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# The measurement of bioaccessible benzo(a)pyrene in contaminated soils using a physiologically based extraction test

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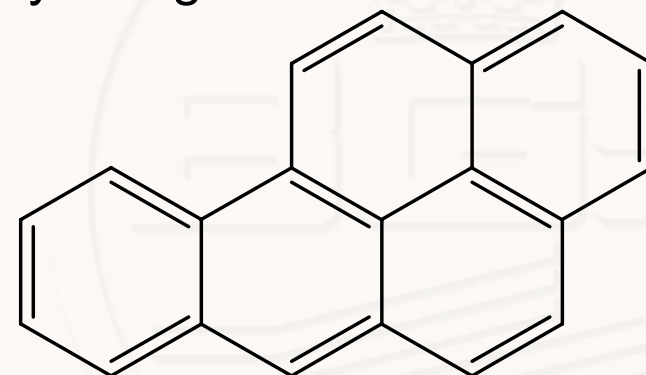
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# B(a)P

- Considered the most toxic of the common environmental PAHs (16 USEPA PAH priority pollutants)
- Carcinogenic and mutagenic effects
- Results from animal testing
  - Liver and kidney enzyme function damage (ATSDR, 1995; Robinson *et al*, 1975)
  - Leukaemia
  - Non-tumour effects of the skin, respiratory and gastrointestinal tract, liver, uterus, ovaries and testes
  - Tumours
    - Forestomach, Lung, Oesophagus





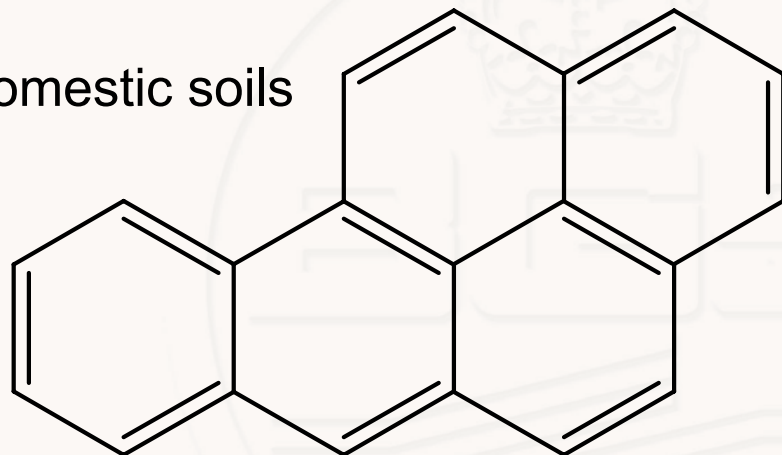
# B(a)P Absorption

- Oral ingestion studies, animal and some human
  - Systemically absorbed!!
- Inhalation studies
  - Clearance from the lungs, but particles are swallowed and ingestion occurs
- Dermal absorption studies
  - Extensive and rapid!!
- Dermal>Oral>Inhalation



# B(a)P in the UK

- B(a)P concentrations significant in domestic soils, present through practices such as BBQ's and bonfires
- Can be high at 'Brown-field' sites, e.g. ex- gas, creosote and coal tar processing works
- DEFRA currently in the process of assigning an SGV (Soil Guideline Value) for B(a)P
- Interim maximum value  $1\text{mg kg}^{-1}$  for domestic soils





# Human Health Risk Assessment

- Currently assume 100% risk – all B(a)P in soil is available for uptake by humans
  - Via oral, respiratory or dermal exposure routes
- Growing need to look at bioaccessibility
  - The solubility of the contaminant in simulated human fluids/systems
    - dependant on the route of exposure
    - Work to date has mainly concentrated on inorganic soil contaminants
    - Investigating Oral exposure route





