

Media Briefings 2020

#2 Environmental Research: Igniting Innovation

Catherine Kaye EMEA PR Manager

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Media Briefing Series

'Igniting Innovation' a catalyst for the advancement of science and technology.

Showcasing the drivers of **Innovation** in today's world:

- **Product innovation** advances in technology offering new approaches that work smarter and faster for the lab of the future
- Sustainability of the lab and operations
- · Collaborations and partnerships that advance science



20 Years of **Agilent** Igniting **Innovation**



#2 Environmental Research: Igniting Innovation Today's Agenda

Speaker	Presentation
Professor Dr. Christian Zwiener University of Tübingen, Germany	Emerging pollutants (focus on per- and polyfluoroalkyl substances (PFAS)) Identifying and characterizing emerging contaminants in water treatment systems and their removal to promote safe drinking water
Professor Jes Vollertsen Aalborg University, Denmark	Microplastics here, there and everywhere Development and improvement of analytical methods to quantify microplastics in environmental matrices, with a focus on airborne microplastics
Professor Fiona Regan Dublin City University, Ireland	Optimising techniques to enhance the detection of Persistent Organic Pollutants (POPs) Emerging concerns for contaminants at low concentrations giving rise to new analytical and preparation technologies for different types of water such as ground level, surface level, and wastewater effluents
Closing remarks session	Audience Q&A and briefing recap







Mathematisch-Naturwissenschaftliche Fakultät





Emerging pollutants - PFAS

Environmental Analytical Chemistry Center for Applied Geoscience



Emerging Pollutants

- > 120,000 chemicals registered (EU)
- > 30,000 REACH chemicals in daily use
- More than 700 pollutants found in EU waters (Norman network)
- \rightarrow Challenge for
 - monitoring programs
 - assessment of fate and risks
- > Multianalyte methods, harmonized
- Low LOQs for highly hazardous compounds
- Prioritization approaches
- PC data on metabolites and pollutants
- Approaches to assess cumulative risks



"Chemicals challenge"

Our research

Occurrence and fate of polar organic pollutants in the environment and in water treatment

- Non-target screening (HRMS)
- Identification of TPs
- Electrochemistry
- Reactive membranes
- Disinfection byproducts











Our instrumentation

\rightarrow Sample preparation

automated and manual SPE (Gilson GX-271 ASPEC)



\rightarrow Target methods for multiple analytes, low LOQ

LC-TQ-MS (Agilent 6490, 6470) GC-Membrane-inlet-MS (Thermo Fisher Scientific, DSQ II MS)

→ Nontarget methods, suspect screening, high resolution and mass accuracy LC-QTOF-MS (Agilent 6550 iFunnel)





Emerging pollutants and their effects

CRC 1253

CAMP

CATCHMENTS AS REACTORS

Environmental Sciences Europe

RESEARCH

Open Access

Combining in vitro reporter gene bioassays with chemical analysis to assess changes in the water quality along the Ammer River, Southwestern Germany

Maximilian E. Müller¹, Beate I. Escher^{1,2}, Marc Schwientek¹, Martina Werneburg¹, Christiane Zarfl¹ and Christian Zwiener^{1*}

→Target methods→Cell-based bioassays

Tramadol (0.1 µg L⁻¹) TCPP (0.5 µg L⁻¹) Benzotriazole Sulfamethoxazole $(1.4 \ \mu g \ L^{-1})$ (0.2 µg L⁻¹) Oxcarbazepine (0.04 µg L⁻¹) Carbamazepine (0.2 µg L⁻¹) Metoprolol (0.1 µg L⁻ Diclofenac (0.6 µg L⁻¹) Methylbenzo-Hydrochlorotriazoles thiazide (0.5 µg L⁻¹) (0.9 µg L⁻¹)





Assessment of N-Oxide Formation during Wastewater Ozonation

Sylvain Merel, Sascha Lege, Jorge E. Yanez Heras, and Christian Zwiener*®

Environmental Analytical Chemistry, Center for Applied Geosciences, Eberhard Karls University Tübingen, Hölderlinstraße 12, 72074 Tübingen, Germany







