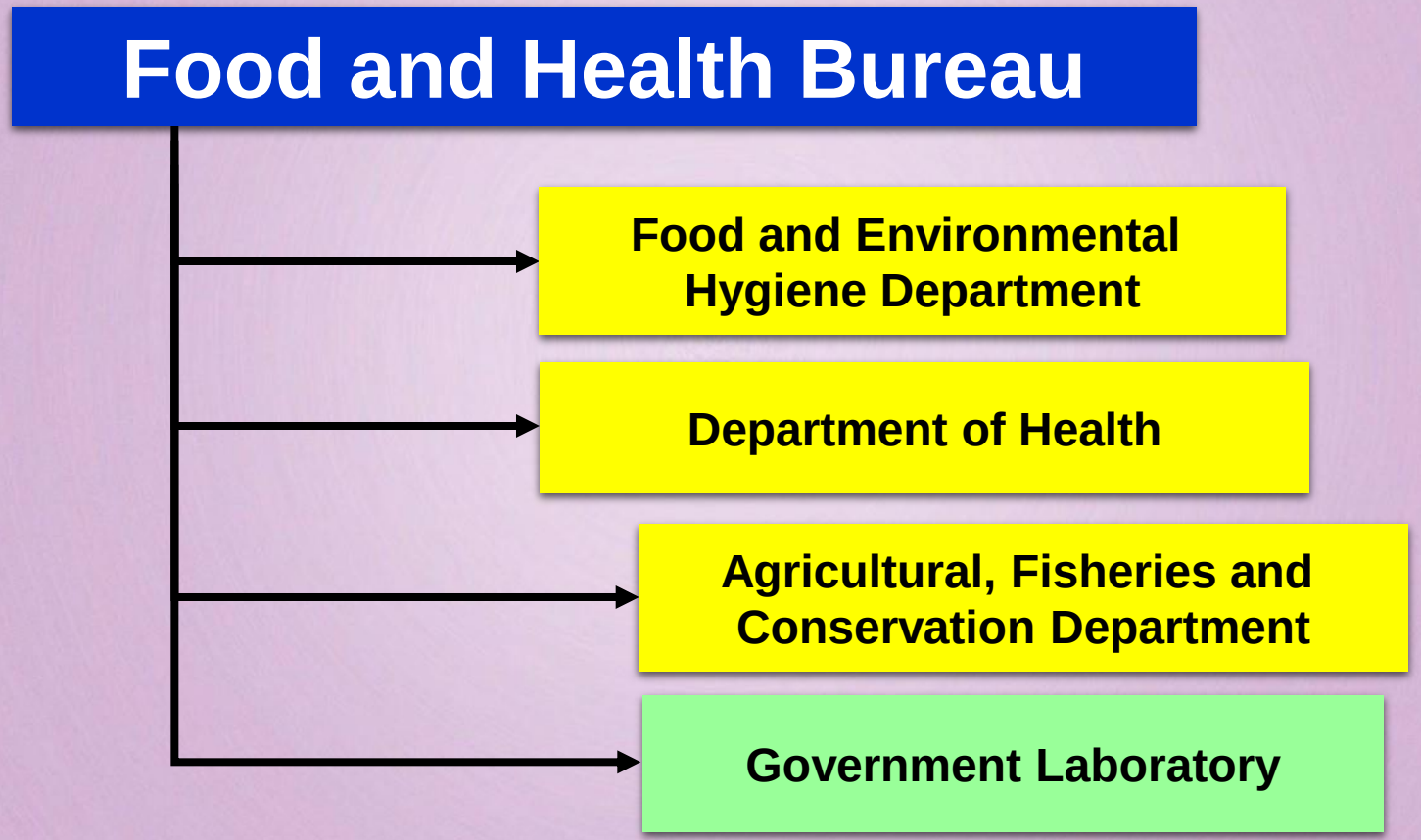
Highlight

1. Authentication of Chinese medicinal food, using Cordyceps sinensis as an example
2. Briefing on the control of common marine toxins in seafood

