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Contact: Karolina Polska

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TABLE OF CONTENTS

Scroll to the title and select a **Blue** link to open a paper. After viewing the paper, use the bookmarks to the left to return to the Table of Contents.

Monday, June 5, 2023

15:00

Remy Franken[1], Neeraj Shandilya[1], Marie-Sophie Barreau[1], Blanca Suarez-Merino[2], Andrea Porcari[3], Daniela Pimponi[3], Keld Alstrup Jensen[4], Wouter Fransman[1]
[1]Netherlands Organisation for Applied Scientific Research (TNO), The Netherlands; [2]TEMAS Solutions GmbH, Switzerland; [3]Italian Association for Industrial Research, Italy; [4]National Research Centre for the Working Environment (NRCWE), Denmark

15:15

Elisa Moschini[1], Panagiotis Isigonis[2], Evert Bouman[3], Shareen Doak[4], Eleonora Longhin[3], Iseult Lynch[5], Ineke Malsch[6], Tommaso Serchi[1], Christoph Steinbach[7], Arno Gutleb[1], Maria Dusinska[3], Riskgone Consortium[8]
[1]Luxembourg Institute of Science and Technology, Luxembourg; [2]Ca' Foscari University of Venice, Italy; [3]Norwegian Institute for Air Research, Norway; [4]Swansea University, UK; [5]University of Birmingham, United Kingdom; [6]Malsch TechnoValuation, The Netherlands; [7]DECHEMA e.V., Germany; [8]RISKGONE project, Norway

15:30

Dalila Antunes, Benjamin Douglas Trump, Igor Linkov
Factor Social / ISCSP - Universidade de Lisboa, Portugal

16:30 – 18:00
Data-driven in Silico Methods

Chairs: Antreas Afantitis, *NovaMechanics Ltd, Cyprus*
Tomasz Puzyn, *QSAR Lab, Poland*

16:30

Tomasz Puzyn[1,2]
[1]University of Gdansk, Poland; [2]QSAR Lab Ltd., Poland

16:45

Ntsikelelo Yalezo, Ndeke Musee
University of Pretoria, South Africa

17:00

Georgia Tsiliki[1,2], Georgios Drakakis[1], Alex Zabeo[3], Vicki Stone[4], Danail Hristozov[5]
[1]Purposeful IKE, Greece; [2]Athena Research Center, Greece; [3]GreenDesicion Srl, Italy; [4]Heriot-Watt University, United Kingdom; [5]Emerge Srl, Bulgaria

17:15

Jeaphianne P.M. van Rijn, Egon L. Willighagen
Maastricht University, The Netherlands

17:30

Ceyda Oksel Karakus, Eyüp Bilgi
Izmir Institute of Technology, Turkey

16:30 – 18:00
Methods, Tools, & Technologies for SSbD Purposes 1
Chairs: Andrew Nelson, *University of Leeds, UK*
Carlos Fito, *ITENE, Spain*

16:30

Guillaume Phan[1], Adeline Tarantini[2], Emilie Jamet-Anselme[1], Vincent Haute[2], Sabine Lam[1], Alexandre Legrand[1], David Suhard[1], Véronique Chamel[2], Céline Bouvier-Capely[1]
[1]Institut de Radioprotection et de Sûreté Nucléaire, France; [2]Commissariat à l'Energie Atomique et aux Energies Alternatives, France

16:45

Lianyong Han[1], Carola Voss[1], Meshal Ansari[1], Qiaoxia Zhou[1], Verena Haefner[1], Ulla Vogel[2], Markus Rehberg[1], Herbert B. Schiller[1], Tobias Stoeger[1]
[1]Helmholtz Zentrum München, Germany; [2]National Research Centre for the Working Environment, Denmark

17:00

Maelle Fontaine, Eline Bartolami, Marion Prono, David Béal, Marie Carrière
Univ. Grenoble-Alpes, CEA, CNRS, IRIG, France

17:15

Ozge Kose[1], Magda Blosi[2], Rossella Bengalli[3], Anna Costa[2], Paride Mantecca[3], Marie Carrière[1]
[1]Univ. Grenoble Alpes, CEA, CNRS, IRIG, France; [2]CNR-ISTEC, Italy; [3]University of Milano-Bicocca, Italy

17:30

Elisa Moschini[1], Antje Biesemeier[2], Naila Rajabli[1], Sabina Burla[1,3], Sebastien Cambier[1], Elorri Igos[1], Ivonne Trebs[1], Thomas Schleeh[1], Ruth Moeller[1,4], Arno C. Gutleb[1,3]
[1]Luxembourg Institute of Science and Technology (LIST), Luxembourg; [2]INVITROLIZE sarl, Luxembourg; [3]Laboratoire National de Santé (LNS), Luxembourg

Tuesday, June 6, 2023

9:45 – 10:45
In Silico Tools & For Comprehensive Modeling
Chairs: Antreas Afantitis, *NovaMechanics Ltd, Cyprus*
Tomasz Puzyn, *QSAR Lab, Poland*

9:45

Alex Zabeo, Gianpietro Basei, Matteo Carisi, Giacomo Chiarot, Fabio Rosada, Danail Hristozov
GreenDesicion Srl, Italy

10:00

Alex Zabeo[1], Gianpietro Basei[1], Nicola Toschi[2], Antonio Pietroiusti[2], Kostas Chairetakis[3], Effie Marcoulaki[3], Lisa Pizzol[1], Matteo Carisi[1], Fabio Rosada[1], Danail Hristozov[4]
[1]GreenDecision Srl, Italy; [2]Univeristà degli studi di Roma Tor Vergata, Italy; [3]NCSR "Demokritos", Greece; [4]East European Research and Innovation Enterprise, Bulgaria

10:15

Hyunjoo Hong[1], Florian Part[2], Bernd Nowack[1]
[1]Empa, Swiss Federal Laboratories for Materials Science and Technology; [2]University of Natural Resources and Life Sciences, Austria

10:30

Janez Štrancar[1], Tilen Koklič[2], Hana Kokot[2], Iztok Urbančič[2]
[1]Infinite Biotech, Slovenia; [2]Jozef Stefan Institute, Slovenia

9:45 – 10:45
Methods, Tools, & Technologies for SSbD Purposes 2

Chairs: Andreas Falk, *BioNanoNet, Austria*
Andrew Nelson, *University of Leeds, UK*

9:45

Victoria Xenaki[1], Ying Xue[1], Stein Atle Lie[1], Mihaela Cuida Marthinussen[1,2], Anne Nordrehaug Åstrøm[1], Daniela Elena Costea[1,3], Mihaela Roxana Cimpan[1]
[1]University of Bergen, Norway; [2]Oral Health Centre of Expertise in Western Norway, Norway; [3]Haukeland University Hospital, Norway

10:00

Lara Faccani[1], Rossella Bengalli[2], Maurizio Gualtieri[2], Felice Carlo Simeone[1], Paride Mantecca[2], Magda Blosi[1], Anna Luisa Costa[1]
[1]CNR-ISSMC, Italy; [2]University of Milano - Bicocca, Italy

10:15

Nienke Ruijter[1], Matthew Boyles[2], Marie Carrière[3], Alberto Katsumiti[4], Ana Candalija[5], Joan Cabellos[5], Rafael Ayerbe[5], Isabel Rodríguez-Llopis[4], Jessica Marshall[2], Flemming R. Cassee[1,6], Hedwig Braakhuis[1]
[1]National Institute for Public Health and the Environment (RIVM), The Netherlands; [2]Institute of Occupational Medicine (IOM), United Kingdom; [3]Univ. Grenoble-Alpes, CEA, CNRS, France; [4]GAIKER Technology Centre, Spain; [5]Leitat Technological Center, Spain; [6]Utrecht University, The Netherlands

10:30

Rossella Bengalli[1], Cinzia Bragato[1], Patrizia Bonfanti[1], Roberta Mazzotta[1], Filipa Oliveira Gomes[2], Adriana Moreira[2], Marcelo Oliveira[2], Juliana Oliveira[2], Mariana Ornelas[2], Maurizio Gualtieri[1], Paride Mantecca[1]
[1]University of Milano Bicocca, Italy; [2]CeNTI-Centre of Nanotechnology and Smart Materials, Portugal

11:15 – 12:30
Characterization of Advanced Materials, Including Nano Materials 1

Chairs: Eva Valsami-Jones, *University of Birmingham, UK*
Miguel Banares, *CSIC, Spain*

11:15

Nathalia S. Barbosa, Cynthia Silva de Oliveira, Giselle Cerchiaro, Amedea B. Seabra
Federal University of ABC (UFABC), Brazil

11:30

11:45

12:00

12:15

12:30

11:15 – 12:30
Methods, Tools, & Technologies for SSbD Purposes 3

Chairs: Andreas Falk, *BioNanoNet, Austria*
Araceli Jimenez Sanchez, *INSST, Spain*

11:15

11:30

11:45

12:00

12:15

Bernd Nowack[1], Jaco Westra[2], Spyros Karakitsios[3], Ivo Iavicoli[4], Denis Sarigiannis[3]
[1]Empa, Swiss Federal Laboratories for Materials Science and Technology; [2]National Institute for Public Health and the Environment (RIVM), The Netherlands; [3]Aristotle University of Thessaloniki, Greece; [4]University of Naples, Italy

12:30

Jeaphianne P.M. van Rijn, Egon L. Willighagen
Maastricht University, The Netherlands

11:15 – 12:30
Harmonization & Standardization in the Context of Regulation 1

Chairs: Elisabeth Heunisch, *Federal Institute of Occupational Safety and Health, Germany*
Flemming Cassee, *RIVM, The Netherlands*

11:15

Yi Zhang, Kathy Nguyen, Andrew Belknap, Mainul Husain, Amanda Bard, Djordje Vladisavljevic
Health Canada, Canada

11:30

Eric A.J. Bleeker[1], Elmer Swart[1], Hedwig Braakhuis[1], Maria Luisa Fernández-Cruz[2], Steffi Friedrichs[3], Ilse Gosens[1], Frank Herzberg[4], Keld Alstrup Jensen[5], Frank von der Kammer[6], Jolinde A.B. Kettelarij[1], Jose María Navas[2], Kirsten Rasmussen[7], Kathrin Schwirn[8], Maaike Visser[1]
[1]National Institute for Public Health and the Environment (RIVM), The Netherlands; [2]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Spain; [3]AcumenIST, Belgium; [4]German Federal Institute for Risk Assessment (BfR), Germany; [5]National Research Centre for the Working Environment (NRCWE), Denmark; [6]University of Vienna, Germany; [7]European Commission, Joint Research Centre (JCR), Italy; [8]German Environment Agency (UBA), Germany

11:45

Sean Kelly[1], Blanca Suárez Merino[2], Kai Paul[3], Lee A. Walker[4]
[1]Nanotechnology Industries Association (NIA), Belgium; [2]Blue Frog Scientific, United Kingdom; [3]UK Center for Ecology and Hydrology, United Kingdom

12:00

Anna Pohl[1], Camilla Delpivo[2], Kathrin Schwirn[3], Elisabeth Heunisch[1], Frank S. Bierkandt[4], Virginia Rodriguez Unamuno[5], Keld Alstrup Jensen[6], Socorro Vázquez[2], Thomas A.J. Kuhlbusch[1]
[1]Federal Institute for Occupational Safety and Health (BAuA), Germany; [2]Leitat Technological Center, Spain; [3]German Environment Agency (UBA), Germany; [4]German Federal Institute for Risk Assessment, Germany; [5]European Chemicals Agency, Finland; [6]National Research Centre for the Working Environment (NRCWE), Denmark

12:15

Steffi Friedrichs
AcumenIST SPRL, Belgium

14:45 – 16:00
Characterization of Advanced Materials, Including Nano Materials 2

Chairs: Eva Valsami-Jones, *University of Birmingham, UK*
Miguel Banares, *CSIC, Spain*

14:45

C. Asbach[1], M. Nothhelfer[1], B. Sutter[2], A. Bescond[3], F. Gaie-Levrel[3], S. Ruiter[4], E. Kuijpers[4], J.P. Lollinga[4], W. Fransman[4], C. Möhlmann[5], A.M. Todea[1]
[1]Institut für Energie- und Umwelttechnik e. V. (IUTA), Germany; [2]Institut National de Recherche et de Securité (INRS), France; [3]Laboratoire national de métrologie et d'essais (LNE), France; [4]Netherlands Organisation for Applied Scientific Research (TNO), The Netherlands; [5]Institute for Occupational Safety and Health (IFA), Germany

15:00

Anna Pohl[1], Carla Ribalta[2], Elisabeth Heunisch[1], Ana Sofia Fonseca[2], Jacques Bouillard[3], Keld Alstrup Jensen[2], Thomas A.J. Kuhlbusch[1]
[1]Federal Institute for Occupational Safety and Health (BAuA), Germany; [2]National Research Centre for the Working Environment (NRCWE), Denmark; [3]L'Institut National de l'Environnement Industriel et des Risques (INERIS), France

15:15

Gottlieb Georg Lindner, Claus-Peter Drexel, Katrin Sälzer, Tobias B. Schuster, Nils Krueger
Evonik Operations GmbH, Germany

15:30

Xenia Knigge[1], Eugenia Valsami-Jones[2], Vasile-Dan Hodoroaba[1], Jörg Radnik[1]
[1]Bundesanstalt für Materialforschung und-prüfung (BAM), Germany; [2]University of Birmingham, United Kingdom

15:45

A. Masion[1], F. Ziarelli[2], S. Viel[3]
[1]CNRS, Aix-Marseille Univ., IRD, INRA, France; [2]Aix-Marseille Univ., CNRS, Centrale Marseille, FSCM, France; [3]Aix-Marseille Univ., CNRS, ICR, France

14:45 – 16:00
Harmonization & Standardization in the Context of Regulation 2

Chairs: Monique Groenewold, *RIVM, The Netherlands*
Elisabeth Heunisch, *Federal Institute of Occupational Safety and Health, Germany*

14:45

Jörg Radnik[1], Giovanni Chemello[1], Vasile-Dan Hodoroaba[1], Tom Howe[2], Neil Vyas[2], Benjamen P. Reed[3], Andrew J. Pollard[3], Charles A. Clifford[3]
[1]Bundesanstalt für Materialforschung und-prüfung (BAM), Germany; [2]Haydale Ltd., United Kingdom; [3]National Physical Laboratory, United Kingdom

15:00

Frederick C. Klaessig
Pennsylvania Bio Nano Systems, LLC, United States

15:15

Keld Alstrup Jensen, Yahia Kembouche
The National Research Centre for the Working Environment (NRCWE), Denmark

15:30

Marta Sousa[1], Pedro Arezes[1], Francisco Silva[1,2]
[1]University of Minho, Portugal; [2]Centro Tecnológico da Cerâmica e Vidro, Portugal

Wednesday, June 7, 2023

9:30 – 10:30
Micro & Nanoplastics Pollution 1

Chairs: Mark Morrison, *Optimat Ltd, UK*
Lesley Tobin, *Optimat / ENAS, UK*

9:30

Vérane Bard[1], Maeva Boulée[1], Aliro Villacorta[2], Sylvie Motellier[1], Alba Hernández[2], Ricard Marcos[2], Marie Carrière[1]
[1]Univ. Grenoble Alpes, France; [2]Universitat Autònoma de Barcelona, Spain

10:00

Mark R. Wiesner[1], J. Sipe[1], L. Ferguson[1], A. Lewis[1], K. Santizo[3], P.M. Pfohl[3], R. Cross[2], G. Adediran[2], A. Praetorius[4], W. Wohlleben[3], Sam Harrison[2], C. Svendsen[2]
[1]Duke University, United States; [2]UK Centre for Ecology and Hydrology (UKCEH), United Kingdom; [3]BASF, Germany; [5]University of Amsterdam, The Netherlands

10:15

Maeva Boulée, Thierry Douki, Vérane Bard, Marie Carrière
CEA Grenoble, France

9:30 – 10:30
Commonalities of the SSbD Paradigm in Innovation

Chairs: Martin Himly, *University of Salzburg, Austria*
Anthony Bochon, *University in Belgium, Belgium*

9:30

Joséphine Steck[1], Apostolos Salmatonidis[2], Camilla Delpivo[2], William Brown[3], Polly McLean[3], James Hanlon[3], Richard Seddon[4], Hasnae Ben Jeddi[5], Henk Goede[5], Sébastien Artous[1], Simon Clavaguera[1]
[1]Univ. Grenoble Alpes, CEA, Liten, DTNM, France; [2]Leitat Technological Center, Spain; [3]Institute of Occupational Medicine (IOM), United Kingdom; [4]Tecnalia Research and Innovation, Spain; [5]Netherlands Organisation for Applied Scientific Research (TNO), The Netherlands

9:45

Lisa Pizzol[1], Arianna Livieri[1], Alex Zabeo[1], Sarah Devecchi[1], Martina Menegaldo[1], Antonio Sellitri[1], Ainhoa Egizabal Luzuriaga[2], Ana Ayerdi Izquierdo[2], Tamara Oroz[3], Oihane Ibarrola Moreno[4], Danail Hristozov[1]
[1]GreenDecision Srl, Italy; [2]Fundacion Tecnalia Research & Innovation (TECNALIA), Spain; [3]Tecnologia Navarra de nanoproductos sl (TECNAN), Spain; [4]Biokeralty, Spain

10:00

Archini Paruthi[1,2,3], Prateek Goyal[2], Jared M. Brown[3], Abhay Raj Singh Gautam[2], Venkata Krishnan[4], Superb K. Misra[2]
[1]Indian Institute of Science, Bangalore, India; [2]Indian Institute of Technology, Gandhinagar, India; [3]University of Colorado, United States; [4]Indian Institute of Technology, Mandi, India

10:15

Elena Cesa[1,2], Paola Ziosi[2], Anna Luisa Costa[3], Dario Fornara[4], Mustafa Culha[5], Giuseppe Valacchi[1,7], Silvia Vertuani[1,2], Stefano Manfredini[1,2]
[1]University of Ferrara, Italy; [2]Ambrosialab Srl, Italy; [3]ISTEC-CNR, Italy; [4]Lamberti SpA, Italy; [5]Sabanci University Nanotechnology Research and Application Center, Turkey; [7]NC State University, USA

11:00 – 12:30
Micro & Nanoplastics Pollution 2

Chairs: Mark Morrison, *Optimat Ltd, UK*
Lesley Tobin, *Optimat / ENAS, UK*

11:00

Miguel A. Bañares[1], Cristina Jiménez-Arroyo[2], Alba Tamargo[2], Natalia Molinero[2], Julian J. Reinosa[3,4], Victor Alcolea-Rodriguez[1], Raquel Portela[1], José F. Fernández[4], M. Victoria Moreno-Arribas[2]
[1]Institute of Catalysis and Petrochemistry, CSIC, Spain; [2]Institute of Food Science Research, CIAL, (CSIC-UAM), Spain; [3]Encapsulae S.L., Spain; [4]Instituto de Cerámica y Vidrio, CSIC, Spain

11:15

Véronique Collin-Faure[1], Bastien Dalzon[1], Anaelle Torres[1,2], Thierry Rabilloud[1]
[1]Univ. Grenoble Alpes, CNRS, CEA, France; [2]Solvay, France

11:30

Marta Micheletto[1], Elisa Gaio[1], Alessandro Perini[1], Michela Zanella[1], Flavio Rizzolio[2,3], Federico Benetti[1]
[1]ECSIN-European Center for the Sustainable Impact of Nanotechnology, Italy; [2]Ca'Foscari University of Venice, Italy; [3]Pathology Unit, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Italy

14:30 – 16:00
Risk Assessment & Risk Management
Chairs: Elisabeth Heunisch, *Federal Institute of Occupational Safety and Health, Germany*
Monique Groenewold, *RIVM, The Netherlands*

14:30

Hedwig Braakhuis[1], Lya Hernandez[1], Sébastien Artous[2], Stephanie Desrousseaux[2], Joséphine Steck[2], Camilla Delpivo[3], Daniel Ganszky[4], Simon Clavaguera[2]
[1]National Institute for Public Health and the Environment (RIVM), The Netherlands; [2]Univ. Grenoble Alpes, CEA, Liten, DTNM, France; [3]Leitat Technological Center, Spain; [4]Geonardo Environmental Technologies, Hungary

14:45

Cecile Philippot[1], Joséphine Steck[1], Sébastien Jacquinot[1], Gilles-Charles Gaillard[1], Soizic Riou[2], Jeremie Pourchez[3], Lara Leclerc[3], Nicolas Albinet[4], Ophelie Kochmann[5]
[1]Univ. Grenoble Alpes, CEA, Liten, DTNM, France; [2]Dassault Aviation, France; [3]EMSE Centre Ingénierie et Santé, France; [4]AddUp Solutions, France; [5]TEKNA, France

15:00

Spyridon Damilos, Stratos Saliakas, Elias P. Koumoulos
Innovation in Research and Engineering Solutions (IRES), Belgium

15:15

Ata Rafiee, Maria B. Ospina, Tona M. Pitt, Bernadette Quémerais
University of Alberta, Canada

15:30

W. Fransman[1], P. Isigonis[2], A. Afantitis[3], K.A. Jensen[4], E.A. Bouman[5], D. Drobne[6], B. Pozuelo Rollón[7], I. Rodríguez-Llopis[8]
[1]Netherlands Organisation for Applied Scientific Research (TNO), The Netherlands; [2]Ca' Foscari University of Venice, Italy; [3]NovaMechanics Ltd, Cyprus; [4]National Research Centre for the Working Environment (NRCWE), Denmark; [5]Norwegian Institute for Air Research, Norway; [6]University of Ljubljana, Slovenia; [7]Technological Institute of Packaging, Transport and Logistics (ITENE), Spain; [8]GAIKER Technology Centre, Spain

Thursday, June 8, 2023

9:45 – 10:45
Implementation of the SSbD Concept in Case Studies 1
Chairs: Socorro Vazquez, *Leitat Technological Center, Spain*
Anna Costa, *CNR - ISTEC, Italy*

9:45

A. Masion[1,2], J. Rose[1,2], M. Auffan[1,2], C. de Garidel-Thoron[2], S. Artous[2], S. Clavaguera[3], G. Brochard[4]
[1]CNRS, Aix Marseille Univ, France; [2]SERENADE, France; [3]Univ. Grenoble Alpes, CEA, LITEN, DTNM, France; [4]ALLIOS, France

10:00

Lisa Pizzol[1], Arianna Livieri[1,2], Beatrice Salieri[3], Lucian Farcal[4], Lya G. Soeteman-Hernández[5], Alex Zabeo[1], Magda Blosi[6], Anna Luisa Costa[6], Willie Peijnenburg[5,7], Stella Stoycheva[8], Neil Hunt[8], Mariajosé López-Tendero[9], Danail Hristozov[10]
[1]GreenDecision Srl, Italy; [2]Ca' Foscari University of Venice, Italy; [3]TEMAS Solutions GmbH, Switzerland; [4]European Commission, Italy; [5]National Institute for Public Health and the Environment (RIVM), The Netherlands; [6]CNR - ISSMC, Italy; [7]Institute of Environmental Sciences (CML), The Netherlands; [8]Yordas Group, Germany; [9]Laurentia Technologies SLL, Spain; [10]EMERGE Ltd, Bulgaria