Article

pubs.acs.org/est

TP identification



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Article

Transformation Products of Fluoxetine Formed by Photodegradation in Water and Biodegradation in Zebrafish Embryos (*Danio rerio*)

Selina Tisler,[†] Florian Zindler,[‡] Finnian Freeling,[§] Karsten Nödler,[§] László Toelgyesi,[∥] Thomas Braunbeck,[‡] and Christian Zwiener^{*,†}

Norfluoxetine Is the Only Metabolite of Fluoxetine in Zebrafish (*Danio rerio*) Embryos That Accumulates at Environmentally Relevant Exposure Scenarios

Florian Zindler,* Selina Tisler, Ann-Kathrin Loerracher, Christian Zwiener, and Thomas Braunbeck





→Metabolite ID→Effects



PFAS

- > 4700 Poly- and perfluorinated substances (PFAS; OECD portal)
- PFAS are **persistent and mobile**, accumulate in humans and in the environment
- **PFOS and PFOA** in Annex A (Stockholm Convention)
- PFOA and PFOS are priority hazardous substances (WFD 2000/60/EC)
- → Proposal of regulating PFAS as class, 0.5 µg/L group limit, 0.1 µg/L for 16 individual PFAS (European Environment Agency 2019)





PFAS contaminaton

"Rastatt Case" – Large-scale PFAS contamination

- **2013 PFC** found by chance in GW & DW in Rauental
- Monitoring showed large-scale contamination of wells
- **PFAS input** on agricultural land
- Compost and paper fibers as input sources
- \rightarrow 7.8 Mio m² agricultural soils contaminated
- → 2 Drinking water works closed, other with enhanced treatment





PFAS in paper and card board

- PFAS are oil and water repellent
- Use in food contact materials, textiles, leather, cleaning agents...
- Commercial **PFAS products** contain homologue series





HRMS screening

- LC-ESI-QTOF-MS screening of soil extract
- Data evaluation:



- Peak finding (MFE)
- mass defect filter,
- Kendrick mass analysis



HRMS screening

Analytical and Bioanalytical Chemistry https://doi.org/10.1007/s00216-019-02358-0

PAPER IN FOREFRONT

LC-MS screening of poly- and perfluoroalkyl substances in contaminated soil by Kendrick mass analysis

Boris Bugsel	Christian Zwiener	0
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Compound	Number of compounds	Mass range	Identification
PFCA	17	263 - 1013	Standard (PFOA)
PFSA	5	399 - 599	Standard (PFOS)
FTUCA	10	357 - 857	Standard (8:2 FTUCA)
FASA	4	398 - 648	Standard (FOSA)
diPAP	9	689 - 1489	Standard (6:2/8:2)
PFPIA	4	751 - 901	Homologue series
n:2 FTCA	3	427 - 527	Standard (8:2 FTCA)
n:3 Acid	6	391 - 641	Standard (7:3 Säure)
PFAL	6	297 - 547	Homologue series
MeFASA	1	512	No homologue series
EtFASA	1	526	No homologue series
diSAmPAP	8	953 - 1303	Standard (C8)
monoPAP	11	343 - 843	Standard (8:2)
EtFASAA	4	534 - 684	Standard (C8)
2H-PFCA	15	345 - 1045	Homologue series
triPAP	>4	n.a.	Homologue series
PF-polyether	4	1071 - 1221	Homologue series



- Most important precursors are diPAPs and diSAmPAP
- Degradation products: PFCAs, PFOS, N-Ethyl FASAA

Future needs and developments

- Sample prep and **separation methods** for polar pollutants
- HRMS: Enhanced resolution and accuracy \rightarrow assignment of chemical formula
- **Data evaluation:** Automated workflows for NTS data
- Cheap and **easy to use (field) analysis approaches** to monitor and control PFAS in remediation and water treatment



Thanks

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Project partners:

- Technology Center Karlsruhe TZW
- Fraunhofer Institute for Process Engineering and Packaging IVV, Freising
- Helmholtz Center for Environmental Research, Leipzig
- Agricultural Technology Center Augustenberg LTZ, Karlsruhe

Students: Boris Bugsel, Selina Tisler, Jonathan Zweigle, Rebecca Bauer, Yudha Haeqal