Introduction of GL

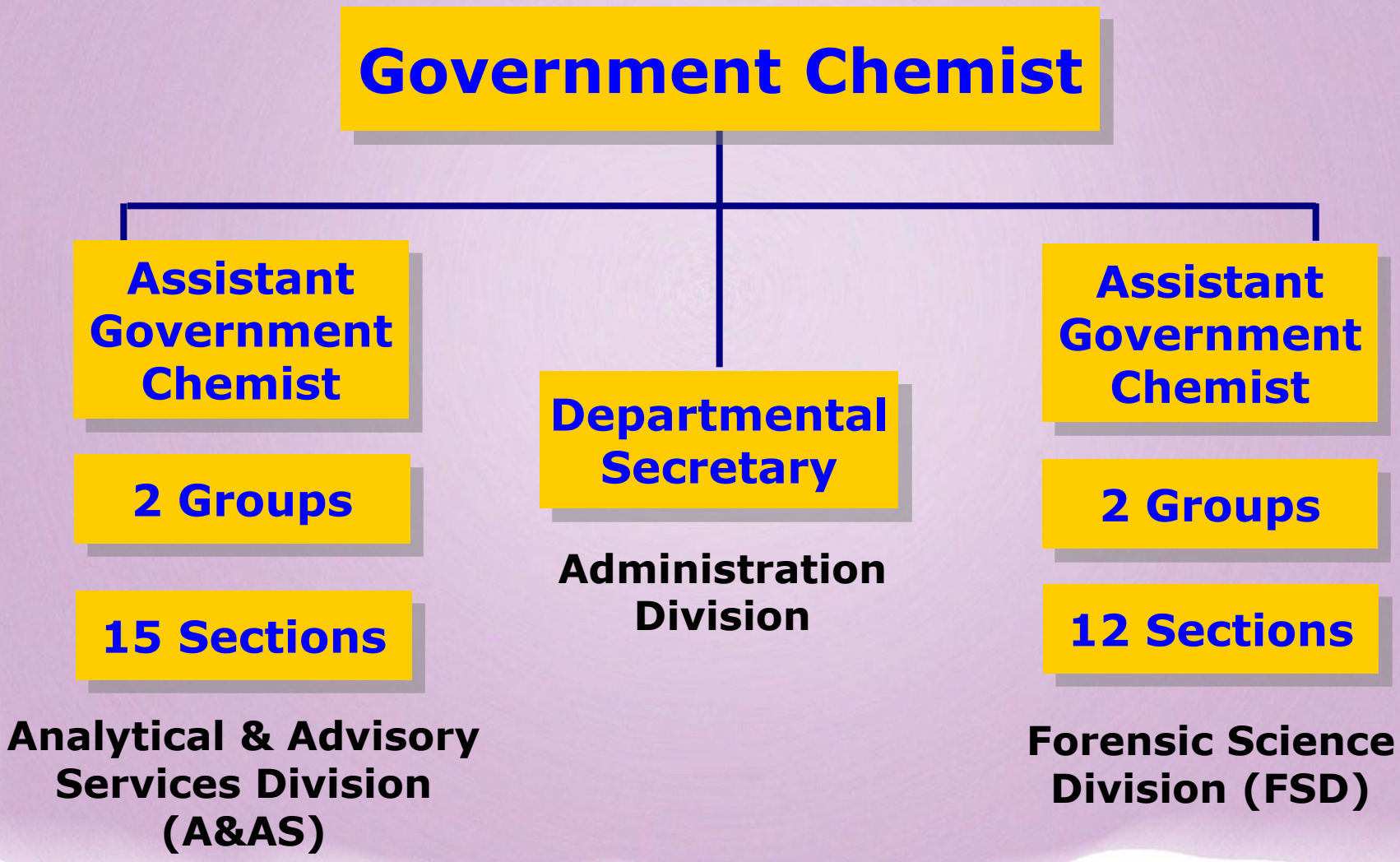


Administration



No. of staff: ~500, Founded in 1913

Organization



HQ & Satellite Labs

1. Headquarters, Homantin
2. Meteorological Station
3. Public Works Central Laboratory Building
4. LCK Govt. Offices
5. Public Health Laboratory
6. Food Safety Laboratory

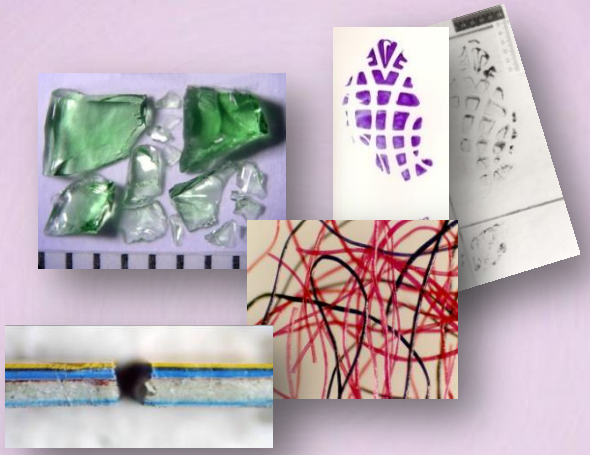
7. Science & Technol. Parks

(Hong Kong Chinese Materia Medica Standards)