10:15

Ana Rita Alberto[1,2], Carla F. Martins[1], João Lanjeira[1], Helena Monteiro[1]
[1]ISQ - Instituto de Soldadura e Qualidade, Portugal; [2]UNL/ENSP, Portugal

10:30

Andrea Brigliadori[1], Magda Blosi[1], Ilaria Zanoni[1], Lara Faccani[1], Simona Ortelli[1], Claudia Vineis[2], Alessio Varesano[2], Valntina Dami[3], Giovanni Baldi[3], Franco Belosi[4], Joonas Koivisto[5], David Burrueco-Subirà[6], Juliana Oliveira[7], Massimo Perucca[8], Irini Furxhi[9], Anna Luisa Costa[1]
[1]CNR-ISSMC, Italy; [2]CNR-STIIMA, Italy; [3]Colorobbia Consulting Research Centre, Italy; [4]CNR-ISAC, Italy; [5]Air Pollution Management APM, Finland; [6]Leitat Technological Center, Spain; [7]CeNTI - Centre for Nanotechnology and Smart Materials, Portugal; [8]Project HUB-360, Italy; [9]Transgero Ltd., Ireland

9:45 – 10:45
Methods, Tools, & Technologies for SSbD Purposes 4

Chairs: Andrew Nelson, *University of Leeds, UK*
Araceli Jimenez Sanchez, *INSST, Spain*

9:45

Magda Blosi[1], Andrea Brigliadori[1], Ilaria Zanoni[1], Simona Ortelli[1], Serena Delbue[2], Paolo Blasi[3], Rossella Bengalli[4], Paride Mantecca[4], Massimo Perucca[5], Claudia Vineis[6], Alessio Varesano[6], Anna Luisa Costa[1]
[1]CNR-ISSMC, Italy; [2]University of Milan, Italy; [3]University of Bologna, Italy; [4]University of Milano-Bicocca, Italy; [5]Project HUB -360, Italy; [6]CNR-STIIMA, Italy

10:00

Hedwig Braakhuis[1], Alberto Katsumiti[2], Elma Lahive[3], Melanie Auffan[4], Rafael Ayerbe[5], Matthew Boyles[6], Joan Cabellos[5], Ana Candalija[5], Marie Carrière[7], Flemming Cassee[1,11], Julia Catalan[8], Adriana Rodríguez-Garraus[8], Richard Cross[3], James Hanlon[6], Armand Masion[4], Polly McLean[6], Elise Morel[3], Shahzad Rashid[6], Isabel Rodríguez-Llopis[2], Nienke Ruijter[1], Felice Simeone[9], Lya Soeteman-Hernández[1], Ralph Vanhauten[10], Socorro Vázquez-Campos[5]
[1]National Institute for Public Health and the Environment (RIVM), The Netherlands; [2]GAIKER Technology Centre, Spain; [3]UK Centre for Ecology and Hydrology (UK-CEH), United Kingdom; [4]CNRS, Aix-Marseille Univ., France; [5]Leitat Technological Center, Spain; [6]Institute of Occupational Medicine (IOM), United Kingdom; [7]Univ. Grenoble-Alpes, CEA, CNRS, France; [8]Finnish Institute of Occupational Health (FIOH), Finland; [9]Institute of Science and Technology for Ceramics (CNR-ISTEC), Italy; [10]ThinkWorks, The Netherlands; [11]Institute for Risk Assessment Sciences (IRAS), The Netherlands

10:15

Irini Furxhi[1,2], Rossella Bengalli[3], Paride Mantecca[3], Ozge Kose[4], Marie Carrière[4], Ehtsham Ul Haq[5], Charlie O'Mahony[5], Magda Blosi[6], Anna Costa[6]
[1]Transgero Ltd, Ireland; [2]Kemmy Business School, University of Limerick, Ireland; [3]University of Milano-Bicocca, Italy; [4]Univ. Grenoble Alpes, CEA, CNRS, Grenoble INP, IRIG, SYMMES, France; [5]Department of Physics, and Bernal Institute, University of Limerick, Ireland; [6]Istituto di Scienza e Tecnologia dei Materiali Ceramici (CNR-ISTEC), Italy

11:15 – 12:30
Implementation of the SSbD Concept in Case Studies 2

Chairs: Anna Costa, *CNR - ISTEC, Italy*
Socorro Vazquez, *Leitat Technological Center, Spain*

11:15

F. Boccuni[1], F. Tombolini[1], C. Natale[2], I. Bellagamba[3], F. Sebastiani[3], R. Ferrante[1]
[1]INAIL - Italian Workers Compensation Authority, Italy; [2]Italian Institute of Technology, Italy; [3]Sapienza University of Rome, Italy

11:30

Anaelle Torres[1], Mickael Crégut[1], Stéphane André[1], Jacques-Aurelien Sergent[2], James Wilson[1], Ana Hipolito[1]
[1]Solvay, France; [2]Solvay, Belgium

11:45

Matt Jellicoe[1], William Stokes[1], Mattthew Simmonds[1], Pia Muller[1], Marti Busquests[2], Aikaterini Anastasopoulou[1], Nicole Hondow[1], Andrew Nelson[1], Nikil Kapur[1], Thomas W. Chamberlain[1]
[1]University of Leeds, United Kingdom; [2]Applied Nanoparticles SL, Spain

12:00

Bianca de Melo Santana[1,2], Amedea Barozzi Seabra[1], Myron Christodoulides[2]
[1]Federal University of ABC (UFABC), Brazil; [2]University of Southampton, United Kingdom

12:15

Anaelle Torres[1], Mickael Crégut[1], Stéphane André[1], James Wilson[1], Jacques-Aurelien Sergent[2], Ana Hipolito[1]
[1]Solvay, France; [2]Solvay, Belgium

11:15 – 12:30
Methods, Tools, & Technologies for SSbD Purposes 5

Chairs: Andrew Nelson, *University of Leeds, UK*
Araceli Jimenez Sanchez, *INSST, Spain*

11:15

Massimo Perucca[1], Andrea Garrone[1], Mattia Costamagna[1], Ahmad Aldaghi[1], Anna Costa[2], Magda Blosi[2], Juliana Oliveira[3], Irini Furxhi[4]
[1]Project HUB-360, Italy; [2]Consiglio Nazionale delle Ricerche - ISSMC, Italy; [3]CeNTI - Centre for Nanotechnology and Smart Materials, Portugal; [4]Transgero Ltd., Ireland

11:30

William Stokes, Nikil Kapur, Andrew Nelson
University of Leeds, United Kingdom

11:45

Sarah Roberts, Richard Cross, Elise Morel, Elma Lahive, Marta Baccaro, David Spurgeon
UK Centre for Ecology & Hydrology (UKCEH), United Kingdom

12:00

Richard K. Cross[1], Dave Spurgeon[1], Claus Svendsen[1], Elma Lahive[1], Simon Little[2,3], Frank von der Kammer[4], Frédéric Loosli[4], Marianne Matzke[1], Teresa F. Fernandes[2], Vicki Stone[2], Willie J.G.M. Peijnenburg[6], Eric A.J. Bleeker[6]
[1]UK Centre for Ecology and Hydrology (UKCEH), United Kingdom; [2]Heriot Watt University, United Kingdom; [3]Veterinary Medicines Directorate, United Kingdom; [4]University of Vienna, Austria; [5]National Institute for Public Health and the Environment (RIVM), The Netherlands

12:15

Ata Rafiee, Emily Quecke, Dariya Sadovska, Mohamed Conteh, Teniola Yomi-Faseun, Bernadette Quémerais
University of Alberta, Canada

14:00 – 15:30
Implementation of the SSbD Concept in Case Studies 3

Chairs: Anna Costa, *CNR - ISTEC, Italy*
Socorro Vazquez, *Leitat Technological Center, Spain*

14:00

Ilaria Zanoni[1], Andrea Brigliadori[1], Simona Ortelli[1], Anna Luisa Costa[1], Mariajosé Lopez Tendero[2], Ana Serrano Lotina[3], Miguel A. Bañares[3], Andrea Brunelli[4], Elena Badetti[4], Willie Peijnenburg[5], Fiona Murphy[6], Helena Prima[7], Angela Saccardo[8], Lisa Pizzol[9], Arianna Livieri[9], Magda Blosi[1]
[1]CNR-ISSMC, Italy; [2]LAURENTIA TECHNOLOGIES, Spain; [3]CSIC Consejo Superior de Investigaciones Científicas, Spain; [4]UNIVE Università Ca' Foscari Venezia, Italy; [5]National Institute for Public Health and the Environment (RIVM), The Netherlands; [6]HWU Heriot-Watt University, United Kingdom; [7]ITENE Centro Tecnológico, Spain; [8]Swansea University, United Kingdom; [9]GreenDecision Srl, Italy

14:15

A. Salmatonidis[1], D. Burrueco[1], C. Delpivo[1], P. Castejón[1], C. Philippot[2], S. Jacquinot[2], S. Clavaguera[2], A. Guiot[2], B. Pellegrin[2], D. Lotti[3], S. Vazquez[1]
[1]Leitat Technological Center, Spain; [2]Univ. Grenoble Alpes, CEA, Liten, France; [3]LATI Industria Termoplastici, Italy

14:30

Elise Morel[1], Melanie Auffan[2], Patricia Solorzano Vives[3], Claire Badder[1], Andrea Carboni[2], Melissa Paula Diogo Dos Santos Faria[3], Richard Cross[1], Armand Masion[2], Jerome Rose[2], Gregory Brochard[4], Elma Lahive[1]
[1]UK Centre for Ecology & Hydrology (UKCEH), United Kingdom; [2]CNRS, Aix-Marseille Univ., France; [3]Leitat Technological Center, Spain; [4]ALLIOS, France

14:45

Sébastien Artous[1], Sébastien Jacquinot[1], Arnaud Guiot[1], Joséphine Steck[1], Neeraj Shandilya[2], Wouter Fransman[2], Mustafa Culha[3], Hulya Yilmaz[3], Helena Prima[4], Calabri Lorenzo[5], Alex Grigoropoulos[6], Mariajose Lopez-Tendero[7], Carlos Fito[4]

[1]Univ. Grenoble Alpes, CEA, Liten, DTNM, France; [2]Netherlands Organisation for Applied Scientific Research (TNO), The Netherlands; [3] Sabanci University Nanotechnology Research and Application Center (SUNUM), Turkey; [4]ITENE, Spain; [5]ART-ER S. cons. p. a., Italy; [6]Creative Nano PC, Greece; [7]Laurentia Technologies SLL, Spain

15:00

A. Cristadoro[1], B. von Vacano[1], L. Hammer[2], G. Scherr[2], A. Huegun[3], E. Ben Mariem[4], P.R. Outon[4], A. Cassel[5], G. Cabrera[6], Pierre Piluso[7], Andrea Tummino[7], Simon Clavaguera[7]

[1]BASF Polyurethane GmbH, Germany; [2]BASF SE, Germany; [3]CIDETEC, Spain; [4]INDRESMAT, Spain; [5]WIPAK GRYSPEERT SAS, France; [6]IPC, France; [7]Univ. Grenoble Alpes, CEA, Liten, DTNM, France

15:15

João Laranjeira, A. Rita Soares, Carla F. Martins

ISQ - Instituto de Soldadura e Qualidade, Portugal

14:00 – 15:30
Methods, Tools, & Technologies for SSbD Purposes 6

Chairs: Andrew Nelson, *University of Leeds, UK*
Carlos Fito, *ITENE, Spain*

14:00

Mustafa Culha[1,2], Hulya Yilmaz[1], Nilay Cicek[1], Sevin Adiguzel[1], Zehra Cobandede[1], Gülnur Sener[1], Irem C. Taskin[3], Tugba Muhlise Okyay[1], Yasemin Yildizhan[4], Yuksel Cetin[4], Julio Gómez Cordón[5], Raquel C. Puelles[5], S. Artous[6], S. Jacquinot[6], Arnaud Guiot[6], Neeraj Shandilya[7], W. Brown[8], Elena Cesa[9], Stefano Manfredini[9,10], Helena Prima[11], Alex Grigoropoulos[12], Maria D. Romero[13], Mariajose Lopez[14], Mariada Malvindi[15], Begoña Espiña[16], Santiago Blanco[17], Alberto Castillo[17]

[1]Sabanci University Nanotechnology Research and Application Center (SUNUM), Turkey; [2]Augusta University, USA; [3]Istanbul Medipol University, Turkey; [4]TUBİTAK-Marmara Research Center, Genetic Engineering and Biotechnology Institute, Turkey; [5]Avanzare Innovacion Tecnologica S.L., Spain; [6]Univ. Grenoble Alpes, CEA, France; [7]Netherlands Organisation for Applied Scientific Research (TNO), The Netherlands; [8]Institute of Occupational Medicine (IOM), United Kingdom; [9]University of Ferrara, Italy; [10]Ambrosialab srl, Italy; [11]ITENE, Spain; [12]Creative Nano PC, Greece; [13]Applynano Solutions, S.L., Spain; [14]Laurentia Technologies SLL, Spain; [15]HiQq- Nano srl, Italy; [16]INL, Portugal; [17]Groupo Antolin Ingeniería, Spain

14:15

Isabella Tavernaro[1], Nithiya Nirmalananthan-Budau[1], Lena Scholtz[1,2], Ute Resch-Genger[1]

[1]Bundesanstalt für Materialforschung und-prüfung (BAM), Germany; [2]Institute for Chemistry and Biochemistry, Freie Universität Berlin, Germany

14:30

Virginia Cazzagon[1], Rubén Martínez[1], Socorro Vázquez-Campos[1], Felice C. Simeone[2], Ilaria Zanoni[2], Polly McLean[3], James Hanlon[3], Ralph Vanhauten[4], Joséphine Steck[5], Simon Clavaguera[5], Camilla Delpivo[1]

[1]Leitat Technological Center, Spain; [2]CNR-ISSMC, Italy; [3]Institute of Occupational Medicine (IOM), United Kingdom; [4]ThinkWorks B.V., The Netherlands; [5]Univ. Grenoble Alpes, CEA, Liten, France

14:45

Kostas Blekos, Effie Marcoulaki
National Centre for Scientific Research "Demokritos", Greece

15:00

Philip Doganis, Argyri Kardamaki, Athanassios Nikolakopoulos, Michalis Kavousanakis, Haralambos Sarimveis
National Technical University, Greece

15:15

Stella Stoycheva[1], Alex Zabeo[2], Lisa Pizzol[2], Danail Hristozov[2]
[1]Yordas Group, Germany; [2]GreenDecision Srl, Italy

14:00 – 15:30
Characterization of Advanced Materials, Including Nano Materials 3

Chairs: Miguel Banares, *CSIC, Spain*
Eva Valsami-Jones, *University of Birmingham, UK*

14:00

Ralph A. Sperling[1], Christoph Bantz[1], Ines Frese[1], Jürgen Nolde[2], Svend Berger[2]
[1]Fraunhofer Institute for Microengineering and Microsystems IMM, Germany; [2]Grace GmbH, Germany

14:15

Victor Alcolea-Rodriguez[1], Raquel Portela[1], Vanesa Calvino-Casilda[2], Miguel A. Bañares[1]
[1]CSIC-ICP Instituto de Catalisis y Petroleoquimica, Spain; [2]UNED, Spain

14:30

Joana Claudio Pieretti, Giovana Marchini Armentano, Bruna Morerira Freire, Bianca de Melo Santana, Bruno Lemos Batista, Marcela Sorelli Carneiro-Ramos, Amedea Barozzi Seabra
Federal University of ABC (UFABC), Brazil

14:45

Taiwo Hassan Akere[1], Aline M.Z. de Medeiros[2,3], Diego Stéfani T. Martinez[2,3], Bashiru Ibrahim[1], Hanene Ali-Boucetta[1], Eugenia Valsami-Jones[1]
[1]University of Birmingham, United Kingdom; [2]Brazilian Centre for Research in Energy and Materials (CNPEM), Brazil; [3]University of São Paulo, Brazil

15:00

Stefan Schymura[1], Lisa Zörner[1], Iaroslav Rybkin[1,2], Sandra Drev[2], Rok Podlipec[2], Ales Lapanje[2], Alexander Mansel[1], Marko Strok[2], Karsten Franke[1]
[1]Helmholtz-Zentrum Dresden-Rossendorf, Germany; [2]Jozef Stefan institute, Slovenia

Tuesday, June 6, 2023

16:30 – 18:30
Methods, Tools, & Technologies for SSbD Purposes 7
Chairs: Sylvie Motellier, *CEA, France*
Olivier Lebaigue, *CEA, France*

Poster Area 1

16:30 – 18:30
Methods, Tools, & Technologies for SSbD Purposes 8

Chairs: Sebastien Artous, *CEA, France*
Herve Fontaine, *CEA, France*

Poster Area 2

16:30 – 18:30
Transferability & Acceptability of the Concept of SSbD

Chairs: Carlos Fito, *ITENE, Spain*
Claire Mays, *Symlog, France*

Poster Area 3

Wednesday, June 7, 2023

11:00 – 12:30
Harmonization & Standardization in the Context of Regulation 3

Chairs: Elisabeth Heunisch, *Federal Institute of Occupational Safety and Health, Germany*
Cecile Philippot, *CEA, France*

Poster Area 1

11:00 – 12:30
Characterization of Advanced Materials, Including Nano Materials 4

Chairs: Bastien Pellegrin, *CEA, France*
Sonia DeSousaNobre, *CEA, France*

Poster Area 2

17:20 – 18:30
Implementation of the SSbD Concept in Case Studies 4
Chairs: Joséphine Steck, *CEA, France*
Arthur Roussey, *CEA, France*

Poster Area 1

17:20 – 18:30
Modelling, Digitalization of Nanosafety & Data Management
Chairs: Tassos Papadiamantis, *NovaMechanics Ltd, Cyprus*
Joséphine Steck, *CEA, France*

Poster Area 2

17:20 – 18:30
Micro & Nanoplastics Pollution 3
Chairs: Bastien Pellegrin, *CEA, France*
Sylvie Motellier, *CEA, France*

Poster Area 3

TOPIC N° A1L-1

FIRST PRINCIPLES CHARACTERIZATION OF BIO-NANO INTERFACE FOR PREDICTIVE NANOSAFETY ASESSMENT

Alexander Lyubartsev[1]

[1]*Department of Materials and Environmental Chemistry, Stockholm University, S-10691 Stockholm, Sweden*

Safe and Sustainable by Design (SSbD) concept requires risk and safety assessment considerations on possible earlier stages of the innovation process. This task is especially challenging in design of advanced materials with novel or enhanced properties, effect of which on human health and environment is not yet known. Recent nanotoxicology- and bioinformatic research are able to link various adverse effects for human health to molecular initiating (triggering) events at bio-nano interface via adverse outcome pathways (AOP). Understanding of molecular interactions and biointerface structure is therefore crucially important for developing of predictive models of nanotoxicity assessment, especially for new developed materials for which not enough characterization and bio-nano interactions data exists.

Here a systematic multiscale modelling approach is presented that allows one to characterize the bionano interface in atomistic details from the first principles without any prior information about the material and thus establish links between the details of the nanomaterials structure to biomolecular–nanoparticle interactions [1]. A sequence of models is built starting from density functional theory for electronic density, which is used to derive an atomistic force field, and then calculate adsorption energies for essential biomolecules such as proteins and lipids. These models generate bio-nano interaction descriptors providing "biological fingerprint" of a material, which can be further linked to specific AOP thus making possible to develop a "mechanisms-aware" scheme of nanotoxicity assessment.

These ideas are illustrated by our modelling of TiO_2 and ZnO nanosurfaces and nanoparticles. *Ab-initio* molecular dynamics simulations provided detailed atomistic information on hydration structure and water reactivity at the surfaces. Classical molecular dynamics simulations with force field parametrized from ab-initio simulation provided adsorption free energy profiles of aminoacids and lipids components at the surfaces. These data were further used to build coarse-grained models characterizing adsorption of proteins, formation of biocorona, and adhession of lipid bilayers. Finally we show how molecular simulations were used for understanding of molecular mechanisms of chronic inflammation associated with inhallation of TiO_2 nanoparticles [2].

[1]I. Rouse, D. Power, E.G.Brandt, M. Schneemilch, K. Kotsis, N.Quirke, A.P.Lyubartsev, V. Lobaskin "First principles characterisation of bio-nano interface" Phys. Chem. Chem. Phys., 23, 13473 (2021)
[2] H.Kokot et al (J.Strancar) "Prediction of Chronic Inflammation for Inhaled Particles: the Impact of Material Cycling and Quarantining in the Lung Epithelium" Advanced Materials, 32, 2003913 (2020)

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

POLYMETALLIC Ce/Zr MIXED CERAMIC NANOCATALYST: IN SILICO CHARACTERIZATION AND TOXICOLOGICAL RISK ASSESSMENT

Sara Rozas[1], Nuria Aguilar[1], Elisabeth Escamilla[2], Carlos Rumbo[2] , Sonia Martel[2], Rocío Barros[2] , Pedro Ángel Marcos[3], Iakovos Yakoumis[4], Alfredo Bol[2, 3], Santiago Aparicio*[1, 2]

[1] Department of Chemistry, University of Burgos, 09001 Burgos, Spain
[2] International Research Center in Critical Raw Materials for Advanced Industrial Technologies (ICCRAM), University of Burgos, 09001 Burgos, Spain
[3] Department of Physics, University of Burgos, 09001 Burgos, Spain
[4] Monolithos Catalysts & Recycling Ltd., 11476 Athens, Greece
*Corresponding author

Safe by Design (SbD) strategy guarantees long-term nanosafety for industrial nanomaterials design, including sustainability and toxicological life cycles. Tools development for Multi-Component Nano-Materials (MCNMs) and High-Aspect Ratio Nanoparticles (HARNs) characterization developed into the Horizon - DIAGONAL project include multiscale modelling approaches and Machine Learning - based tools, which allow the identification of the hazardous indicators based on (nano)mono and multicomponent descriptors, as well as Adverse Outcome Pathways (AOP) and Molecular Initiating Events (MIE) – initial key event linked to an adverse outcome – , using novel predictors.
In this work, an *in silico* characterization and toxicological risk assessment of a commercial polymetallic ceramic substrate used in automotive catalytic converters was accomplished using multiscale modelling methods. Cu, Pd, Pt and Rh metal nanoparticles were considered for coating mixed CeO_2/ZrO_2 nanocatalyst. Density Functional Theory (DFT) quantum mechanics first principle approach was used for structure and properties effect characterization, obtaining DFT-based nanodescriptors, such as energy gap, hardness, electronegativity, etc. Regarding safety studies, classical Molecular Dynamics (MD) simulations were carried out for release and exposure features evaluation. Model lipid bilayer systems were used as membranes for studying the interaction with selected coated nanocatalyst in aqueous medium. Hence, plasma membrane nanoscopic descriptors were obtained. Additionally, interactions with further biological systems such as selected relevant proteins were also studied. Finally, training processes for machine learning predictive models development were performed.
Thus, suitability of multiscale modelling methods for molecular descriptors identification and molecular initiating events recognition was provided for the CeO_2/ZrO_2 commercial nanocatalyst used in automobile car converters. The suitability of this approach was probed and it may be further extended for *in silico* design of safe materials.