Microplastics here, there and everywhere

Development and improvement of analytical methods to quantify microplastics in environmental matrices, with a focus on airborne microplastics

Jes Vollertsen, Professor, Aalborg University, Denmark

Environmental Engineer with focus wastewater and stormwater

Came into microplastics research in 2016

An estimated 4.8 and 12.7 million tonnes

of plastic is emitted into the marine environment every year

How much is elsewhere? in the air you breath, the food you eat and the water you drink? What harm does it cause? What can we do about it? To answer these questions we need good analytical methods

Microplastics research grew out of the field of Marine Biological Sciences

The first quantification methods were analytically inadequate

Prohibiting comparison of data on microplastics occurrence

Today the microplastic analysis slowly moves into the field of Analytical Chemistry

The microplastic team at Aalborg University

Key lab infrastructure µFTIR imaging ATR-FTIR Pyrolysis GC-MS µRaman Single-cell ICP-MS FlowCam Clean-lab facilities Field monitoring equipment







Professors

Jes Vollertsen Asbjørn H Nielsen Diana A Stephansen

Postdocs

Alvise Vianello Inga Kirstein Claudia Lorenz Fan Liu

PhD students

Nikki van Alst Marta Simon Kristina B Olesen Lasse A Rasmussen Rupa Chand Jeanette Lykkemark Lucian Iordachescu Marziye Molazadeh Luca Maurizi Yuanli Liu

Technicians Jytte Dencker

Henrik Koch

Research assistants

Konstantinos Papacharalampos Fides Hensel









And out comes the data monster



We typically scan an area of 10x10 mm at 5.5 μ m pixel resolution to create a map of 3,211,264 individual spectra We then compare each spectrum with several hundreds reference spectra

We build our own software together with a German research group: siMPle

(MP analysis for non-nerds)



Data crunching





MP identifier	Coordinates [pixels]	Coordinates [µm]	Polymer group	Area on map [µm	2] 4ajor dimension [µm	Minor dimension [µm]	Volume [µm*]	Mass [ng]	-	What to report
MP_1	[10;36]	[55;198]	PET	2148	64.0	43.4	37775	52.129		 Show only MP Show all particle
MP_2	[31;324]	[171;1782]	PET	938	62.3	19.8	7666	10.578		
MP_3	[43;22]	[237;121]	PET	817	39.6	27.2	9222	12.726	l	Delete Part
MP_4	[50;198]	[275;1089]	PET	393	25.3	21.3	3606	4.976		
MP_5	[68;35]	[374;193]	PET	7109	123.4	73.7	210389	290.337		
MP_6	[59;406]	[325;2233]	PET	787	52.4	19.8	6481	8.944		
MP_7	[64;363]	[352;1997]	PET	605	45.5	17.8	4513	6.228		
MP_8	[68;94]	[374;517]	PET	1694	66.1	33.2	22898	31.600		
MP_9	[79;59]	[435;325]	PET	9347	169.3	70.5	264542	365.068		
MP_10	[88;406]	[484;2233]	PET	787	39.6	26.2	8575	11.833		
MP_11	[89;225]	[490;1238]	PET	1845	87.6	27.2	20443	28.211		
MP_12	[103;369]	[567;2030]	PET	908	49.7	24.0	9017	12.443		
MP_13	[109;334]	[600;1837]	PET	1029	43.4	31.1	13156	18.156		
MP_14	[137;259]	[754;1425]	PET	1876	89.1	27.2	20749	28.633		
MP_15	[145;353]	[798;1942]	PET	1059	49.1	28.2	12289	16.958		
MP_16	[158;492]	[869;2706]	PET	787	45.0	23.1	7556	10.427		
MP_17	[161;473]	[886;2602]	PET	2057	61.2	43.4	36266	50.047		
MP_18	[159;296]	[875;1628]	PET	242	22.9	15.1	1649	2.276		
MP_19	[175;117]	[963;644]	PET	4417	113.4	49.9	88831	122.587		
MP_20	[174;491]	[957;2701]	PET	2995	77.8	49.5	59870	82.621		
MP_21	[203;500]	[1117;2750]	PET	3842	115.6	42.7	66071	91.177		
					50 B		10074			

www.simple-plastics.eu

MP in indoor air



A study of microplastics in indoor air









www.nature.com/scientificreports

SCIENTIFIC **REPORTS**

OPEN Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin

Alvise Vianello 1, Rasmus Lund Jensen 1, Li Liu 2 & Jes Vollertsen 1

Received: 22 November 2018

Published online: 17 June 2019

Accepted: 29 May 2019

Humans are potentially exposed to microplastics through food, drink, and air. The first two pathways have received quite some scientific attention, while little is known about the latter. We address the exposure of humans to indoor airborne microplastics using a Breathing Thermal Manikin. Three