# *In-vitro* Bioaccessibility Testing

- Bioaccessibility – via oral exposure route  
Defined as ‘the fraction that is soluble in the gastrointestinal environment and is available for absorption’
- Surrogate of Bioavailability testing  
Can only be carried out by animal testing!!
- We currently only simulate oral exposure
  - So risk from dermal and inhalation routes still needs to be accounted for in HHRA



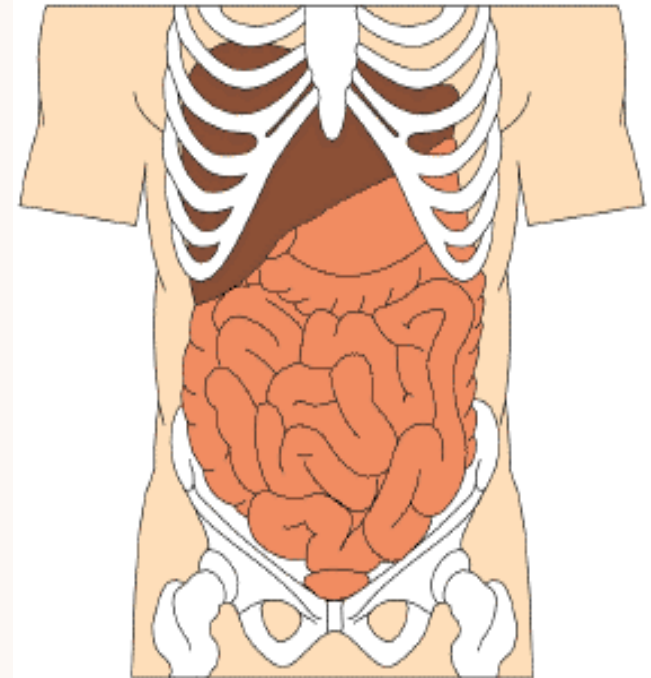


# *In-vitro* Bioaccessibility Testing II

- Simulate accidental ingestion of soil using a Physiologically Based Extraction Test (PBET)
  - Simulates leaching of a solid matrix in the human GI tract (stomach + small intestine)
  - Mimic kinetic and chemical changes in the human GI environment

## AIM

- Measure the amount of contaminant, bound to the soil, that is actually soluble in the human GI environment, and thus available for uptake
- As a result give a more reasonable estimate of risk than the use of 100%

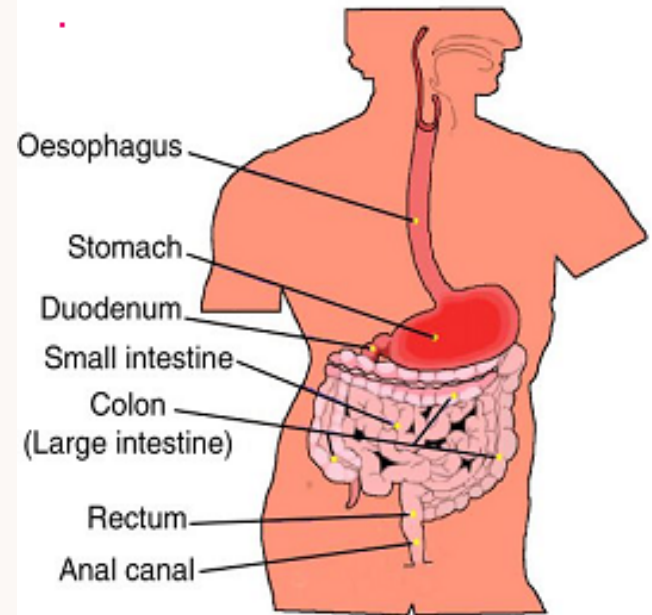




# How do we carry out a bioaccessibility testing on soils?

- Physiologically based extraction test
- Simulate the GI tract of a 2-3 yr old child
  - In most cases the at risk receptor
- Simulate gastric and gastrointestinal solutions
  - Stomach pH @ 2.5, 1 hr
  - Small Intestine pH @ 7.0, 4 hrs
- 37°C
- Simulate the worst case scenario
  - Inorganics = fasted state
  - Organics = fed state

