Incident Investigation - chemicals

What we would expect to see in a large toxic pollution incident

- Contaminant of concern in the water, biological and sediment samples outside 'historic' detections
- Dilution mitigation (exposed, mixed coast) - we would expect this to have a big part to play in reducing any impact form a point source in time and space
- Other species obviously affected

Chemicals will be detected when we analyse environmental samples but are they the cause of the mortalities?

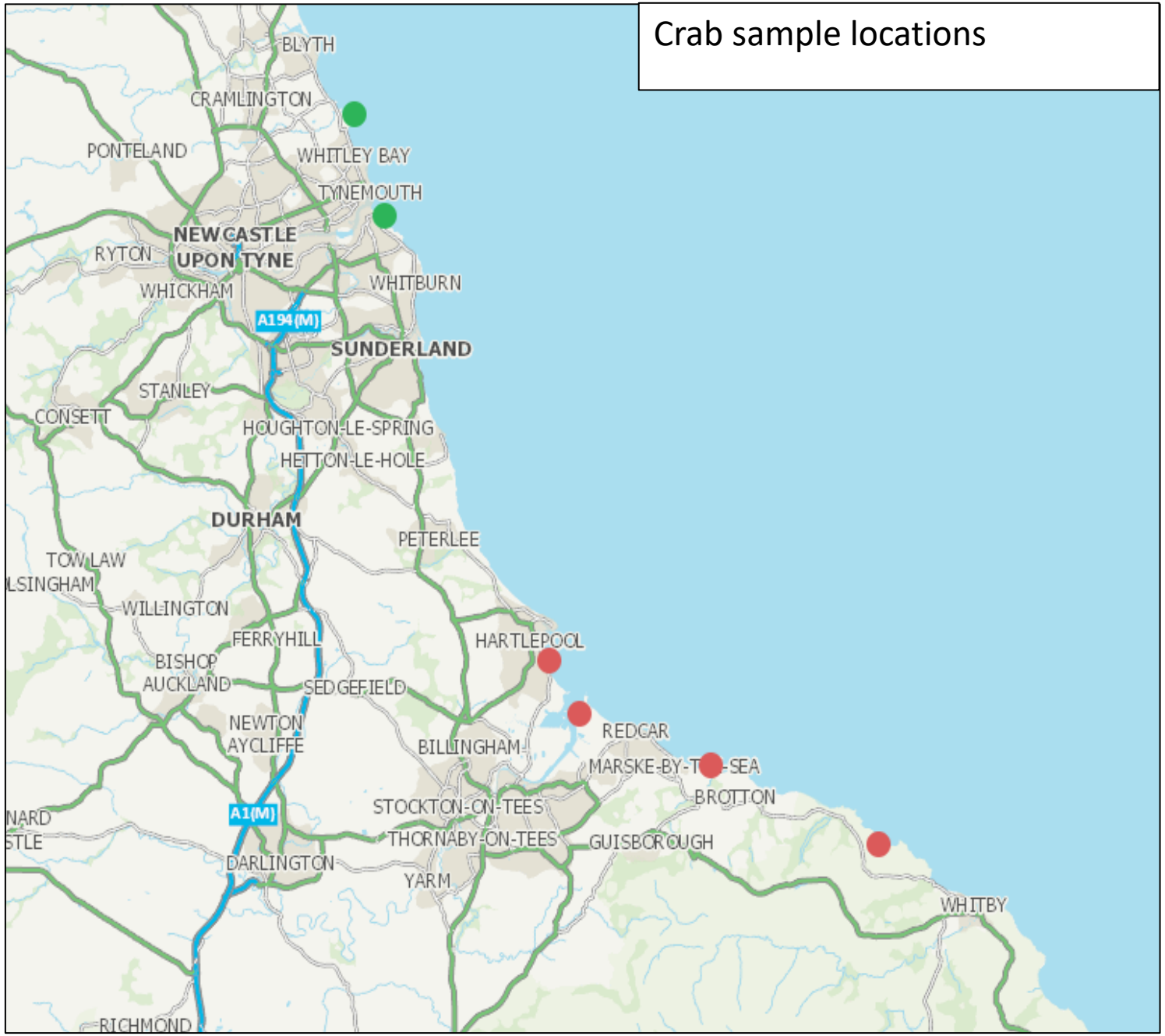
Environment Agency

Incident Investigation: Could pyridine be the cause?