Analytical Services

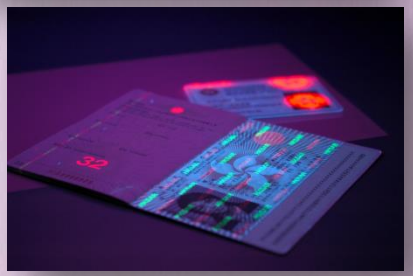
Forensic Services

Scene of Crime Investigation



Trace Contact Evidence

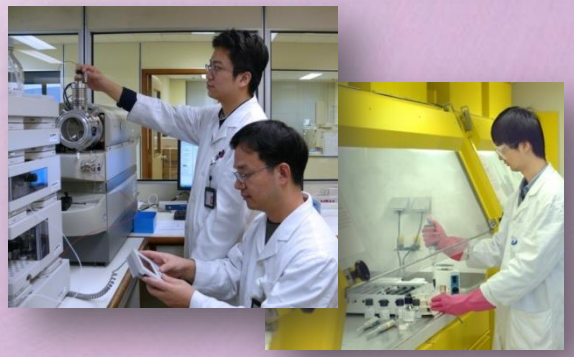
DNA



Questioned Documents

Physical Investigation

Controlled Drugs



Forensic Toxicology

Our Role

To provide advisory and scientific testing services to various government departments in Hong Kong to uphold:

- **Law and Order**
- **Public Health and Food Safety**
- **Environmental Protection**
- **Consumer Protection**
- **Government Revenue**

1. Chinese Medicinal Food

- **What is Cordyceps??**

Endoparasitic fungus

HOST

Infection

Development of mycelia

Growth

CARCASS

WHOLE FUNGUS

Cordyceps

- **Over 400 species, distributed worldwide. Mostly abundant in tropical forests**

Cordyceps cicadea (cicada)

Cordyceps unilateralis (ant)

Cordyceps formosana (beetle)

Cordyceps amazonica (grasshopper)

Cordyceps Sinensis

SPRING

Germination

EGG

Development of mycelia

GHOST MOTH

LARVA

5-year

Infected by spores

PUPA

SUMMER

- **Winter worm summer grass or caterpillar fungus**

Cordyceps Sinensis

- Well known Chinese medicinal herb, used as food or dietary supplement to improve immune system & body health today
- Having an appearance of a plant and an worm
- Only found in Tibet Highland

Cordyceps Sinensis

- Ghost moths live in Tibet area at 4,500 to 6,000 m above sea level

Cordyceps Sinensis

- Has been used for more than 1,500 years as an important Chinese and Tibetan medicine
- Became popular when two Chinese female broke the world records in 1500, 3000 & 10000 meters running in 1993
- Negative doping, but admitted taking cordyceps sinensis
- Since then, the price has sky-rocketed. It increased > 1000% within 10 years from 2003. Retail price is ~ USD 35,000 per kg

Cordyceps Sinensis

- Hunting for fungus in spring to summer time

Fake Items

Fungus and Insect Host
Cordyceps militaris + Lepidoptera pupa
Cordyceps liangshanensis + Lepidoptera larva
Cordyceps ophioglossoides
Cordyceps hawkesii + Lepidoptera larva
Cordyceps barnesii + Lepidoptera larva
Cordyceps ramosa + Lepidoptera larva

Others
Plant Radix Stachys geobombycis & Radix Stachys sieboldii
Flour, plaster and pigments

Genuine vs Fake

	Genuine	Fake
Appearance	2 pairs of feet close to the head and tail are flat, middle 4 pairs are more obvious	Feet are neatly arranged; or no foot
Colour	Natural brown	Turbid, pale yellow
Odour	Mushroom flavour	Wood-like smell
Immerse in water	Colour remains	Lose pigment, sticky

How about these?

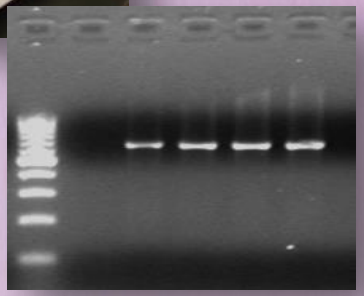
- **Commercial products: Powder in capsules and many claim to contain cordyceps sinensis. Really?**
- **Relied on a DNA-based method**

Workflow of DNA Sequence Analysis for Cordyceps Authentication

Raw materials or powder

DNA Extraction

PCR Amplification of DNA markers



Sequence Analysis

Cordyceps sinensis

- Partial ribosomal RNA gene (ITS1) ~350bp
- Partial RNA polymerase II largest subunit gene (RPB1) ~700bp

