Microplastics Analysis using Accelerated Solvent Extraction (ASE) and Pyrolysis Gas Chromatography / Mass Spectrometry (Pyr-GC/MS)

Chris Shevlin

Scientific Affairs Manager

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The microplastic problem

Thermo Fisher SCIENTIFIC

Microplastics detected in meat, milk and blood of farm animals

Particles found in supermarket products and on Dutch farms, but human health impacts unknown

Scientists found microplastics in 75% of meat an
in their pilot study. Photograph: David Kelly

Microplastic contamination has been re
time, as well as in the blood of cows and

Microplastics found in human blood for first time

**Exclusive: The discovery show
body and may lodge in organs**

Microplastics cause damage to huma
Kelly/Photograph David Kelly

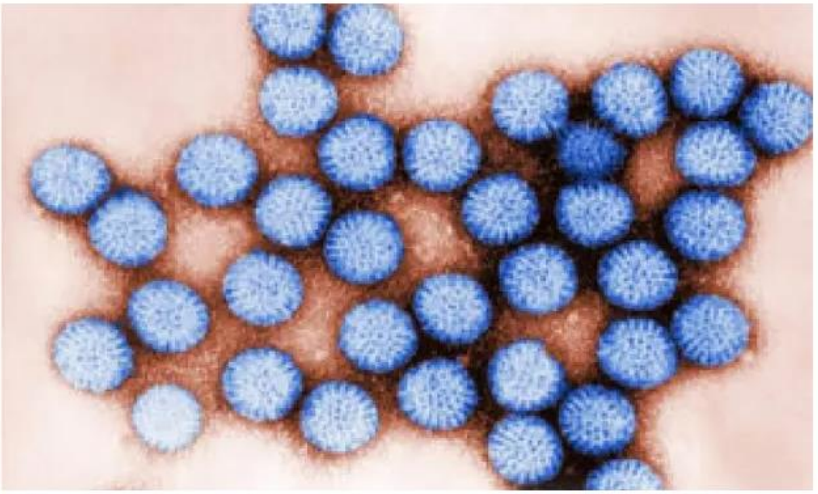
Microplastics found in freshly fallen Antarctic snow for first time

**New Zealand researchers identified tiny plastics, whic
toxic to plants and animals, in 19 snow samples**

Research published in The Cryosphere journal identified microplast
Antarctica for the first time. Photograph: Alex Aves

Viruses survive in fresh water by 'hitchhiking' on plastic, study finds

Intestinal viruses such as rotavirus were found to be infectious for up to three days by attaching to microplastics, research shows



Human enteric, or intestinal, viruses survive for days on plastic particles easily swallowed by swimmers, Stirling University researchers found. Photograph: GJ Flick and DD Kuhn/Virginia Tech

Source: www.guardian.co.uk

Analysis of microplastics: challenges

Sample collection and preparation

- Critical to understand distribution, composition, size
- Automation where feasible to reduce costs and accelerate sample throughput
- Include sub 1µm size (nanoplastic)

Technique	Attribute
FTIR	• Widely used particle number concentration • Polymer identification (> 10 µm) • Spectra match to library
Raman micro-spectroscopy	• Size and shape to 1 µm
Py-GC-MS	• Provides molecular level information to determine chemical composition/structure <u>regardless of size</u> • Polymer determination • Contaminants on MP surface • Quantitative and unknowns

Microplastics analysis

Sample preparation

↓

Pyrolysis

↓

GC separation

↓

MS detection

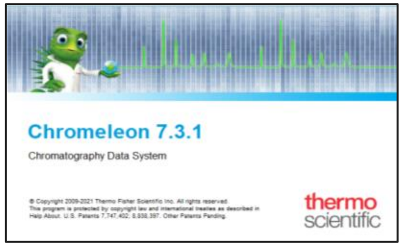
↓

Data processing

Microplastics analysis

Analysis Goal

Target analysis



- Detection of target polymers
- Quantitation

Non-targeted

- Detection of non-target polymers
- Identification of unknowns

Accelerated solvent extraction (ASE) Pyr-GC-MS

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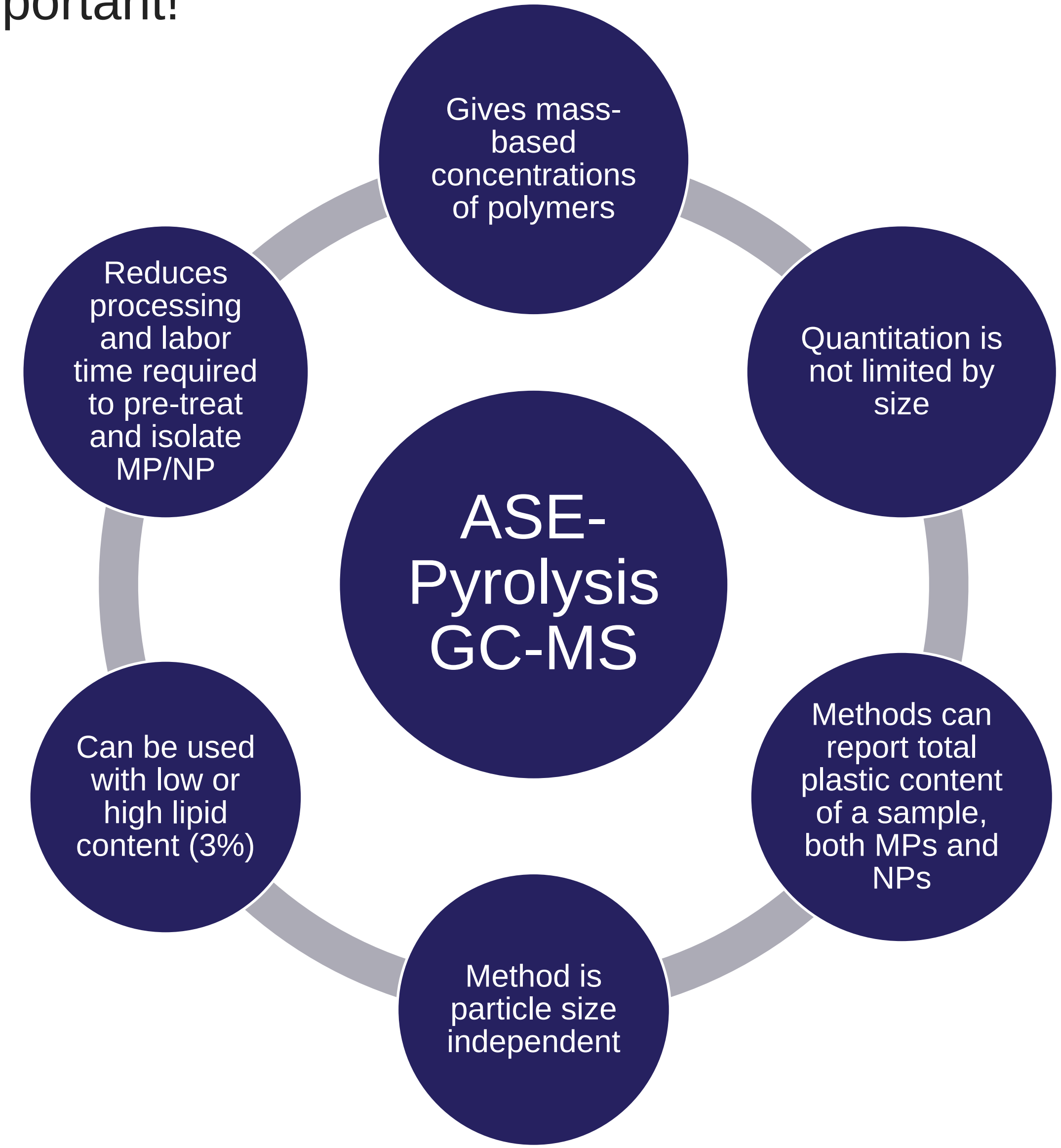
Sample prep is extremely important!



