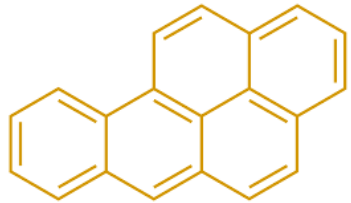
Technical briefing

Testing of Benzo[a]pyrene in Food

17 January 2023

CHOI Lai-sheung, Timmy 蔡麗嫦
Chemist / ACC Section
Government Laboratory

Introduction – benzo[a]pyrene



- **Benzo[a]pyrene (BaP)** is one of the polycyclic aromatic hydrocarbons (PAHs)
  - PAHs are compounds containing two or more fused aromatic hydrocarbon rings
- PAHs are formed during pyrolytic processes such as incomplete combustion of organic matters

  e.g. Forest fire, fuel burning, barbecuing and roasting
- Foods may be contaminated by environmental pollution and/or processing steps
- BaP is classified as carcinogenic to humans (Group 1) by IARC
- JECFA: one of the major contributors to PAHs intake is **vegetable fats and oils**

Food incidents of BaP

Gutter oil 地溝油

- Recycled cooking oil from restaurant waste and slaughterhouse waste
- In Dec 2012, cooking oil samples were found containing BaP up to 17 µg/kg in Hong Kong
- CFS has established a provisional action level of 10 µg/kg for BaP in cooking oil
- In Sep 2014, Taiwanese companies were found selling gutter oil

Instant noodle

- In Oct 2012, excess amount of BaP was found in the powdered soup seasoning in instant noodles Korea

Coconut oil

- In Feb 2019, a coconut oil was found containing 47 µg/kg of BaP in Hong Kong

Regulations on BaP (I)

Hong Kong

*The Harmful Substances in Food (Amendment) Regulation 2021 (Cap. 132AF) **

Description of food 食物類別	**Maximum concentration** 最高濃度 **(μg/kg)**
Oil or fat or any mixture of oil and fat 油或脂肪或兩者的混合物	5
Infant formula and follow-up formula intended to be consumed principally by persons under the age of 12 months 擬主要供不足12 個月大的人食用的嬰兒配方產品及較大嬰兒及幼兒配方產品	1

* 1 June 2023 come into operation

Regulations on BaP (II)

China

GB 2762-2017 食品中污染物限量

食品類別(名稱)	限量 (μg/kg)
油脂及其制品	10

EU

EC 1881/2006 – setting maximum levels for certain contaminants in foodstuffs

Foodstuffs	**Maximum levels (μg/kg)**
Oils and fats (excluding cocoa butter and coconut oil) intended for direct human consumption or use as an ingredient in food	2
Coconut oil intended for direct human consumption or use as an ingredient in food	2
Infant formulae and follow-on formulae, including infant milk and follow-on milk	1

Standard methods

	Oils and fats	Infant formula
GB 5009.265-2021 食品中多环芳烃的测定	✓	✓
GB 5009.27-2016 食品中苯并(a)芘的测定	✓	
BS EN ISO 16619:2015 Food analysis — Determination of benzo[a]pyrene, benz[a]anthracene, chrysene and benzo[b]fluoranthene in foodstuffs by GC-MS	✓	✓
CEN/TS 16621:2014 Food analysis — Determination of benzo[a]pyrene, benz[a]anthracene, chrysene and benzo[b]fluoranthene in foodstuffs by HPLC-FD	✓	✓
BS EN ISO 15302:2017 Animal and vegetable fats and oils — Determination of benzo[a]pyrene — Reverse-phase HPLC	✓	
BS EN ISO 15753:2016 Animal and vegetable fats and oils — Determination of PAHs	✓	
BS EN ISO 22959:2009 Animal and vegetable fats and oils — Determination of PAHs by on-line donor-acceptor complex chromatography and HPLC-FD	✓	

Analytical approaches

Purification:

- Solid phase extraction
  - e.g. Alumina / C18 & Florisil bonded-phase / molecular-imprinted polymer (MIP) SPE
- Liquid-liquid extraction
- Pressurised liquid extraction
- Soxhlet extraction
- Size exclusion chromatography
- Saponification

Instrument:

- HPLC - fluorescence detection
- GC-MS

Methods for oils and fats

ISO 15302:2017

Crude or refined oils and fats
- 0.4 g sample
- Add petroleum ether
- Add **benzo[b]chrysene** as IS

↓

Alumina column cleanup
- Pre-wash neutral alumina column with petroleum ether
- Load sample solution onto column
- Elute with petroleum ether

↓

- Evaporate solvent and reconstitute

↓

HPLC-FD analysis

GB 5009.27-2016

Oil and its products
- 0.4 g sample
- Add hexane, vortex

↓

Alumina column cleanup
- Pre-wash neutral alumina column with hexane
- Load sample solution onto column
- Elute with hexane

OR

Molecular-imprinted polymer (MIP) SPE cleanup
- Condition MIP-SPE with DCM & hexane
- Load sample solution onto MIP-SPE cartridge
- Wash with hexane
- Elute with DCM

↓

- Evaporate solvent and reconstitute
- Filter

↓

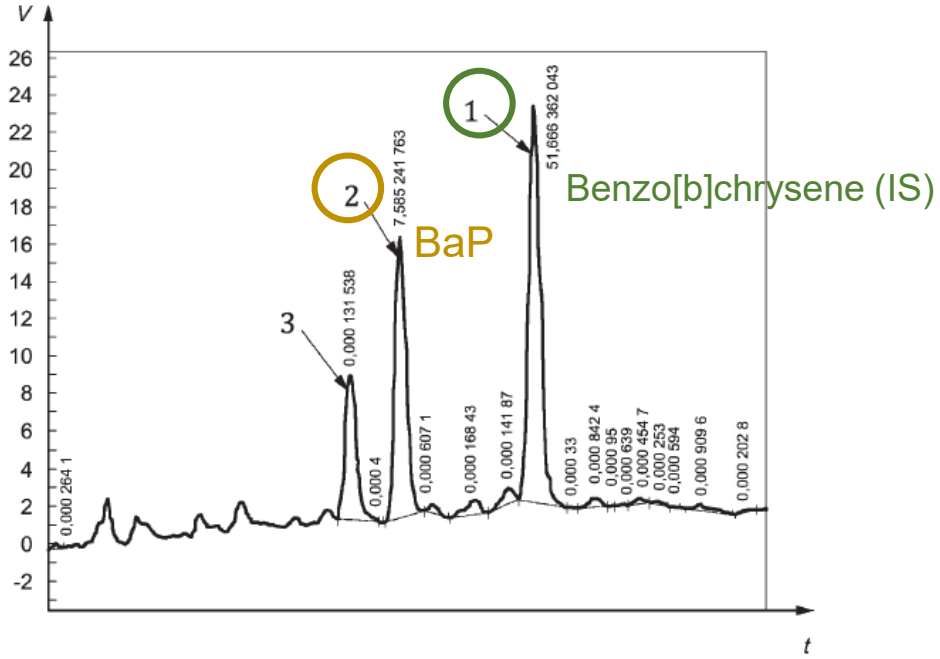
HPLC-FD analysis

LoQ
0.5 µg/kg

ISO 15302:2017

HPLC settings

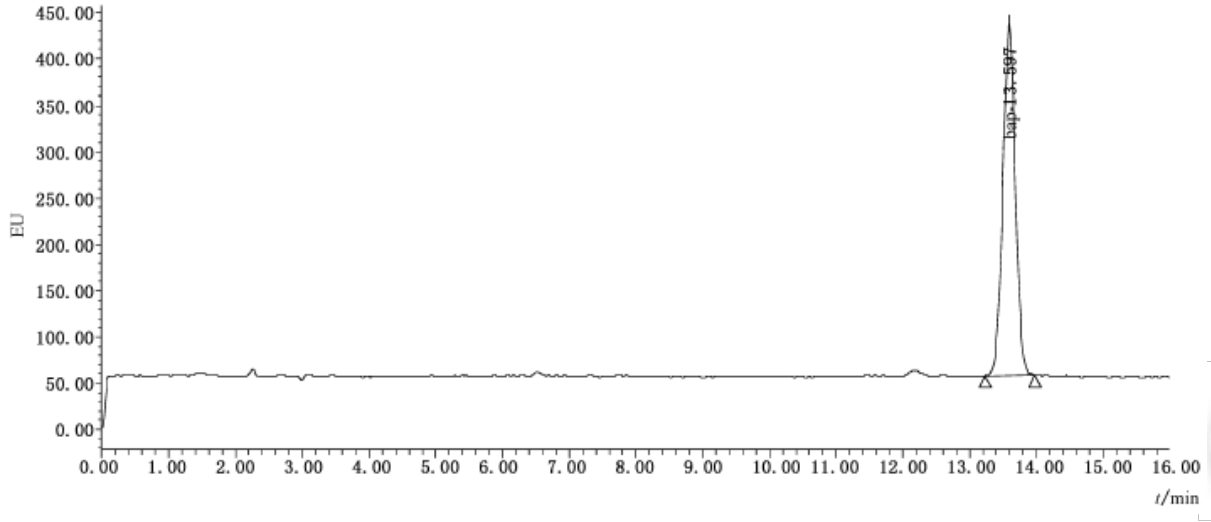
Column	:	RP-C18 column (250 mm × 4.6 mm, 5 µm) or equivalent
Column temperature	:	35 °C
Mobile phase	:	MeCN : Water (88:12)
Flow rate	:	1.0 mL/min
Injection volume	:	20 - 50 µL
Solvent programme	:	Isocratic



Fluorescence detector settings

Excitation wavelength	:	384 nm
Emission wavelength	:	406 nm

GB 5009.27-2016



ISO 15753:2016 (I)

Animal and vegetable fats and oils
- 2.5 g sample

↓

Pre-concentration
- Extract with 10 mL **MeCN / acetone (6:4)** x 3
- Evaporate solvent

↓

Liquid-liquid extraction
- Extract with 2 mL **MeCN / acetone (6:4)** x 3

↓

C18 SPE cleanup

↓

Florisil column cleanup

↓

HPLC-FD analysis

LoQ
0.2 µg/kg

ISO 15753:2016 (II)