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

ZINC OXIDE NANOPARTICLES: IN SILICO CHARACTERIZATION AND TOXICOLOGICAL RISK ASSESSMENT

Nuria Aguilar[1], Sara Rozas[1], Elisabeth Escamilla[2], Carlos Rumbo[2], Sonia Martel[2], Rocío Barros[2], Pedro Ángel Marcos[3], Alfredo Bol[2,3], Santiago Aparicio*[1,2]

[1]Department of Chemistry, University of Burgos, 09001 Burgos, Spain
[2]International Research Center in Critical Raw Materials for Advanced Industrial Technologies (ICCRAM), University of Burgos, 09001 Burgos, Spain
[3]Department of Physics, University of Burgos, 09001 Burgos, Spain
**Corresponding author*

The aim of this theoretical study is to characterize the relationship between the structure and the physicochemical properties of zinc oxide nanoparticles (ZnO NPs) and Mn doped ZnO NPs to assess the toxicological impact of these materials. In order to do so, a multiscale modelling approach is applied. Different nanoparticles – in terms of geometry, aggregation and size – are characterized by their electronic properties using quantum mechanics methodologies (i.e., DFT, Density Functional Theory). To evaluate the toxicology impact of ZnO NPs in human health safety, the interaction between the material and biological target systems are modelled. Applying classical molecular dynamics methodologies, the interaction between a set of selected human proteins with the NPs are tested. Based on the Adverse Outcome Pathway (AOP) concept, identifying the Molecular Initiating Event (MIE) – the first event that triggers the myriad of events that cause the adverse outcome – allow to develop Safe by Design materials. In this scheme, the interaction of the studied nanoparticles with model human cell membranes is proposed as a pivotal MIE, which is studied through Molecular Dynamics Simulations for different ZnO NPs in terms of shape, size and concentration. The interaction between the NPs and model cell membranes is quantified in terms of membrane nanodescriptors which are scaled up through Machine Learning–based methods for developing simple and predictive tools.
This work is included as a case study for the Horizon - DIAGONAL project which aims to develop Sustainable and Safe By Design (SSbDs) materials, process and technologies to guarantee the long-term nanosafety for Multicomponent Nanomaterials (MCNMs) and High Aspect Ratio Nanoparticles (HARNs) in their whole life cycle.

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

FIELD TESTING OF LOW-COST PARTICLE SENSORS FOR INLINE MONITORING OF NANOMANUFACTURING PROCESSES, IN DIGITAL TWIN APPLICATIONS

Jesus M. Lopez de Ipiña[1], Alberto Lopez[2], Alejandro Gazulla[2], Gabriel Aznar[2], Franco Belosi[3], Joonas Koivisto[4], Richard Seddon[5], Paweł Durałek[6], Antonios Vavouliotis[7], Grigorios Koutsoukis[7], Karmele Lopez de Ipiña[8,9], Sonia Florez[5], Anna Costa[10]

[1] *TECNALIA, BRTA, 01510 Miñano, Spain*
[2] *TECNALIA, BRTA, Zaragoza, Spain*
[3] *Consiglio Nazionale delle Ricerche - ISAC, 40129 Bologna, Italy*
[4] *Air Pollution Management APM, 33610 Tampere, Finland*
[5] *TECNALIA, BRTA, 20009 Donostia-San Sebastian, Spain*
[6] *TMBK Partners Sp. z o.o., 02106 Warsaw, Poland*
[7] *Adamant Composites Ltd, 26504, Patras, Greece*
[8] *University of Cambridge, Cambridge CB2 3PT, United Kingdom*
[9] *University of the Basque Country, UPV/EHU, 20018 Donostia-San Sebastian, Spain*
[10] *Consiglio Nazionale delle Ricerche - ISSMC, 48018 Faenza, Italy*

Low-cost particle air sensors (LCS) are a rapidly expanding technology that can be used for informational and supplemental non-regulatory air quality outdoor and indoor monitoring applications. The advantages offered by LCS, in terms of functionality and lower cost, contrast with some limitations identified by ongoing research, such as variability in data quality, the effect of thermohygrometric conditions or the drift in sensor response over time. The application of this technology for monitoring industrial manufacturing processes is still in its early days but shows great potential.
The European project ASINA investigates the use of Digital Twin (DT) technologies in the optimization of nanoprocesses, with the aim of achieving safer and more sustainable nanomanufacturing processes by design, which reduce the consumption of nanoforms, as well as minimising NOAA air emissions and derived occupational exposures. Complex digital systems, such as the ASINA-DT, require a robust data capture layer (DCL). ASINA has tested LCS technologies as part of the DCL, intended for inline monitoring of nanomanufacturing processes. The project developed a set of LCS-Internet of Things nodes and an LCS multi-sensor node, to monitor hotspots and workers, and to test LCS from different manufacturers respectively. The data captured by the LCS are the basis for the design and development of soft sensors for the ASINA-DT.
This paper presents the results of field performance evaluations carried out by ASINA with LCS, in four pre-commercial and commercial pilot lines that manufacture nano-enabled products, belonging to the ASINA and OASIS H2020 projects. Field tests consisted of deploying LCS in process, measuring in parallel with collocated reference instruments for inter-comparison. The cross-analysis of the data collected has enabled the assessment of the performance of LCS in real manufacturing conditions.
Preliminary results include: 1) Significant correlation (0.51-0.76) between the concentrations measured by the LCS and the reference instrumentation; 2) Poor correlation between concentrations measured by LCS, when worn by workers, and personal monitors, as predicted; 3) Concentrations recorded by the reference instruments higher than the concentrations recorded by the LCS (bias); 4) Minor failures in the data capture firmware (e.g. missing or duplicate data), corrected in successive software versions.

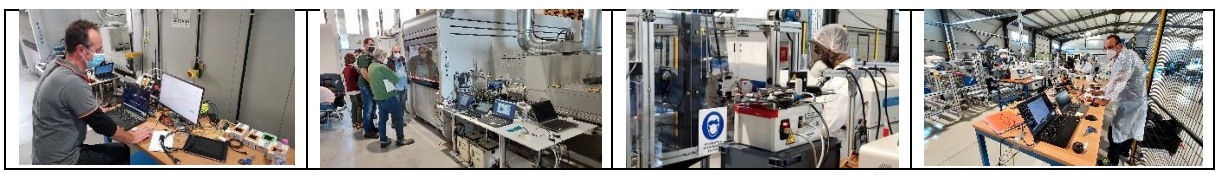

Figure 1. Testing LCS in ASINA and OASIS pilot lines

ASINA and OASIS have received funding from the European Union's Horizon 2020 research and innovation programme, under grant agreements No 862444 and 814581, respectively. This paper reflects only the author's view, and the Commission is not responsible for any use that may be made of the information it contains.

TOPIC N° 5 – From nanomaterials risk assessment to risk governance, a journey through regulation and standardization

Silver Nanoparticles Induced Cytotoxicity on Human Alveolar Adenocarcinoma Cells by Inhibiting the Ubiquitin Proteasome System

Bashiru Ibrahim[1,2], Taiwo Hassan Akere[1,2], Eugenia Valsami-Jones[2], and Hanene Ali-Boucetta[1]

[1]Nanomedicine, Drug Delivery & Nanotoxicology (NDDN) Laboratory, School of Pharmacy, Institute of Clinical Sciences, University of Birmingham, Birmingham, United Kingdom

[2]School of Geography, Earth and Environmental Sciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom

The growing use of silver nanoparticles (AgNPs) in consumer products has increased exposure to these nanoparticles, with subsequent risks to human health and the environment. The cytotoxicity of 10 nm AgNPs on the ubiquitin proteasome system (UPS) with a particular focus on deubiquitinating enzymes (DUBs) in human lung adenocarcinoma (A549) cells was studied. It was found that AgNP exposure reduced the percentage of cell viability, mitochondrial membrane potential (MMP) and increased the production of reactive oxygen species (ROS) in a dose-dependent manner. Similarly, the expression of ubiquitin specific proteases (USP7, USP8, and USP10), and ubiquitin carboxyl-terminal hydrolase-1 (UCHL-1) was significantly downregulated upon treatment with 1.25-10 µg/mL AgNPs. The higher concentration (10 µg/mL) had a greater ability to decrease the expression of the proteins compared to the lower concentrations (1.25, 2.5 and 5 µg/mL). Moreover, the expression of caspase-3, caspase-9, and poly (ADP-ribose) polymerase was upregulated. The results showed that AgNPs can induce cell death in A549 cells through UPS inhibition.

TOPIC N° X – YYY

TRAAC FRAMEWORK AND ONLINE TOOL FOR REGULATORY ACCEPTANCE AND WIDER USABILITY OF TOOLS AND METHODS FOR SAFE INNOVATION AND SUSTAINABILITY OF MANUFACTURED NANOMATERIALS

Remy Franken[1], Neeraj Shandilya[1], Marie-Sophie Barreau[1], Blanca Suarez-Merino[2], Andrea Porcari[3], Daniela Pimponi[3], Keld Alstrup Jensen[4], Wouter Fransman[1]

[1] TNO, Princetonlaan 6, 3584 CB Utrecht, Netherlands
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Despite growing tool and method developments for assessing health risks on the use or manufacture of nanomaterials (MNs), the level of awareness and use by stakeholders is still limited. Regulatory compliance and acceptance, reliability and trust, user-friendliness and compatibility with the users' needs are important factors traditionally known to hinder their widespread use. Therefore, a framework is presented to quantify the readiness of different tools and methods towards their wider regulatory acceptance and downstream use by different stakeholders. The framework diagnoses barriers which hinder regulatory acceptance and wider usability of a tool/method based on their Transparency, Reliability, Accessibility, Applicability and Completeness (TRAAC framework). An online TRAAC tool is being developed within HARMLESS that will be freely available on https://diamonds.tno.nl/#traac. Fourteen tools and methods were assessed using the TRAAC framework as proof-of-concept. The results provide insights into any gaps, opportunities, and challenges in the context of each of the 5 pillars of the TRAAC framework.

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

RISKGONE - SCIENCE-BASED RISK GOVERNANCE OF NANO-TECHNOLOGY

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Due to their unique properties, the use of engineered nanomaterials (ENMs) in innovative products and technologies has highly increased. The complexity of understanding their potential risks versus their benefits have urged policy makers, stakeholders and the research community to shift from traditional risk assessment (RA) methodologies to more complex and holistic risk governance efforts. The RiskGONE project, funded under the EC H2020 programme, aimed at developing specific support tools to better predict the impact of ENMs on human health and environment, to manage the decision-making process and to shape a more holistic safety policy for nanomaterials. [1]
Six main pillars have been identified, under which the project activities have been organized: i) risk governance framework implementation, ii) preparation of tools for risk benefit analysis of ENMs, iii) technical guidance (TG) on physicochemical properties, fate, and dosimetry of ENMs, iv) TG on human hazard assessment of ENMs, v) TG on ecotoxicological hazard assessment of ENMs, and vi) risk communication and training. The project activities were balanced between experimental and theoretical work, in an integrated effort to balance uncertainty, threats, and risks and benefits of ENMs.
Significant efforts towards standardization and validation process for ENMs have been undertaken through the evaluation, optimization and pre-validation of SOPs/TGs and their integration into the risk governance framework. This was done within the technical WPs through the implementation of round robin exercises for interlaboratory comparison of harmonized procedures direct to physicochemical characterization of ENMs, and human and environmental hazard characterization. The applicability of existing SOPs/TGs (e.g., TG318; TG111 and others) on the selected ENMs, as well as potential adaptations have been addressed in the specific project deliverables. Data collection and management have been organized by following FAIR principles aiming to help the harmonization of the experimental approach beyond the project.
Draft guidelines have been produced for the quantification of lifecycle risk indicators, the societal acceptance of ENMs considering risk and benefit perceptions, the assessment of ethical impacts, the correct implementation of risk transfer in insurance models and the quantification of macro-economic benefits. These guidelines aim at improving the stakeholders' understanding of benefits and risks which are considered crucial for the correct governance of ENMs.
The RiskGONE consortium has aimed at developing a flexible and solid public policy framework for risk governance of ENMs, associated with the user-friendly RiskGONE Cloud platform for its operationalization by stakeholders [2]. The framework supports stakeholders through the early adoption of scientific advances and emerging data, and their translation via functional tools (e.g., decision trees, technical guidance, risk benefit guidelines, toolboxes, databases, training material and more), all within a transparent, guided decision scheme considering their needs and expectations.
This project has received funding from the European Union's Horizon 2020 program (Grant Agreement no 814425).

[1] https://riskgone.eu/
[2] Isigonis, P. et al.: Risk Governance of Emerging Technologies Demonstrated in Terms of its Applicability to Nanomaterials. Small 2020, 16, 2003303. https://doi.org/10.1002/smll.202003303

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

ENHANCED GOVERNANCE FOR EMERGING TECHNOLOGIES: MERGING TRL AND SAFETY-BY-DESIGN

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In 2014, the European Union formally adopted Technology Readiness Levels (TRLs) into the Horizon 2020 Program (H2020). While the expansion of TRL into such diverse scientific domains helps European agencies benchmark progress in technology development, some potential shortcomings linger regarding the ability of TRLs to address the range of formal and informal needs and expectations of European regulators, governments, and society. Key amongst such limitations are three critical concerns, including (a) possible ambiguity regarding how progress is measured across different technologies at different TRL stages, (b) a classification of which elements of a given technology must meet specific advancement standards in order to advance to the next TRL stage (all components of a technology, versus only those on the critical path to technology maturation), and (c) downstream safety, security, and societal implications associated with the technology's entrance to the marketplace. Collectively, each of these concerns relates to a corresponding financial pitfall: how can developers anticipate the cost of progressing through the nine established TRL stages, and what further risks or liabilities might be assumed upon technology commercialization? And, subsequently, what actions might be taken in earlier stages of technology development to minimize development costs and potential liabilities or regulatory challenges, and improve the financial sustainability of innovation?
These concerns and questions have few easy answers, although raise the importance of fortifying TRLs to better suit the European Union's diverse innovation governance needs. One possible complement includes the use of Safety-by-Design (SBD), which incentivizes technology developers to 'engineer-out' potential risks early in the development process. The ability to anticipate downstream safety, sustainability, and other social implications of technology commodification by successful SBD would improve developer prioritization of research and development and foster a more socially and economically viable set of product alternatives.

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

EVOLUTION OF THE NANO-QSAR PARADIGM

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First nano-QSAR models were based on directly applying the classic QSAR paradigm to nanotoxicological data. Thus, simple endpoints (e.g., toxicity to bacteria or human cell lines) were modelled as a combination of descriptors calculated for molecules representing the chemical identity of the nanomaterial. With the progress in the methodology of nanomaterials' characterization, new descriptors (coming from the experiments) were exploited. In this context, special attention was put on the relationship between the properties of nanoforms and their supramolecular characteristics, like size, shape, porosity, and surface. In parallel, enormous effort has been taken to appropriately describe multicomponent nanomaterials (consisting of more than one chemical compound) and the relationship between the components. More recently, we concluded that the system (environment) significantly influences the (nano)structure and, consequently, its phys-chem properties and toxicity. Thus, system-dependent properties (e.g., zeta potential, protein corona) became, on the one hand, the commonly modelled properties. But, on the other one, they were used as descriptors important for modelling toxicity. The successful modelling, in this case, required particular nano-QSARs to be lined in Structure-Activity Predicting Networks (SAP-Nets), where the output from one model is used as the input to another one. Finally, in the paper *Representing and describing nanomaterials in predictive nanoinformatics,* experts from NanoSolveIT and NanInformaTIX projects tried to standardize how the nanomaterials should be characterized in nano-QSAR.
Along with developing experimental toxicology testing, especially those focused on closing the gap between animal and human results, nano-QSAR models evolved from predicting simple endpoints to more comprehensive ones. For example, applying nano-QSAR to predict the response at the transcriptomics level (e.g., pathways) made it possible to observe how the modifications of the (nano)structure change the chronic toxicity at low doses. And the toxic effects of nanoparticles can be predicted early in advance before observing symptoms at the organism level. Moreover, an exciting and challenging direction of nano-QSAR is applying this technique to predict the results of three-dimensional (3D) *in vitro* tests. This is important because most *in vitro* data used for modelling so far come from two-dimensional (2D) cell cultures, where the information on changes in cell morphology, function, proliferation, loss of polarity, etc., is not included.
This lecture aims at showing how the nano-QSAR paradigm evolved under the influence of evolving methodological toolbox available for studying the properties and toxicity of nanoparticles. Since the correlation is clear, we would deliberate on the possible further directions and challenges of nano-QSAR based on the trends of modern nanotechnology. What could we expect from the future? Should nano-QSAR become more dynamic (time-dependent) as the (nano)structure varies in time? What type of endpoints should be predicted and how? How to design new models to deliver answers relevant to new ideas (e.g., Safe and Sustainable-by-Design, SSbD)? I hope the lecture opens the discussion.

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

DATA-DRIVEN IDENTIFICATION OF PREDICTIVE VARIABLES FOR ENGINEERED NANOPARTICLES AGGREGATION IN FRESHWATER-LIKE SYSTEMS

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Over the last two decades, numerous fate studies have documented data aimed to identify the significant driving variables for the engineered nanoparticles (ENPs) aggregation in freshwater-like systems. Review of original research and review articles has identified the explanatory variables found to play a significant role in the aggregation of ENPs (e.g. natural organic matter, ionic strength, ENPs concentration, etc.). This raises the question; can the literature documented significant variables deemed to control ENPs aggregation also carry good predictive power? In this work, we demonstrate using heterogeneous, multi-dimensional, non-linear, and highly fragmented aggregation data for ENPs sourced from the published literature that at present the reported highly significant variables are not essentially the highly predictive variables for ENPs aggregation. To this end, we demonstrate this phenomenon using datasets of titanium dioxide ($nTiO_2$) and zinc oxide (nZnO) aggregation in freshwater-like systems. Results herein were derived using different machine learning (ML) techniques. ML results showed that the algorithms of random forest regression (RFR), support vector regression (SVR), and artificial neural network (ANN) using three quantitative input parameters (time, pH, and zeta potential – identified as good predictors) can aid to develop robust predictive models for the aggregation of ENPs. The developed model based on highly predictive variables is useful and can support robust decision-making on the likely exposure potential of ENPs to aquatic organisms.

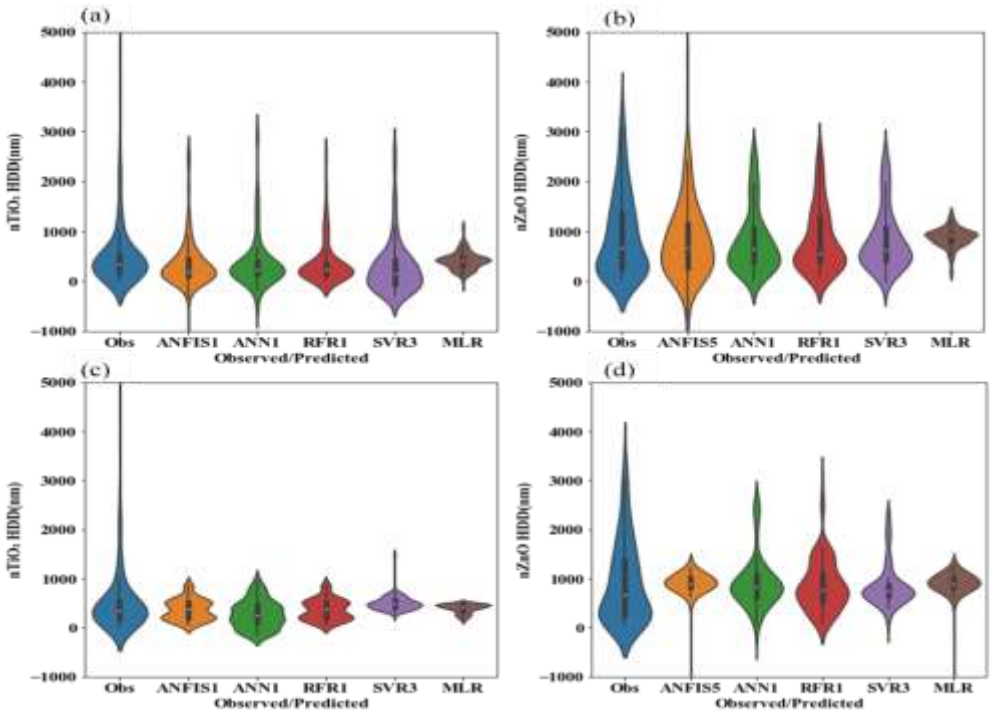

Figure 1: Violin plots with density mass distribution of predicted values using ML models against the independent dataset (Obs). (a) and (b) are based on ζ, pH, and time as model inputs, (c) and (d) are based on NOM, size, IS, and ENP concentration as input variables. White dots on violin plots depict the mean of each dataset. The wide regime signifies high probability distribution, whereas, in the skinner regime, the converse holds. The box bound is the inter-quintile range (IQR) (25th, 50th, and 75th quartiles). The ends of solid black points depict the highest (95th) and lowest values (5th).

TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

A SIMILARITY ASSEESSMENT METHOD FOR GROUPING MULTI-COMPONENT NANOMATERIALS

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Multicomponent nanomaterials (MCNMs) are complex materials that are synthesised by combining at least two components with distinctive material composition, shape or size and with the purpose of creating or improving a certain functionality. However, apart from their clear benefits, MCNMs new or enhanced functionalities could also have an impact on the risk for human health or the environment, for example, by affecting the release, fate, toxicokinetics or hazard. We present a computational method to identify what are the properties that are close related to new or enhanced functionality of MCNMs, and prioritize them with respect to their impact in grouping MCNMs for safety purposes. To this end, the use of existing information of the single components or of similar MCNMs are incorporated together with any available information that would increase the power of the method to efficiently rank and group MCNMs with respect to their safety. Similarities of MCNMs are estimated based on experimental, functional and physicochemical data properties, as well as toxicological endpoints, tailored by predefined grouping hypotheses. An integration approach was deemed preferable in this case, since different and diverse data were available for MCNMs, however limited in volume. Applications to use cases data are presented together with challenges that still remain to be addressed. Particularly, data standardization and scaling issues are considered, together with data filtering to retain only information valuable to grouping. The methodology envisages to serve as a valuable tool in risk assessment for grouping and read-across to leverage safety information for MCNMs.

Funding acknowledgement European Commission Horizon 2020 for GRACIOUS, SUNSHINE, Grant Agreements No. 760840, 952924 respectively.