Accelerated Solvent Extraction

- Automates sample preparation for solid and semisolid samples using solvents at elevated temperatures and pressure.
- Operates above the boiling point of extraction solvents by using pressurized sealed extraction cells.
- Ensures complete dissolution of all polymer materials

Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor

Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system

Extraction and analysis of rice samples

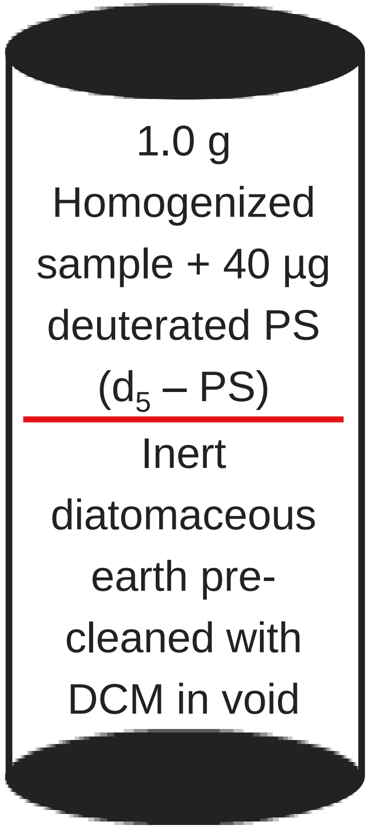
Sample Preparation Procedure

Matrix spiked store-bought rice

- Polyethylene (PE)
- Polystyrene (PS)
- Polypropylene (PP)
- Polyethylene Terephthalate (PET)
- Polycarbonate (PC)
- Polyvinyl Chloride (PVC)

Samples freeze dried then milled to fine powder for 30 min.

Glass fiber filter



Glass fiber filter

10mL ASE Cell

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ASE extraction conditions

Parameter	Extraction parameters
Cell type	Stainless steel
Extraction solvent	Dichloromethane (DCM)
Extraction/Oven temperature (°C)	180
Static time	5
Cycles	2
Rinse volume (%)	80
Purge time (s)	75
System rinse volume (mL)	9
Heating time (min)	9
Pressure (psi)	1,500

After Extraction

- 80 µL extract transferred into a pyrolysis cup
- Evaporated for 30 min in fume hood
- Loaded onto autosampler for Pyr-GC-MS

The six polymers investigated remained sufficiently dissolved for over 2.5 hours post extraction

Pyrolysis GC-MS parameters

Parameter	Setting
Micro-furnace Multi-Shot Pyrolyzer™ (Double-Shot analysis) EGA/PY-3030D (Frontier Lab)	
First-shot furnace temperature (thermal desorption)	Ramped from 100 °C → 20 °C /min → 300 °C (1 min)
Second-shot furnace temperature (pyrolysis)	650 °C
Interface temperature	320 °C
Pyrolysis time	0.20 min (12 s)
GC	
Column	Ultra Alloy™ 5 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (Frontier Lab)
Injector port temperature	300 °C
Column oven temperature program	40 °C (2 min) → (20 °C /min) → 320 °C (14 min)
Injector mode	Split/splitless (split 50:1)
Carrier gas	Helium, 1.0 mL/min, constant linear velocity
MS	
Ion source temperature	250 °C
Ionization energy	Electron ionization (EI); 70 eV
Scan range	40 to 600 *m/z*

Polymer targets and recoveries

Plastic type	Pyrolysis product	Indicator ions (*m/z*)	Molecular ion (*m/z*)	LOQ (µg/g)
PP	2,4-dimethyl-1-heptene	70, 83, ***126***	126	1.25
PS	5-hexene-1,3,5-triyltribenzene (styrene trimer)	***91,*** 117, 194, 312	312	0.94
PET	Dimethyl terephthalate* Vinyl benzoate	194, ***163*** ***105,*** 77, 148, 51	194 148	2.86
PC	Bisphenol A (BA)	***213,*** 119, 91, 165, 228	242	1.73
PE	n-alkene (C_{10}, C_{12}, C_{14})	***83,*** 111, 140	140, 196	3.95
PVC	Naphthalene	***128,*** 132, 146, 116, 102	128	3.97
Internal standard				
Polystyrene-d_5	Styrene monomer	***109,*** 82, 54, 107, 108		

*Only after TMAH treatment

	PE	PP	PET (VB)	PET*	PS	PVC	PC
Spike 1	95	96	99	91	86	80	128
Spike 2	75	75	97	66	96	73	131
Spike 3	86	78	97	87	90	93	132
Avg	**85**	**83**	**97**	**81**	**90**	**82**	**130**
St Dev	**10**	**11**	**1**	**13**	**5**	**10**	**2**

*After TMAH treatment, VB: quantification using vinyl benzoate

Real world rice samples

Sample	Concentration of polyethylene (μg/g dw)				Concentration of polypropylene (μg/g dw)				Concentration of polyethylene terephthalate (μg/g dw)				Overall (μg/g dw)			
	Not shaken		Shaken		Not shaken		Shaken		Not shaken		Shaken		Not shaken		Shaken	
	Not washed	Washed	Not washed	Washed	Not washed	Washed	Not washed	Washed	Not washed	Washed	Not washed	Washed	Not washed	Washed	Not washed	Washed
1	317	143	207	166	3	3	10	<LOD	<LOD	<LOD	<LOD	<LOD	320	146	217	166
2	314	132	260	209	3	<LOD	3	<LOD	4	<LOD	8	17	322	132	272	226
3	64	47	55	51	105	<LOD	<LOD	3	<LOD	<LOD	<LOD	<LOD	168	47	55	54
4	94	51	56	51	14	10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	108	61	56	51
5	60	45	56	45	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	64	45	56	45
6	57	48	67	53	3	<LOD	7	<LOD	<LOD	<LOD	<LOD	<LOD	61	48	74	53
7	55	58	55	54	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	59	58	55	54
8	60	46	57	46	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	63	46	57	46
9	60	54	57	54	3	<LOD	3	<LOD	<LOD	<LOD	<LOD	<LOD	63	54	61	54
10	63	53	54	50	0	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	63	56	54	50
11	79	51	47	45	3	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	82	54	47	45
12	68	52	63	56	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	71	52	63	56
13	51	51	48	60	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	54	51	48	60

dw = dry weight

Find out more:


Customer note | 001201

Sample preparation

Extraction and analysis of plastics in rice samples using accelerated solvent extraction and pyrolysis-gas chromatography-mass spectrometry

Authors
Jake O'Brien, Elvis Okoffo, Cassie Rauert, Kevin Thomas
University of Queensland, Brisbane, Australia

Keywords
Accelerated solvent extraction (ASE), Dionex ASE 350 Accelerated Solvent Extractor, microplastics, nanoplastics

Goal
To describe a new method for the extraction and analysis of microplastics using accelerated solvent extraction

Introduction
Increased production and use of plastics have resulted in growth in the amount of plastic debris accumulating in the environment. This plastic debris can potentially fragment into smaller pieces, with particles <5 mm and <0.1 µm defined as microplastics (MPs) and nanoplastics (NPs), respectively.[1] Over the past decades, an increasing number of studies have reported the occurrence of MPs/NPs in the aquatic and terrestrial environments, including oceans, rivers, lakes, air, soil, and dust.[2-6] Most of the previously reported studies have typically relied on visual inspection and spectroscopic imaging approaches, reporting data on the size, shape, color, number, and polymer type of particles. These measurements may not reflect the total mass concentration of polymers in samples because the approaches are typically limited by size. The number of studies now reporting mass-based concentrations of common plastics is growing, as these methods can report the total plastic content of samples, including plastics in both the