HPLC settings

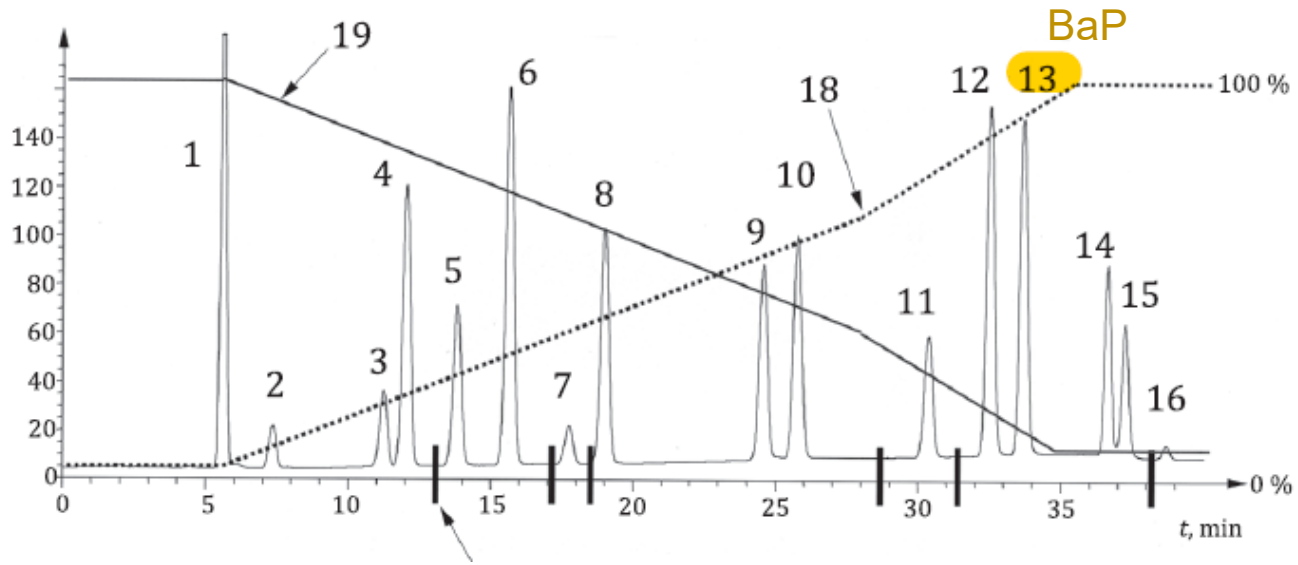
Column	:	RP-C18 column (250 mm × 4.6 mm, 5 µm)
Column temperature	:	25 °C
Mobile phase	:	A: MeCN B: MeCN : Water (1:1)
Flow rate	:	1.2 mL/min
Injection volume	:	20 µL

Solvent programme :

Time (min)	%A	%B
0	0	100
5	0	100
27	60	40
36	100	0
41	100	0
43	0	100
45	0	100

Fluorescence detector settings

Excitation wavelength	:	292 nm
Emission wavelength	:	410 nm



Methods for infant formulae

as well as oils and fats

ISO 16619:2015 (I)

Edible oil, Infant formula

- 5 g sample
- Add polyacrylic acid and sand, mix till homogeneous
- Add $^{13}C_4$-BaP

↓

Hexane or cyclohexane extraction

- Either Pressurised liquid extraction (PLE) or Soxhlet extraction

↓

SEC cleanup

- Column: 50 g of strene-divinylbenzene polymer with 3% cross-linkage, bead size 40 – 80 µm
- Eluent: cyclohexane : EA = 1:1
- Flow rate: 4 mL/min

↓

SPE cleanup

- Silica SPE (500 mg, 4 mL)

↓

GC-MS analysis

LoQ

0.9 µg/kg

ISO 16619:2015 (II)