- First results, when the area of impact was still localised, led to presumption that pyridine was the cause
- Literature searches for information including the ecotoxicology and background levels of, and impact of, pyridine in crabs and lobsters, were carried out.
- A potential source of the contaminant was sought. This included taking a formal water discharge sample (9/11/2021).
- No significant pyridine was found to be present. No significant source could be identified.
- As the impacted area and length of time of the Incident increased, ***with no dilution mitigation***, a contaminant source became increasing improbable
- Comparison crabs from outside the known impacted area were sourced to provide an indication of the ‘background’ levels of pyridine in crab tissues.
- Pyridine was analysed for in other materials in the area – water, sediment and blue mussels. Pyridine was detected at low levels by the screening method in blue mussels but not in all the sediment samples. Pyridine was not generally detected in the water samples.

Could pyridine be linked to biological processes in the crab tissue - Consequence rather than cause?

Environment Agency



- Focus was initially on the analysis of dying crabs from the shore (red dots)
- Samples were taken of healthy crabs (green dots) to help interpret the results
- Further comparison crabs: Penzance (1 sample) & Norfolk Wash (2 samples)
- Just because chemicals can be detected in the tissue does not mean that they are the cause of the crab deaths

Environment Agency

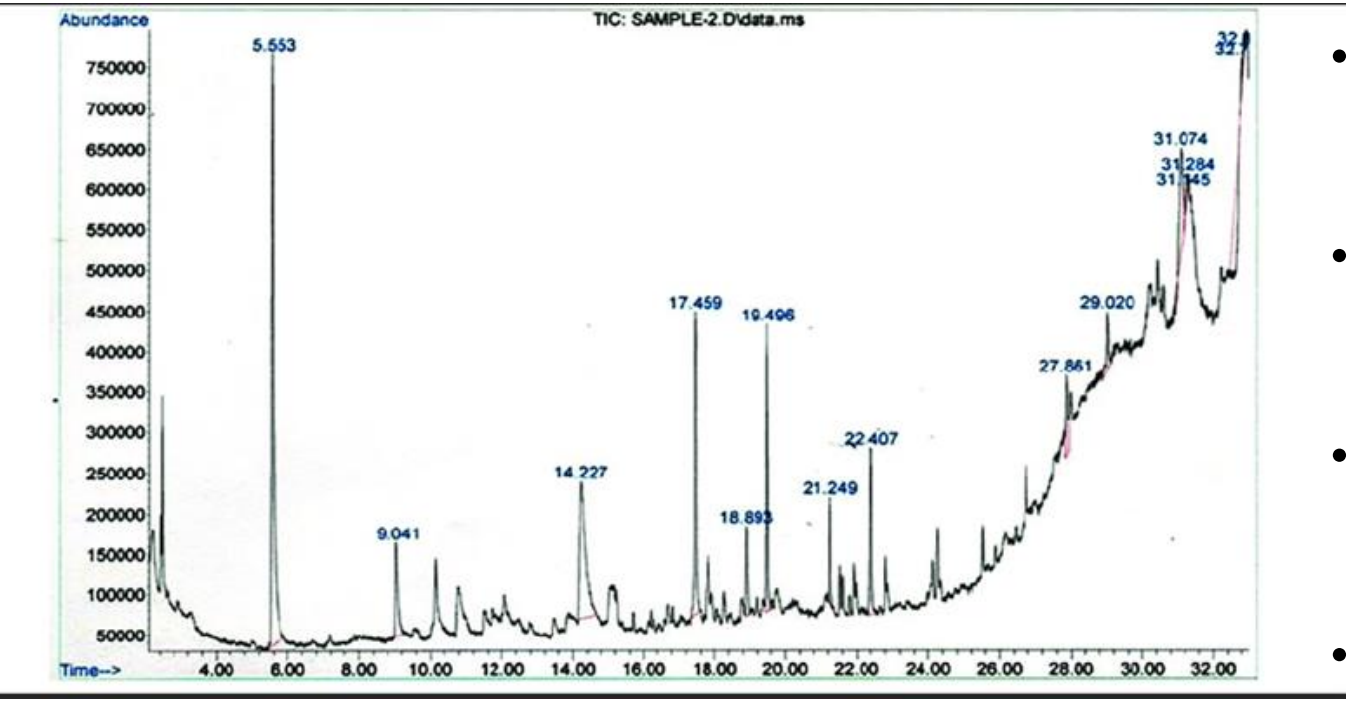
Pyridine in crab samples (mg/kg)

- Saltburn 439
- Bran Sands 255 (08/10/21)
- Seaton 204
- Norfolk Wash (Eastern IFCA 3) 195
- St Mary's Lighthouse 78
- South Shields 35
- Runswick 20
- Penzance 6
- Norfolk Wash (Eastern IFCA 1) 3

Comparison crabs in green

Numbers are indicative only – see method discussion on GCMS screening

Incident Investigation: **gas chromatography-mass spectrometry** (GCMS)
Interpreting the numbers from the crab tissue analysis