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

THE SEMANTIC LANDSCAPE: AN EVER GROWING, PUBLICLY AVAILABLE OVERVIEW OF NANOSAFETY KNOWLEDGE

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Finding the right knowledge or prediction for the right situation is a challenge and current solutions require a significant time investment from researchers. To shorten this time we are developing the Semantic Bioactivity Landscape using FAIR (findable, accessible, interoperable, reusable) approaches. For this we need detailed annotation of the biology and chemistry a resource captures. This can be achieved with ontologies and specific identifiers. That is, we need a knowledge graph.

The knowledge graph content comes from many sources, such as other projects, public databases, literature text mining, etc, that need harmonization into a single representation. Here, semantic web technologies are used, particularly the Resource Description Framework (RDF), ontologies, identifiers (e.g. ERM, NanoInChI), shape expressions (ShEx), and the SPARQL query language. Data summaries of the landscape are compiled into an ebook.

This effort is seeded by the overview of prior knowledge developed by the SbD4Nano project. As is customary, the project started by creating an overview, in the form of a spreadsheet, with prior knowledge which the partners deem relevant for the project. Naturally, doing this for each project is not the best way to spend project resources, nor is it the favorite pastime for the partners creating the overview. Especially since updating the information and links in the overview and detailed ontological annotation of sources with all relevant data is (time) expensive.

With the FAIR principles in mind, we set out to create a publicly available overview of databases and datasets, models (e.g. nanoQSAR), adverse outcome pathways (AOPs), key event relationships (KERs) and molecular initiating events relevant for nanomaterials (nano-MIE), which in time could be reused between projects. We have developed a modular platform that can pull in data and metadata from diverse sources, unify this with semantic web approaches (RDF, ontologies, and shape expressions), and make this accessible as FAIR data itself (SPARQL). Other harmonization attempts exist, for example FAIRsharing, bio.tools, etc, but these are still scattered, isolated and not integrated for our purpose.

One problem is that the content of the landscape can often not be shared during the project. However, "shapes", indicating which information should be reported for the different data types, can be shared. This allows us to later merge the information from the different projects, creating an ever growing, publicly available Semantic Landscape of knowledge relevant for nanosafety research.

Here we would like to present examples of such shapes; showcase the ebook which automatically shows the content of the landscape and some example questions we can answer with the landscape, such as: "Give me all nano-QSAR models about TiO_2 that can say something about ROS production".

This research was funded by the European Union Horizon 2020 Programme (H2020) under grant agreement nº 862195 (SbD4Nano).

TOPIC N°7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

MACHINE LEARNING-ASSISTED NANOPARTICLE SYNTHESIS AND NANOTOXICITY PREDICTIONS

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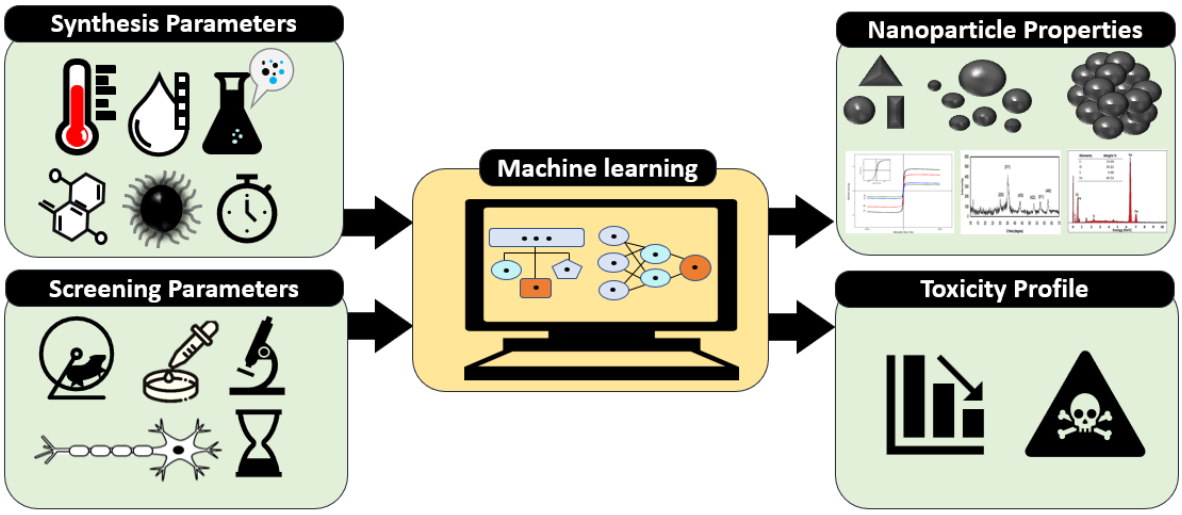
The substantial differences in the conduct or design of nanomaterial synthesis and nanotoxicity screening studies have led to the generation of conflicting research findings that may be accurate in their own narrative but fail to provide a complete picture of parameters affecting both physicochemical characteristics and toxicological behaviour of nanoparticles. One strategy to maximize the use of individual findings with potentially biased estimates is to homogenize results across several studies and to increase the generalizability and relevance of their findings. Here, we present two different case studies with metallic nanoparticles to demonstrate how the synergetic use of meta-analytic approaches and machine learning algorithms can boost the precision of conclusions drawn from accumulated data in both nano-synthesis and nanosafety domains. An exhaustive literature search yielded 250+ data (points) from individual experiments in each case study, which were independently used to identify the parameters that drive the physicochemical and toxicological properties of nanoparticles. Two different machine learning approaches, decision tree (DT) and artificial neural network (ANN), were employed to develop predictive models with reasonably high accuracies. The relative importance of experimental conditions for the response variable (nanoparticle characteristics or toxicity potential) was determined using different algorithms. Our results highlighted the important role machine learning-assisted efforts could play in designing safe and commercially viable nanoparticles. Such models represent an important step toward maximizing the use of accumulated scientific data to predict the final properties of nanoparticles that have not been synthesized yet, an important prerequisite for the successful implementation and realization of both quality-by-design (QbyD) and safety-by-design (SbyD)concepts.



TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

STUDY OF TITANIUM DIOXIDE NANOPARTICLES DIFFUSION THROUGH THE SKIN AND DECONTAMINATION IN VITRO

Guillaume Phan[1], Adeline Tarantini[2], Emilie Jamet-Anselme[1], Vincent Haute[2], Sabine Lam[1], Alexandre Legrand[1], David Suhard[1], Véronique Chamel[2] and Céline Bouvier-Capely[1]

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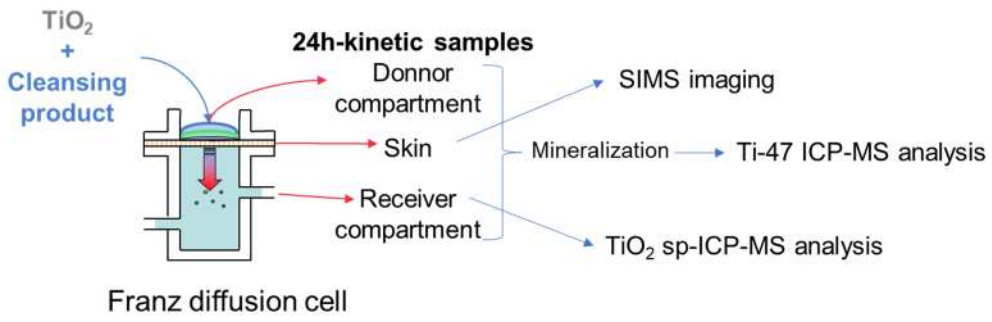
There is a growing concern regarding occupational skin exposure to metallic nanoparticles (NP) such as titanium dioxide (TiO_2) and health consequences, due to an increased use of these raw materials in the industry and in consumption products. Risk management procedures related to skin exposure to nanomaterials are currently based on generic hygiene measures in terms of protection and decontamination and lack of specificity. Although NP possible diffusion through the skin has been well described in the literature, a limited number of studies are available on the evaluation of decontamination protocols and products. In this context, the aim of this study was to adapt an experimental model based on Franz diffusion cells and porcine skin explants to characterize the diffusion of TiO_2 NP and to compare the efficacy of different cleansing products as function of the time of treatment.
TiO_2 NP were characterized both in the commercial suspension and in the different compartments and fluids after 24h-kinetic experiments with TEM microscopy, dynamic light scattering (DLS) or single particle ICP-MS (sp-ICP-MS). The distribution of the NP in the skin depth was observed by SIMS spectrometry and Ti-47 was quantified in the different compartments by ICP-MS. The efficacy of skin rinse with two different cleansing products, soapy water and a calixarene cleansing nanoemulsion, were compared to pure water, when applied 30min or 6h after skin exposure to TiO_2.
While TiO_2 NP tend to form agglomerates in aqueous solutions, a diffusion through the skin was confirmed as particles were detected in the receiver medium of Franz cells using sp-ICP-MS. In the absence of treatment, SIMS images showed for the first time the accumulation of TiO_2 agglomerates in the epidermis, the dermis and around hair follicles. Decontamination assays showed that both tested products exhibited comparable efficacy to limit Ti penetration, whatever the time of treatment. However, only calixarene nanoemulsion was statistically more efficient than water to retain TiO_2 in the donor compartment (>89%), to limit the retention within the skin (<1%) and to prevent NP diffusion through the skin (<0.13%) when treatments were initiated 30min after skin exposure. When decontamination was delayed from 30min to 6h, the amount of Ti diffusing and retained in the skin increased. To conclude, this study demonstrates that TiO_2 NP may diffuse through the skin following exposure. Thus, effective decontamination using cleansing products should be performed as soon as possible.



In vitro experiment model for nanoparticles cutaneous diffusion and decontamination studies

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

LUNG SINGLE CELL TRANSCRIPTOMICS TO GUIDE THE DEVELOPMENT OF AOP ANCHORED CELL-BASED ASSAYS IN RESPONSE TO NANOMATERIAL EXPOSURE

Lianyong Han[1], Carola Voss[1], Meshal Ansari[1], Qiaoxia Zhou[1], Verena Haefner[1], Ulla Vogel[2], Markus Rehberg[1], Herbert B. Schiller[1], Tobias Stoeger[1]

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Inhaled nanomaterial (NM) can cause acute and chronic inflammation, with the latter considered a crucial step for the pathogenesis of pulmonary fibrosis. Accordingly, the adverse outcome pathway (AOP) framework for NM inhalation generated fibrosis relies on a strong involvement of the inflammatory component. Here we applied single cell transcriptomics to identify NM-specific cellular perturbation pathways and underlying key cell types to inform AOP anchored *in vitro* studies. We exposed mice intratracheally to carbon black (CNP, 20 µg), tangled double-walled (DWCNT, 50 µg) and because of their pathogenicity rigid, multi-walled carbon nanotubes (MWCNT, 15 µg), all dispersed in lung surfactant. All lungs underwent single-cell RNA sequencing (scRNA-seq), histology and bronchoalveolar lavage (BAL) analysis.

At the dose levels chosen, all three NMs induced comparable levels of acute inflammation, i.e. airspace neutrophilia at 12 h, which increased further until 6 days only for the two CNTs. Elevated levels at 28 days however were only observed for MWCNT, allowing a differentiation of the key events (KE) acute and chronic inflammation. To understand which cells contribute to the initiation of inflammation, we compared 12 h BAL cytokine levels with scRNA-seq profiles. Interestingly, the fate of inflammation was characterized in a NM-specific manner, already at this early time-point. CNP uniquely triggered GM-CSF and CXCL1 release into the airspace, with *Csf2* and *Cxcl1* mRNAs induced mainly in alveolar epithelial cells. Gene set enrichment analysis (GSEA) revealed CNP triggered pathways related to 'neutrophil chemotaxis' and 'cytokine production' in alveolar epithelial cells. DWCNT caused a release of the monocyte attractants CCL2, -3 and -4, involving interstitial macrophages and monocyte RNA signatures and associated with acutely elevated lung monocyte and at day 6 high airspace macrophage numbers. MWCNT in contrast caused the most extensive BAL cytokine response, involving different cell types and specifically induced the Th2 cytokines CCL11 and IL10, seen as a crucial KE in fibrosis AOPs. Cell communication analysis demonstrated MWCNT-specific networks involving alveolar and bronchiolar epithelial cells, fibroblasts, and macrophages.

To test cell communication *in vitro*, we exposed murine alveolar epithelial cells (LA4 cell line) and macrophages (MH-S cell line), at single and co-culture conditions, to doses up to 250 ug/ml. However, a pro-inflammatory response could only be detected for MWCNT, indicating challenges of a too simple *in vitro - in vivo* translation.

Our study demonstrates that depending on NM quality different cell types can be involved already for the induction of early KEs and suggests that more complex cell-cell communications might have to be considered to recapitulate NM-specific cell perturbations and KE response pattern at the *in vitro* level and thereby guide AOP predictive cell-based testing strategies.

TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

DNA DAMAGE ASSESSMENT VIA HIGH THROUGHPUT P53-BINDING PROTEIN 1 FOCI COUNTING AS A NEW APPROACH METHODOLOGY FOR GENOTOXICITY TESTING

Maelle Fontaine[1], Eline Bartolami[1], Marion Prono[1], David Béal[1], Marie Carriere[1]

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Designing safe and sustainable materials requires the ability of rapidly screening the impact of the newly developed materials, using cost-effective methods that only require small quantities of the material.
The aim of this work was to develop a high throughput method for screening DNA damage induced by nanomaterials, using automated microscopy, and which could be coupled to the evaluation of other cell toxicity endpoints through high content analysis. In this context, we focused on the p-53-binding protein 1 (53BP1), which is one of the first proteins recruited on DNA double strand breaks as they occur, forming large foci around the damage and initiating its repair.
An assay based on immunostaining and automatic counting of 53BP1 foci using a high throughput screening/high content analysis (HTS/HCA) platform was developed, using the HCT116 colon carcinoma cell line as model. First, the sensitivity of the assay was evaluated using a series of acknowledged genotoxic agents chosen from the list of substances recommended by the EU reference laboratory for alternative to animal testing (EURL ECVAM) to be tested when a new genotoxicity assay is developed. Then, the assay was applied to a series of nano(bio)materials with various compositions, including metal and metal oxide nanoparticles, organic and mineral materials.
Our results show that the assay appropriately detected the DNA damaging potential of the genotoxic substances from the ECVAM list, which are known to induce genotoxicity via distinct mechanisms, including DNA alkylation, aneugenicity, topoisomerase inhibition and production of DNA adducts. Most of these substances led to a concentration-dependent increase of 53BP1 foci count. Conversely, among the tested nano(bio)materials, only some of the silica nanoparticles showed significant genotoxicity.
Therefore, this assay could be used in the future as an alternative high throughput genotoxicity assay among New Approaches and Methodologies.

This work was funded by the European Union's Horizon 2020 Research and Innovation program under grant agreement no. 760928 (BIORIMA) and no. 862419 (SAbyNA).

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M. Fontaine et al., Nanomaterial genotoxicity evaluation using the high-throughput p53-binding protein 1 (53BP1) assay. *Submitted*.

TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

AN AOP-ORIENTED TESTING STRATEGY FOR THE ASSESSMENT OF SILVER NANOPARTICLE TOXICITY TO HUMAN INTESTINAL CELLS, AFTER IN VITRO SIMULATED DIGESTION

Ozge Kose[1], Magda Blosi[2], Rossella Bengalli[3], Anna Costa[2], Paride Mantecca[3] and Marie Carriere[1]

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Silver nanoparticles (AgNPs) are widespread used in food and consumer products, suggesting that they may be ingested by humans and lead to adverse effects in the gastrointestinal tract. This study aimed at evaluating the toxic impact of AgNPs on an intestinal cell model, using an adverse outcome pathway (AOP)-derived methodology, as part of New Approach Methodologies (NAMs) that are described in SSbD frameworks.
First, the literature and AOPwiki database were searched for AOPs that could reflect the most reported toxic effects of AgNPs. This led to the selection of four AOPs that served as a basis for the design of a toxicity testing strategy. This strategy was then applied to HCT116 epithelial intestinal cells exposed to three AgNPs, either uncoated or coated with polyvinylpyrrolidone (PVP) or hydroxyethycellulose (HEC). The latter has been demonstrated as showing improved functionality and low toxicity, making it an interesting safer-by-design candidate for AgNP antimicrobial application. In an attempt to increase the realism of this in vitro study, these particles were submitted to in vitro digestion prior to cell exposure.
During the different stages of in vitro digestion, the physico-chemical characteristics of AgNPs evolved, with series of agglomerations and disagglomeration processes. The digestion only mildly affected the toxic effects of the three particles, as it only increased their genotoxicity, but it did not affect their cytotoxicity, the intracellular reactive oxygen species levels that they generate and the mRNA expression of inflammatory markers that they trigger. Finally, these particles, both undigested and digested, affected the cell cycle but did not trigger apoptosis. Although the intracellular accumulation of these three particles did not significantly differ, HEC-coated AgNPs showed lower toxicity compared to uncoated and PVP-coated AgNPs.
Taken together, these results indicate the potential toxicity of digested Ag NPs towards intestinal cells, which varied depending on their coating but did not significantly differ from that of non-digested NPs. It highlights the relevance of an AOP-based approach to design a toxicity testing experimental plan, as well as the lower toxicity and better functionality of HEC-coated AgNPs.

This work was funded by the European Union's Horizon 2020 Research and Innovation program under grant agreement n. 862444, ASINA (Anticipating Safety Issues at the Design Stage of NAno Product Development)

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

USE OF REALISTIC ADVANCED IN VITRO METHODS FOR SAFETY ASSESSMENT OF MCNMs/HARNs

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DIAGONAL project's objective is to bring Safe by Design (SbD) knowledge and tools to a development stage which can be implemented in multicomponent nanomaterials (MCNMs) and High-Aspect Ratio Nanoparticles (HARNs) related industries. To better address this challenge, industrial test cases have been selected and state-of-art nano-tailored testing protocols (SOP & draft OECD TGs/GDs) have been applied to gather meaningful results for the understanding of the biological consequences of the exposure to MCNMs and HARNs (and derived NEPs) along their life cycle. The first step has been the identification of potential workplace and environmental scenarios (as well as potential human exposure routes) to which either workers or consumers may be exposed to. The second step has been moving to the hazard assessment of relevant materials through advanced and realistic human *in vitro* cellular models, representative for the most critical exposure routes.
A tetraculture *in vitro* model mimicking the human alveolar region and set up to investigate the sensitization potential of chemicals has been previously established at LIST. The model has been already applied in several nanosafety-related studies aiming at understanding the biological effects induced by advanced materials upon inhalation. In the frame of DIAGONAL, the *in vitro* system has been used to screen common toxicological effects induced by selected MCNMs/HARNs (e. g. AgNWs, SWCNTs, MnZnO, etc.), like cytotoxicity, inflammation, reactive oxygen species (ROS) generation, genotoxicity as well as immune system activation. Different exposure methods (submerged condition vs air-liquid interface combined with aerosol exposure) have been compared to verify their suitability for the administration of the selected compounds to the *in vitro* model. Then, the interaction between the nanomaterials and the cellular components has been investigated by using advanced microscopic techniques.
Different toxicity profiles were observed depending on the exposure method applied to deliver the compounds to the tetraculture system for most of the investigated NMs. A slight dose and time-dependent increase in reactive oxygen species generation was detected after exposure to AgNWs, however this translates only in a negligible cytotoxicity at 24h post exposure. In order to investigate whether this effect is buffered by the cellular antioxidant defense systems, or it is evolving in adverse pathways, the evaluation of the same end points after repeated exposures or by applying longer exposure times might be advised.
However, the approach used so far to investigate the different case studies has allowed to 1) identifying current gaps in the existing Standard Operating Procedures (SOPs) for the characterization of physicochemical properties of MCNMs and HARNs along the life cycle; 2) drafting adaptations of existing protocols (originally addressed to NMs) for their application to MCNMs/HARNs toxicity characterization; 3) generating new data about relevant advanced materials and their NEPs; 4) feeding novel *in silico* models for the prediction of advanced materials toxicity; 5) increasing awareness on SSbD concepts at industrial scale through the implementation of the project strategy in the specific case studies.
This project has received funding from the European Union's Horizon 2020 program (Grant Agreement no 953152).

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TOPIC N° 7 - MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

The SUNSHINE e-Infrastructure

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Due to their inherent complex nature and interactions Multicomponent (advanced) nanomaterials (MCNM) pose questions regarding their possible health and environmental risks. Because of this, Safe & Sustainable by design (SSbD) strategies need to be developed and implement for products enabled by MCNM.
To facilitate the development and implementation of such SSbD strategies, the EU H2020 SUNSHINE project has developed a digital e-infrastructure that provides a platform for industries to exchange information along MCNM supply chains, and to obtain access to safety and sustainability analysis data and tools by connecting to the SUNSHINE Open and FAIR database and to modelling approaches included in the system.
The SUNSHINE e-infrastructure is based on the Safe Innovation Approach (SIA)[1]. It is designed to: (1) facilitate collaboration and information exchange between actors along nanotechnology supply chains (developers, producers, downstream users) to promote the development and implementation of SSbD strategies for MCNM-based materials, products and processes; (2) support SMEs and large industries in the selection and application of simple, robust and cost-effective experimental, modelling and grouping/read-across approaches to acquire/generate the data needed to test the effectiveness of the SSbD strategies; (3) enable risk-benefit analysis of the SSbD-modified materials and products at each stage of the *Agile Stage Gate*[2] innovation process to ensure that they are safe and sustainable without compromising their technical functionality and commercial probability of success.

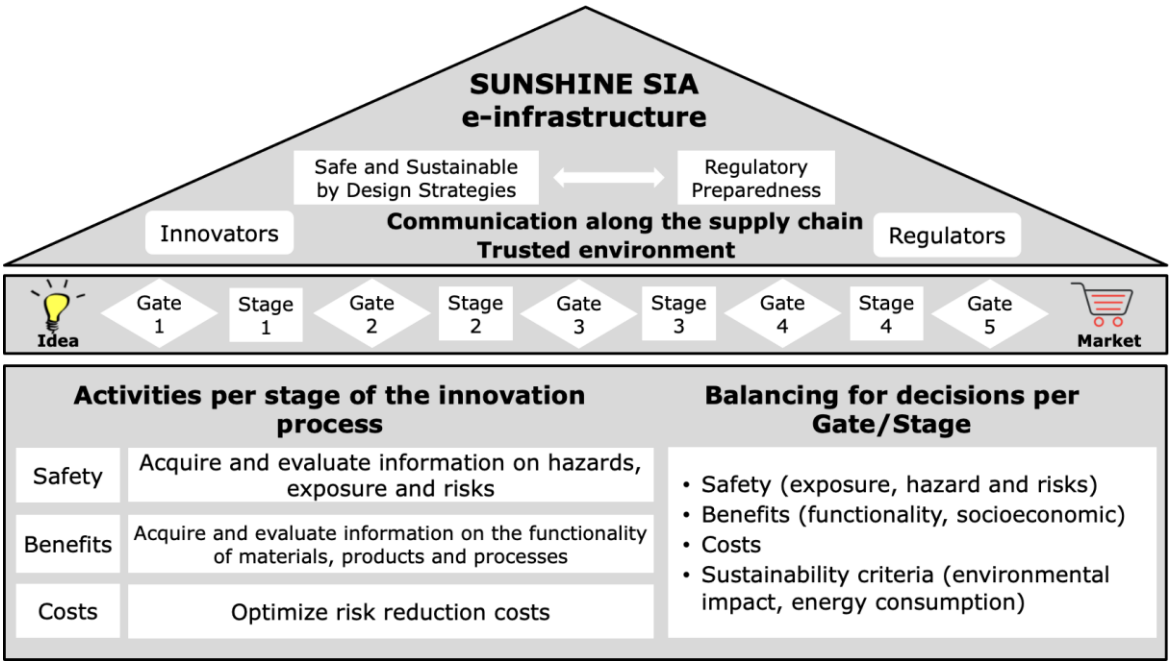

Figure 1 SUNSHINE Safe Innovation Approach (SIA) e-infrastructure general framework.