Gray: Natural materials Colored: Plastics

Composition of the organic material in the dust



- Proteins, for example skin and hair
- Cellulose, for example cotton
- TOT MP: all sorts of microplastics

Variations between the 3 flats



Another study of microplastics in indoor air

Microplastics in a flat in Copenhagen – a part of a Danish TV production

We measured the microplastics in the air

Air was filtered for 8 hours, analyzed on the Cary 620/670









How much microplastics does a human take in?

The comparison is done on data from Aalborg University only, so we are sure it is comparable (part is still unpublished)

20,000 pieces of plastic per year 20 pieces of plastic per year 1,500 pieces of plastic per year ? pieces of plastic per year (likely less than soft drinks?)

Indoor air ≈ 4000 m³/year Drinking water ≈ 1000 L/year Soft drinks ≈ 400 L/year Food ≈ 400 kg/year

Outlook and challenges

Routine monitoring Commercial laboratories

Standardized methods High reproducibility Low cost Fast analysis

Microplastics research Universities, research institutions, ...

High flexibility Challenge method boundaries Price and time less important

not **The End** Lets together work for that goal

Optimising techniques to enhance detection of persistent organic pollutants

Professor Fiona Regan School of Chemical Sciences

Director, Water Institute

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Introduction

DCU Water Institute

Our mission is to support society by carrying out research and development that generates innovative solutions that help to address major water challenges.



Phthalates and human health

Research Questions

- To what degree are humans exposed to phthalates?
- What do we know about how phthalates affect human health?
- Is wastewater-based epidemiology an effective tool for risk assessment of these chemicals?
- With this information what recommendations can be made for future phthalate legislation?









What is driving research efforts

Phthalate research and legislation

1.1972-Teratogenicity of phthalates first studied (rat model) 2.1973-Monesters as phthalate metabolites first (rat model) 3.1978-Monoesters as phthalate metabolites first studied (humans) 4.1982-First review on carcinogenicity of DEHP, BBP published by IARC 5.1999-First use of monoesters for human biomonitoring 6.2005a-Phthalate exposure first associated with teratogenicity in humans 13 (epidemiological cohort study n=85, OR=6.95[1.03 to 42.2]) 3000 7.2005b-EU ban on certain products containing DEHP, DBP BBP, DINP, DIDP and 2800 11. DNOP 2 2600 8.2008-US ban on certain products containing DEHP, DBP BBP, DINP, DIDP and DNOP 2400 9.2011- Canada ban on certain products containing 2200 2000 2000 DEHP, DBP BBP, DINP, DIDP and DNOP 9 10.2012-Updated DEHP review from IARC and US EPA Phthalates Action Plan 1800 8 11.2014a-US EPA Issue Significant New Use Rule for DnPP 1600 12.2014b-CHAP Report from US Consumer Product Safety Commission 6,7 **Jacobia** 1400 1200 1000 13.2015-DEHP banned in medical devices used for neonates 800 600 400 200 0 1970 1975 1980 1985 1990 1995 2000 2005 2010 2015 Year —Phthalate Adverse Effects Phthalate Biomonitoring Phthalate Sewage



đ

European Chemicals Agency (ECHA)

- Recommendation to the European Commission

- ECHA has submitted a recommendation to the European Commission to amend Authorisation List (Annex XIV of REACH) entries by adding the endocrine disrupting properties of four phthalates.
- Helsinki, 10 July 2019 ECHA has prepared a recommendation to amend the Authorisation List to include endocrine disrupting properties into the respective entries of:
- bis(2-ethylhexyl) phthalate (DEHP) (EC 204-211-0, CAS 117-81-7)
- benzyl butyl phthalate (BBP) (EC 201-622-7, CAS 85-68-7)
- dibutyl phthalate (DBP) (EC 201-557-4, CAS 84-74-2)
- diisobutyl phthalate (DIBP) (EC 201-553-2, CAS 84-69-5).