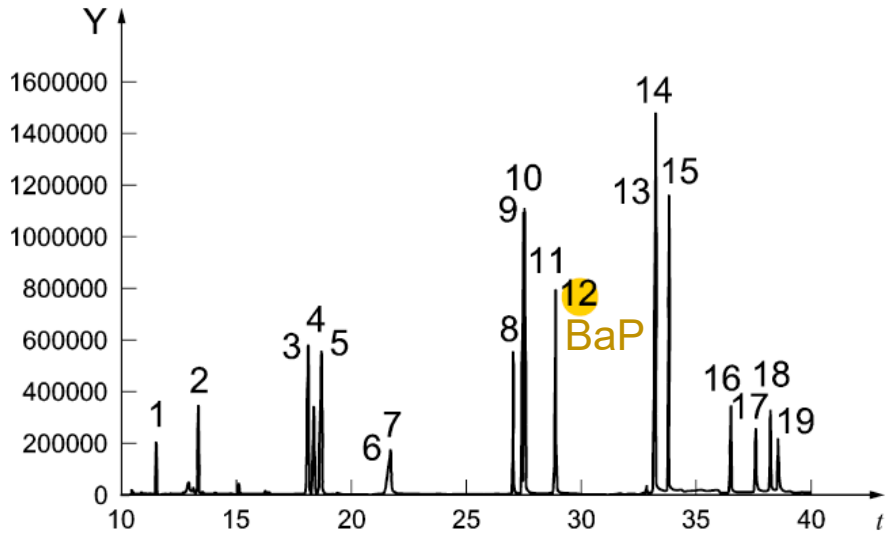
GC settings

Column	:	Select PAH (15 m × 0.15 mm, 0.10 μm)
Injector temperature	:	300 °C
Injector mode	:	Splitless
Injection volume	:	1 μL
Column flow	:	Helium at 1 mL/min
Oven programme	:	70 °C (hold for 1 min), 60 °C/min to 180 °C (hold for 0 min), 4 °C/min to 230 °C (hold for 10 min), 28 °C/min to 280 °C (hold for 5 min), 60 °C/min to 340 °C (hold for 5 min).
Solvent delay	:	7 min

PTV injector can also be used.

MS settings (SIM)

Ionization mode	:	EI (70 eV)
Transfer line temperature	:	325 °C
Ion source temperature	:	300 °C



GB 5009.265-2021 (I)

Infant formulae
- 2 g sample, add d_{12}-BaP

Animal and vegetable fats and oils
- 0.5 – 1 g sample, add d_{12}-BaP

Fat extraction
- Add water and 25% NH_3, incubate at 65 °C
- Add EtOH, Et_2O and petroleum ether, vortex
- Centrifuge and separate the upper layer
- Evaporate solvent to dryness

Saponification
- Add KOH in EtOH, incubate at 70 °C
- Add water and hexane, vortex
- Centrifuge and separate the upper layer

SPE purification
- Styrene-divinylbenzene copolymer SPE

GC-MS analysis

LoQ

Fats and oils: **2 µg/kg** (1 g sample)

Infant formulae: **0.5 µg/kg** (2 g sample)

GB 5009.265-2021 (II)

GC settings

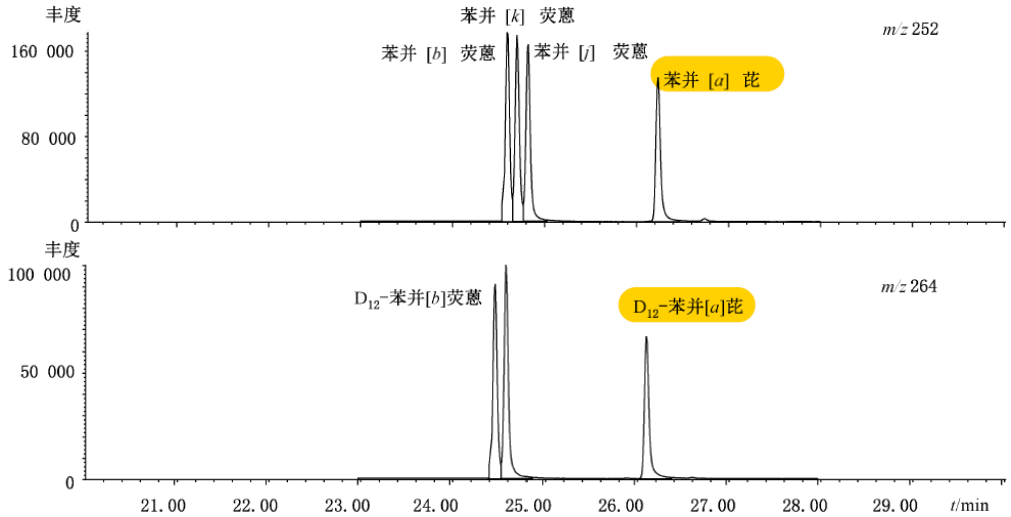
Column	:	DB-EUPAH (20 m × 0.18 mm, 0.14 μm) or equivalent
Injector temperature	:	280 °C
Injector mode	:	Splitless
Injection volume	:	1 - 2 μL
Column flow	:	Helium at 0.7 mL/min (hold for 32 min), increase at 5 mL/min to 1.5 mL/min.
Oven programme	:	80 °C (hold for 2 min), 10 °C/min to 250 °C (hold for 2 min), 8 °C/min to 315 °C (hold for 4 min), 20 °C/min to 320 °C (hold for 5 min).
Solvent delay	:	16.5 min