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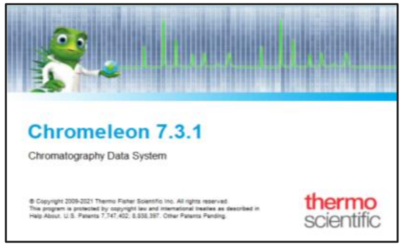
- www.thermofisher.com/microplastics

Microplastics analysis

Analysis Goal

Target analysis



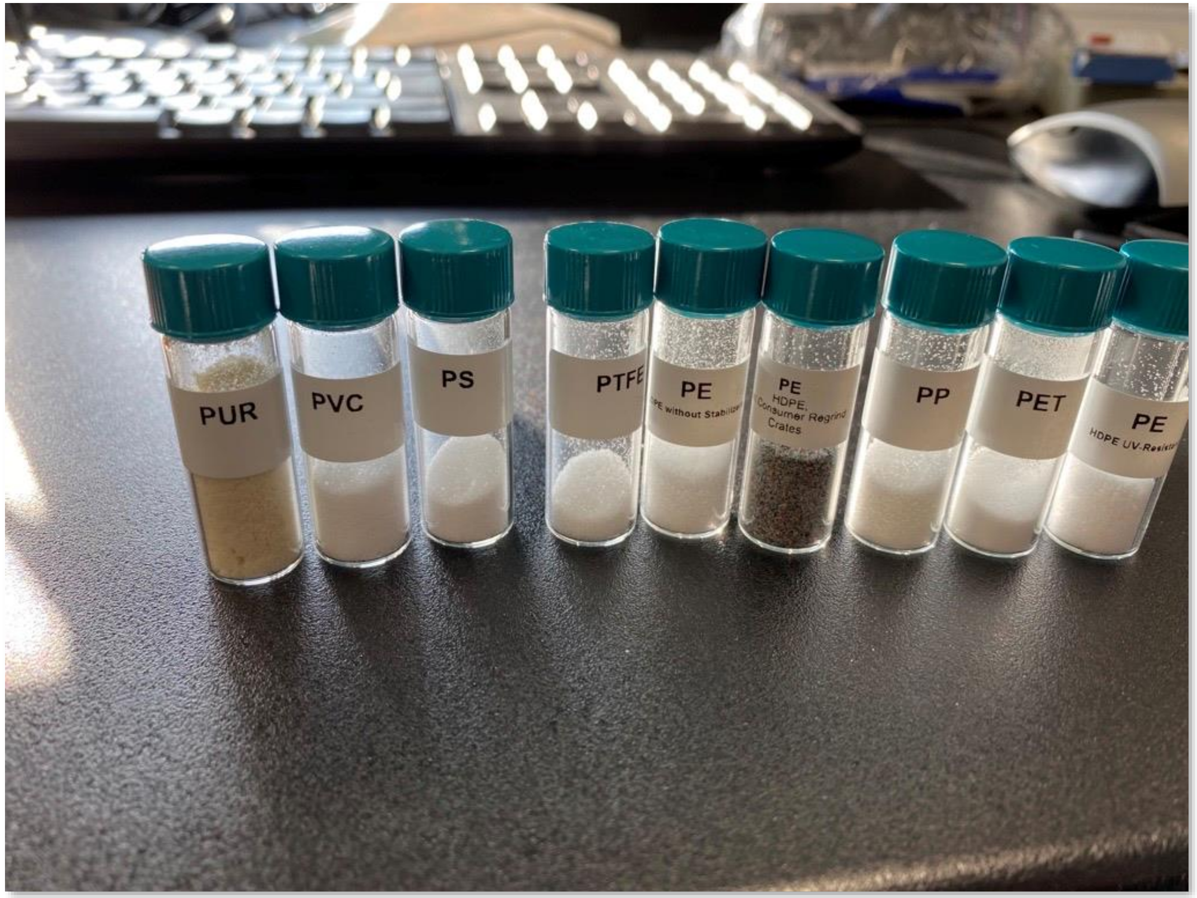
- Detection of target polymers
- Quantitation

Non-target analysis

- Detection of non-target polymers
- Identification of unknowns

Polymer standards & samples

Supplied by NILU & University of Queensland



- Polymer standards supplied by NILU
- Enables pyrolysis products to be determined
- HRAM spectrum obtained and added to library

- Two types of samples:
  - Stormwater samples (Filtration)
  - Milk & meat samples (ASE)

Preparation of milk and meat samples

Freeze dried and milled for 30 min. → Homogenize on overhead shaker at 140 rpm for 2 hours → 1g sample spiked with d_5-PS → Accelerated Solvent Extraction with ASE 350

Extraction parameter	Setting
Cell type	5 mL Stainless Steel
Extraction solvent	Dichloromethane (DCM)
Extraction temperature	180 °C
Static time	5 min.
Extraction cycles	3
Pressure	1,500 psi

- 80 µL extract transferred into a pyrolysis cup
- Evaporated in fume hood
- Loaded onto autosampler for Pyr-GC-HRAM

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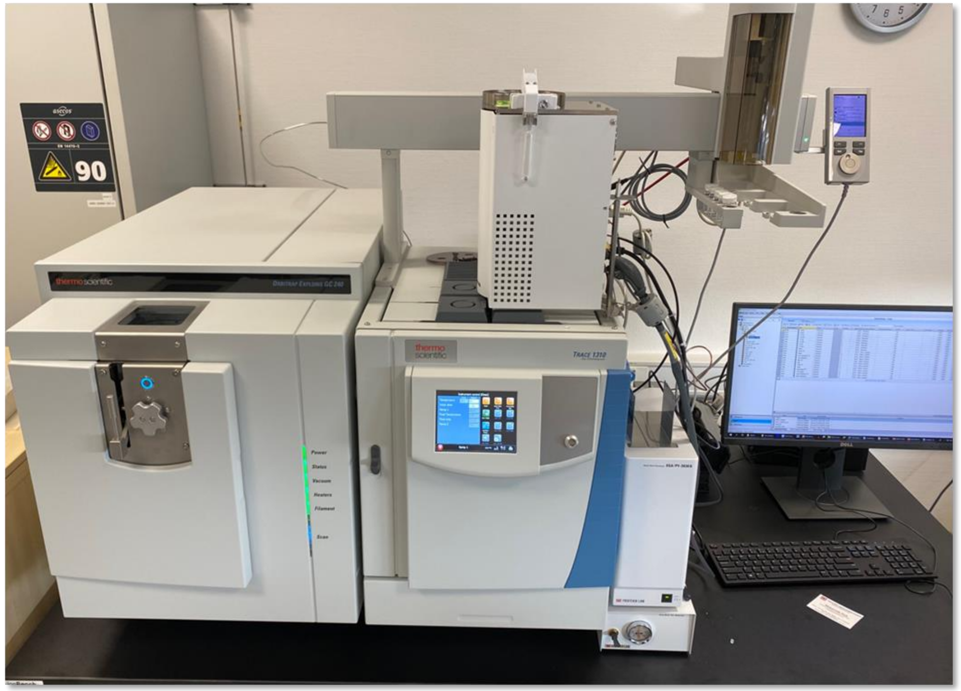
Method parameters

Multi-Shot Pyrolyzer EGA/PY-3030D parameters	
Analysis type	Double-shot analysis
Thermal desorption	
Initial (°C)	100
Initial (min)	0
Rate (°C /min)	20
Final (°C)	300
Final (min)	1
Total time (min)	11
Pyrolysis	
Initial (°C)	650
Initial (min)	0.2
Interface temperature °C	320