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Funding information

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TOPIC N° 7 - MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

The NanoInformaTIX platform

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The NanoInformaTIX platform is a sustainable data management and multi-scale modelling framework to optimise the hazard and exposure assessment of engineered nanomaterials (ENMs) for both risk assessment and safe by design purposes. This framework was implemented as a web-based platform specifically tailored to the needs of stakeholders from industry, regulatory bodies, and academia.
The NanoInformaTIX platform is a digital hub designed to enable easy access to nanosafety databases (e.g., eNanoMapper, NanoPharos) and models for prediction of properties, emissions, exposure, biodistribution and hazard (i.e., material models, Material Flow and Environmental fate models, Physiologically Based Pharmacokinetic (PBPK) models, Quantitative-Structure-Activity Relationships (QSAR), System Biology). The data repositories and the models are linked to the platform by means of a common Application Programming Interface (API). Moreover, the system enables creation of chains (i.e., pipelines) of the databases and different models to enable higher-level analyses such as dose-response assessment, grouping, in vitro-in vivo extrapolation (IVIVE). This requires high level of interoperability, which was achieved by extending and applying the Modelling Data (MODA) scheme of the European Materials Modelling Council. The outputs generated by models are fed as input to subsequent models, following the MODA scheme enabling "chains" of models which fulfils a specific task. The results of the analyses are presented as both dynamic charts and textual reports.
The architectural organization of the platform follows a microservice infrastructure based on Amazon AWS services as presented in Fig. 1.

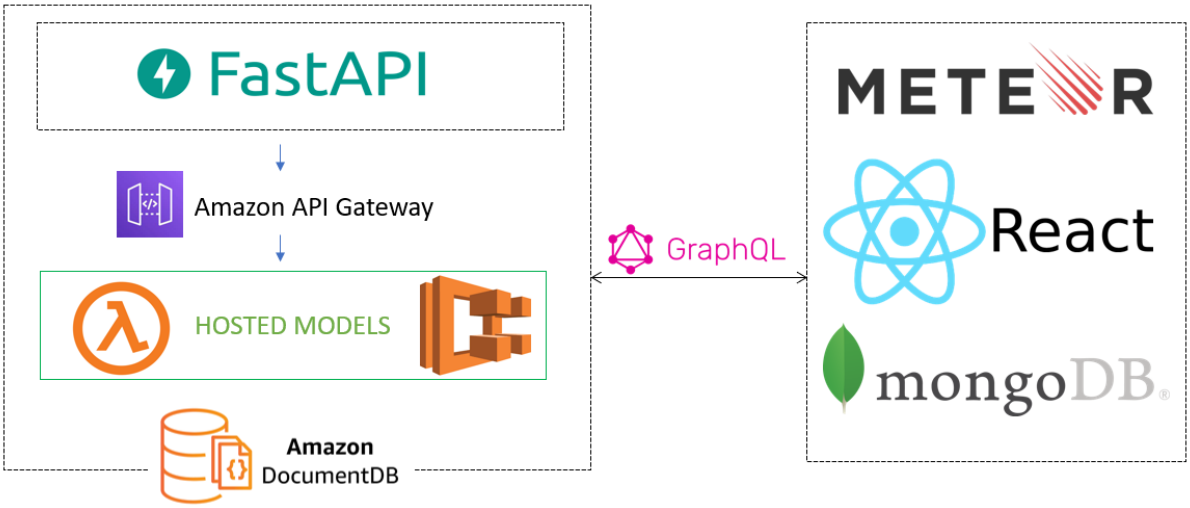

Figure 1: Overview on the platform infrastructure.

Funding information
NanoInformaTIX project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 814426.

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

MATERIAL FLOW MODELLING OF GRAPHENE-BASED PRODUCTS IN EUROPE FROM 2004 TO 2030

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The 2D nanomaterial graphene has unique material properties and is used in lithium-ion batteries, conductive inks (e.g. colour-changing car paints), supercapacitors and various composite materials. Graphene and its derivatives are also used for lightning dissipating wind turbine blades or drilling fluids in the oil industry due to their excellent mechanical, electrical and chemical properties. Although the production of graphene-based materials (GBM) has been increasing since 2004, very little is known about the potential exposure and the amounts released into the environment. Therefore, an exposure estimation model using dynamic probabilistic material flow analysis (MFA) was developed to calculate predicted release concentrations (PRC values) of graphene. The transfer coefficients of the model, which are needed to partition GBM flows after specific processes (e.g. during use or recycling), were estimated based on the physicochemical properties of GBMs and their behaviour in technical compartments such as waste incinerators and during GBM release into the environment. Based on market analysis and literature review, production volumes were estimated to increase from 140 t in 2020 to 3460 t in 2030. Taking into account the lifetime of GBP (e.g. 20 years of use in wind turbine blades) and the release rates during the use and end-of-life phases, it was possible to estimate graphene mass flows and releases to the environment. The DPMFA model showed that in 2030 most of the graphene will remain in the products (in-use stock), while a total annual release of 430 t is predicted, of which about 14 % will be exported, 17 % landfilled and 61 % disposed of in waste incinerators. The remainder is diffusely emitted to soils and surface waters. The model has resulted in PCR values of 1.4 ng L-1 in surface waters, 16 ng L-1 in urban soils and 19 ng L-1 in soils treated with sewage sludge. These results can be further used for environmental risk assessment and determination of predicted environmental concentrations (PEC values), insofar as the developed DPMFA model is combined with fate models to consider possible transformation processes of the released graphene in the future.

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

IN VIVO VALIDATION OF ANIMAL-FREE PREDICTION OF CHRONIC INFLAMMATION AFTER NANOMATERIAL INHALATION

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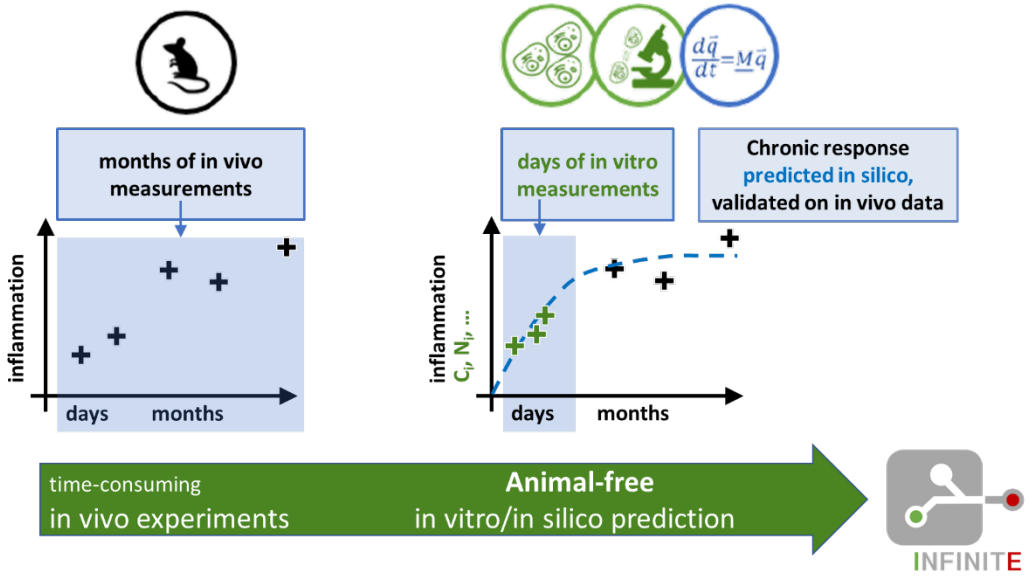
Inhalation of nanomaterials can cause lung inflammation. When nanomaterials are inhaled, they can deposit in the lungs and trigger an inflammatory response, which can lead to various respiratory diseases, including asthma, chronic obstructive pulmonary disease, and lung cancer.

In vivo testing plays an essential role in the development and regulation of nanomaterials, ensuring that they are safe and effective for use in various applications. However, there are more nanoparticles being continuously introduced than can be tested by time-consuming in vivo testing.

We have recently shown that the mechanism of lung inflammation involves the activation of immune cells in the lungs, and the formation of aggregates of nanomaterials, lipids, and proteins originating from lung epithelial cells, as well as continuous cycling of nanoparticles from epithelial to immune cells. Once the inflammatory response is triggered, it can persist for a long time, which can cause accumulation of nanoparticles and organic debris (nano quarantining) in alveoli, and a chronic influx of neutrophils in vivo [1].

After calibration against in vivo data, we show that inhalation-related chronic lung inflammation can be reliably assessed with animal-free in-vitro-to-in-silico-coupled tests, wherein in vitro part relies on time-lapse live-cell microscopy measurements of the above-mentioned cellular events for up to 2 days, and in silico modelling involves time propagation of dynamics of early key events. We tested the model on a diverse set of nanomaterials, including metal oxide and carbonaceous nanomaterials.

This new safety assessment approach allows the prediction of lung inflammation associated with nanomaterial inhalation only in vitro measurements and in silico modelling. Because of its profound implications for animal-free predictive toxicology, this work paves the way to a more efficient and hazard-free introduction of numerous new advanced materials into our lives.



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TOPIC N° 1– METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

EFFECT OF TITANIUM DIOXIDE NANOPARTICLES ON ORAL AND LUNG CELLS AND 3D ORAL MUCOSA MODELS

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Nanotechnology and its products have already become a part of our everyday life. It has been successfully applied in many sectors, including medicine and dentistry. Nano-TiO_2 is widely used and its putative toxic effects on oral health need to be investigated. The aim of this study was to assess the effects of selected TiO_2 nanoparticles (NPs) on the viability and proliferation of normal gingival fibroblasts (NGF) and on 3D oral mucosa models and compare these with the effects on lung epithelial cancer cells (A549). The integrity of the 3D oral mucosa models was also evaluated.
Spherical 40nm, rutile (American Elements®, USA) and spindle-shaped 40 x 10nm, rutile (American Elements®, USA) TiO_2 NPs were used for exposure (24 h) at concentrations of 0.2, 0.8, 10, 80, and 100 µg/ml for cells and 5, 20 and 2000 µg/ml for 3D models. The NPs were physico-chemical characterized (S_{BET}, D_{BET}, crystal structure and size, IEP, Z-potential, hydrodynamic diameter, polydispersity index). The NGF and keratinocytes were obtained from buccal mucosa of healthy patients after wisdom tooth extraction and used to construct 3D oral models *in vitro.* Label-free live impedance-based monitoring of NGF and A549 cells was performed using the xCELLIgence system (Agilent). Immunohistochemical markers of proliferation (Ki-67, 1:100, Dako), apoptosis (Cleaved-caspase 3, 1:200, Cell Signaling Technology), and tissue integrity (E-cadherine, 1:25, Dako) were used to study the effect of TiO_2 NPs exposure on 3D models. Quantification of positively stained cells was performed in QuPath.
TEM revealed that TiO_2 NPs were taken up by cells and were mostly present as agglomerates in the cytoplasm and occasionally in nucleus. According to DLS, spherical NPs had larger hydrodynamic diameter (418.7 ± 4.55 nm) and polydispersity index (0.210 ± 0.023) compared to the spindle-shaped NPs. Impedance-based monitoring revealed concentration-dependent (from 10 µg/ml and above) decrease in fold-change versus control for both types of NPs in NGF (fold change < 1), indicating cell death after 24h of exposure. However, fold-change in A549 cells exposed to both types of NPs was very close to controls (fold-change = 1), indicating no effect. Immunohistochemistry revealed a slight decrease in cell proliferation in the basal layer of 3D models exposed to both types of NPs at the highest concentration of 2000 µg/ml (3.3 ± 1.8% and 3.4 ± 2.6% for spherical and spindle-shaped NPs, respectively), however, not significantly different from the control (6.1 ± 2.2%). An increased percentage of apoptotic cells in the basal layer was found in tissues exposed to spherical NPs at 2000 µg/ml and spindle-shaped at 20 µg/ml and 2000 µg/ml, the differences were though not statistically significant. The integrity of reconstructed epithelium was not affected by TiO_2 NPs.
The study showed that exposure to both types of TiO_2 NPs impaired the growth of NGF but not of A549 cells in monolayers. Exposure to the highest concentration of TiO_2 NPs lead to decreased cell proliferation and increased cell death in 3D oral mucosa models, however the difference did not reach statistical significance.

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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

COMPARISON ABIOTIC-BIOTIC TESTS FOR ASSESSMENT PRO-OXIDATIVE POTENTIAL ON NANOPARTICLES

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In 1956 Denham Harman introduced the concept of oxidative stress: a pathological condition where the physiological balance between oxidants and antioxidants is broken. When the redox balance is altered, free radicals are produced. It is when production becomes excessive that the process of oxidative stress starts, which today is associated with many human pathologies [1].
With the exponential growth of engineered nanomaterials (NMs), there is an increasing need to find abiotic, fast, predictive and reproducible methods to test the ability of these materials to induce oxidative stress in cells. In many European projects the scientific community has studied the mechanisms of action underlying the adverse responses of the organism and has created tools (i.e. NanoReg - GA: 646221) (i.e. NanoInformaTIX - GA: 814426) to predict the effects caused by new materials already in the design phase of the material itself. This concept is called Safe by Design (SbD) and allows developers to create new intrinsically safe engineered materials.
In this work we compared abiotic colorimetric tests and cytochrome C (cyt C) tests with a cellular test to evaluate the production reactive oxygen species (ROS) due to different nanoparticle (NP) designs. During the ASINA EU project (GA: 862444) different design strategies for silver nanoparticles (Ag-NPs) with different biopolymeric coatings: Naked (NKD, without coating), HydroxyEthylCellulose (HEC), Polyvinylpyrrolidone (PVP) and Curcumin (CUR) has been compared.
The goal is to correlate the biological responses with the pro-oxidative potential (OP) assessed through the evaluation of the consumed moles of glutathione (GSH/GSSG) or p-nitroaniline (RNO), a molecular probe specific for the detection of OH•. Furthermore, the acellular assay cyt C was performed to determine the intrinsic OP of the NPs. This assay could be considered for the prediction of reactivity and toxicity by using electron transport chain proteins [2]. The intracellular production of ROS was further investigated through the DCFDA-DH assay in A549 cells exposed for 90 min to the different NPs.

From the intracellular ROS tests, results show that AgHEC NPs do not induce an increase in ROS levels in A549 cells, while the ROS production increases in the case of exposure to AgNKD and AgPVP NPs. On the counterpart, AgCUR NPs do not induce ROS production, possibly due to the antioxidant properties of the CUR coating. Interestingly, the exposure to AgHEC NPs induce oxidative DNA damage at higher levels than the other NPs [3]. The same trend was also found in the case of the abiotic test with the GSH assay where we have AgNKD (24% moles of GSH consumed) > Ag PVP (23%) > AgHEC (0%) = AgCUR (0%).
As regards the tests of the intrinsic OP evaluated through the cyt C reduction method, the trend is reversed, with AgHEC NPs with higher intrinsic OP in comparison to the other AgNP tested. This follows what was found by the consumption test of the RNO molecule, specific for OH• where we have AgHEC (32% moles of RNO consumed) > AgCUR (13%) > AgNKD (7%) > AgPVP (3%).
The resulting strong correlation between the OP evaluated with GSH/GSSG test and DCFDA-DH assay in A549 cells on one side and between the RNO and cyt C assay on the other side, is very promising because provides a proof of concept for the use of abiotic tests for the evaluation of one of the main relevant mechanisms related to NPs toxicity.

This research received support from the EU H2020 ASINA (Anticipating Safety Issues at the Design Stage of NAno Product Development) n° 862444.

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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

Finding optimal methods for testing pro-inflammatory potential of SiO_2 nanomaterials within a SbD hazard testing strategy

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Abstract
The concept of safe-by-design (SbD) can help innovators and manufacturers to produce safer products, implement safer production methods, and ensure a safer use and end-of-life. As part of SbD, the hazard potential of a new nanomaterial (NM) is identified in the early stages of product development. For this, simple yet predictive toxicity assays are crucial. It was previously reported that predictivity of *in vitro* models greatly depends on the tested type of NM and their mode of action. In this work, we investigated the suitability of several *in vitro* models and exposure methods to predict the pro-inflammatory potential of silica NMs.

Four silica NMs were selected of which *in vivo* pulmonary toxicity has previously been assessed. Their ranking in terms of potency to induce pulmonary inflammation in rodents is as follows: DQ12 (quartz) > NM-203 (fumed silica) > Colloidal silica > Silanized colloidal silica. Several cell types including a human primary bronchial epithelial cell model, immune cell lines, and lung epithelial cell lines, either in mono- or in co-culture were exposed to the four NMs either in a submerged setting or at the air-liquid interface (ALI). After the exposure, cytokine response was assessed using either qPCR, 13-plex LegendPlex, or ELISA and toxicity rankings were compared.

The results indicate differences in response between cell models and exposure methods. It is commonly believed that more physiologically relevant models give the best prediction of *in vivo* effects. However, we find that simple models are capable of predicting *in vivo* effects of silica NMs, as long as the assay is focused on the expected mechanism of action of the NM. Submerged exposure of a cell-line and the hemolysis assay seem suitable for assessing pro-inflammatory potential of silica NMs. These assays also fit very well in a SbD hazard testing strategy because of their simplicity and cost-effectiveness. For NMs that are not compatible with submerged testing, ALI exposure might be needed.

Funding information
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TOPIC N° 1 - METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

IN VITRO EVALUATION OF THE SAFETY OF BIO-SILICA NANOPARTICLES USED AS NANOFILLER FOR THE PRODUCTION OF SUSTAINABLE PUR FOAMS

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Most of the polyurethane (PUR) foam-based products, used for building, construction, bedding and automotive industries, are based on petrochemical precursors and usually lack of enabling functionalities, such as antimicrobial, antifungal, flame retardant properties and sustainability. Bio-based composites, derived from renewable resources such as biomass wastes, are nowadays applied in the above-mentioned markets to answer to the increasing requirements for the development and application of Safe and sustainable by design (SSbD) chemicals and materials. In this perspective bio-silica nanoparticles (NPs) derived from rice husk (SiO_2-RHSK) were used as nanofillers in the production of greener and sustainable bio-PUR foams. However, the human and environmental safety associated to the exposure to these new bio-nanomaterials (NMs), as pristine materials or once inside the final products, is poorly investigated. Moreover, the literature lacks data about the safety of using bio-based NMs compared to conventional ones (e.g. commercial metal oxide NPs). The aim of this work is to evaluate, by *in vitro* investigations, the safety of SiO_2-RHSK, in comparison to commercially available SiO_2 NPs (Aerosil 200) largely used for several applications. Some modifications of SiO_2-RHSK, by the addition of hydroxyl groups and additives (e.g., polyols), were also investigated. The different NPs were characterized for their physico-chemical properties, by FTIR, TEM, DLS and ζ-potential analyses. As *in vitro* models representative of human exposure, cell lines of the alveolar region (A549) and of the immune system (THP-1) were used to assess the cytotoxicity, by MTT or Alamar Blue assay, the oxidative stress and the inflammatory response, by RT-qPCR tests and ELISA tests, of the different NMs. Moreover, zebrafish (*Danio rerio*) embryos were used to evaluate the safety of the two selected SiO_2-NPs through FET test (OECD 236) and RT-qPCR. This model represent a biological system with a higher level of complexity and it is useful to assess the potential environmental toxicology of the SiO_2-based nanofillers. The results showed that, while the exposure to commercial SiO_2 NPs induce cytotoxicity, inflammatory and oxidative stress responses in A549 and THP-1 cells, SiO_2-RHSK NPs resulted safer at the same tested concentrations (10, 50, 100 and 200 µg/mL). However, preliminary results also showed that specific modifications of SiO_2-RHSK with additives, applied to improve their properties for enabling the incorporation in PUR-foams, also modified the *in vitro* toxicity profiles of the bio-based NMs. On the counterpart, the rate of mortality, malformations and hatching of zebrafish embryos are not affected by the exposure to Aerosil 200 and SiO_2-RHSK NPs, showing their safety on whole organisms and from an ecotoxicological perspective. Experiments are ongoing to determine if the newly developed NMs modulates the expression of selected genes involved in oxidative stress and inflammation pathways in both human cell lines and zebrafish model. This study gives new insights on the safety profiles of different nanocomposites used as fillers and provide promising results for the safe application of bio-NMs in PUR foams. Further research will be focused on the safety assessment of the final products (e.g. semi-rigid PUR panels) enabled with the tested bio-based nanofillers.

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BIOSYNTHESIS, CHARACTERIZATION AND CITOTOXICTY ACTIVITY OF COPPER OXIDE NANOPARTICLES OBTAINED BY ESSENTIAL OILS

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Copper (Cu) is a cheap material, stable in terms of chemical and physical properties, which has been studied for its antimicrobial, antifungal, antitumor properties, among others. This study investigated the biosynthesis approach for the preparation of copper oxide nanoparticles (CuO NPs) obtained by a biogenic route using essential oils derived from *Cymbopogon citratus* – Lemon Grass Essential Oil (CuONPs-CP) or *Schinus terebenthifolia* – Pink Pepper Essential Oil (CuONPs-PR) plants that has components with various pharmacological actions including anticancer, antifungal, antibacterial, antiviral, and antioxidant properties. CuONPs-CP and CuONPs-PR have a mean hydrodynamic size of 209.45 ± 28.65 nm and 242.73 ± 45.77 nm and PDI values of 0.260 ± 0.091 and 0.287 ± 0.058, respectively. The cytotoxicity of both types of CuONPs were evaluate against malignant melanocyte cell lines stage III, derived from the lymph node of the metastatic (VMM39) and against fibroblasts (FN1) (Figure 1). The significant cytotoxic potentials of nanoparticles were determined by MTT assay by measuring cellular metabolic activity as an indicator of cell viability and proliferation. The results demonstrate that both CuONPs-CP and CuONPs-PR nanoparticles have a concentration dependent toxicity against both cell lines at the tested concentrations (0.49 µg/mL to 250 µg/mL). Importantly, both nanoparticles were found to be more toxic to VMM39 compared to fibroblast. For instance, half maximal effective concentration (EC50) values of CuONPs-CP were found to be 3.24 and 28.12 µg/mL for tumoral and non-tumoral cell lines, respectively. Similarly, EC50 values of CuONPs-PR were found to be 0.90 and 4.40 µg/mL for tumoral and non-tumoral cell lines, respectively. These results indicate higher toxicity of the essential oil synthesized CuONPs to melanoma cells, compared to fibroblast cells, suggesting a safe window for the potential use of these nanoparticles. Moreover, CuONPs-PR nanoparticles were found to be more toxic to both cell lines, compared to CuONPs-CP, indicating that the chemical nature of the essential oil used in the nanoparticle biosynthesis confer additional biological effect due to the caping effect onto the nanoparticle surface.