Investigation

No.	Products	Sample nature	Claims	DNA Results
1		Raw material	Cordyceps sinensis	Fungus from the genus *Metarhizium* ✗
2		Powder	Cordyceps sinensis	*Cordyceps sinensis* ✓
3		Powder	100% Cordyceps	*Tolypocladium inflatum* ✗
4		Powder	Cordyceps Mycelia	*Paecilomyes* genus ✗
5		Powder	100% Cordyceps Mycelia	Fungus from the family *Trichocomaceae* ✗

(i) Dried Abalone

About 100 catties (~ 60.5 kg) of suspected fake dried abalone slices from 31 dried seafood retail shops

The items carrying trade descriptions of:

High Quality Abalone slices

Australian abalone slices

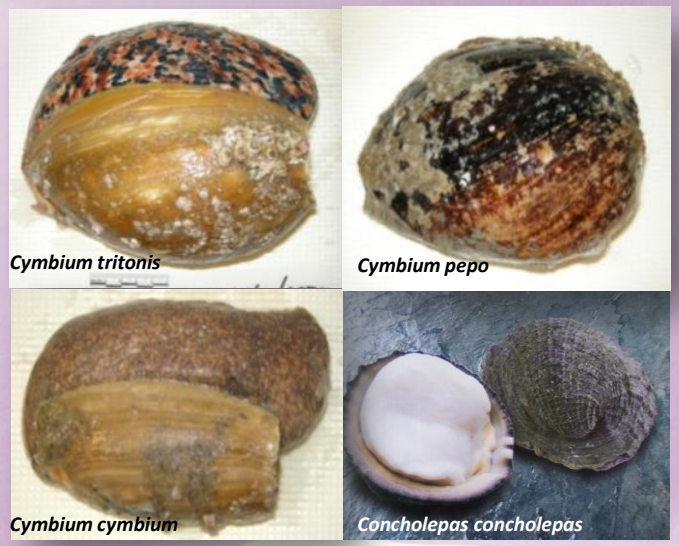
Japanese abalone slices

American abalone slices

South African abalone slices

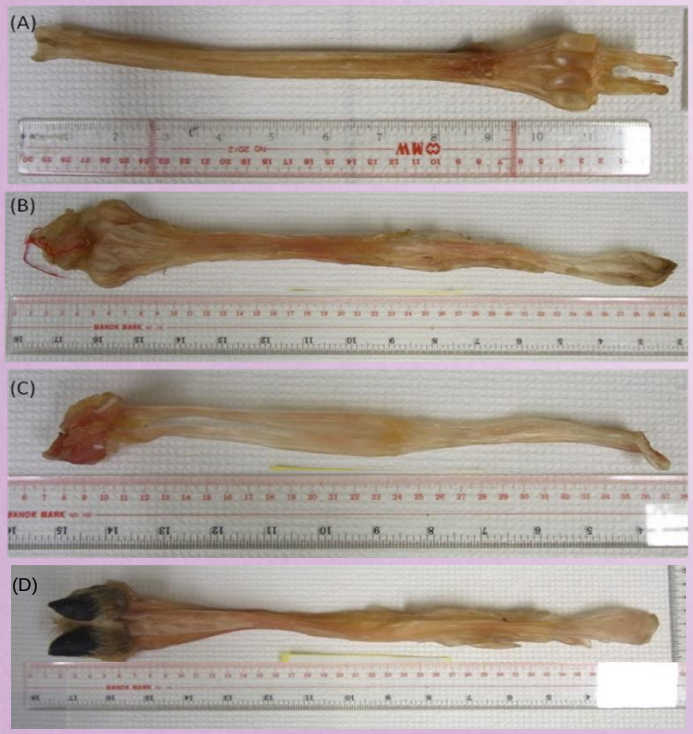
All slices were found to be of CONCH origin.

A total of 33 retailers and 5 wholesalers were convicted and fined a total of HKD446,000 (£36,000). Some convicted parties were sentenced to 4 months and 1 month suspended imprisonment.



(ii) Dried Deer Tendon

Fifty one pieces of suspected fake dried deer tendons (~£65 each) from several dried food retailers



Deer (2/51)

Cattle (41/51)

Water buffalo (2/51)

Deer + Cattle (6/51)

Choice magazine 15.3.2012

Sale of cattle tendons for deer tendon

The retailers shops were fined a total sum of HKD 338,500 (£27,000).