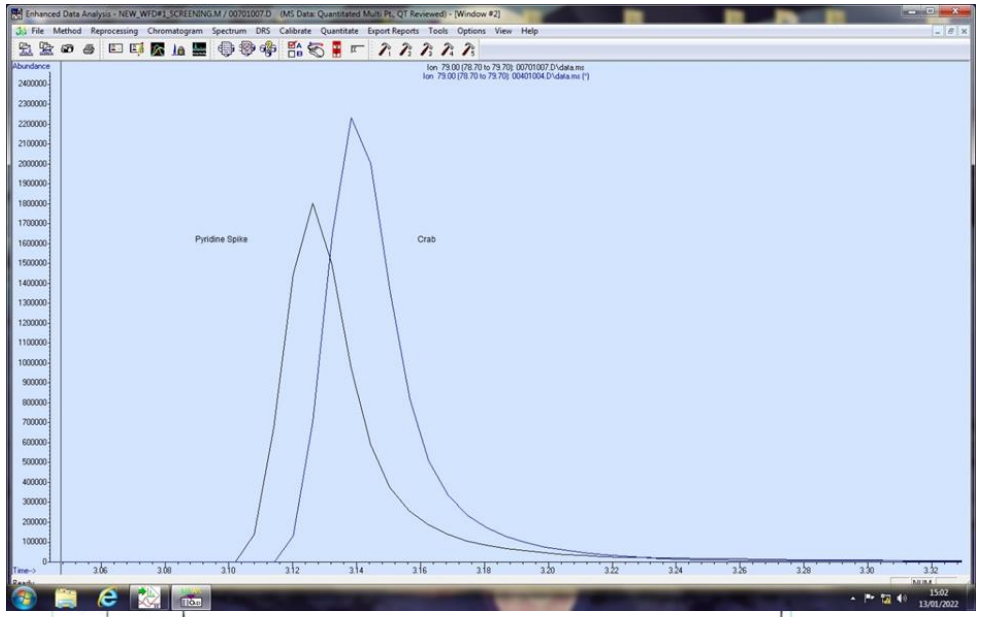
- accredited **water** screening methodology developed for the identification of substances including pyridine.
- In response to this seriousness of this incident the method was adapted for the screening of biota and sediment.
- Uses part of an established method e.g solvent extraction, but has not been tested in these materials.
- Done to provide as much information as possible about potential chemical leads.

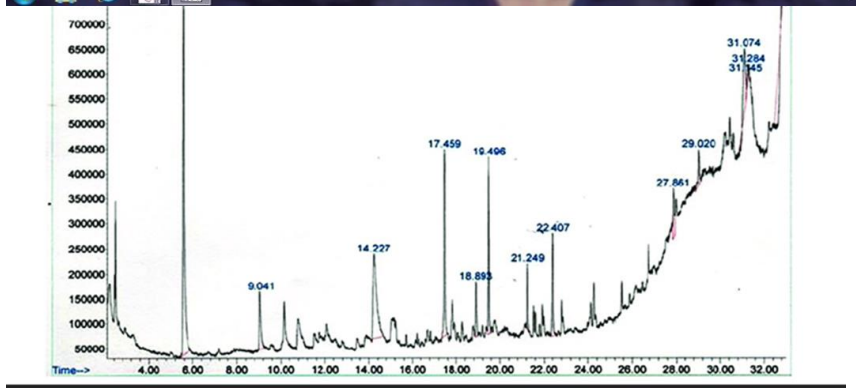
We don't know how this method behaves in analysing biological tissue
We don't have a baseline for pyridine in crab tissue

Environment Agency

Incident Investigation: How far can you push a GCMS semi quantitative screening method developed for water?

- GCMS screening is an accredited **water** screening methodology developed for the identification of substances including pyridine.