Trace 1310 GC System parameters	
Injector type	SSL with an adapter kit for gas injection
Injection mode	Split
Temperature (°C)	300
Split ratio	200:1
Carrier gas (mL/min)	He, 1
Oven temperature program	
Temperature 1 (°C)	40
Hold time (min)	2
Rate (°C /min)	20
Temperature 2 (°C)	320
Hold time (min)	14

Method parameters

Orbitrap Exploris GC 240 MS parameters	
Transfer line temperature (°C)	300
Ionization type	EI
Ion source temperature (°C)	280
Electron energy (eV)	70
Emission current (µA)	50
Acquisition mode	Full scan
Mass range (*m/z*)	40–600
Resolving power setting	60,000
Lock masses (*m/z*)	133.01356; 207.03235; 225.04292; 281.05114; 299.06171; 355.06993



Thermo Scientific™ Orbitrap Exploris™ GC 240

Orbitrap Exploris GC 240 with Frontier multi shot pyrolizer installed on GC

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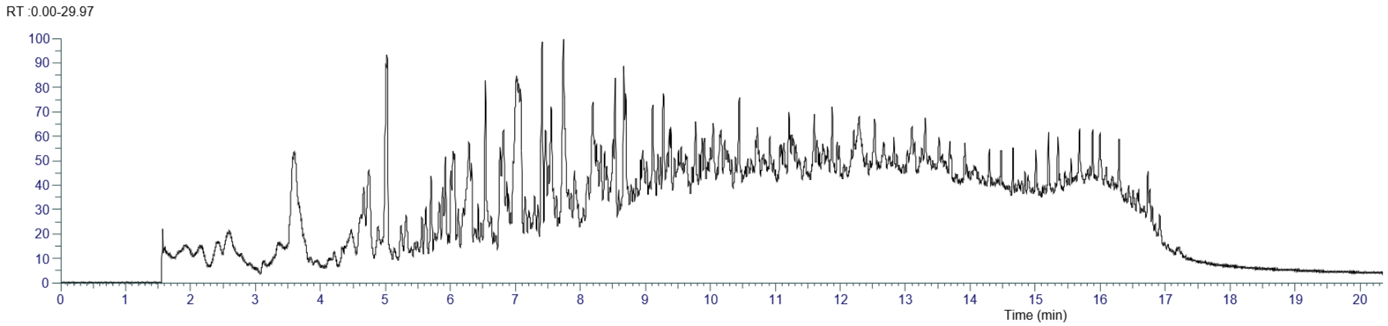
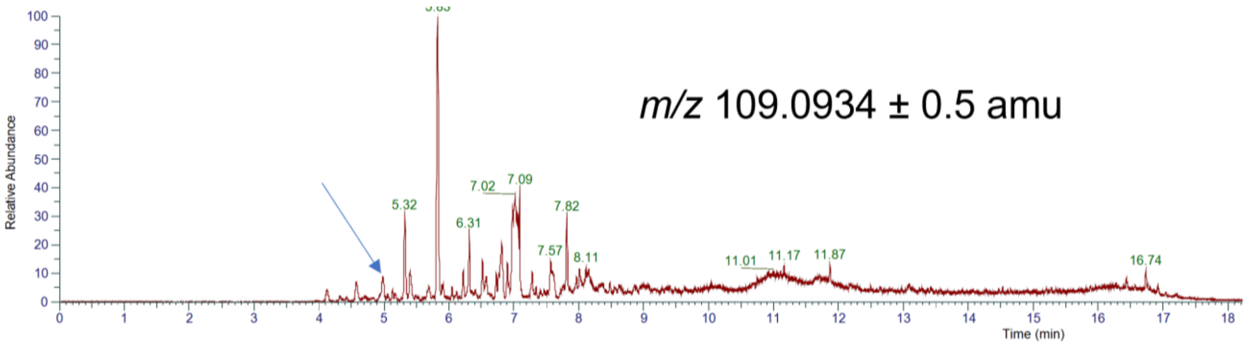
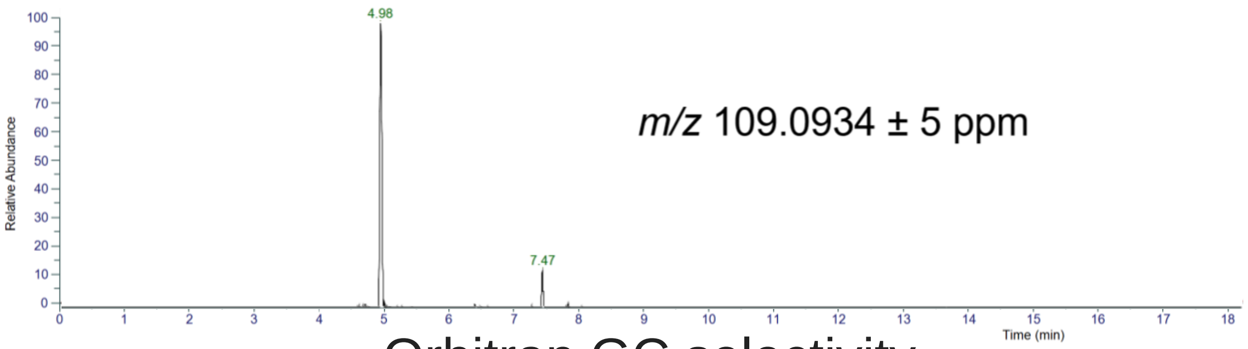
Interferences