## The Gastrointestinal Tract

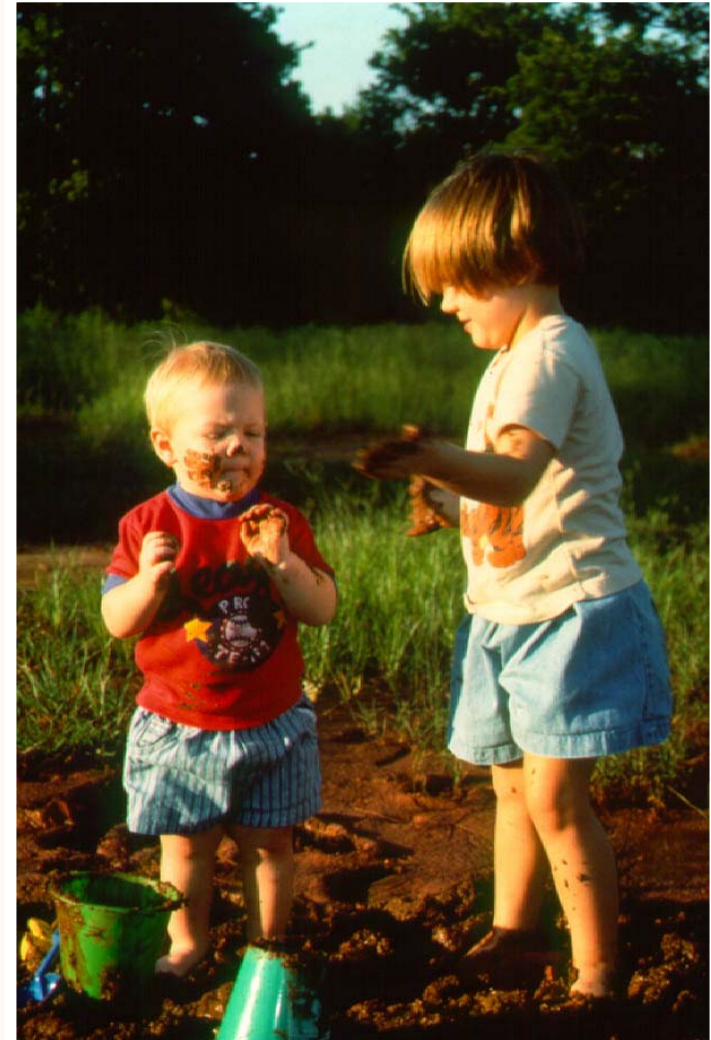






## Inorganic Contamination

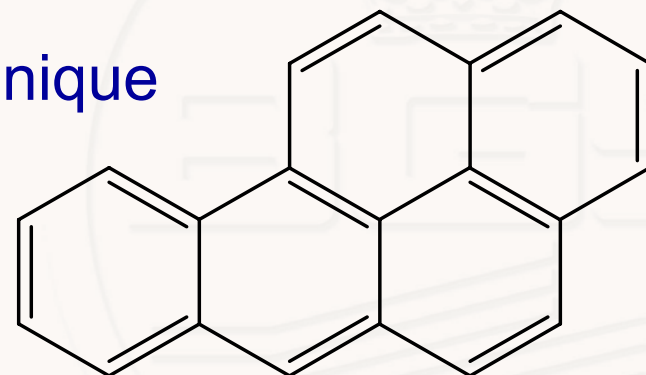
- Bioaccessibility testing can provide a very useful screening tool for risk assessment
- Current research is allowing the uncertainties associated with *in-vitro* tests to be quantified
- As long as the uncertainties are known, the data can provide an important input into the risk assessments (e.g. Bioaccessibility)
- This is where we want to get to for organic contamination
  - But there is a long way to go!