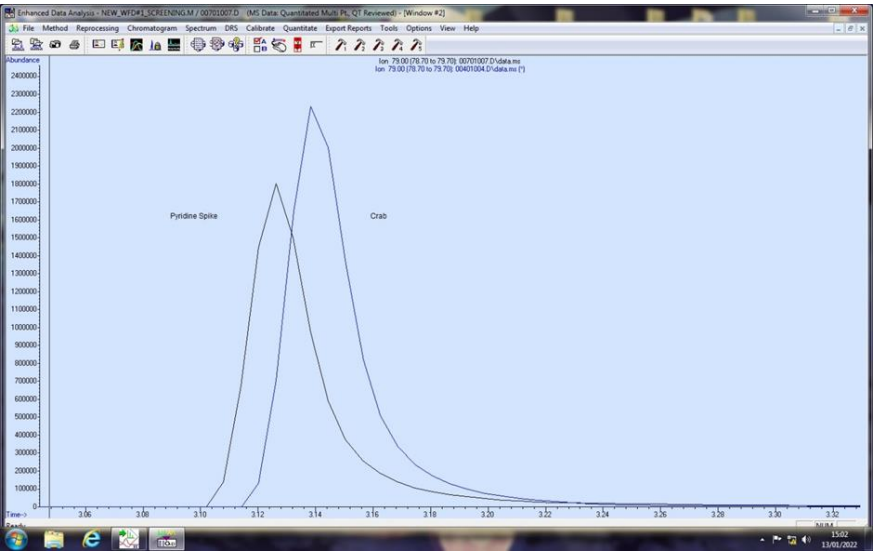
The measuring of Pyridine is two parts (i) the identification of pyridine and (ii) the determination of how much pyridine is present

Identification

Pyridine is measured by putting the sample of water or biota (following some preparation) into an instrument. The pyridine molecule is then broken apart by firing electrons at it. Pyridine will always break apart (we call this fragmentation) in the exact same way every time and we can compare the pattern it forms against a library picture of a known picture of pyridine. Like fingerprints, no two fragments will be the same, this means that when a sample is measured, if we see the pyridine fragment, we know it's pyridine

A purchased pyridine standard was run in Jan 2022 which confirmed that we were detecting pyridine

Incident Investigation: GCMS screening – can we estimate concentrations in crab tissues?



Measuring the amount – part 1

When the instrument sees pyridine the instrument responds. The more pyridine present the more the instrument responds. We report this as a number and call it a concentration. In the case of pyridine we use the number of milligrams of pyridine in every kilogram of crab (mg/kg). A mg/kg is equivalent to, one drop of food dye in 16 gallons of water or 1 inch in 16 miles

Measuring the amount – part 2

Whilst we have screened water samples for Pyridine before, we've never done this in crabs or sediment. We have used understanding from water analysis to estimate the concentration in the crab. This is unlikely to the very accurate, but we don't have the data to confirm this.

Confidence in the measuring and concentration

Although we know the instrument measured pyridine, we don't know the following things - to do so would require a lot more testing:

- We don't know whether the solvent has removed 'captured' all the pyridine from the crab (the Recovery or BIAS)
- We don't know how consistently it does this (the Precision)

Initially the lab only reported levels as <1000 mg/kg but we asked for further breakdown in order to aid the investigation. *We have to recognise that 'concentrations' are indicative only*

Pyridine in crab samples (mg/kg)

- Saltburn 439
- Bran Sands 255 (08/10/21)
- Seaton 204
- Norfolk Wash (Eastern IFCA 3) 195
- St Mary's Lighthouse 78
- South Shields 35
- Runswick 20
- Penzance 6
- Norfolk Wash (Eastern IFCA 1) 3

Comparison crabs in green

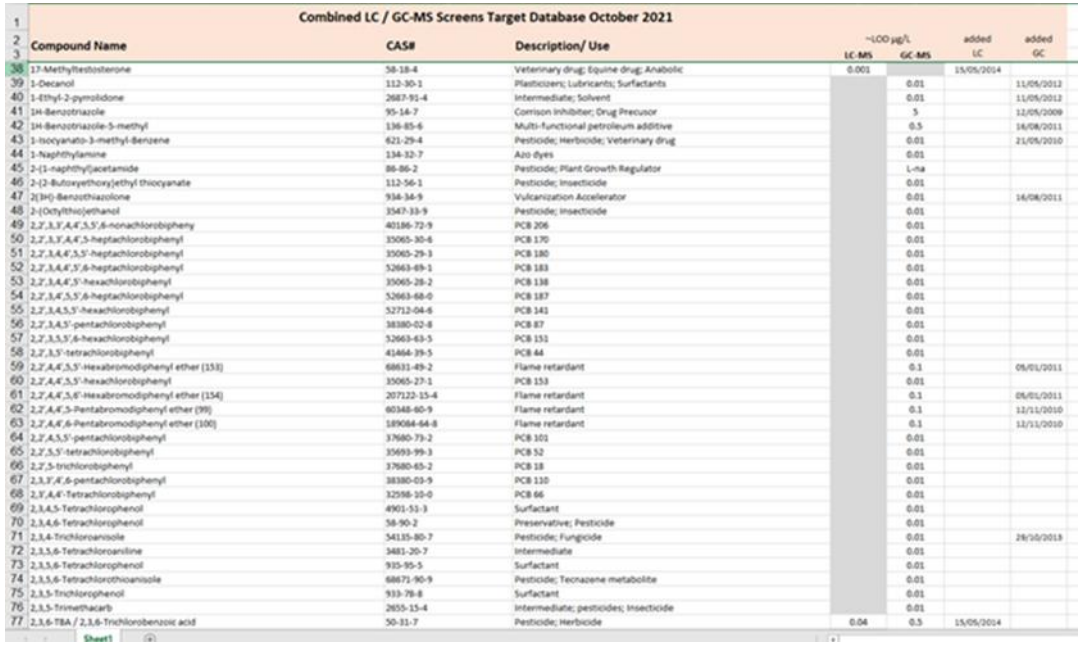
Could pyridine be linked to biological processes in the crab tissue - Consequence rather than cause?