Chinese Medicinal Foods

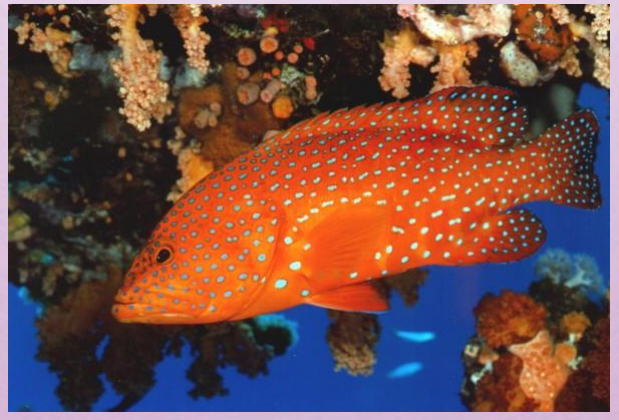
DNA tests assist authentication

- Fish maws
- Shark fins
- Sea cucumber
- Edible Bird's nest
- Ginseng
- Crocodile meat

2. Marine Toxins

- Most marine toxins are originated from toxic planktonic algae, some are from bacteria. Among the 5,000 species of marine planktonic algae, some 300 species can cause red tides, while only ~80 species have the capacity to produce potent toxins.
- Marine toxins can accumulate in seafood through the food chain.

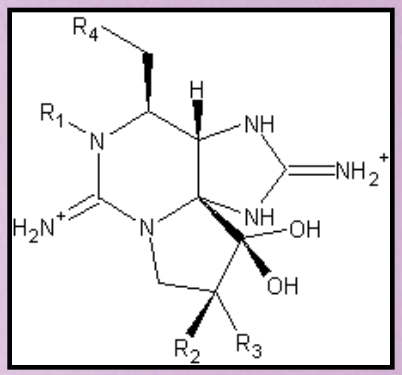
- **Shellfish Toxins**
- **Ciguatoxins**
- **Tetrodotoxin**



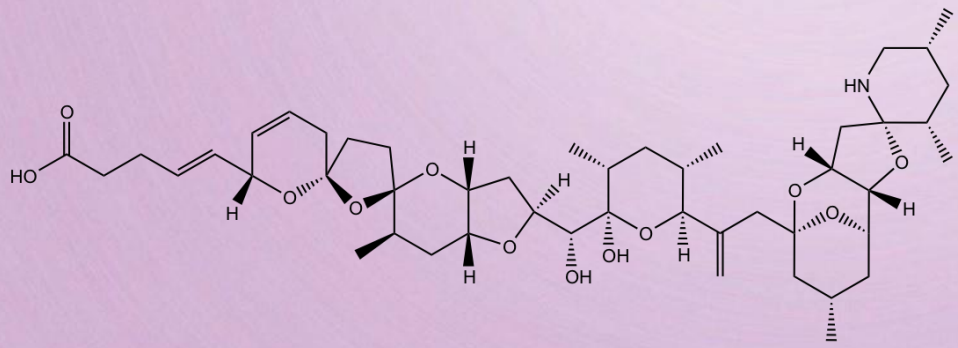
Shellfish Toxins

Five main types of shellfish toxins

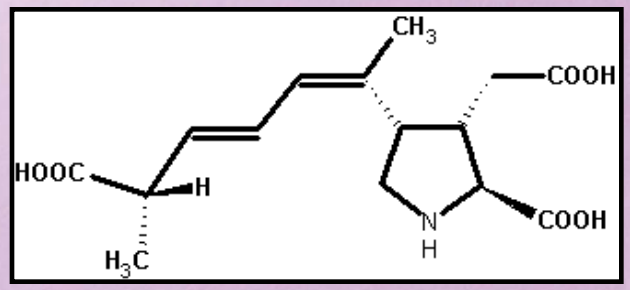
- Paralytic Shellfish Poisoning Toxins, PSP
- Amnestic Shellfish Poisoning Toxins, ASP
- Neurotoxic Shellfish Poisoning Toxins, NSP
- Diarrhetic Shellfish Poisoning Toxins, DSP
- Azaspiracid Shellfish Poisoning Toxins, AZP



(PSP)



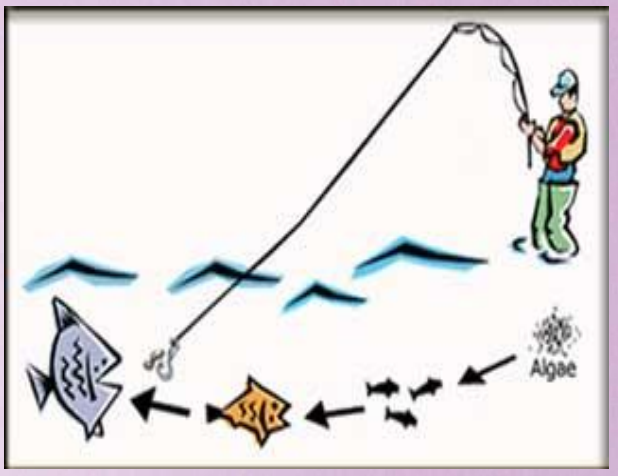
(AZP)