# Challenges for bioaccessibility testing of organic contaminants

- B(a)P is hydrophobic
- A dirty aqueous matrix
  - Lipid emulsion (from baby milk), organics (pepsin, pancreatin, bile salts etc), colloidal soil minerals, humic materials etc
- Question: How to extract B(a)P from the matrix?
- Substantial challenge for HPLC coupled with fluorescence detection
- **Development of an analytical technique to assess bioaccessible B(a)P in contaminated soils**





# B(a)P Contaminated Site

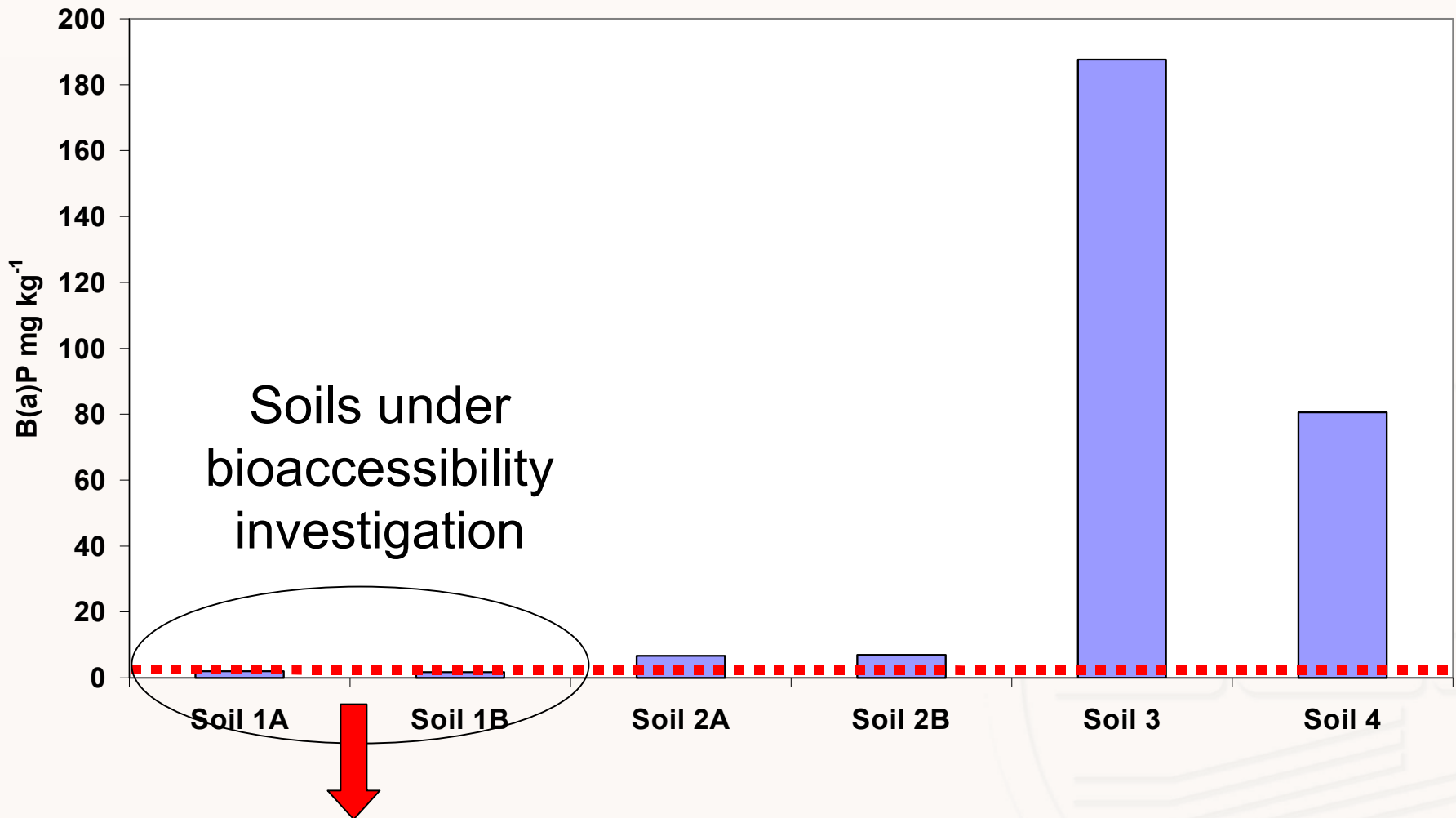
- Keighley, West Yorkshire
- Provided by National Grid Properties (formerly Second Site)
- Late 19<sup>th</sup> Century Gasworks site
  - Gas production house
  - Gas cooling and cleaning apparatus
  - Storage tanks for gas and by-products
  - Meter or valve house to monitor gas pressure and quality