Water

To see whether there are any unusual chemical signals in the water. *What is different compared to samples seen historically in the area?*

Focus on organics due to mode of action on crabs – used validated screening methods developed for water.

- Liquid Chromatography Mass Spectrometry LCMS (ca. 740 substances)
- Gas chromatography-mass spectrometry GCMS (ca. 1040 substances)



Combined LC / GC-MS Screens Target Database October 2021						
Compound Name	CAS#	Description/ Use	~LOD µg/L		added	added
			LC-MS	GC-MS	LC	GC
17-Methyltestosterone	58-18-4	Veterinary drug; Equine drug; Anabolic	0.001		15/05/2014	
1-Decanol	112-30-1	Plasticizers; Lubricants; Surfactants		0.01		11/05/2012
1-Ethyl-2-pyrrolidone	2687-91-4	Intermediate; Solvent		0.01		11/05/2012
1H-Benzotriazole	95-14-7	Corrison Inhibiter; Drug Precusor		5		12/05/2009
1H-Benzotriazole-5-methyl	136-85-6	Multi-functional petroleum additive		0.5		16/08/2011
1-Isocyanato-3-methyl-Benzene	621-29-4	Pesticide; Herbicide; Veterinary drug		0.01		21/05/2010
1-Naphthylamine	134-32-7	Azo dyes		0.01		
2-(1-naphthyl)acetamide	86-86-2	Pesticide; Plant Growth Regulator		L-na		
2-(2-Butoxyethoxy)ethyl thiocyanate	112-56-1	Pesticide; Insecticide		0.01		
2(3H)-Benzothiazolone	934-34-9	Vulcanization Accelerator		0.01		16/08/2011
2-(Octylthio)ethanol	3547-33-9	Pesticide; Insecticide		0.01		
2,2',3,3',4,4',5,5',6-nonachlorobipheny	40186-72-9	PCB 206		0.01		
2,2',3,3',4,4',5-heptachlorobiphenyl	35065-30-6	PCB 170		0.01		
2,2',3,4,4',5,5'-heptachlorobiphenyl	35065-29-3	PCB 180		0.01		
2,2',3,4,4',5',6-heptachlorobiphenyl	52663-69-1	PCB 183		0.01		
2,2',3,4,4',5'-hexachlorobiphenyl	35065-28-2	PCB 138		0.01		
2,2',3,4',5,5',6-heptachlorobiphenyl	52663-68-0	PCB 187		0.01		
2,2',3,4',5,5'-hexachlorobiphenyl	52712-04-6	PCB 141		0.01		
2,2',3,4,5'-pentachlorobiphenyl	38380-02-8	PCB 87		0.01		
2,2',3,5,5',6-hexachlorobiphenyl	52663-63-5	PCB 151		0.01		
2,2',3,5'-tetrachlorobiphenyl	41464-39-5	PCB 44		0.01		
2,2',4,4',5,5'-Hexabromodiphenyl ether (153)	68631-49-2	Flame retardant		0.1		06/01/2011
2,2',4,4',5,5'-hexachlorobiphenyl	35065-27-1	PCB 153		0.01		
2,2',4,4',5,6'-Hexabromodiphenyl ether (154)	207122-15-4	Flame retardant		0.1		06/01/2011
2,2',4,4',5-Pentabromodiphenyl ether (99)	60348-60-9	Flame retardant		0.1		12/11/2010
2,2',4,4',6-Pentabromodiphenyl ether (100)	189084-64-8	Flame retardant		0.1		12/11/2010
2,2',4,5,5'-pentachlorobiphenyl	37680-73-2	PCB 101		0.01		
2,2',5,5'-tetrachlorobiphenyl	35693-99-3	PCB 52		0.01		
2,2',5-trichlorobiphenyl	37680-65-2	PCB 18		0.01		
2,3,3',4',6-pentachlorobiphenyl	38380-03-9	PCB 110		0.01		
2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	PCB 66		0.01		
2,3,4,5-Tetrachlorophenol	4901-51-3	Surfactant		0.01		
2,3,4,6-Tetrachlorophenol	58-90-2	Preservative; Pesticide		0.01		
2,3,4-Trichloroanisole	54135-80-7	Pesticide; Fungicide		0.01		29/10/2013
2,3,5,6-Tetrachloroaniline	3481-20-7	Intermediate		0.01		
2,3,5,6-Tetrachlorophenol	935-95-5	Surfactant		0.01		
2,3,5,6-Tetrachlorothioanisole	68671-90-9	Pesticide; Tecnazene metabolite		0.01		
2,3,5-Trichlorophenol	933-78-8	Surfactant		0.01		
2,3,5-Trimethacarb	2655-15-4	Intermediate; pesticides; Insecticide		0.01		
2,3,6-TBA / 2,3,6-Trichlorobenzoic acid	50-31-7	Pesticide; Herbicide	0.04	0.5	15/05/2014	