(ASP)

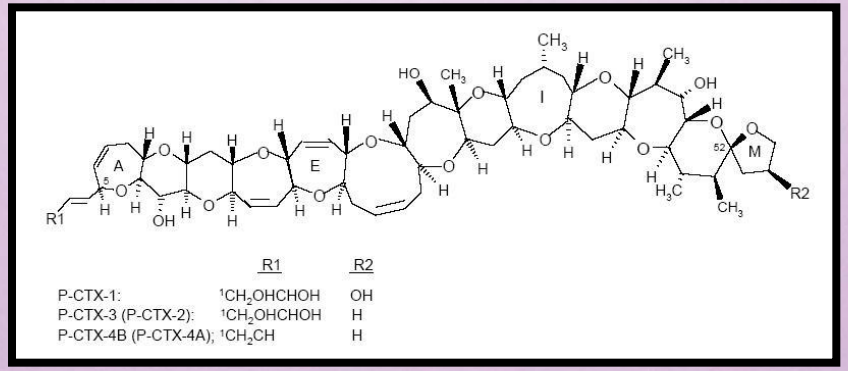
Ciguatoxins

- Ciguatoxins that cause ciguatera poisoning are produced by dinoflagellates.
- Particularly high concentrations in some large predatory tropical coral fish.
- Grouper, Sea Bass, Snapper and Barracuda are commonly associated with ciguatoxin poisoning.



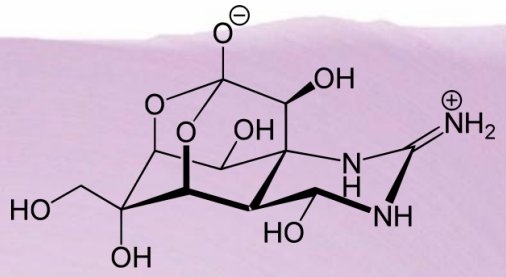
Ciguatoxins

- **Three different classes of Ciguatoxins have been identified.**
  - Pacific Ciguatoxins (neurological symptoms predominate)
  - Caribbean Ciguatoxins (gastrointestinal symptoms are a dominant feature)
  - Indian Ciguatoxins (cause a cluster of symptoms reminiscent of hallucinatory poisoning)



Pacific Ciguatoxins

Tetrodotoxin



- Tetrodotoxin (TTX) has been isolated from different animal species, including newts, toads, blue-ringed octopuses, several sea stars and certain angelfish. Also common in **puffer fish**, a delicacy in Asia
  - TTX is roughly 100 times more poisonous than potassium cyanide (LD_{50}: 334 μg/kg)
  - TTX block the sodium ion channel, causes paralysis of voluntary muscles and loss of sensation

Control of Marine Toxins in HK

FAO Report (2011):

Consumption: 505,553 tonnes seafood

> 70 kg per person per year

Ranking per capita:

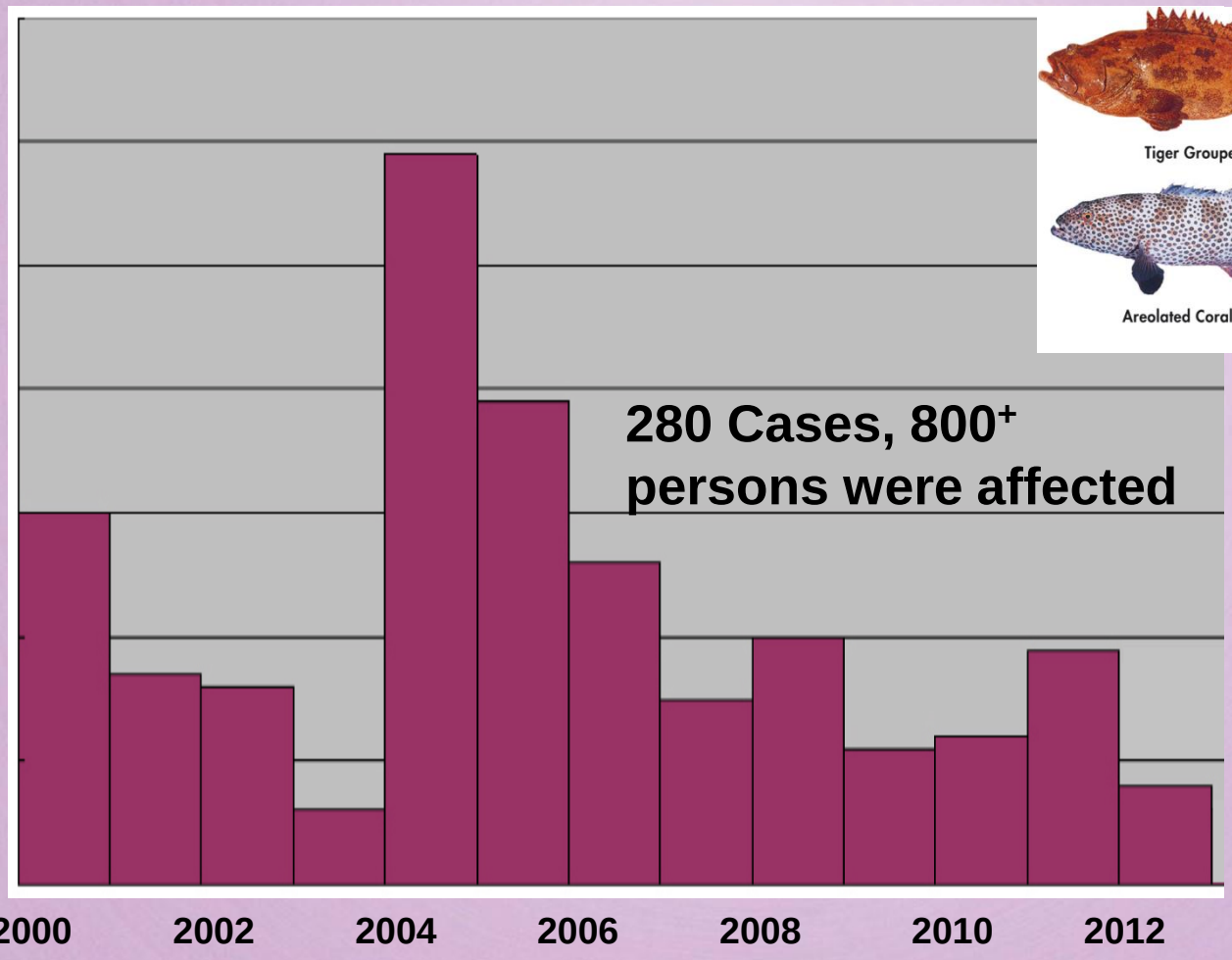
2nd in Asia

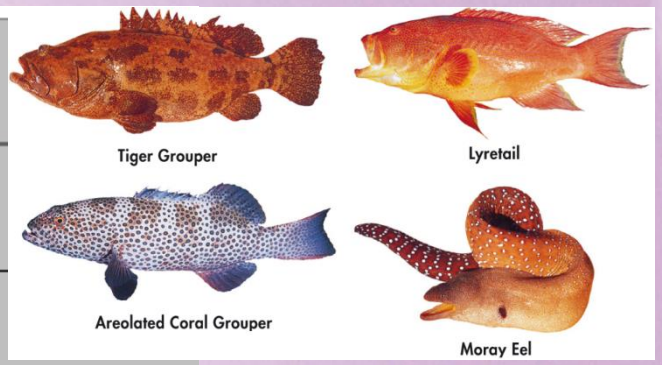
7th in the world

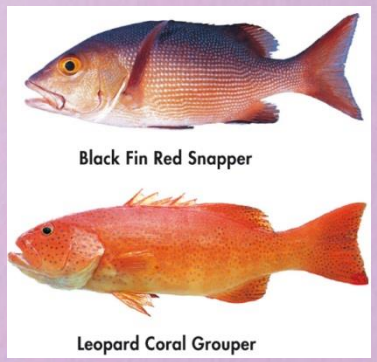
Control of Marine Toxins in HK

- **Routine Surveillance Programme**
  - Shellfish Toxins
- **Ad-hoc Projects**
  - In response to overseas food alert to conduct risk assessment ad-hoc project on other marine toxins
- **Urgent Testing Services**
  - In response to local food incident to provide urgent testing services to prevent further spreading of the questionable seafood to the market and consumers
  - Testing scope: Shellfish toxins, Tetrodotoxin and Ciguatoxins (in 2014)