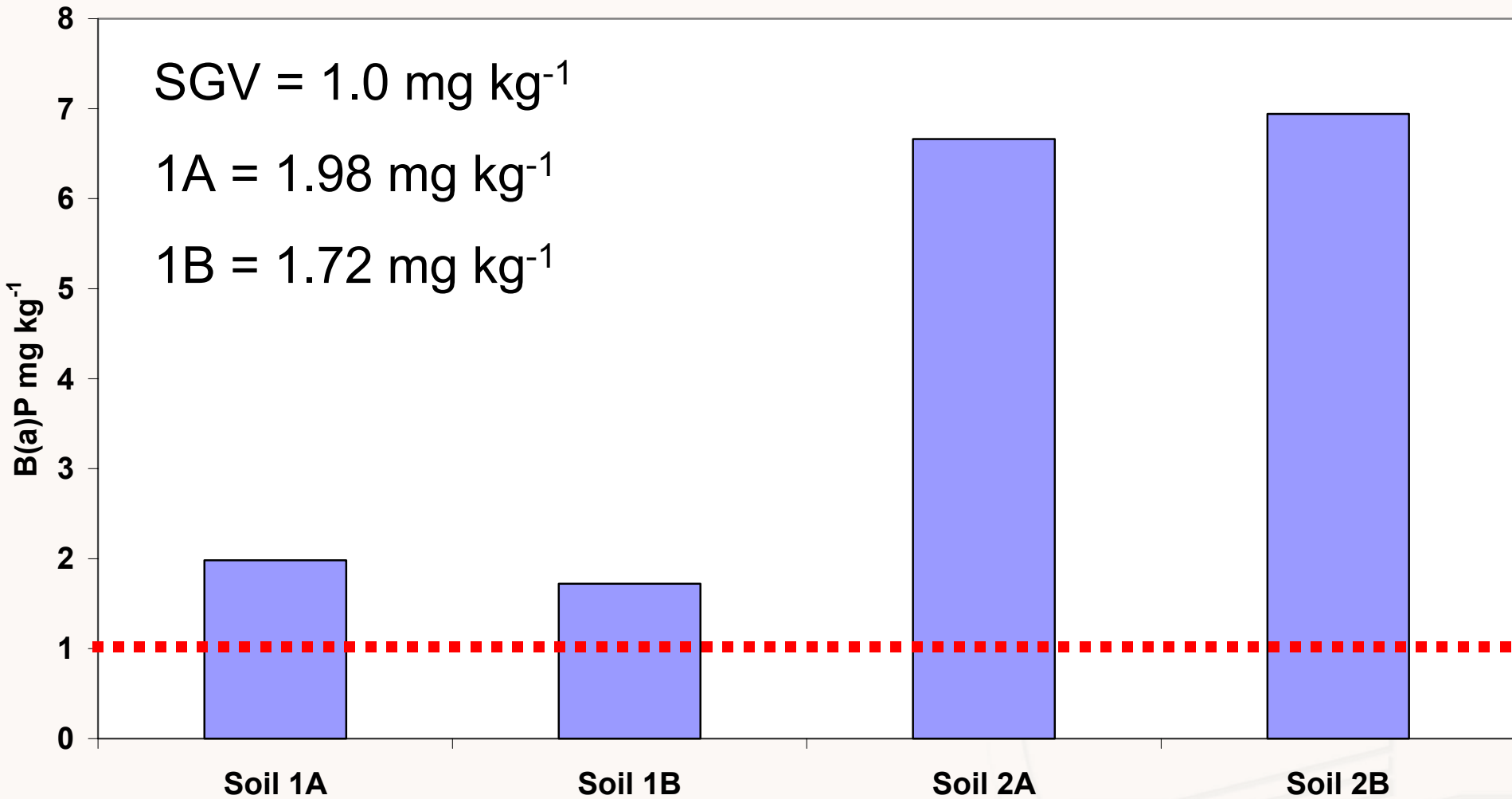


**Preliminary study to develop the method – Priority = collection of B(a)P contaminated soils**



# B(a)P Contamination



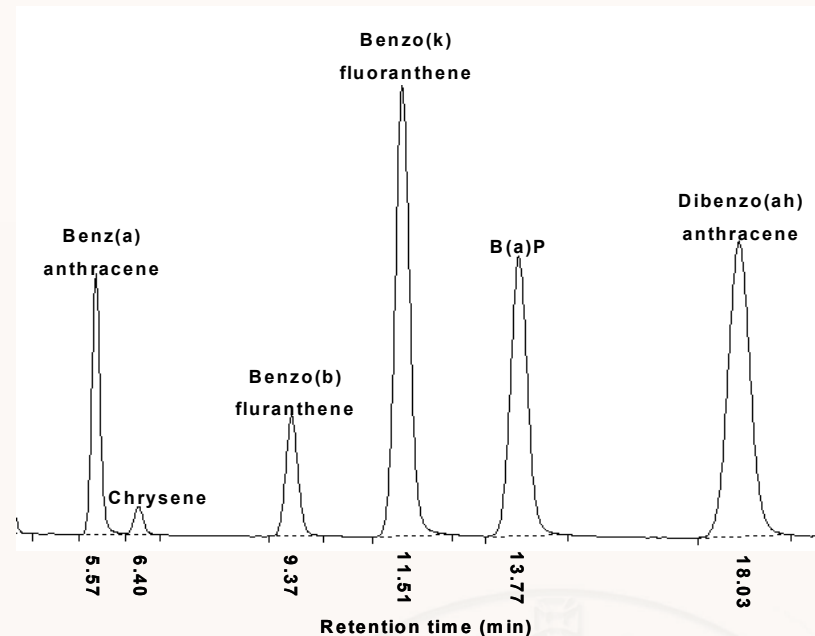




# Soil Choice

- Based on experience of bioaccessible inorganic contaminants
- Total B(a)P concentration in the 1-5 mg kg<sup>-1</sup> range (up to 5x the SGV)
- Sampling duplicates
- 1 sample extracted in duplicate





## Gastro-intestinal Extraction

- Gastric pH 2.5, 1 extraction sample

Pepsin, lactic acid, acetic acid, malate, citrate, milk powder

- Intestine pH 7.0, 2 extraction samples

Gastric solution, pancreatin, bile salts, plus  $\text{NaHCO}_3$  (for pH neutralisation)