Environment Agency

Pyridine – Tees GCMS since 2012

Screening_Method_Details	CAS_Number	unit	Concentration	Compound_Name	USE	LOD	method	month_and_year
Target screening (EA NLS database)	110861	ug/l	1	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Jan-12
Target screening (EA NLS database)	110861	ug/l	2.4	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Oct-12
Target screening (EA NLS database)	110861	ug/l	1.4	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	May-12
Target screening (EA NLS database)	110861	ug/l	0.4	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Jul-12
Target screening (EA NLS database)	110861	ug/l	0.3	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Jul-12
Target screening (EA NLS database)	110861	ug/l	0.15	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	May-15
Target screening (EA NLS database)	110861	ug/l	0.06	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Apr-15
Target screening (EA NLS database)	110861	ug/l	0.04	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Apr-15
Target screening (EA NLS database)	110861	ug/l	0.05	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Apr-15
Target screening (EA NLS database)	110861	ug/l	2.3	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Feb-16
Target screening (EA NLS database)	110861	ug/l	1.5	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Jan-16
Target screening (EA NLS database)	110861	ug/l	1.2	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Feb-16
Target screening (EA NLS database)	110861	ug/l	0.485	Pyridine	Precursor; Agrochemicals; Pharmaceuticals	0.01	GCMS	Aug-18

Nov 21 – in over 20 water samples, only 3 had positive detects of <0.5 ug/l (one was outside the impact zone, surface water south of Flamborough Head)

Note: We do occasionally see positive detects in saline waters elsewhere e.g. Southampton water, Orwell

(GCMS = Gas chromatography–mass spectrometry)