Single quadrupole vs Orbitrap



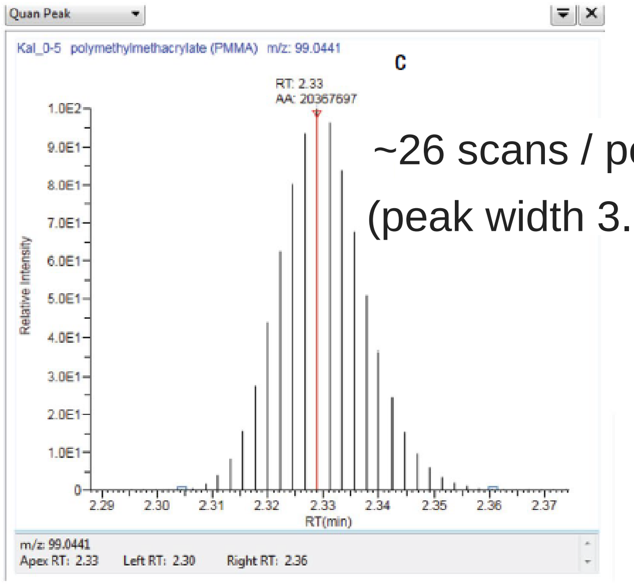


Single quad selectivity



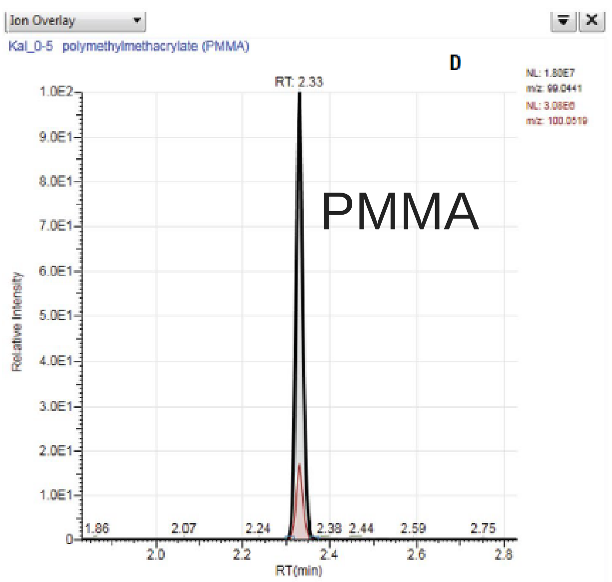
Orbitrap GC selectivity

Chromatography and recovery

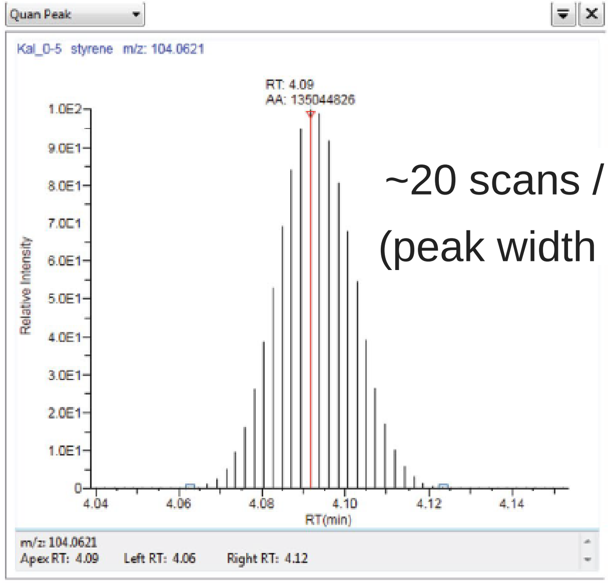


~26 scans / peak

(peak width 3.5 s)

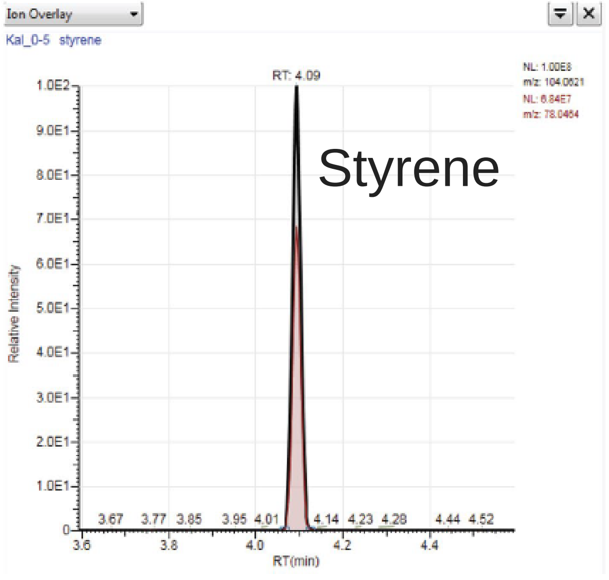


PMMA



~20 scans / peak

(peak width 3.5 s)



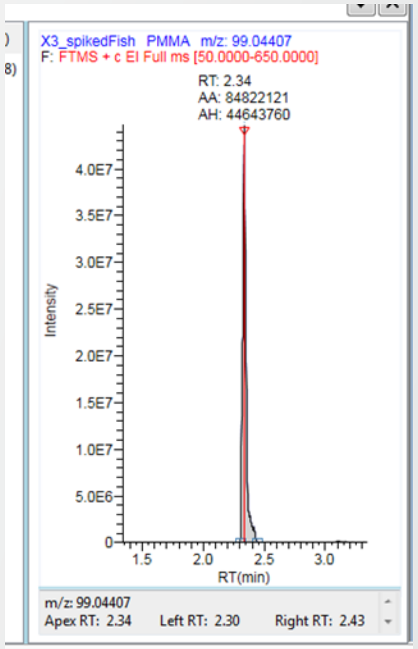
Styrene

Spiked fishmeal

Calculated amount

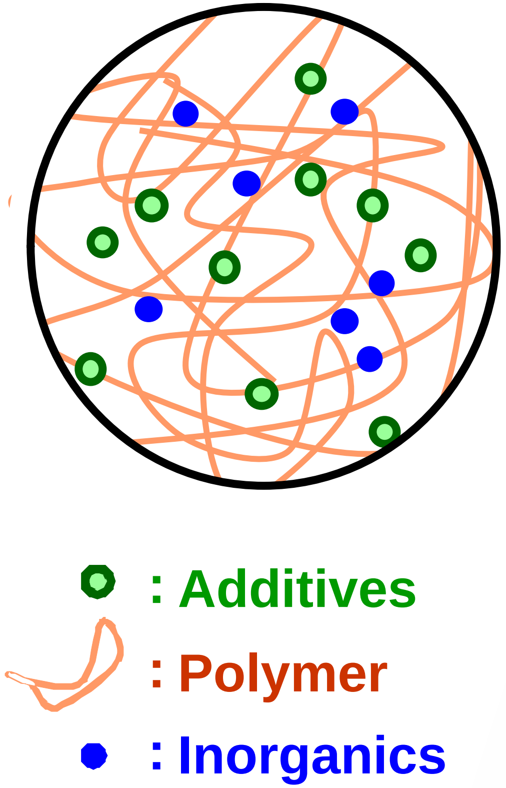
= 2.2 mg PMMA

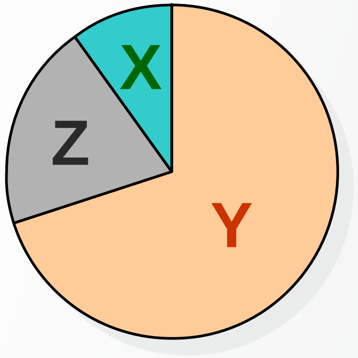
(spiked at 2.5 mg)



Pyrolysis products

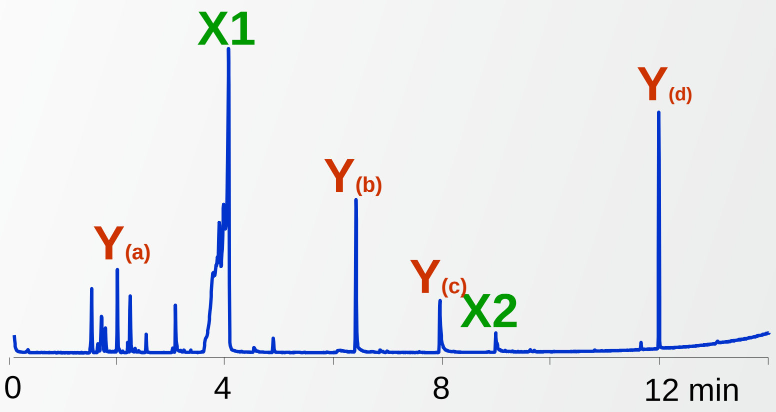
Pattern diagram of typical polymeric material





X: Additives
Y: Polymer
Z: Inorganic

Pyrolysis (Single-Shot)