Legislation

- They were identified as substances of very high concern (SVHCs) due to endocrine disrupting properties with effects on human health.
- DEHP was also identified for its effects on the environment.
- The Candidate List entries for these substances were updated accordingly in 2014 and 2017.
- These four phthalates had earlier been identified as SVHCs (in 2008 and 2009) and subsequently added to the Authorisation List in 2011 and 2012 due to their classification as toxic for reproduction.



Cycle of Phthalates and their Metabolites in the WWTP

Phthalates are ubiquitous synthetic organic compounds

- Plasticizers
- Endocrine disruptors
- Banned/limited in manufacturing

Exposure routes:

- Ingestion
- Inhalation
- Absorption

Health Impacts:

- Male Birth Defects
- Impaired neurological development in children
- Obesity







Identifying and characterizing POPs



Environmental Analytical Approach

Rationale	Proposed			
Small scale WWTP	Influent (diester and monoester), Effluent, Sludge			
Medium scale WWTP	Influent (diester and monoester), Effluent, Sludge			
Large scale WWTP	Influent (diester and monoester), Effluent, Sludge			
Surface water catchment	12 months grab			
Soil catchment	1 x winter			
(2 x farms, Tolka Park, industrial estate)	1 x summer			
	Starting July 2018			
Landfill + groundwater	4 quarterly samples starting Aug 2018			
Recycling plant	1 of each			
Green, brown, black	Dec. 2017			
Passive samples	2 deployments winter and			
	summer			

Contamination Control in Phthalate Analysis

Reduce Contamination:

- Extensive glassware preparation with quality controlled solvents
- Minimal handling/analysis steps
- Quality checks on each batch of solvent

Engineering Controls:

- Stainless steel fittings,
- Delay column fitted
- Multi-wash system and 3 analytical blanks are run between each sample

Eliminate Residual Background: Subtract Procedural Blank



Analytical Blanks Multi-wash



Delay Column

Method Development - Extraction

Solid Phase Extraction (SPE):

water, leachate, sludge (after preprep)

Strata-X Teflon cartridges

- Condition: 20 mL of ACN
- Equilibrate: 20 mL of water
- Load: Sample (500 mL 1 L of sample and matrix very slowly)
- Wash: 20 mL of (40:60) Methanol:Water
- Dry: 10 min under full vacuum
 - Elute: 20 mL of ACN



Sample Extraction



Soxhlet Extraction (SE): solids, plastics

- 1 g of material
- Place in round bottom flask and add 100 mL dichloromethane
- Assemble SE apparatus
- Bring to 40°C and maintain for 16 h





Phthalate target ions Log Kow 1.6 - 10

Peak No.	Compound	Precurser Ion	Product Ion
1	Dimethylphthalate	195.1	162.9
	(DMP)	195.1	77
0	Benzylbutylphthalate	313.2	91
Ζ	(BBP)	313.2	148.9
R	Diisobutylphthalate	279.2	205.1
0	(DIBP)	279.2	149
4	Dibutylphthalate	279.2	205
	(DBP)	279.2	148.9
5	Diisopentylphthalate	307.18	149
5	(DIPP)	307.18	77.1
6	Dipentylphthalate	307.2	219
0	(DNPP)	307.2	148.9

Peak No.	Compound	Precurser Ion	Product Ion
7	Dihexylphthalate	335.2	233
1	(DHP)	335.2	148.9
	Diethylhexylphthalate	391.3	279
8	(DEHP)	391.3	148.9
	Di-n-octvlphthalate	391.3	166.9
9	(DNOP)	391.3	148.9
10	Diisononylphthalate	419.31	148.9
10	(DINP)	419.31	71.1
11	Diisodecylphthalate	447.3	141.1
11	(DIDP)	447.3	85.1



Diester Separation

Column: 2.1 x150 mm, 2.7 μ m internal diameter Poroshell, temperature 60^o C

Mobile Phase: Water 50:50 MeOH:ACN Gradient: 0 min 60%B, 2.0 min 80% B, 5.0 min 100%B, 9.1 min 60% B