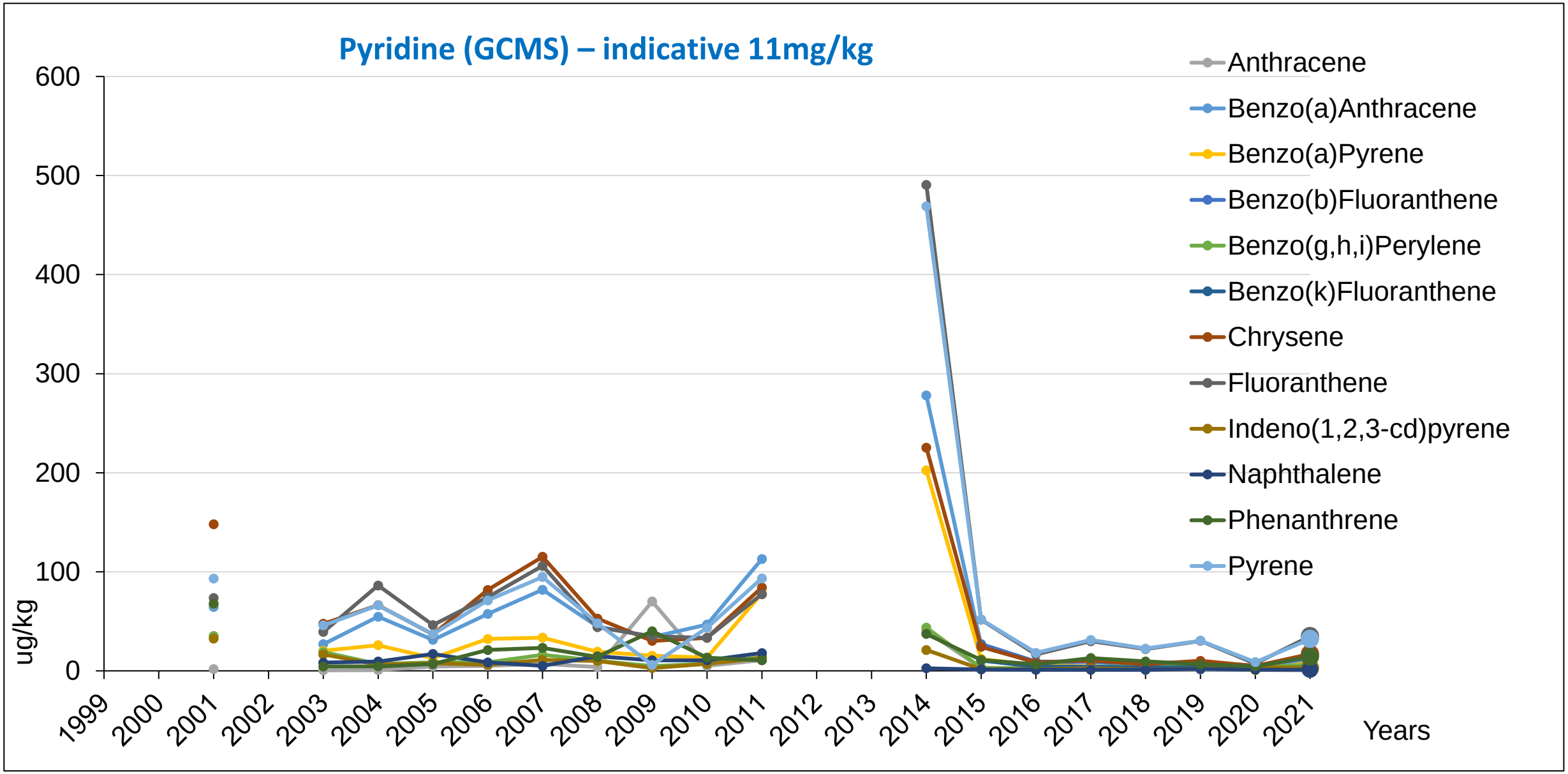
Sediment

- GCMS screening was run on 3 samples from Bran Sands
- Method limitations similar to crab tissue – indicative results only
- Surface sediment scrape only - investigation focused on newly deposited/disturbed sediment
- Not looking at historic contaminant levels in deeper sediments – focused only on recent disturbance
- Detection of pyridine only in one sample (>80% spectral fit - 0.018 mg/kg)

- *Note*: Sediment samples frozen for future analysis if needed

Environment Agency

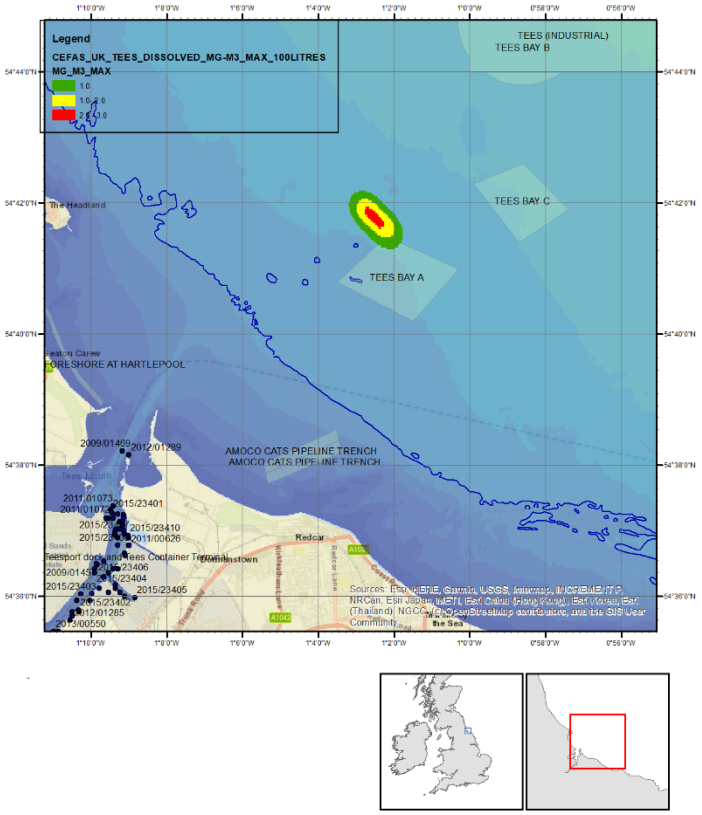
Blue Mussels – Phillips Buoy/Bran Sands- 08/10/21