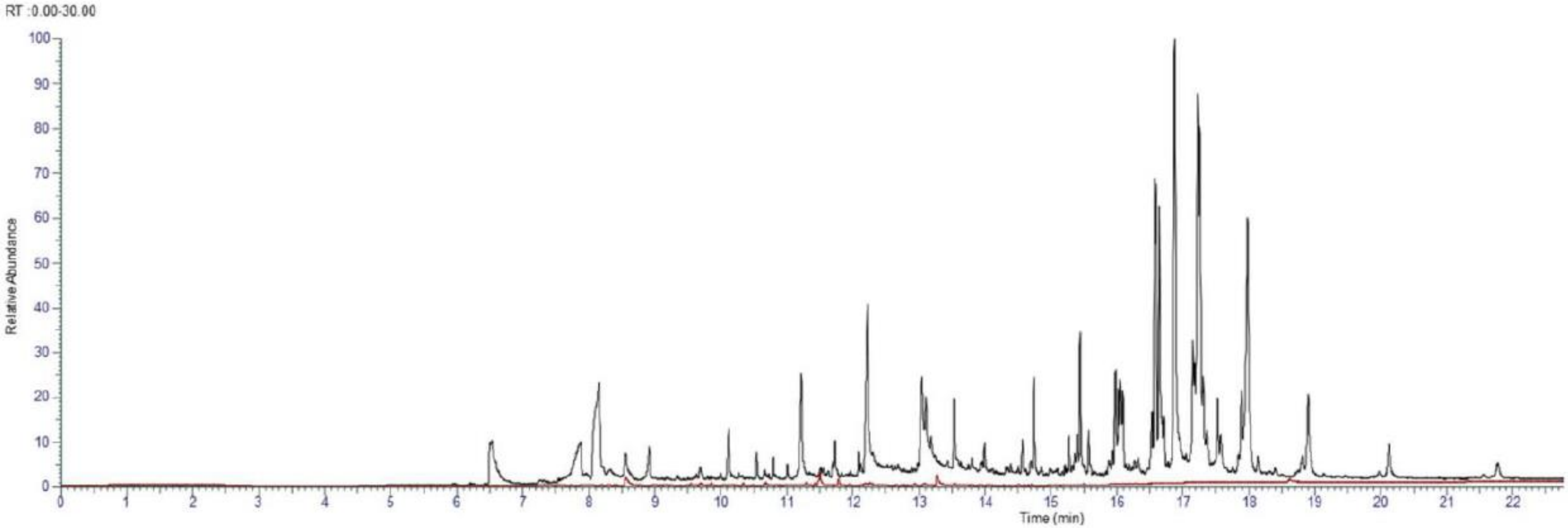


Mixed Information of polymer and volatiles

Sometimes difficult to interpret results !!

Slide by courtesy of Frontier Laboratories

Total ion chromatogram vs blank after TD



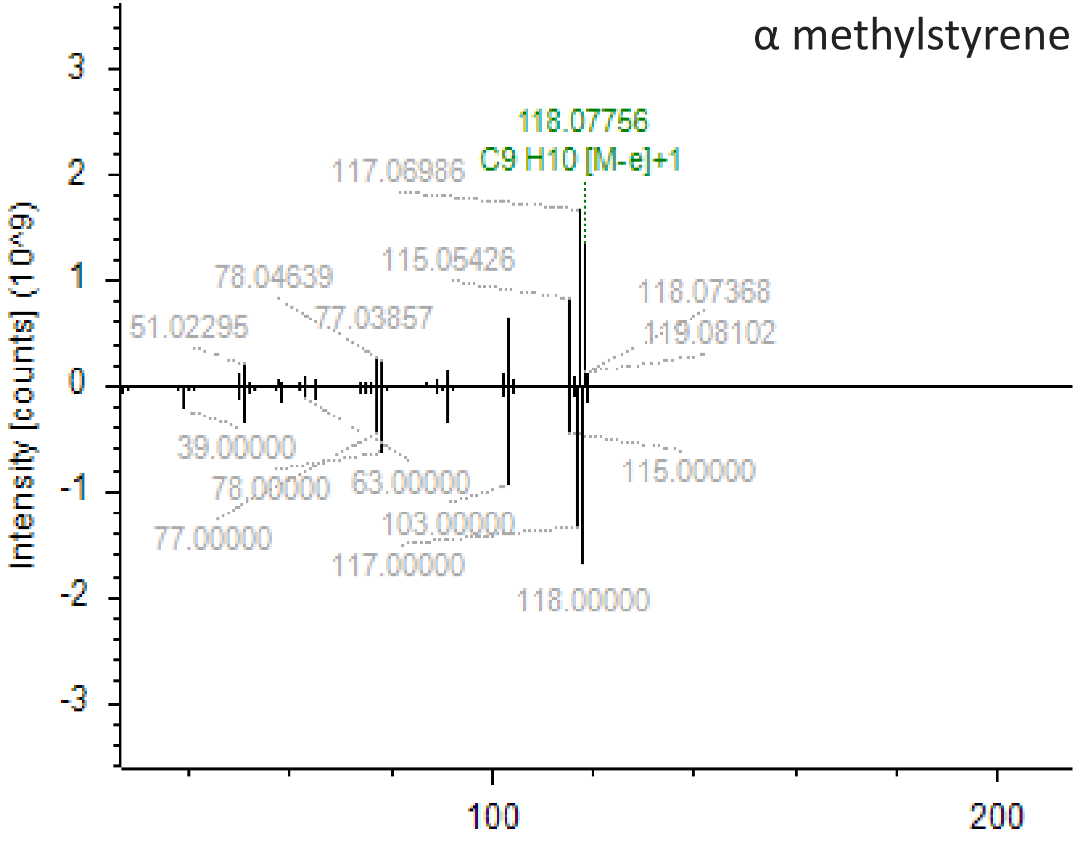
Total ion current chromatogram (m/z 40–600) obtained for a milk sample (black chromatogram) compared with a solvent standard of a mix of polymers (red chromatogram) after the thermal desorption (TD) step.

Polymers found in real samples

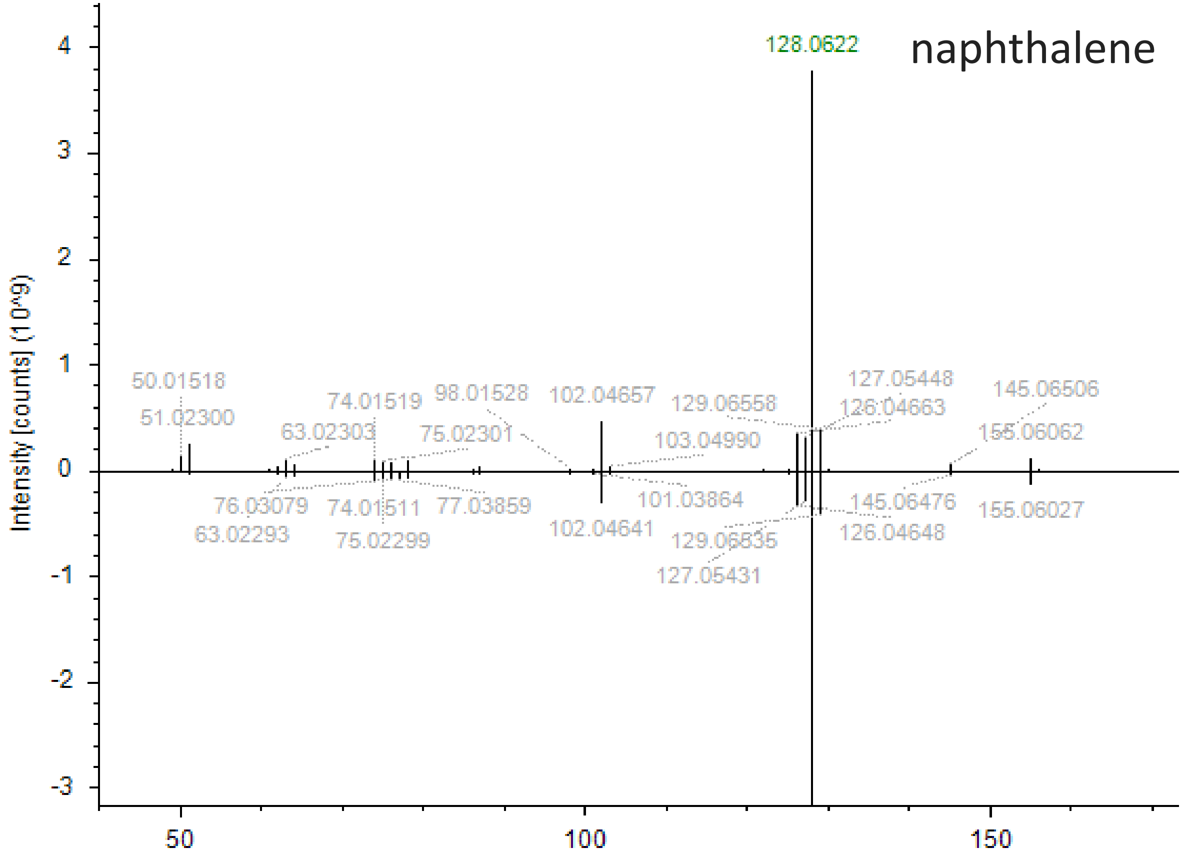
Polymer	Pyrolysis products
Polystyrene (PS)	Styrene; styrene dimer; styrene trimer; allylbenzene; α-methylstyrene; toluene
Polypropylene (PP)	2,4-dimethyl-1-heptane; 3-5-dimethyl-1-hexane
Polyvinyl chloride (PVC)	Benzene, naphthalene, fluorene
Polymethyl methacrylate (PMMA)	Methyl methacrylate
Polycarbonate (PC)	Bisphenol A
Polyethylene terephthalate (PET)	Vinyl benzoate