Ciguatera in HK







Ciguatoxins Analysis

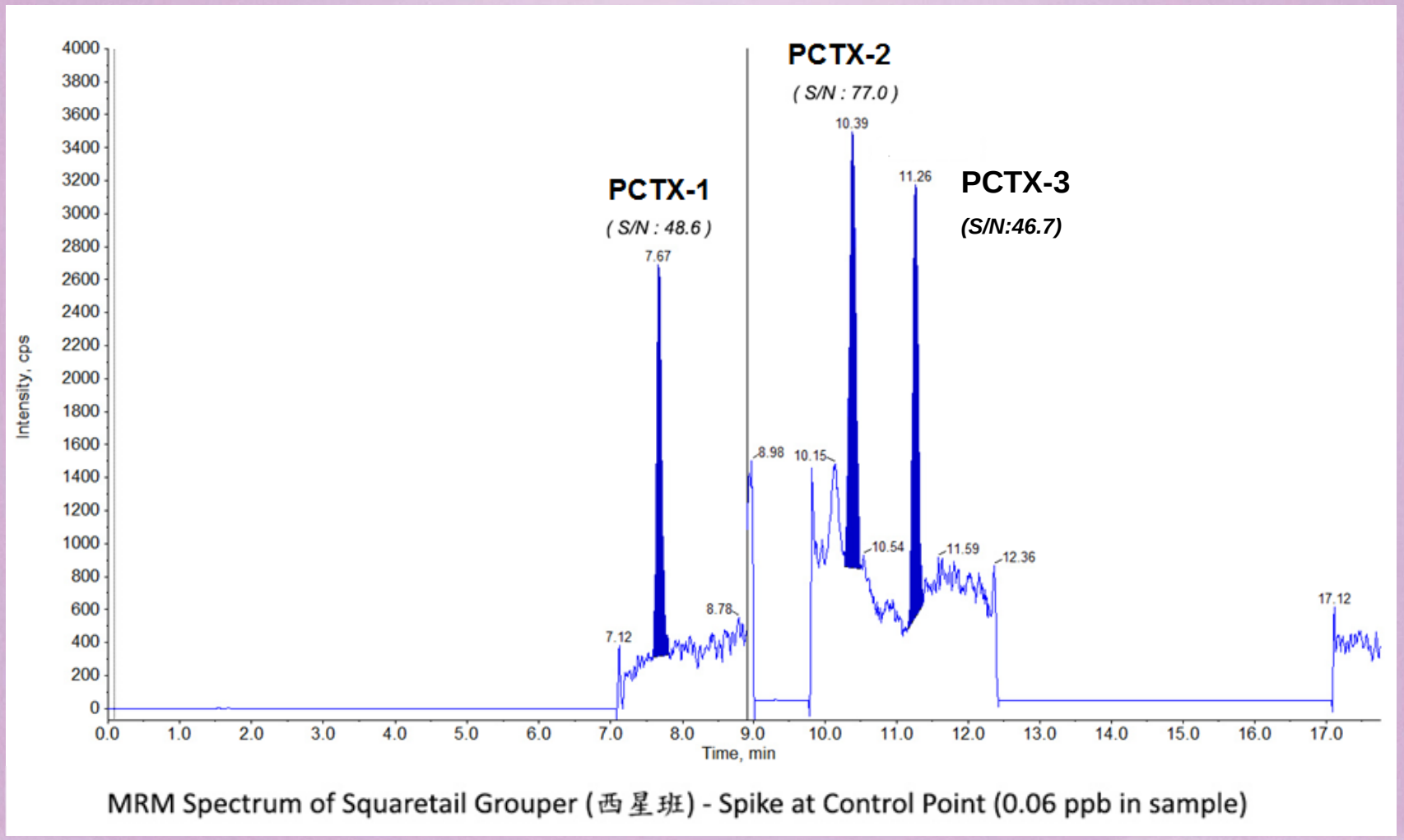
- **Ciguatoxin Standards**
  - Ciguatoxin standards cannot be directly obtained from algae.
  - It should only be extracted from fish containing ciguatoxin.
  - The production cost of ciguatoxin is extremely high.
  - US$1 per 1 ng (i.e. 10ug ➔ US$10,000)

- Pacific Ciguatoxin standards obtained from a toxin research team at the University of Queensland, Australia
- Conventional quantification method (using calibration curve) is not suitable for Ciguatoxin analysis

LC-MS/MS method for Pacific Ciguatoxins

- Screening was done by comparing the responses of the MRM peaks of P-CTX1, P-CTX2 and P-CTX3 in sample to the corresponding peak responses (control points) set out by the positive control samples
- The control point was a cut-off between a “negative” and a “positive” result, is set at 0.06 µg/kg
- Any positive sample will be quantified by using **standard addition method**

MRM Chromatogram



MRM Spectrum of Squaretail Grouper (西星班) - Spike at Control Point (0.06 ppb in sample)

Previous Case : Tetrodotoxin in Grilled Grouper Snack

- In a routine DNA sequencing testing of snack, a sample of dried fish meat was found not to match its claim. It contained pufferfish (*Lagocephalus lunaris*) instead of grouper
- Suspect food item might also contain tetrodotoxin
- LC-MS/MS showed that the sample contained tetrodotoxin ranging from 0.31 μg to 1.7 μg/kg
- Centre for Food Safety immediately recalled and warned the public to stop consuming the product

Lagocephalus lunaris

THANK YOU