Consideration of dilution - Hypothetical pyridine release from dredge disposal

Cefas Chemical Release Modelling
Chemmap - Pyridine 5th Oct 2021 @1200

Jon Rees 16th Dec 2021



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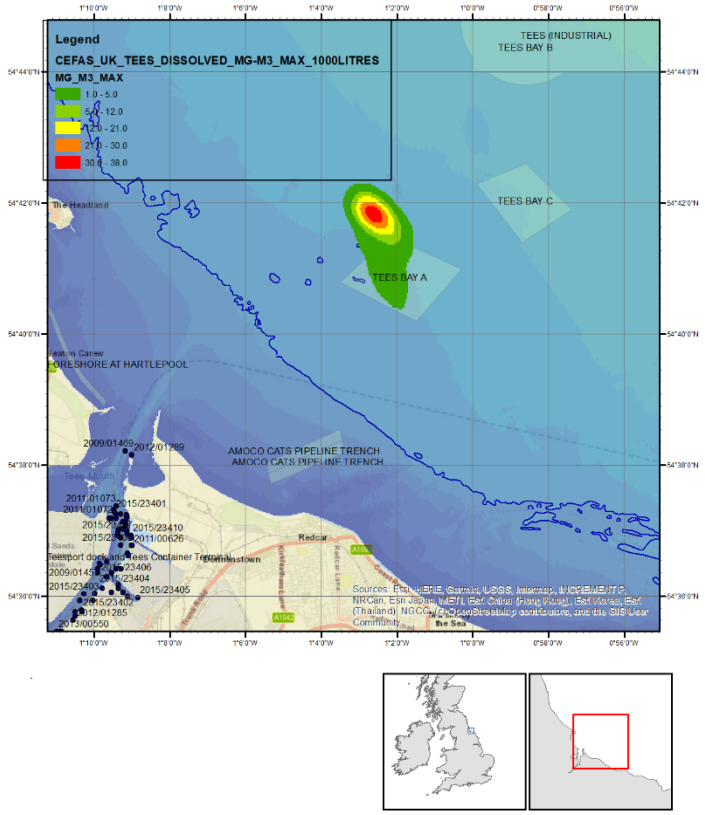
Cefas

Cefas Chemical Release Modelling
Chemmap - Pyridine 5th Oct 2021 @1200

Jon Rees 16th Dec 2021



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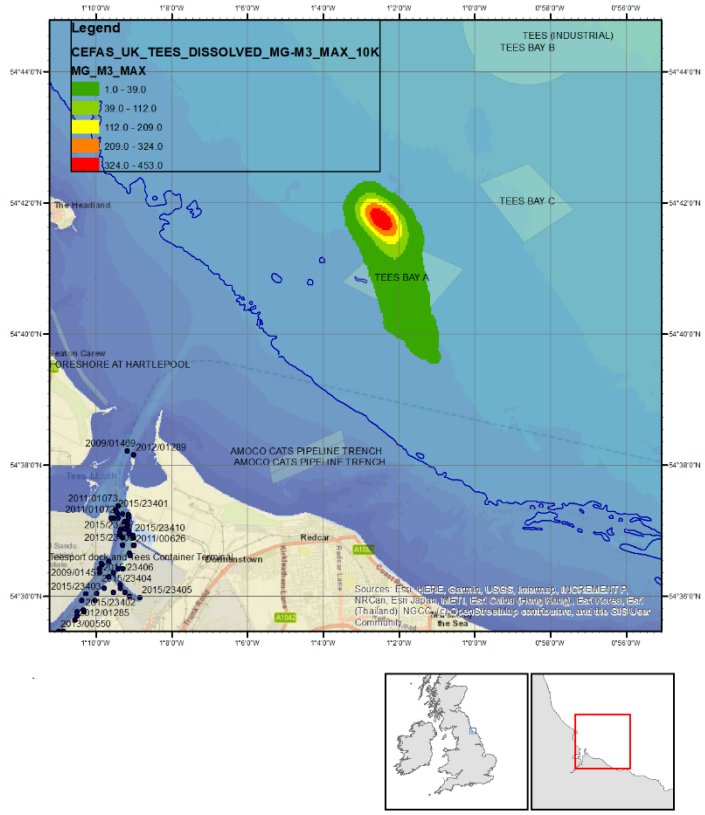

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Cefas

Release volume (litres)	Maximum Dissolved concentration (mg/m³)
100	3
1,000	38
10,000	453

Recap – Weight of Evidence around Pyridine

- Pyridine in crab tissue – but in impacted and comparison crabs (indicative method only)
- No obvious pyridine in surface sediment
- No significant pyridine signal in water (above historical samples)
- (Pyrene in blue mussels within historical levels in Tees)
- Huge dilution factor in exposed, well mixed coastal waters
- No findings of levels of chemical concentrations that would be needed to impact over the time and space of this Incident

Environment Agency