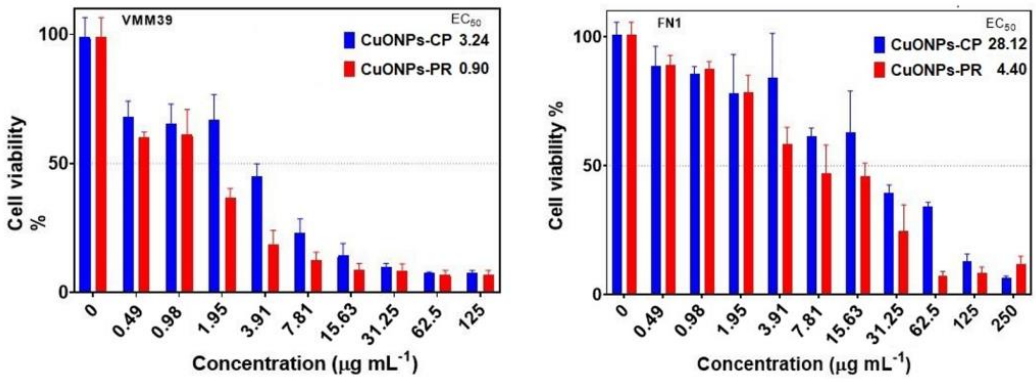

Figure 1- Cell viability of malignant melanocyte cell lines stage III, derived from the lymph node of the metastatic (VMM39) and against fibroblasts (FN1) incubated with CuONPs-CP and CuONPs-PR

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

STRUCTURAL CHARACTERIZATION AND CYTOTOXIC EVALUATION OF PARENT COMPOUND-COVERED SUPERPARAMAGNEITC IRON OXIDE NANOPARTICLES

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Cancer is one of the most complex public health problems faced by health systems around the world, given its epidemiological, social, and economic magnitudes. It is well known that combretastatin A4 (CA-4), isolated from the *Combretum caffrum*, is used to inhibit angiogenesis, which is related to the growth of new blood vessels from the existing ones. However, depending on the dose administered to the patient, it can cause some side effects. The parent compound LASSBio-1735, an *N*-acylhydrazone derivative, was evaluated for its cytotoxic effects using MTT assays against tumor cells of HL-60 (human leukemia), SF-295 (human glioblastoma), MDA-MB435 (melanoma), and HCT-8 (ileocecal adenocarcinoma) showing promising results.
Developing proper drug carriers, which can deliver the drug to a specific target, has been the driving force of our research group. We have been synthesizing superparamagnetic iron oxide nanoparticles (SPIONs), functionalized with biocompatible ligands, and evaluating their *in vitro*/*in vivo* cytotoxic effects. Here, the structural characterization and cytotoxicity of LASSBio-1735 attached to L-cysteine-covered SPIONs were carried out. Using X-ray powder diffraction data and the Rietveld method, we could perform a quantitative phase analysis of the formed system, which consisted of anhydrous and dihydrate forms of LASSBio-1735, L-cystine, and magnetite. It is worth noting that L-cysteine turned into L-cystine in the solid state.
To evaluate the antineoplastic action, tumor cells obtained from the ascitic fluid of Balb/c mice with Erlich's ascitic tumor were used, at a concentration of 2.10^5 cells/mL, in the lateral region of the back of each animal. After 30 days of treatment, we observed a tendency to reduce tumor mass when treating animals with LASSBio-1735-covered NPs.

Acknowledgments

We are thankful for the financial support provided by FAPESP (proc. 2021/03640-7) and CAPES (Finance Code 001).

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

ANTIOXIDANT ACTION OF L-CYSTEINE ANCHORED ON THE SURFACE OF MAGNETITE NANOPARTICLES

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This work describes the synthesis, characterization, and evaluation of the antioxidant action of the L-cysteine (Cys) molecule when anchored to superparamagnetic iron oxide nanoparticles such as magnetite (Fe_3O_4). Fe_3O_4 nanoparticles are obtained by the coprecipitation method, in which the salts iron chloride II ($FeCl_2{\cdot}4H_2O$) and iron chloride III ($FeCl_3{\cdot}6H_2O$) are solubilized in an aqueous solution by the addition of ammonium hydroxide (NH_4OH). The surface of SPIONs is functionalized with Cys to increase its biocompatibility and evaluate its antioxidant action and the possibility to use these systems as drug carriers, once the SH groups act as coordination points to biomolecules and drugs. L-Cys-functionalized Fe_3O_4 nanoparticles are produced by changing the reaction times and mass proportions between Fe_3O_4 nanoparticles and the L-Cys molecule to investigate the dimerization of L-cysteine into Cystine.

Therefore, this study is separated into two aspects: the first one, intends to observe the dimerization of the Cys molecule into cystine and evaluates and improves the functionalization process of the SPIONs surface with Cys, by varying parameters such as reaction time and mass proportions (NPs:Cys). The samples are characterized by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) and magnetic curves. Our study corroborates that the cysteine molecules are anchored on the nanoparticle's surface by their carboxylate groups since the presence of free SH groups for dispersed nanoparticles is verified and quantified by DTNB titration (0.062 mmol per gram of nanoparticles). However, it is observed that for these nanoparticles in the solid state, cysteine dimerization leads to the cystine crystal structure; therefore, there are no free SH groups in this case. We also propose that there is physical adsorption, i.e., an interaction governed by van der Waals interactions between the carboxylate group and the surface of the particles since no relevant displacements of the bands associated with the CO and COO stretching are observed. In addition, the results show that the nanoparticles have a magnetite structure with a crystallite average size of 12 nm and superparamagnetic behavior.

The second part of this work focuses on verifying the antioxidant action of Cys by performing biological tests *in vitro* and *in vivo*. The results demonstrate that Cys anchored in SPIONs presents antioxidant action, helping in cell regulation and preservation. This study demonstrated the effective application of thiol-functionalized SPIONs to be applied in biomedical applications.

TOPIC N°6**.** CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

POLYVINYLPYRROLIDONE (PVP) INTERACTION WITH BIOMEMBRANE-LIKE LAYERS

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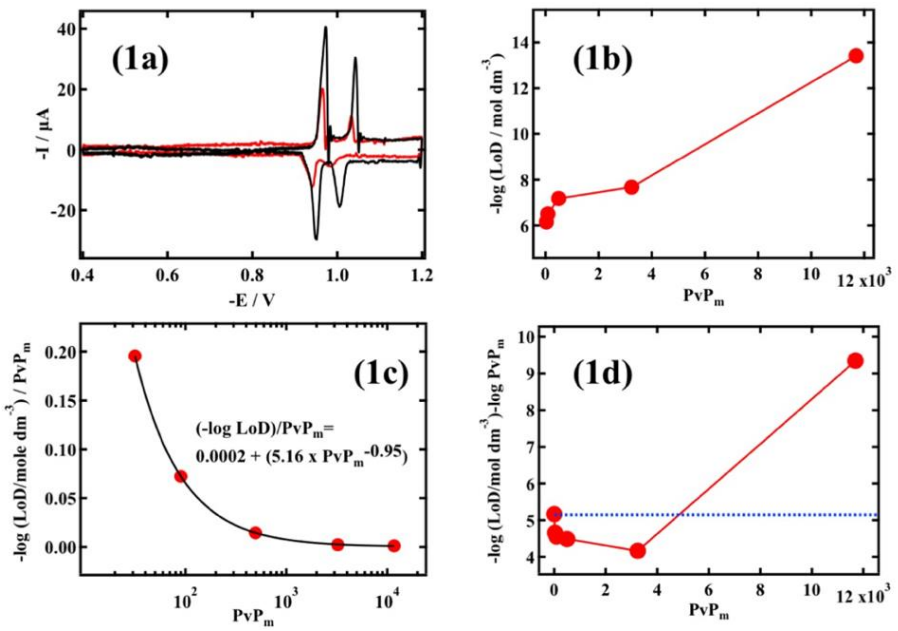
Polvinylpyrrolidone (PvP) is a water soluble polymer made from the monomer *N*-vinylpyrrolidone (*N*-vP) and has extensive use in the synthesis and stabilisation of nanomaterials. Recently, this laboratory found that PvP interacted very strongly with the HISENTS biomembrane sensor, a response which is directly related to cell membrane damage by compounds. Comprehensive studies were therefore instigated to look at the effect of PvP on the HISENTS biomembrane sensor element.

5 lengths of PvP were investigated of monomer number ($PvP_{\mathbf{m}}$) from 31 to 11697 and dissolved in MilliQ water as stock solutions. Working solutions were prepared therefrom in phosphate buffered saline (PBS) at pH 7.4 and screened on the dioleoyl phosphatidylcholine (DOPC) sensor element. Interactions of PvP with the sensor element are shown as depressions of the two capacitance current peaks on the rapid cyclic voltammogram **(1a)**. The limit of detection (LoD) metric of the PvP affinity for the sensor element is the lowest solution concentration of PvP, which can structurally modify the DOPC layer. Calibration curves are linear for low $PvP_{\mathbf{m}}$ (31, 90) but Langmuirean for higher $PvP_{\mathbf{m}}$ (495, 3239, 11697) indicating that PvP is adsorbing on and damaging the DOPC layer surface and structure.

Plots of -log LoD versus $PvP_{\mathbf{m}}$ show an increase corresponding to a greater interaction of the PvP with the DOPC layer with increasing length **(1b)**. However a plot of (-log LoD)/$PvP_{\mathbf{m}}$ versus $PvP_{\mathbf{m}}$ gives a reciprocal relationship **(1c)** from which a realistic value of LoD for *N*-vP can be extracted. A plot of log (1/(PvP_m x LoD)) versus $PvP_{\mathbf{m}}$ shows a sharp decrease at low $PvP_{\mathbf{m}}$ values and an increase at higher PvP_m values **(1d)**. If the physical nature of the PvP plays no role in the PvP interaction with DOPC, the value of log (1/($PvP_{\mathbf{m}}$ x LoD)) should remain constant (blue line in **1d**). The reason for the decrease at low $PvP_{\mathbf{m}}$ values is due to the decrease in entropy as the PvP chain moves from a 3D to a 2D environment. The increase in values at higher $PvP_{\mathbf{m}}$ is caused by the increased self-assembly forces between the longer chains on the DOPC surface.



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TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

SYNTHESIS AND CHARACTERIZATION OF FIBROIN-CONTAINING LAYERED DOUBLE HYDROXIDE MICRONEEDLES AS DRUG-DELIVERY SYSTEMS

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During the last few years, researchers have been investigating new nanomaterials focusing on developing drug-delivery systems, including mesoporous silica nanoparticles (NPs), liposomes, polymeric nanoparticles, and layered double hydroxides (LDHs), to improve the therapeutic effect of drugs and reducing their side effects. LDHs (represented by the general formula $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}\left[A^{n-}_{x/n}\cdot mH_2O\right]$, where M^{2+} and M^{3+} are di- and trivalent cations and A^{n-} is an ion) have been attracting attention in biomedical therapeutics and cancer therapy as drug-delivery agents, due to their biocompatibility, wide area surface, good chemical and mechanical characteristics, and suitable magnetic characteristics.
Another fascinating system being used is based on transdermal microneedles (TMNs). TMN patches penetrate the skin barrier and can deliver proteins, peptides, vaccines, and small molecules. Such patches are a painless method of drug administration, offering a preferred route of administration over parenteral and oral ones. Arrays of silk fibroin-based TMNs have been used for the successful delivery of vaccines against the human immunodeficiency virus (HIV), influenza, *Clostridium difficile*, Shigella, sustained-release contraceptives, insulin, and photosensitizers. However, microneedles made from unmixed fibroin are prone to breakage, and rapid dissolution can lead to sudden drug release. Thus, some modifications are needed to improve the tenacity and dissolution time and ultimately extend the drug release time from the microneedles.
In this work, we developed a new combination of materials subjected to structural modifications and delivery control. In this context, we chose LDHs (hydrotalcite type) and silk fibroin (SF) for manufacturing TMNs, investigated their structures, and evaluated the release and controlled delivery of rhodamine and methylene blue as agents. Patches containing 10×10 grids of square-based pyramidal microneedles were successfully produced using different contents of SF, LDH, and PVA (polyvinyl alcohol). The TMNs are 700 μm long, 200 μm wide at the base, and 25 μm wide at the tip. They are characterized by their penetration depth performance and release kinetics of methylene blue and rhodamine. This study indicates that these hybrid arrangements of SF, LDH, and PVA could overcome the barrier function of the skin and ensure the transdermal delivery of molecules for future applications.

Acknowledgments

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TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

ACCOUNTING FOR CONSTITUENT PARTICLE POLYDISPERSION IN THE IDENTIFICATION OF NANOMATERIAL POWDERS BASED ON THEIR VOLUME SPECIFIC SURFACE AREA

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Introduced by Kreyling et al. (2010), the Volume Specific Surface-Area (VSSA) is to be pointed out as a relevant and alternative method to electron microscopy to determine whether a material is a nanomaterial or not, in addition to being mentioned in the definition from the European Commission. Indeed, this parameter was recently integrated as a tier 1 screen in the JRC decision trees (Mech et al., 2020).

VSSA is an integral measurement method that provides particle size indirectly. In practice, d_{VSSA} is derived from VSSA according to (1):

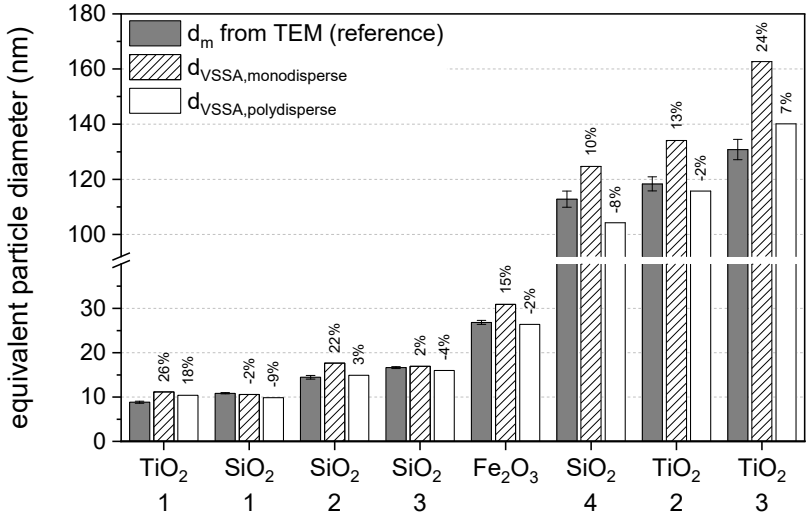
$$d_{VSSA}\ (\text{nm}) = \frac{2000D}{VSSA\ \left(\frac{\text{m}^2}{\text{cm}^3}\right)} = \frac{2000D}{SSA\ \left(\frac{\text{m}^2}{\text{g}}\right)\rho\left(\frac{\text{g}}{\text{cm}^3}\right)} \quad (1)$$

where D = 3 for spherical shape, D = 2 for fiber-like particles and D = 1 for platelet-like particles.

When the conversion from specific surface area to particle diameter is performed, the constituent particles (individual object) are assumed to be spherical and monodisperse. This strong hypothesis is far from reality.

The study consisted in assessing the influence of the polydispersion of the constituent particles of a material on its specific surface area, and in proposing a methodology letting it to be considered in the conversion of the VSSA into equivalent diameter of constituent particles.

A correction factor was theoretically established, for both normal and lognormal size distributions of the constituent particles. This correction was applied to eight industrial nanopowders, with a count median diameter between 9 and 130 nm, and under the assumption of a size distribution according to a normal law. The results indicate that taking into account the polydispersion improves the determination of the equivalent diameter, the relative deviations being between -9% and 18% compared to the reference measurements in electron microscopy.



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TOPIC Nº 1 - METHODS, TOOLS, AND TECHNOLOGIES FOR SSbD PURPOSES

SAbyNA SbD Guidance Platform: Guiding industry to design and develop safer nanomaterials and nano-enabled products

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Safe-by-design (SbD) is not a new concept, it has been used for years in industry, anticipating safety impacts of materials or products, and incorporating management measures into the design and production phases of their innovation processes. In the context of Nanotechnology, implementation of the SbD concept requires a good understanding of the properties influencing nanoforms (NF) or nano-enabled products (NEP) safety as well as influencing technical functionality (performance-oriented properties), and a comprehensive guidance on how to exploit this knowledge into SbD opportunities during the innovation process.
SAbyNA is developing an integrative user-friendly Guidance Platform with optimal workflows to support the development of SbD of products (NF and NEP) and processes over the whole life cycle (Fig. 1). A panel of SbD strategies and risk mitigation measures are incorporated in the Guidance workflows with hierarchies and decision trees to facilitate the identification of most suitable approaches for each case. SAbyNA will also develop specific modules of such Guidance Platform tailored to the Paints and Additive Manufacturing sectors, to showcase how usability can be increased by targeting specific industrial sectors. A continuous dialogue with different stakeholders and end-users maximizes the added value of the Guidance Platform.
The main structure and content of the Guidance Platform will be presented in its current development stage, consisting of two parts; Part 1: A screening phase (for using in early stages of the innovation processes) which will allow the identification of safety concerns based on basic information inputs and providing SbD interventions towards safer solutions or directing the user to a more detailed assessment in Part 2, which guides the user into a more thorough safety assessment to identify the risks along the life cycle and decide which SbD interventions will be needed.

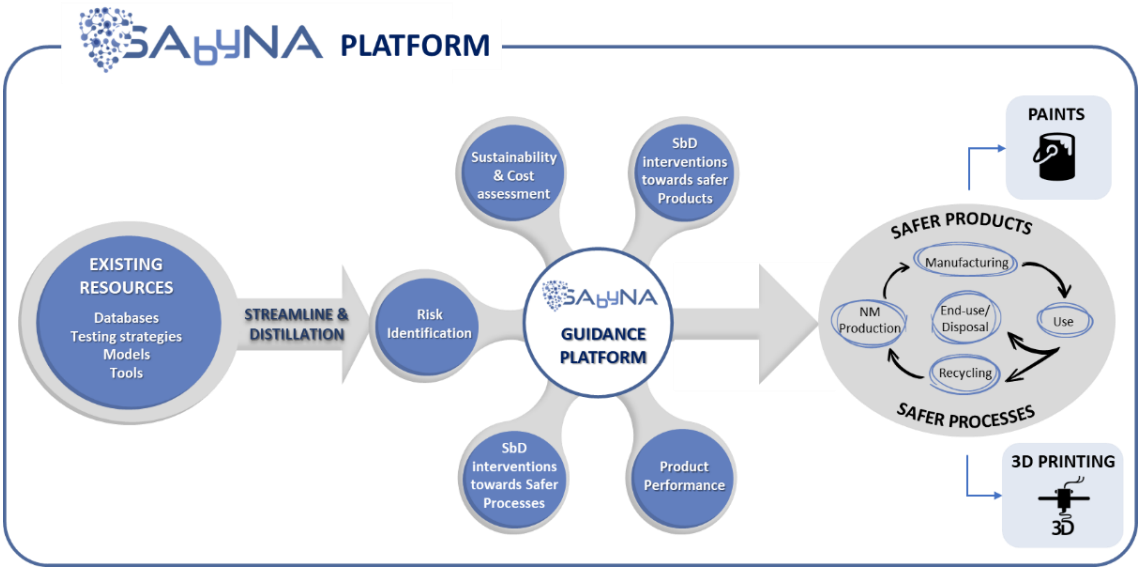

Figure 1: Scheme of SAbyNA Platform components

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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

BETTER EARLY THAN NEVER: INCORPORATING SUSTAINABILITY, RISK AND SAFETY ASSESSMENT IN THE EARLY STAGES OF THE DEVELOPMENT OF NANOMATERIALS

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The field of nanotechnology is experiencing a rapid expansion in the last decades, with nanomaterials being continuously developed with different compositions (e.g., carbon, organic, inorganic, hybrid), dimensions (from zero-dimensional to three-dimensional), porosities (from nano to macroporous), and for different applications (e.g., medicine, agriculture, food, feed, consumer products, catalysis, energy, technology, manufacturing, etc.).[1] Despite being very promising for virtually every aspect of human life, significant criticism has also accompanied the development of nanomaterials, considering their potentially harmful impacts on health and the environment upon release, the uncertainty regarding disposal or recycling at the end of their lifecycle, as well as the resource-intensive methodologies often required for their manufacture.[2]
Different methodologies can be used to assess the risks and impacts of nanomaterials, such as Risk Assessment (RA) and Life Cycle Assessment (LCA). Importantly, several researchers have previously proposed a combination of RA and LCA methodologies, as well as the application of such techniques as early as possible in the research and development stage, so that sustainability and risk criteria can guide the technological development of novel nanomaterials. Nevertheless, some inherent differences in the methodological approach of RA and LCA can inhibit their integration into a concise Decision Support methodology. [3]
The objective of this work is to guide practitioners in establishing a combined RA and LCA methodology, concerning nanomaterials at early stages of their development using open access sources. In addition, the importance of applying a common methodology at early stages of development is presented, and some practical examples of its implementation in nanomaterial research are demonstrated, using literature and taking into account the whole life cycle of nanomaterials.
Finally, some useful tools and databases for LCA and RA are presented, focusing on open access sources and methodologies that are easy to apply for practitioners with limited expertise on LCA and RA. While specialized tools would undoubtedly provide more robust results, a focus on useability and accessibility is expected to improve the transferability and acceptability of a combined RA and LCA approach, and thus the concept of Safe and Sustainable-by-Design (SSbD).
This joint approach will be tested during the four years of Nickeffect project that aims to develop novel ferromagnetic Ni-based coating materials to replace the scarce and costly Platinum and ensure high efficiency in key applications.