High resolution spectra

Matching pyrolysis products with NIST 2020 & HRAM contaminants library



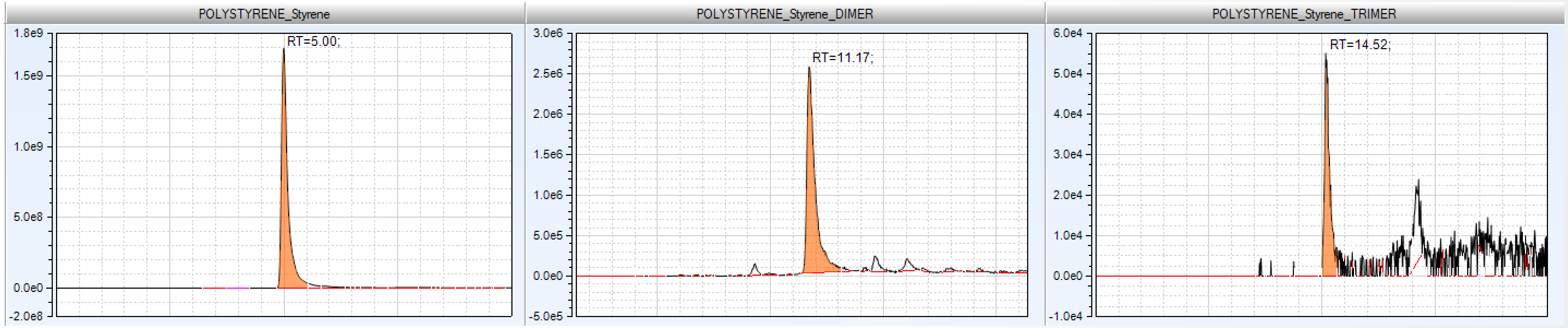
- NIST nominal mass library



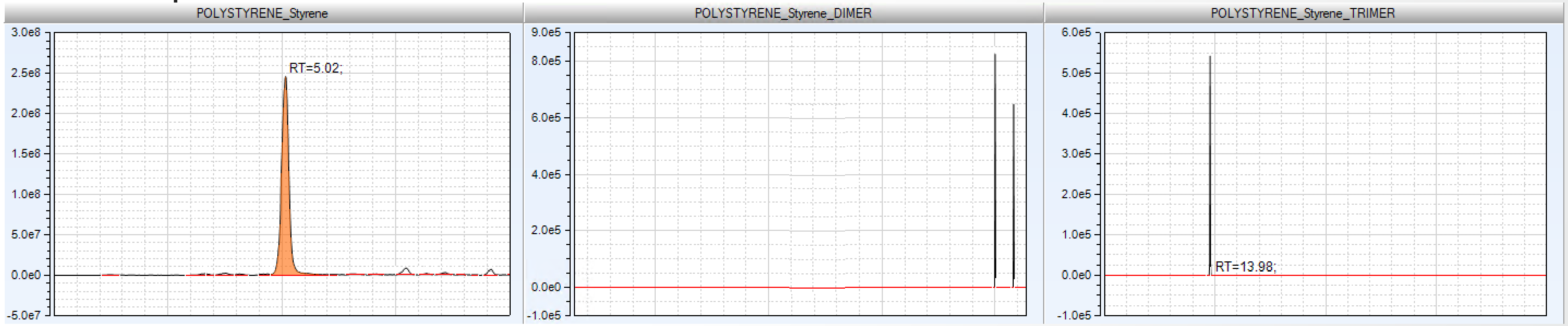
- Internal Library

Milk samples

Standard

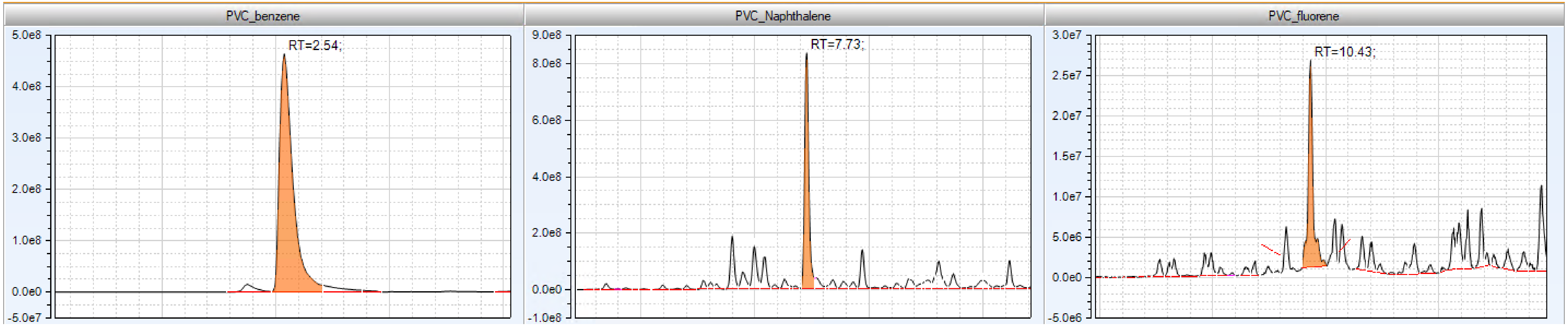


Milk sample

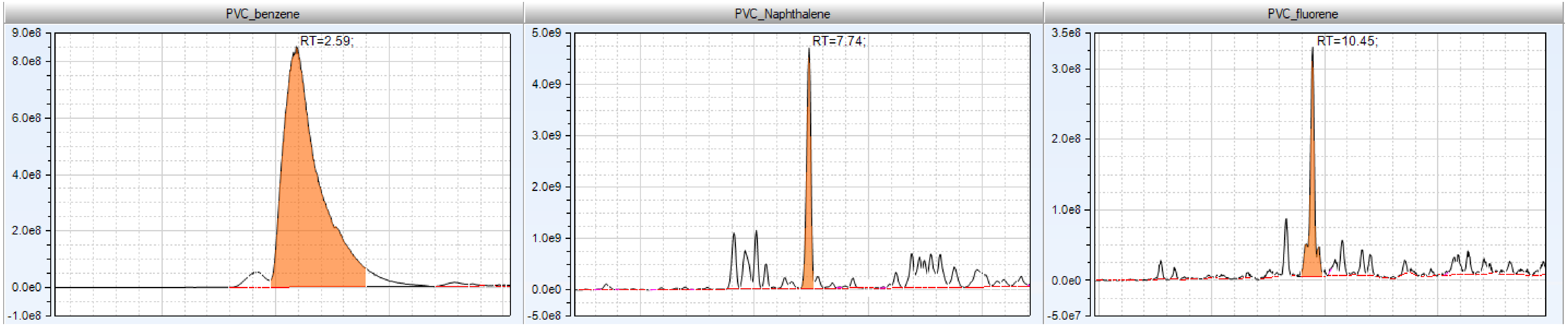


Environmental samples

Standard



Storm water sample



Conclusions

Targeted Analysis:

- Accelerated solvent extraction can extract a variety MPs/NPs including PS, PP, PE, PP, PC, and PVC from rice samples.
- Reduces processing and labor time needed to prepare MP/NP particles from samples
- Effective extraction of MPs/NPs from a wide range of environmental samples
- Rice samples that were found to contain between 17 and 317 µg/g dw of MPs/NPs and this approach has been applied elsewhere

Untargeted Analysis:

- Positive confirmation of the presence and identity of microplastics in different sample types.
- High selectivity and sensitivity were achieved by using the unique characteristics of the Orbitrap mass spectrometer, in combination with a targeted screening
- Combination of automated sample prep and automated analysis using the pyrolizer and targeted data processing enables an automated analysis of environmental samples.