Flow Rate: 0.4 mL/min Injection Volume: 2 μL



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Application to soil

Peak ID

1. DMP 8. DEHP 11 DIDP





Phthalate Biomarkers

DCU

Parent Phthalate	Phthalate Biomarker	Cas No.	Structure	Molecular weight (g/mol)	Log Kow	РКа	Peak No
Benzylbutyl phthalate (BBP)	Mono-benzyl phthalate (MBzP)	2528-16-7		256.26	3.14	3.22	3
Dibutyl phthalate (DBP)	Monobutyl phthalate (MBP)	34-74-2	С С С С С С С С С С С С С С С С С С С	222.09	2.65	3.292	2
Diisobutyl phthalate (DiBP)	Monoisobutyl phthalate (MiBP)	30833-53-5		222.09	2.5	3.08	1
Diethylhexyl phthalate (DEHP)	Monoexylhexyl phthalate (MEHP)	4376-20-9	CH CH	278.35	4.3	3.266	4
Di-n-octyl phthalate (DNOP)	Mono-n-octyl phthalate (MNOP)	5393-19-1		278.35	4.32	3.29	5
Diisononyl phthalate (DINP)	Monoisononyl phthalate (MINP)	68515-53-7	J SH J	292.38	4.65	3.289	6

Monoester Calibration



Samples are pre-concentrated by a factor of 100





Innovation and technology as an engine for environmental research

Future challenges of POPs monitoring

- Real need for easy to use sample handling systems

 large volume samples
 needed for environmental
 assessment
- Innovate in effect-based tools that can link chemistry with toxic effect or risk



Dulio et al. Environ Sci Eur (2018) 30:5



How do we monitor into the future?

ANNEX

Watch list of substances for Union-wide monitoring as set out in Article 8b of Directive 2008/105/EC

Name of substance/group of substances	CAS number (1)	EU number (2)	Indicative analytical method (3) (4)	Maximum acceptable method detection limit (ng/l)
17-Alpha-ethinylestradiol (EE2)	57-63-6	200-342-2	Large-volume SPE - LC- MS-MS	0,035
17-Beta-estradiol (E2), Estrone (E1)	50-28-2, 53-16-7	200-023-8	SPE - LC-MS-MS	0,4
Macrolide antibiotics (5)			SPE - LC-MS-MS	19
Methiocarb	2032-65-7	217-991-2	SPE - LC-MS-MS or GC-MS	2
Neonicotinoids (⁶)			SPE - LC-MS-MS	8,3
Metaflumizone	139968-49-3	604-167-6	LLE - LC-MS-MS or SPE - LC-MS-MS	65
Amoxicillin	26787-78-0	248-003-8	SPE - LC-MS-MS	78
Ciprofloxacin	85721-33-1	617-751-0	SPE - LC-MS-MS	89

(1) Chemical Abstracts Service

(2) European Union number - not available for all substances

(*) To ensure comparability of results from different Member States, all substances shall be monitored in whole water samples.

(4) Extraction methods:

LLE — liquid liquid extraction

SPE — solid-phase extraction

Analytical methods:

GC-MS — Gas chromatography-mass spectrometry

LC-MS-MS — Liquid chromatography (tandem) triple quadrupole mass spectrometry

(⁵) Erythromycin (CAS number 114-07-8, EU number 204-040-1), Clarithromycin (CAS number 81103-11-9), Azithromycin (CAS number 83905-01-5, EU number 617-500-5)

in the second second

NEED: new biosensor technologies and effect-based biomonitoring tools





Wastewater process streams a biomarker for human health

Aim: To study the value of phthalates as a biomarker of human health in human toxicology.

Deliverables:

Analysis of phthalate levels in wastewater process streams as indicated by phthalate metabolites Assessment of phthalates in wastewater process streams informing on human exposure, body burden and health



- @dcuwater
- www.dcuwater.ie

Thankyou.

Questions & Answers

Please use the Q&A chat function or "raise your hand" to ask a question to our panelists.





Thank You!

Look out for your invitation to:

#3 Efficiency & Sustainability: Igniting Innovation