[1] Harisch *et al.*, 2022, DOI: 10.3390/nano12183226;
[2] Nizam *et al.*, 2021, DOI: 10.3390/nano11123324;
[3] Guinée *et al.*, 2017, DOI: 10.1038/nnano.2017.135

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

GUIDANCE ON SAFE-AND-SUSTAINABLE-AND-RECYCLABLE-BY-DESIGN PLASTICS: FIRST STEP TOWARDS OPERATIONALISING, WITH HEALTH, ENVIRONMENTAL IMPACT AND COST ASSESSMENTS

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Plastics are predominantly made from fossil feedstock and a substantial portion is quickly discarded as waste. Almost 70% of the collected plastic waste in the EU are currently incinerated, landfilled or exported to other countries, causing harm to the economy, wildlife and eventually to human health. Societal urgency dictated by those health and environmental impacts has motivated innovators to develop new solutions to make them safer, more sustainable and more recyclable by design. The challenge is anticipating beyond material and process, the life cycle of future products, their end-of-life (EoL) and potentially their inclusiveness in circular economy.
However, the lack of targeted guidance and holistic vision, in addition to the existence of multiple definitions, regulations and standards, results in ambiguity and multiplication of criteria. The aim of the European SURPASS project is to overcome this global challenge by developing a safe-, sustainable-, recyclable-by-design (SSRbD) Assessment and guidance dedicated to polymeric materials. The main objective is to provide European SMEs with a digital guiding tool that will impart knowledge and provides SSRbD assessment & guidance to support them in their development of innovative products incorporating polymers. The tool will be built on the results from three Case Studies (CS) related to strategic sectors representing 70% of European plastics field. In order to operationalise sets of criteria, adapt and develop methodologies and develop user-friendly scoring strategies to assess the three domains of sustainability, inventories of physico-chemical properties of additives, by-products, processing aids, degradation products and contaminants detected in plastics need to be compiled and their relationship to functionality, human and environmental impacts investigated.
Regarding, the operationalising of health, environmental impact and cost assessment, the first step to conduct the evaluation is to establish the Life Cycle Inventory (LCI). The challenge is that case studies TRL are low level (3) at the start of the project. Thus, the LCI collection have to be conducted in parallel of the eco-ideation and eco-selection steps of the innovative development of plastics. The solution adopted and presented here is to implement the JRC Safe and Sustainable by Design (SSbD) framework for chemicals and materials in a global practical approach that takes into account the development of complex systems in industry. To do so, work is carried out on two fronts. Firstly, a detailed description of several routes of the SSbD options, the baseline process and the reference process is established with the CS leaders. Secondly, within the SSbD team, work is carried out to harmonise the methodology for Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) in order to create a single data collection template. In a second phase of the project, this template will be adapted to integrate hazard and exposure aspects. This unified template will serve as a basis for the construction of the digital guiding tool and provide a holistic SSRbD assessment guidance.

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

HARMLESS DECISION SUPPORT SYSTEM FOR SSBD

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Potential users of Safe and Sustainable by Design (SSbD) approaches, in particular small and medium sized enterprises, struggle to find their way in the sheer amount of existing knowledge, testing methods and tools. To better guide them in making decisions throughout their entire design process of advanced nanomaterials, a user-friendly online intelligent SSbD Decision Support System (DSS) is developed within the HARMLESS project.
Previous experience and stakeholder analysis is used to integrate existing as well as numerous newly generated NAM-based data & models and integrated approaches to testing and assessment (IATAs) into a unified HARMLESS SSbD framework. We propose a modular approach in which all data, knowledge and models are captured in stand-alone, interconnected FAIR components. HARMLESS will A) use eNanoMapper as a database to store experimental data, B) launch a new FAIR Data Point to capture all knowledge and decision rules, C) launch a novel Model FAIRpoint with runnable algorithms and D) launch a new Decision Support System interface.
The DSS interface connects to the HARMLESS FAIR components, but can also connect to external components (e.g. NanoCommons Data Warehouse, Jaqpot, NanoInformaTIX). The decision support process is based on a well-established concept of Risk Informed Decision Making adopted by NASA and US NRC, based on the fusion of deterministic and probabilistic risk assessment. To this end, Bayesian network modelling is used to integrate existing and new knowledge, data and models into an integrative reasoning model that captures uncertainty. This information is presented in an interactive visualization presenting an overview of all important decision criteria augmented with SSbD advice allowing the user to interactively work with the tool to improve their design.

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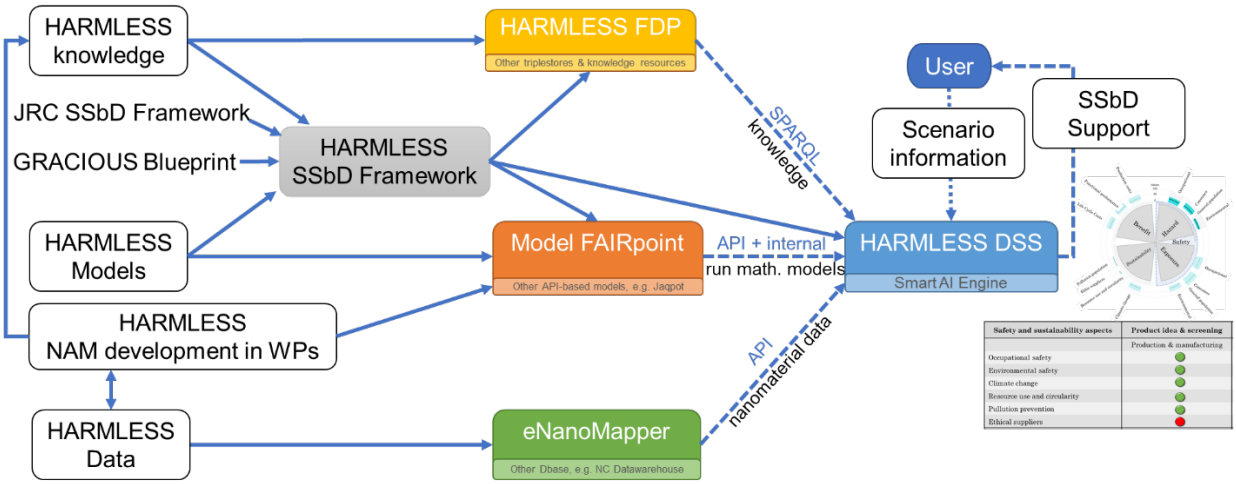

Figure 1 Schematic Diagram of the Modular and FAIR IT-architecture. Besides the four technical components (right side), this diagram also indicates how information generated within HARMLESS flows into each IT-component (left side).

TOPIC N° 1 – TOPIC 1. METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

THE PARC TOOLBOX TO OPERATIONALIZE THE SSbD CONCEPT FOR CHEMICALS AND MATERIALS

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The EU Chemicals Strategy for Sustainability has the aim to improve human and environmental health as part of an ambitious approach to reach a pollution-free environment. The European Commission developed a framework for the definition of SSbD criteria. The "Partnership for the Risk Assessment of Chemicals" (PARC) has been designated by the EC to play a central role in operationalizing the SSbS concept. In particular, PARC has the aim to translate the SSbD criteria into a toolbox, integrating tools for safety and sustainability assessment and setting up a support structure to help making SSbD operational. The toolbox will cover both chemicals and materials/products by addressing in particular the 'by-design' aspect as well as chemical or material use in products. As learned from the development and implementation of the SbD concept for nanosafety, assessment in the early stage of development is vital for SSbD operationalization. Using the SSbD toolbox, the "sustainability" requirements can be quantified, taking into account life cycle assessment (LCA) techniques, risk assessment and socio-economic assessment methodologies. Finally, the toolbox will be available to both regulatory and industrial stakeholders, with the former assessing whether a chemical, material, or product meets the SSbD criteria and the latter using the toolbox to develop chemicals, materials, and products that meet the SSbD criteria at the early stage of innovation or during their development.
As a first step, an inventory of the applicability of existing tools has been be made, focusing on existing initiatives on SbD for nano- and advanced materials, Green Chemistry, Sustainable Products Initiative, the CEFIC-LRI project DOREMI, HBM4EU and others. Additional state-of-the-art knowledge on developments and instruments applicable for SSbD assessment will be based on the experience of previous EU projects where toolboxes have been developed. In particular, the experience on nanomaterials and chemical mixtures in terms of safe-by-design will be translated to broader applications on chemicals and materials. This work will result in an alpha version of the toolbox to be ready in year 2 of PARC based on the conceptual design that has been developed in year 1.
The aim of this presentation is to provide an overview of the current state of the PARC toolbox, the review process of available tools and frameworks and the conceptual design of the PARC toolbox.

TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

A NETWORK OF CAUSAL RELATIONSHIPS LINKING THE CHEMISTRY OF A NANOMATERIAL TO THEIR TOXICOLOGY

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The concept of safe-by-design starts with the notion that the safety is a function, an effect, of the chemical structure and properties of the material. Consequently, a computing infrastructure for the definition and implementation of the safe-by-design approaches therefore needs to be based on a machine readable representation of the relationship that links the chemistry with the toxicology.

In order to describe this link, we have to break the problem into smaller pieces. For example, to link the diameter of a TiO_2 particle to the photoreactivity, we first say the diameter defines the particle size, which in turn governs the surface area, which in turn correlates with the photoreactive. By defining these relationships, a network can be built, linking the chemistry with the toxicology. These causal relationships may be defined by a nano-QSAR model, a research paper, or by a data set where the effect is measured of a structural change.

Knowledge about causal relationships exists, but is scattered over literature and other data sources, which is a problem we address in a different study "The Semantic Landscape: an ever growing, publicly available overview of nanosafety knowledge". Finding sources, however, involves manual curation and so does extracting causal relationships and annotation of the biology and chemistry a resource captures.

When enough "gold standard" datasets have been manually created, these can be used to train text mining solutions to extend the available networks of causal relationships, though capturing the nuances remains challenging. We here speak of networks, because not all relationships are applicable for all nanomaterials, similar to how one model works for metal oxides and another for a series of coated TiO_2s. Since not all relationships are equally well described in literature, we apply a tiered approach. Here Tier 1 corresponds to the causal relationships being directional (e.g. the surface area of a TiO_2 particle positively correlates with the photoreactivity), while for Tier 2 we aim to increase the precision of the safe by design suggestions we can do based on the network by quantifying the relationships.

Earlier we developed a proof of principle of implementing semantic web approaches for reporting NMs, their physicochemical characteristics and their interactions with biomolecules and biological systems. Creating the link between the molecular effects described in literature and key event (KE) identifiers from AOP-Wiki. A similar approach is implemented here, where the link to AOPs provides a connection from the chemistry to adverse outcomes.

Here we present examples of networks we can create for different nanomaterials with their value and limitations. We show the difference between a Tier 1 and Tier 2 approach and the influence it has on the predictions we can make for safe-by-design purposes.

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TOPIC N° 5 - FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

CANADA'S APPROACH TOWARDS ASSESSMENT OF MANUFACTURED NANOMATERIALS IN COMMERCE UNDER THE CANADIAN ENVIRONMENTAL PROTECTION ACT, 1999

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Health Canada and Environment and Climate Change Canada have undertaken an initiative to assess manufactured nanomaterials (NMs) that are in commerce in Canada for their potential risks to human health and the environment under *the Canadian Environment Protection Act* (CEPA, 1999), and take appropriate control measures when required. Building on the Government of Canada's recent publications of a data gap analysis and a draft Framework for the Risk Assessment of Manufactured Nanomaterials under *the Canadian Environmental Protection Act*, *1999* (RAF), this presentation will detail the science-based approach used for assessing the potential risks of NMs to human health and the Canadian environment. The presentation will also cover the methodologies used, the challenges encountered, and lessons learned from ongoing screening risk assessments of NMs.

Previously, 53 NMs listed on the Domestic Substances List were identified in commerce in Canada based on the information obtained from a mandatory survey under section 71 of CEPA, as well as additional information gathering initiatives. A data gap analysis of available human health-related information (exposure and hazard) for each of the 53 NMs was used to identify the exact data needs associated with each for conducting regulatory risk assessment. The outcomes of the exercise were presented in a document published in October 2021, which mapped out the available information and data needs for each NM.

In concert with the data gap analysis, a draft RAF was developed and published in June 2022 to describe key principles, approaches and considerations for risk assessment and risk management of NMs within the Canadian regulatory context. The RAF was established on the same basis as the traditional risk assessment paradigm for chemicals, but further takes into account unique aspects of NMs that may affect the risks they pose to human health or the environment. This includes explicit considerations of physical-chemical properties (size, shape, surface chemistry, etc.), potential alterations in environmental fate and exposure throughout the lifecycle, read-across between nanoforms (e.g., variants of size, shape, surface modification within a single CAS Registry Number), as well as the use of alternative test methods for hazard identification. The RAF applies to assessment of existing NMs (i.e., in commerce in Canada), as well as NMs that are considered new to Canada and subject to notification under the New Substances Notification Regulations (Chemicals and Polymers). A summary of public comments received through a 60-day public comment period, along with an updated RAF, aim to be published in 2023.

Following the development and publication of the draft RAF, Canada has initiated the regulatory screening risk assessment of NMs identified in commerce in Canada to evaluate their potential risks to human health and the environment under CEPA. On the basis of the principles and approaches highlighted in the RAF, the first assessments on NMs are underway.

All these ongoing initiatives will help ensure that a framework and key considerations for risk assessment of in-commerce and new NMs are considered. This will allow the Government of Canada to determine whether risk management measures are needed, and if so, what appropriate risk management actions would be required to reduce or prevent risks to human health and the environment.

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO GOVERNANCE, A JOURNEY THROUGH REGULATION & STANDARDIZATION

TOWARDS HARMONISATION OF TESTING OF NANOMATERIALS FOR EU REGULATORY REQUIREMENTS ON CHEMICAL SAFETY – A PROPOSAL FOR FURTHER ACTIONS

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Over the recent years, EU chemicals legislation, guidance and test guidelines have been developed or adapted for nanomaterials to facilitate safe use of nanomaterials in society. However, so far the priority needs for development and adaptation of Test Guidelines (TGs) and Guidance Documents (GDs) are largely tailored towards the needs for the adaptation of the REACH legislation. This raises the question of whether other (EU) regulatory areas have specific needs for nanomaterials that have been overlooked in earlier assessments and that may require additional adaptations or developments of OECD TGs or GDs. The aim of this study was to identify potential needs for further action to develop or adapt guidance and test guidelines to nanomaterials.

In this presentation we first provide an overview of the information requirements across different EU regulatory areas. These comprise the food and feed sector (including plant protection products), cosmetics, biocides, medical devices, medicinal products for human use, and veterinary medicinal products. For each information requirement, a group of 22 European experts on nanomaterial safety identified the potential needs (from an EU perspective) for further action to develop or adapt guidance and test guidelines to nanomaterials. In total, 136 information requirements across different EU regulatory areas were identified. A need for further action was identified for in total 62 information requirements. Eleven different priority needs for action were identified, capturing twenty-two information requirements that are specific to nanomaterials and relevant to multiple regulatory areas. These were further reduced to three overarching topics: 1) resolve issues around nanomaterial sample preparation, agglomeration, dispersion stability and dosing in toxicity testing, in particular for human health endpoints 2) further develop tests or guidance on degradation and transformation of organic nanomaterials or nanomaterials with carbon components to better assess environmental fate of this group of nanomaterials and 3) further develop tests and guidance to measure (a)cellular reactivity of nanomaterials, which will be critical, e.g. for the development of NAMs and in high-throughput systems needed for assessing the ever increasing diversity of (newly) developed (advanced) nanomaterials.

We propose to prioritize work towards these three topics. Efforts towards addressing these overarching issues will result in better fit-for-purpose test methods for (EU) regulatory compliance. Moreover, it secures validity of the hazard and risk assessments of nanomaterials. The results of this study underscore the need for increased (innovation) policy – science – regulatory interaction to optimally exploit the full potential of nanomaterials and their contribution to technological solutions to address societal challenges.

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO GOVERNANCE, A JOURNEY THROUGH REGULATION & STANDARDIZATION

NANOMATERIALS IN CONSUMER PRODUCTS: IDENTIFICATION AND ASSESSMENT CHALLENGES

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In 2022-23, the Nanotechnology Industries Association (NIA) led a consortium carrying out a research project for the UK government, assessing on the presence of nanomaterials in a variety of consumer products. Our submission covers the results of this work:

1. **The difficulties in identifying the relevant nanomaterials:** while the presence of nanomaterials in a given product can generally be ascertained with testing, this route is not always 100% reliable and is also unpractical and expensive. Inventories of nanomaterials in consumer products therefore rely primarily on the information provided by manufacturers (either voluntarily or as part of their regulatory obligations) or made available by registers managed by government agencies or third parties. With the partial exception of cosmetics, this means that it can be extremely challenging to compile an exhaustive list of the nanomaterials present in products that are available to consumers on the European market;
2. **The assumptions that can be made on the presence of those nanomaterials identified with reasonable certainty:** a small number of substances (e.g. titanium dioxide, nanosilver) make up the vast majority of the nanomaterials identified. The reasons for this include availability; price and production scale considerations; and the nature of the relevant products and supply chains;
3. **The safety and regulatory aspects:** the current regulatory landscape in both the EU and UK is discussed in terms of the legislation applicable to i) the individual substances and ii) the finished products. Depending on the respective frameworks' evolution in diverging directions, potential differences could emerge. Finally, the standards and best practices available to support manufacturers in meeting their obligations are also examined;
4. **The role of consumers:** as online retail becomes an increasingly important channel for products to reach households, making consumers *de facto* importers, the questions of market surveillance and safety become more complex.

The following conclusions can be drawn from the study:

- It is difficult to identify and quantify which consumer products contain nanomaterials;
- The largest category of consumer products that can be identified as containing nanomaterials are cosmetics; this is not surprising considering that nanomaterials need to be identified as ingredients under the EU Cosmetic Products Regulation, and that those nanomaterials found in cosmetic products are more readily available to manufacturers;
- There are some knowledge gaps around the measurement of hazard and risk for consumer products that contain nanomaterials;
- There are also gaps in the standards and test guidelines available to manufacturers to aid them when introducing nanomaterials into consumer products, and in being compliant with regulations.

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO GOVERNANCE, A JOURNEY THROUGH REGULATION & STANDARDIZATION

RELEASE TESTS APPLICABLE TO NANOMATERIALS – A NEW OECD GUIDANCE FACILITATING THE CHOICE OF APPROPRIATE RELEASE TESTS

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Release of manufactured nanomaterials (MNs) is the first step towards exposure of workers, consumers and the environment to MNs. MNs can be released during different processes from MN-containing powders, formulations, composites or other MN-containing products. Processes, which potentially release MNs can be assigned to different mechanisms, such as low-energy or high-energy mechanical impact as well as thermal and chemical stresses. Examples for processes with potential release are bagging of MN-containing powder, cutting of MN-containing composites or washing of fabrics with embedded MNs. In addition, mixed release mechanisms occur in many scenarios, where two or more mechanisms are combined.

Exposure assessment is, along with hazard assessment, indispensable for regulatory risk assessment. Release tests provide fundamental data regarding release of a substance from powders, formulations, composites or other products to determine potential exposure. Validated and harmonised release test methods provide information for a more in-depth exposure assessment and, hence, reduce uncertainty in regulatory risk assessment.

To date, release tests for several processes are available. However, no detailed guidance on already existing standardised release tests suitable for MNs is available, so far. The objective of the presented project is to help producers, processors and (commercial) users of MNs and MN-containing products, as well as institutions, which assess the safety of consumer products, to choose appropriate release test methods by developing an OECD Guidance.

Release test data can also be used for other regulatory assessments such as life cycle assessments or for pre-regulatory approaches like safe and sustainable by design (SSbD) concepts. In order to obtain release data that are of use for regulatory assessment, the conditions under which release tests are performed, the input parameters as well as the output parameters need to be harmonised and suitable to the subsequent use. Release tests provide quantitative information about the released MNs and the release rate during a specific release process. Qualitative information about the released fragments such as information on the form of released MNs or the matrix material to which MNs are attached to or embedded in are obtained by corresponding analysis of the released fragments.

The results of this project will be an OECD Guidance including a comprehensive overview of standardised and not yet standardised release tests linked to release processes implemented in a decision framework to facilitate the choice of appropriate release tests. In addition, a conceptual link from release test data to regulatory assessments will be provided.

B2L-3: HARMONIZATION & STANDARDIZATION IN THE CONTEXT OF REGULATION 1

MACRAMÉ: ADVANCED CHARACTERISATION METHODOLOGIES TO ASSESS AND PREDICT THE HEALTH AND ENVIRONMENTAL RISKS OF ADVANCED MATERIALS

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The MACRAMÉ Project will develop and demonstrate of novel methodologies, and by advancing their harmonisation & standardisation on **three MACRAMÉ Material Families** of **inhalable carbon-based AdMas** of various morphologies and dimensions (Tiwari et al. (2012)), beyond spherical particles: **(a) graphene-related material** (GRM), **(b) carbon nanofibres** (CNFs), e.g., carbon nanotubes (CNTs), and **(c) Poly Lactic-co-Glycolic Acid (nano)particles** (PLGA). The focus on carbon-based AdMas addresses unsolved detection and characterisation issues, especially in complex media. In doing so, MACRAMÉ builds on >15 years of research and innovation (R&I) and knowledge pooling in nanosafety, formed through numerous European and international collaborations.
The **MACRAMÉ R&I Approach** (Figure 1) aims to widen the development of harmonised test guidelines (TGs) and guidance documents (GDs) (OECD) and standards (CEN, ISO) to **market-relevant AdMas in their complex product matrices**.

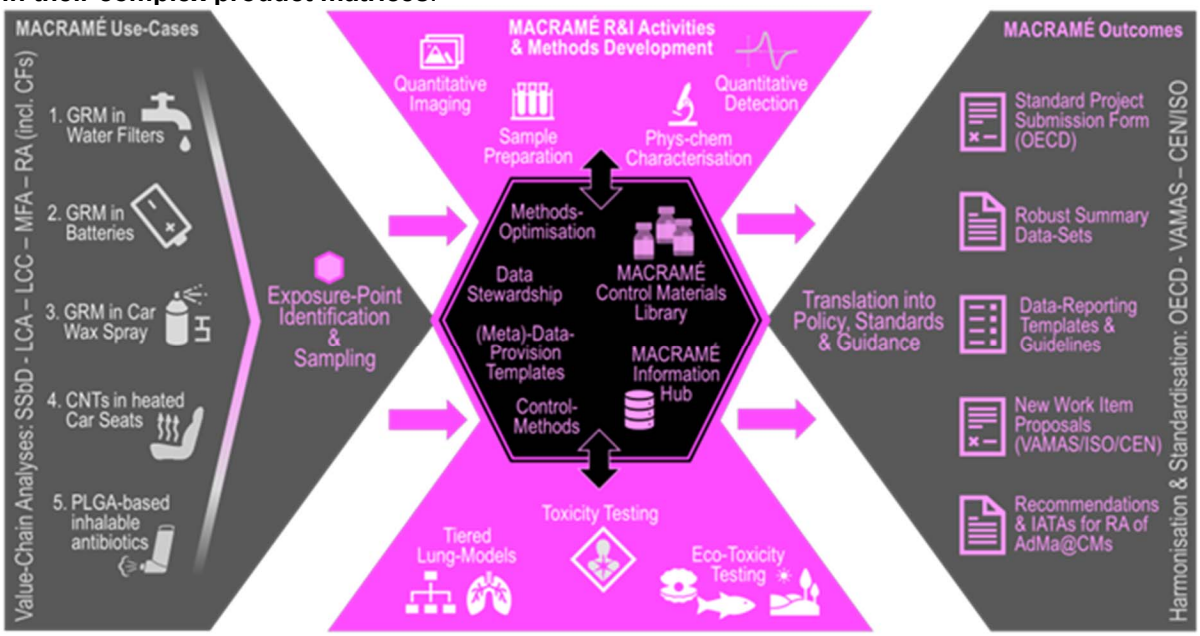

Figure 1: Illustration of the MACRAME R&I Approach.