Find out more:


Technical note | 001155

Environmental

Identification of microplastic particles in water and samples using pyrolysis GC combined with high re Orbitrap mass spectrometry

Authors
Łukasz Rajski[1], Daniel Kutscher[1], Dominic Roberts[2]
[1]Thermo Fisher Scientific, Bremen, Germany
[2]Thermo Fisher Scientific, Hemel Hempstead, United Kingdom

Keywords
Orbitrap Exploris GC, mass resolving power, mass accuracy, Orbitrap technology, gas chromatography, microplastics, pyrolyzer

Goal
The objective of this technical note is to demonstrate the utility of p chromatography-Orbitrap™ mass spectrometry for the detection an common polymers in food and environmental samples.

Introduction
Plastics include a wide range of synthetic and semi-synthetic poly physical and chemical characteristics, and are used in products fo day-to-day life. Large-scale production of synthetic polymers starte by the end of the last decade, it was estimated that the global plas surpassed 400 million tons per year, out of which approximately 85

Microplastics are small particles made from synthetic polymers wit ranging between 5 mm and 1 µm;[2] nanoparticles cover particles si Two sources of microplastics can be distinguished. The primary so medical products, where microparticles—typically polypropylene, p polystyrene—were added deliberately. The secondary source is de the fragmentation of larger items made from synthetic polymers th environment through inadequate disposal. The fragmentation occu stress and atmospheric conditions.[2] Some legal steps have been t of microplastics in cosmetic products; however, secondary source major contributor to microplastic pollution.[3]

Today, microplastics are present in the terrestrial and aquatic envir their small size they can migrate very quickly and can be easily inco chain.[4] Microplastics may consist of not only the pure synthetic poly residuals of the monomer, plasticizers, flame retardants, and many that can have a negative impact on human health.[5] Microplastic par incorporate other contaminants found in the environment over time.

Microplastics

Identification of microplastics in water and food using pyrolysis GC with high resolution Orbitrap mass spectrometry

Łukasz Rajski[1], Nicholas Warner[1], Vladimir Nikiforov[2] Dorte Herzke[2] and Dominic Roberts[3]
[1]Thermo Fisher Scientific, Bremen, Germany, [2] NILU, Tromso, Norway, [3]Thermo Fisher Scientific, Hemel Hempstead, United Kingdom.

Objective

Introduction

Materials and methods

Results

Conclusions

Acknowledgements

Trademarks/licensing

Learn more at thermofisher.com/OrbitrapExplorisGC240

- www.thermofisher.com/microplastics
- www.thermofisher.com/OrbitrapExplorisGC240

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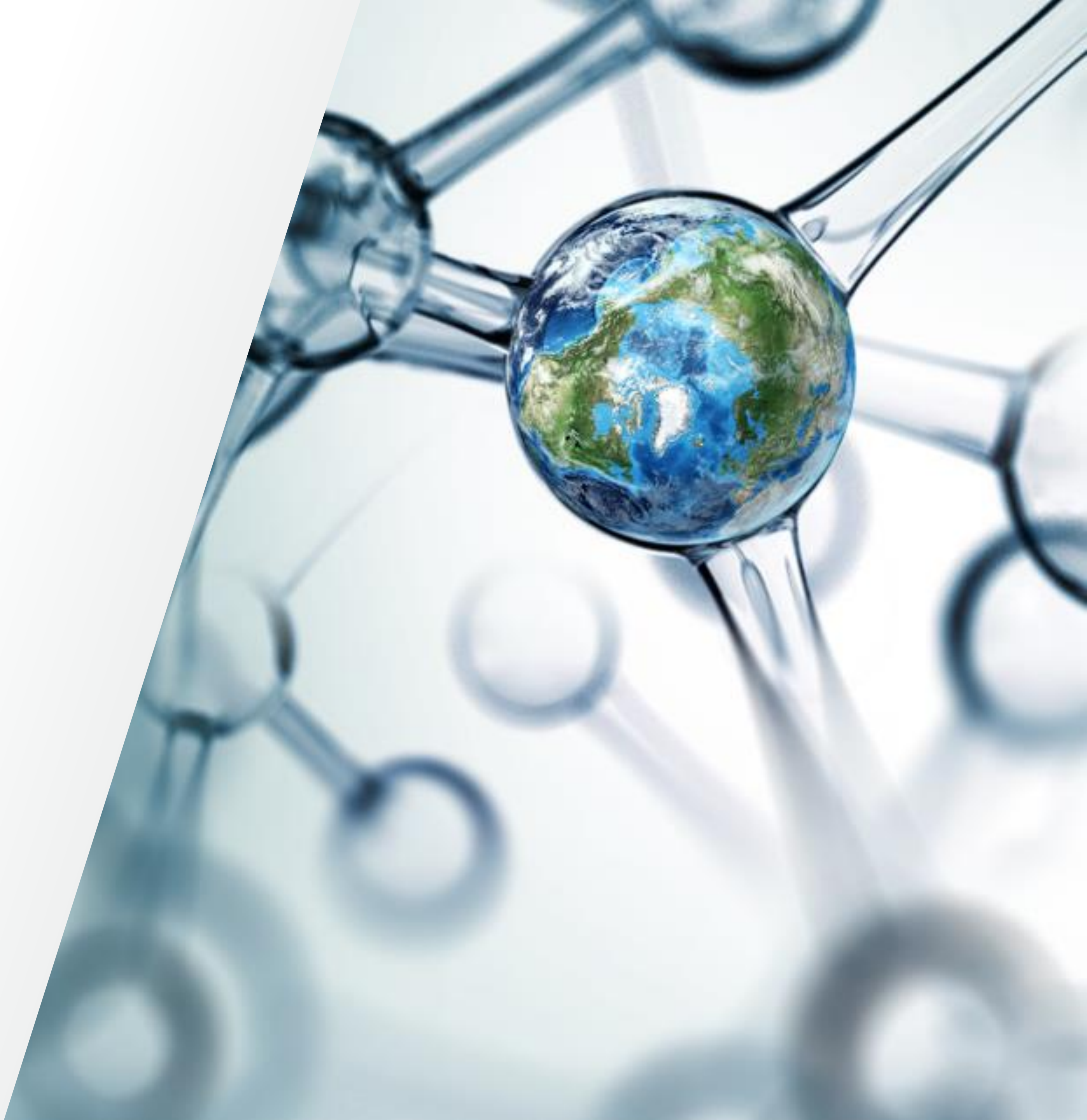
- An extra thank you to Dr Cassie Rauert for supplying milk and meat samples

NILU

- Dr. Vladimir Nikiforov and Dr. Dorte Herzke

Thank you!
Are there any questions?



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