MS settings (SIM)

Ionization mode	:	EI (70 eV)
Transfer line temperature	:	280 °C
Ion source temperature	:	230 °C

	Ions monitored	RT (min)
BaP	252*, 250, 253	26.24
d_{12}-BaP	264*, 260	26.14

** Quantifying ion*



Method in GL (I)

Infant formula
- Weigh sample
- Add ***d*$_{12}$-BaP**

Oil and fat
- Weigh sample
- Add ***d*$_{12}$-BaP** and cyclohexane

Fat extraction
- Add EtOH, water and 25% NH_3, incubate at 65 °C.
- Cool to r.t. Add EtOH and transfer to Mojonnier flask
- Extract with **Et_2O** and **petroleum ether**
- Evaporate solvent and reconstitute with cyclohexane

Molecularly-imprinted polymer (MIP)-SPE cleanup
- Condition **MIP-SPE** with cyclohexane
- Load sample onto MIP-SPE column
- Wash with cyclohexane
- Elute with EA
- Evaporate eluate and reconstitute with pentane

Further cleanup
- Load sample onto **deactivated silica gel column**
- Collect eluate and further elute with pentane
- Evaporate eluate to about 0.2 mL
- Add d_{12}-perylene as injection standard

GC-MS/MS analysis

Method in GL (II)

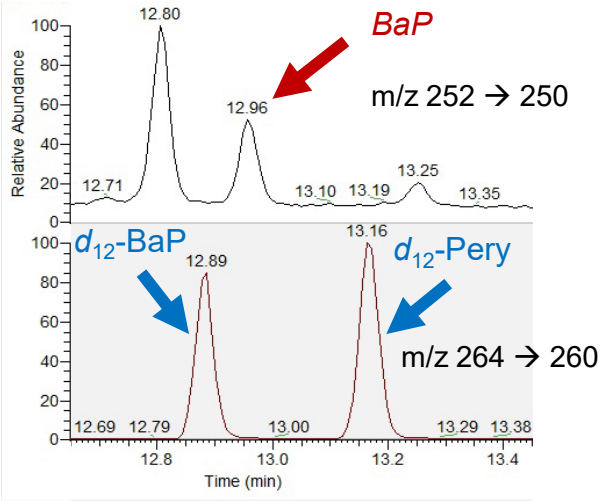
GC-MS/MS analysis

GC settings

Column	:	DB-35ms (30 m × 0.25 mm, 0.25 μm)
Injector temperature	:	280 °C
Injector mode	:	Splitless
Injection volume	:	1 μL
Column flow	:	Helium at 1.2 mL/min
Oven programme	:	150 °C (hold for 1 min), 20 °C/min to 320 °C (hold for 15 min)

MS settings

MS system	:	TSQ 8000 Evo
Ionization mode	:	EI
Transfer line temperature	:	280 °C
Ion source temperature	:	300 °C



Analytes	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
d_{12}-BaP	12.9	264	260	25
BaP	13.0	252	250	30
			251	15
d_{12}-Perylene	13.2	264	260	25

Reference Materials

Certified Reference Materials

BaP – NMIJ

Internal Standards

For GC-MS:

d_{12}-BaP – Cambridge Isotope Laboratory

$^{13}C_4$-BaP (ISO 16619:2015) – LGC

For HPLC-FD (ISO 15302:2017):

Benzo[b]chrysene – LGC

Proficiency testing programmes

PT programmes for PAHs including BaP

Title	Organiser	Matrix	Product code	Item code
Fapas	Fera Science Ltd, UK	Olive oil	FCCE1-OIL22	06127 (May 2023)
BIPEA	International Bureau for Analytical Studies	Milk powder	44b	Pending for 2023

The END

Thank you