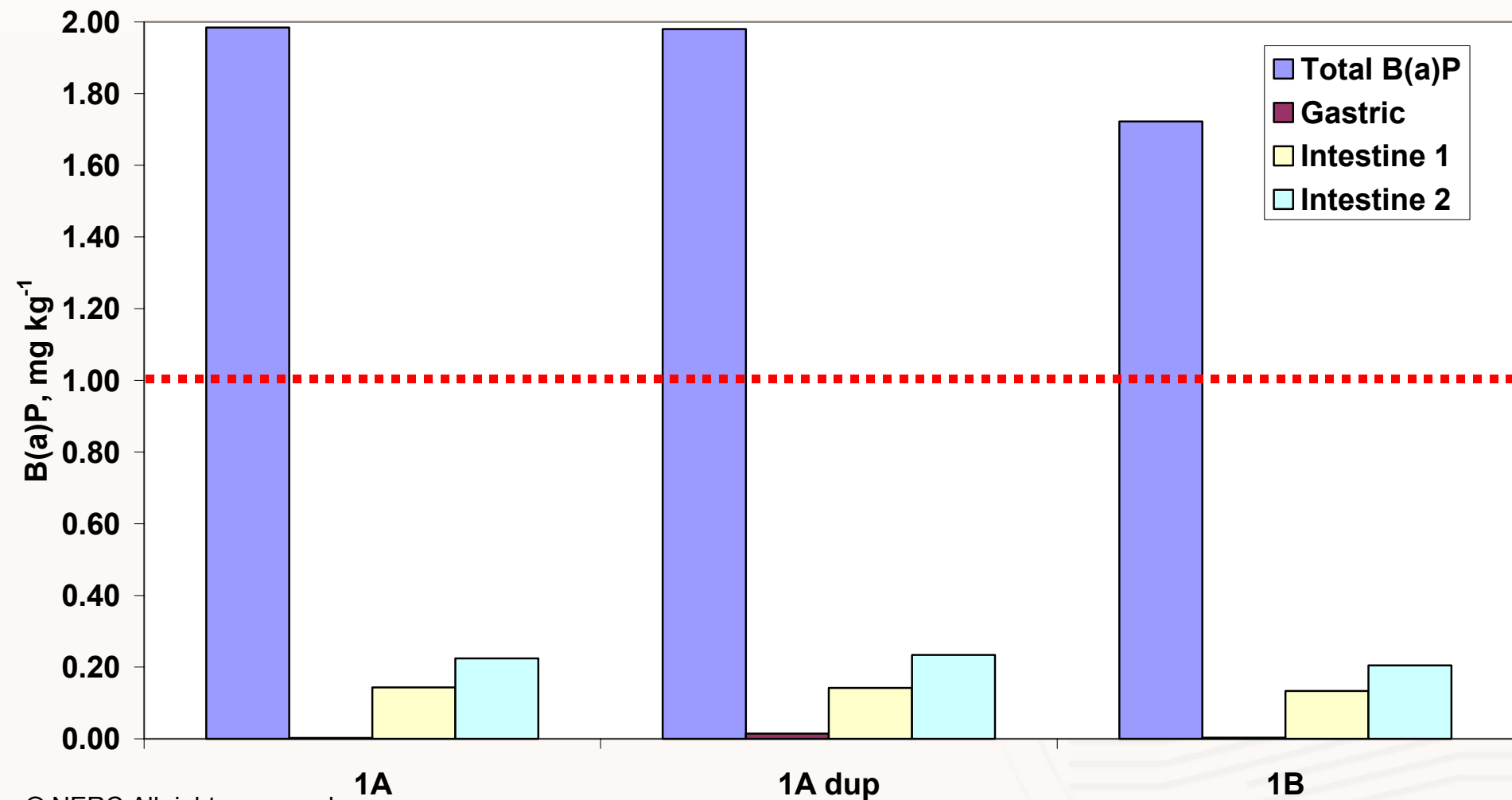
## HPLC-Fluorescence Detection

- Detection-spectrofluorimetric :  
Excitation 296 nm / Emission 408 nm

- RT = 13.77 minutes



# Bioaccessible B(a)P

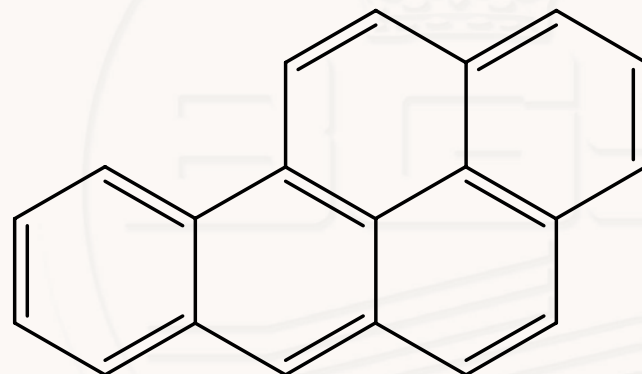






# Results

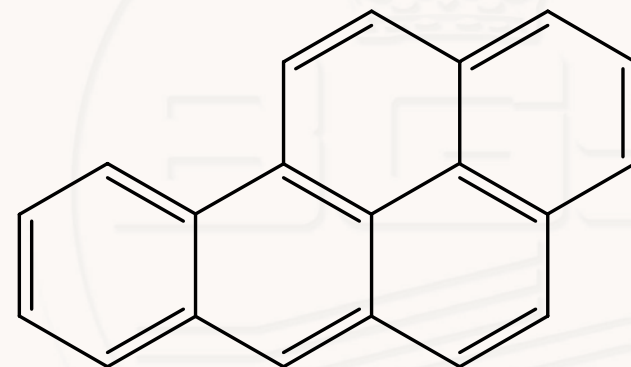
- Bioaccessibility = based on the highest of the 3 results obtained
  - Gastric, Intestine 1 or 2
- Only a small proportion of B(a)P partitions into the gastric phase
- Bioaccessible B(a)P is in the intestine phase
  - Intestine contains bile salts and fatty acids (milk powder)
    - Formation of mixed micelles, which act as a transport vehicle for B(a)P (Bioaccessible)
- Absorption occurs in small intestine





# Conclusions

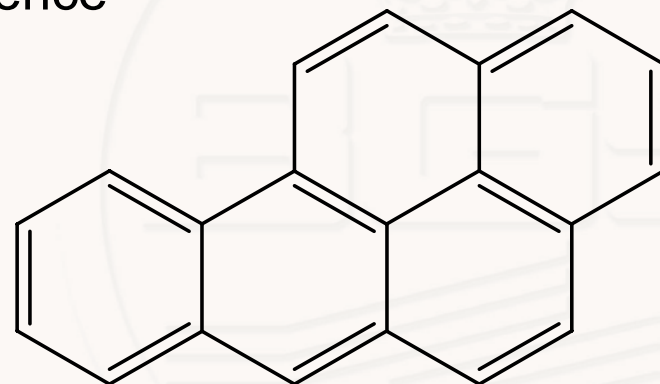
- Bioaccessibility is significantly less than the total B(a)P concentration
  - We may be able to reduce the risk from the oral exposure route
- BUT – Need to look at other exposure routes (Dermal, respiratory) to reduce the risk from 100%
- Still a long way to go, but a good start!!!!
- Equilibrium in small intestine not reached
  - Needs to be addressed
- Potential tool for HHRA





# Further work

- Investigate when the Intestine phase reaches equilibrium
- Look into ways of validating the methodology
- Investigate the B(a)P concentration range where bioaccessibility may still be a useful tool
- Set up methods for the dermal and respiratory exposure pathways
  - Dermal – brand new to BGS
  - Respiratory – some inorganic experience





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# Acknowledgements

- Paddy
- Env & Health





- European Network
- Human Bioaccessibility of priority contaminants
- Gastrointestinal tract
- As, Cd and Pb
- [www.bgs.ac.uk/barge](http://www.bgs.ac.uk/barge)

Aim: To provide robust and defensible data on bioaccessibility that can be used in human health risk assessment and policy making





# Distribution

- persisted in the kidney and testes (Yamazaki and Kakiuchi, 1989).
- the stomach and small intestine, and, as these declined, in the large intestine and caecum (Mitchell, 1982).