This will be achieved by defining the R&I Strategy through life-cycle assessment for **five market-relevant industrial MACRAMÉ Use-Cases.** These **define the selection** of the **MACRAMÉ R&I Activities** and development of **MACRAMÉ Methods**, and the benchmarks chosen for monitoring the progress R&I. MACRAMÉ R&I Activities include a range of novel sample preparation techniques and ambitious quantitative detection and imaging methodologies that support reliable and reproducible determination of AdMas in different complex matrices (**AdMa@CMs**) and using **inhalation as their main exposure route**. By applying, combining and evaluating both established and novel inhalation toxicity tests a tiered approach to toxicity testing will be developed that will provide data on state-of-the-art characterised control materials for the **MACRAMÉ Control Material Library**. The library will serve future AdMa toxicological research.

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANOMATERIALS

INVESTIGATIONS ON THE USABILITY OF LOW-COST PM SENSORS FOR MONITORING NOAA CONCENTRATIONS IN WORKPLACES

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The permanent monitoring of spatio-temporal concentrations of nano-objects, their agglomerates and aggregates (NOAA) in workplaces is typically hindered by the high cost of the necessary measurement equipment. With the advent of low-cost particulate matter (PM) sensors around ten years ago, new possibilities arose to set up sensor networks that measure dust concentrations with high spatio-temporal resolution at reasonable costs. These sensors are all based on the measurement of light, scattered by particles inside a measurement volume. Depending on the complexity of the sensors, they can deliver either a single size-integrated concentration metric, e.g. the $PM_{2.5}$ mass concentration (photometer), multiple size integrated number and/or mass concentrations (advanced photometer) or particle size distributions (spectrometer). However, the usability of low-cost sensors for measuring NOAA concentrations can be limited in link with the particle size range that can be detected.

The possibilities and limitations for the use of low-cost PM sensors for monitoring NOAA concentrations were scrutinized by each partner in a pre-normative research project for CEN. Multiple specimens of six different sensor types, including advanced photometers and spectrometers, were selected and used in laboratory investigations. The response of the sensors to a variety of different particle materials (refractive indices) and sizes were investigated with monodisperse and polydisperse aerosols. Figure 1 exemplarily shows the results of four sensor types for the size dependent PM_{10} detection efficiency of PM sensors, measured using monodisperse particles.

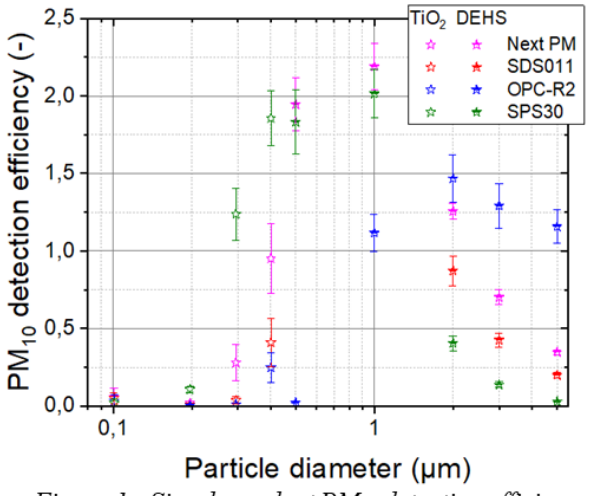

Figure 1 : Size dependent PM_{10} detection efficiency

In addition, possible measurement artefacts due to high or low humidity and temperature were investigated in a climate chamber and potential effects due to fouling were determined by exposing the sensors to high NOAA concentrations. Additionally, the sensors from all partners were used in a round robin test, in which they were exposed to a variety of different test aerosols in order to determine the comparability of the sensor output.

The results indicate that the sensors are not capable of detecting pure nanoparticles due to their small sizes, whereas the concentrations of their agglomerates and aggregates can be detected with reasonable accuracy. The use of low-cost sensors for measuring the spatio-temporal distribution of NOAA-concentrations thus appears feasible in workplaces, where the majority of the airborne particle consist of agglomerates and aggregates.

Results from the laboratory investigations and round robin test will be presented and discussed in view of possibilities and limitations of low-cost PM sensors for monitoring NOAA concentrations in workplaces.

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

DUSTINESS OF HIGH ASPECT RATIO (NANO)MATERIALS – HARMONISATION OF TEST METHODS TOWARDS AN OECD TEST GUIDELINE

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High aspect ratio nanomaterials (HARNs), including nanoscaled fibres and tubes, possess outstanding properties that are advantageous for various products from different fields, such as automotive, aerospace, medical and energy industries. Aside from enabling innovative applications, concerns are raised due to possible harmful effects of HARNs. HARNs releasing respirable bio-persistent fibre dusts can be a risk to human health. Fibrous objects with a length longer than 5 µm, a diameter smaller than 3 µm and an aspect ratio equal to or greater than 3 are defined as WHO-fibres and are seen as possible carcinogens. Due to the raised concern towards fibre dust, dustiness is an important property to determine the associated exposure potential of released fibre dust and thus exposure risk. Dustiness describes the propensity of a material to release airborne dust following agitation. In order to investigate the dustiness of manufactured nanomaterials, standardized and harmonized dustiness tests valid for nanomaterials are required to fulfil regulatory needs.

Currently, an OECD Test Guideline for dustiness testing of manufactured nanomaterials, for both granular nanoparticles and HARNs, is under development lead by France and Denmark. The OECD Test Guideline is planned to provide a comparison of different dustiness methods for which the optimized and harmonized standard operating procedures (SOPs) will be validated. Intra- and interlaboratory comparisons will be conducted in close collaboration with different institutes and industrial partners of various OECD Member countries.

For HARNs, an intra- and interlaboratory comparison was started with 15 international partners with the focus on three different dustiness test methods: small rotating drum, fluidizer and venturi setup. Harmonised SOPs were developed for these three dustiness test methods. The qualitative and quantitative analysis of the generated aerosol will be performed by using scanning electron microscopy, since aerosol measurement instruments are not yet calibrated for fibrous materials. The generated aerosol will be sampled on filters suitable for scanning electron microscopy investigations. Subsequently, electron micrographs will be taken and the airborne micro- and nanoscale (fibrous) objects on these micrographs will be counted and classified according to their morphology following harmonised counting rules. The development of the scientific background for the HARN-part of this OECD Test Guideline is part of the Horizon 2020 project NanoHarmony.

By developing this validated OECD Test Guideline on dustiness testing, a harmonized framework for testing and risk assessment of airborne fibrous dust will be built. A ranking scheme that enables the comparison of different test methods with the potential to be used for regulatory risk management of HARNs is the long-term goal. This OECD Test Guideline will contribute to the safe use and the safe and sustainable by design assessments (SSbD) of HARNs in industrial products.

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TOPIC N° 5 FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

NANOSTRUCTURED BIOGENIC AMORPHOUS SILICAS VERSUS NANOSTRUCTURED SYNTHETIC AMORPHOUS SILICAS – COMPARISON BY SEM, TEM AND SPECIFIC SURFACE AREA -

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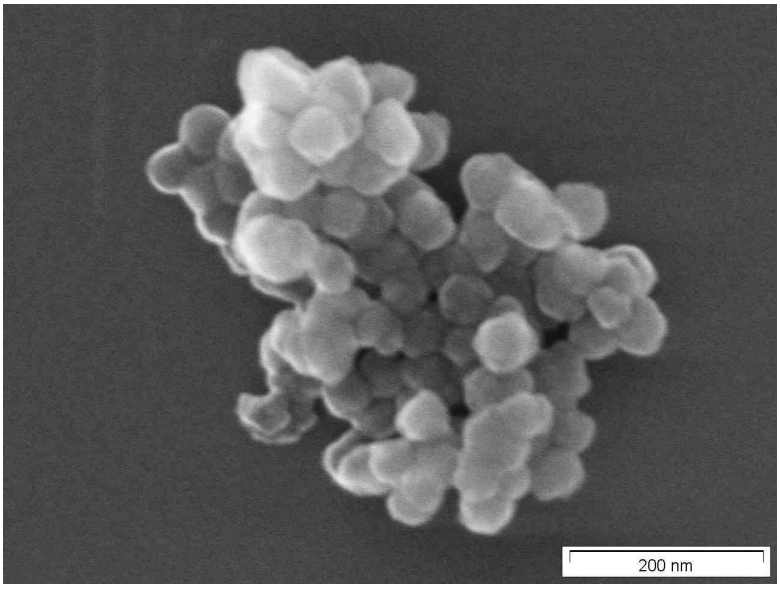
This presentation compares the very nature of the two types of amorphous silicon dioxide (SiO_2), namely, biogenic amorphous silicas (BAS) being ubiquitous in the flora, and synthetic amorphous silicas (SAS). Furthermore BAS is present in marine biota such as diatoms, radiolaria, silicoflagellates, and siliceous sponges. Upon employing electron microscopy techniques like SEM and TEM, both BAS and commercially available SAS can indeed be viewed as being nanostructured materials that cannot be differentiated from one another. Additionally, the similarity is supported by the specific surface area (BET). As examples oat husk, common horsetail and sugar cane bagasse have been chosen.
One may find the ubiquitous BAS in a wide variety of food products such as bread, oat flakes and many more – as seemingly all plants need amorpous silica for their growth and stability. Especially all grasses (e.g. oat, wheat, rice, sugar cane) apparently contain rather high amounts of BAS. Due to the omnipresence of amorphous silica on earth one can assume all organisms are being well adopted to the presence of this substance. Moreover, BAS is considered being beneficial for human health as described in many publications.
SAS is being produced since more than 70 years by means of various processes, namely by a wet process, yielding precipitated silica, silica gel or colloidal silica and by a vapour-phase hydrolysis process, yielding pyrogenic (fumed) silica. Interestingly, those SAS have been known as exhibiting a 'milli micron' (= nano) structure since 1947.
SAS is used, e.g., in specific applications like food, feed, and cosmetics. In this context, the findings as shown in this presentation are of particular interest to underline the safety of these uses.



Nanostructure of BAS as found in sugar cane bagasse

Remark: This presentation is partly based on Lindner GG, Drexel C-P, Sälzer K, Schuster TB and Krueger N (2022) Comparison of Biogenic Amorphous Silicas Found in Common Horsetail and Oat Husk With Synthetic Amorphous Silicas. Front. Public Health 10:909196. doi: 10.3389/fpubh.2022.909196

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

COMPLEMENTARY ANALYSIS OF STERILIZED NANOPARTICLES WITH SEM/EDS AND XPS/HAXPES

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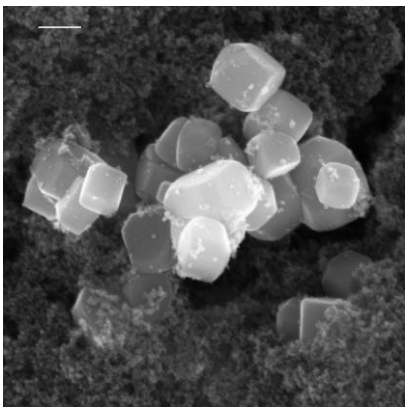
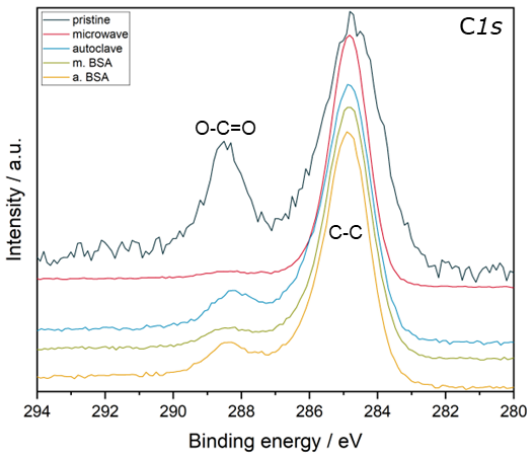
Since there is a lack of knowledge about the effects of nanomaterials on human health and the environment, and in order to get safe- and sustainable-by-design nanomaterials, it is necessary to find an easy way to assess their properties, without having to perform time-consuming experiments each time. In the European project NanoSolveIT, the behavior of nanoparticles is to be derived from a nanomaterial fingerprint database with standardized physico-chemical properties of nanomaterials. The key element for these grouping and reading across approaches is the collection of standardized information about nanomaterials in combination with modelling and simulations. As parameters the particle size, particle shape, chemical composition and surface chemistry are discussed here.
Measurements of the shape and size were performed using scanning electron microscopy (SEM). For the chemical composition energy dispersive X-ray spectroscopy (EDS) and for the surface chemistry X-ray photoelectron spectroscopy (XPS) were used. As an additional method hard X-ray photoelectron spectroscopy (HAXPES) with a higher information depth than XPS was used, thus we were able to include information not only from the top surface, but also from deeper regions of about 30 nm. Therefore, this method is complementary to EDS.
All these methods have been correlatively used to study nanoparticles of different chemical composition that have been treated differently by sterilization. Such a sterilization step is common, before testing for toxicity. To date, the sterilization step has not been considered in establishing the structure-activity relationship of the nanomaterial.
The effects of sterilization are discussed on exemplary samples. The results demonstrate the influence of sterilization on all investigated properties, indicating a restructuring of the nanoparticles. This implies that samples that have been sterilized after synthesis, may show different toxicity from those used in applications without a sterilization step.

Acknowledgement: This research is part of the project "NanoSolveIT", which has received funding from European Union Horizon 2020 Programme (H2020) under grant agreement no 814572. (https://nanosolveit.eu/)



SEM image of pristine $Co_{0.75}Fe_{2.25}O_4$ (scale bar: 100nm)



High resolution XPS spectrum of the C*1s* peak

TOPIC 6 – CHARACTERIZATION OF ADVANCED MATERIALS INCLUDING NANO MATERIALS

NMR: AN UNDERRATED SPECIATION TOOL FOR THE SAFE BY DESIGN OF ADVANCED MATERIALS

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In the context of Safe by Design of an advanced material, the challenges that need to be faced are adequetely characterizing them and the risks associated with them. This often involves low concentrations during the manufacturing process (e.g. doping), and always trace amounts at the end of the life cycle. The available technology (e.g. isotope labelling, modern ICP-MS…) has the capability of establishing an elemental composition at (sub-)ppt concentrations. However, material functionality and the risks associated with the use- of end-life phases are a function of the chemical nature, i.e. the speciation. Methods informing about the speciation typically operate at concentrations a few orders of magnitude above those dedicated to simple detection. As opposed to synchrotron XAS methods that become inoperative for elements Z<20 and requiring multi-billion facilities, NMR can provide the same 0.1 ppm sensitivity, especailly for "light" elements in a 5x5 m area for a fraction of the cost. The example chosen for this presentation is the case of sun-protection cosmetics who could be characterized at different stages of the life cycle, and revealed some concerns is during short term aging.

TOPIC N° 5 - FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

RELIABLE CHEMICAL CHARACTERIZATION PROTOCOLS FOR INDUSTRIAL GRAPHENE-RELATED MATERIALS

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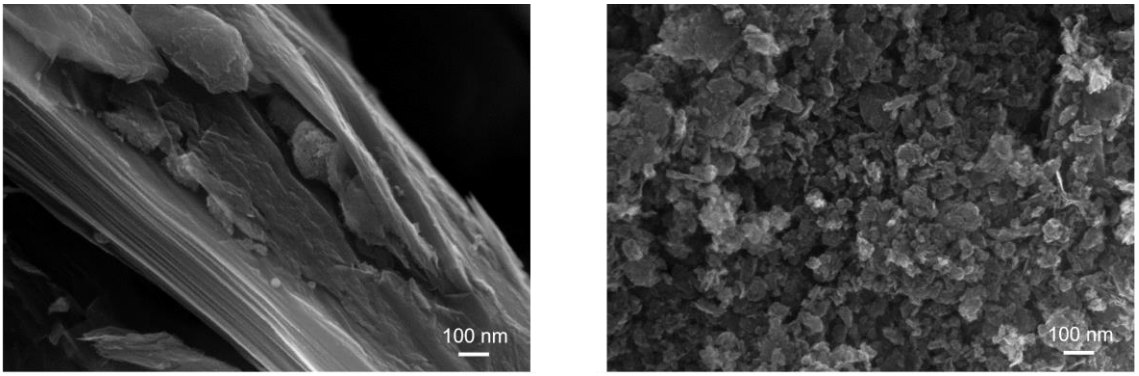
Since its isolation, graphene has received growing attention from academia and industry due to its unique properties. Promising opportunities for applications are discussed in different field like electronics and optoelectronics, detection, and sensing devices, biosystems or chemical and environmental corrosion inhibition. Here, functionalization with elements like oxygen, nitrogen or fluorine can broaden the application, for example in composite materials. However, lack of generally accepted operation procedures hinders the commercialization, the so-called "what is my material" barrier. Therefore, first efforts were done to develop common, reliable, and reproducible ways to characterize the morphological and chemical properties of the industrially produced material.
In this contribution, our efforts in the development of reliable chemical characterizations protocols for functionalized graphene are presented. An ISO standard for the chemical characterization of graphene-related (GRM) is under development with X-ray photoelectron spectroscopy (XPS) having a prominent role. With its information depth of around 10 nm, which is the similar length scale as the thickness of particles of 2D materials consisting of a few monolayers, XPS seems to be highly suitable for the quantitative analysis of (functionalized) GRM. Thereby, different sample preparation methods like pressing the powders onto adhesive tapes, into recesses, or into solid pellets result in inconsistencies in the quantification. Furthermore, different morphologies like stacks of graphene layers (left figure) or irregular particles (right figure) lead to different analysis results for the chemical composition.
For the validation of the quantification with XPS and the further development of standards an international interlaboratory comparison was initiated under the head of the "Versailles Project on Advanced Materials and Standards" (VAMAS). First results are reported showing the suitability of the protocols. Finally, the XPS results are compared with the elemental composition results obtained after quantification with energy-dispersive X-ray spectroscopy (EDS) as a fast analytical method which is usually combined with electron microscopy.

This study has received funding from the European Metrology Programme for Innovation and Research (EMPIR) as part of the ISO-G-SCoPe, 19NRM04, project.



Scanning electron microscopy images of fluorine-functionalized powders of graphene particles.

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

SOME OPEN QUESTIONS IN UNDERSTANDING AND PREDICTING NANOMATERIAL DISSOLUTION, PART II

Frederick C. Klaessig

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Several open questions in interpreting dissolution rates were discussed in a keynote address at NanoWeek 2022. It was shown that literature data from dynamic flow-through testing were zero order, but were being fitted by many authors to a first order model, which was then being used to arrive at questionable half life values for nanoparticle dissolution in biologically relevant media. In that case, the reported results were not interoperable in that one could not recover the original data from the modeled dataset. This presentation extends the analysis to static batch testing, the ATempH stirred batch reactor. With zero order data, i.e. where mass loss is linear with time, the two methods yield similar results. For mixed and higher order data, the Michaelis-Menten equation, conventionally employed with enzyme kinetics, has been used with static batch test results. Though interoperable, the relationship to an underlying dissolution mechanism remains open. The published data for alumina will be examined from a combined modeling and mechanistic perspective, leading to a commentary on the relative merits of static and dynamic dissolution testing for gastric, lung lining and phagolysosomal fluids. Low particle loadings, where complete dissolution should occur, are of particular interest for nanoparticle release from nano-containing products into US drinking waters.

In view of the OECD activities for standardizing dissolution/solubility testing, this talk is probably most applicable to Topic 5 (From nanomaterials risk assessment to risk governance, a journey through regulation and standardization), but might also fit with modeling aspects of Topic 7. (Modelling, digitalization of nanosafety and data management).

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

DEVELOPMENT AND DOCUMENTATION OF AN ATMOSPHERE-TEMPERATURE-PH-CONTROLLED STIRRED BATCH REACTOR APPROACH FOR STANDARD TESTING OF SOLUBILITY AND DISSOLUTION RATE IN WATERS AND BIOLOGICAL SIMULANT FLUIDS

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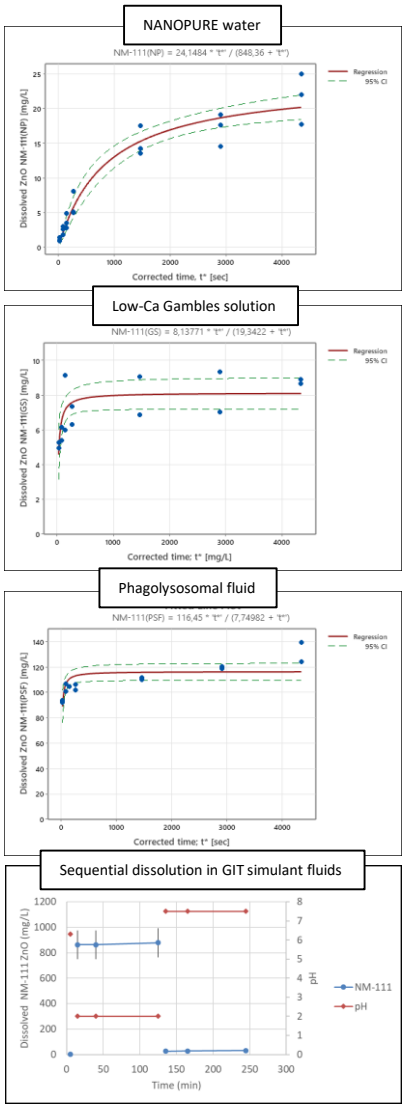
An Atmosphere-Temperature-pH-controlled stirred batch reactor (ATempH SBR) system was developed. The system addresses all dissolution and solubility limit (S_o) testing relevant for grouping and read-across of substances on nanoform in REACH Appendix R.6-1 and is a candidate for inclusion in a forthcoming OECD Guidance document on solubility and dissolution testing of manufactured nanomaterials in water and biologically relevant media.

The ATempH SBR was subject to intralaboratory validation by dissolution testing of 9 different materials (γ-Al_2O_3, TiO_2 (NM-104, glycerine and Al_2O_3 coated), ZnO (NM-110 and NM-113, uncoated, NM-111 triethoxycaprylsilane coated), SiO_2 (NM-200, synthetic amorphous silica), CeO_2 (NM-212), $BaSO_4$ (NM-220), and bentonite (NM-600) in Nanopurified water (NANOPURE), Gambles solution (pH 7.4; 37°C) and Phagolysosomal fluid (pH 4,5; 37°C) (Holmfred et al., *Nanomaterials* **2022**, *12*(3), 517 and new studies herein). The Gambles solution is a simulant for the lung-lining fluid and the phagolysosomal fluid is a simulant for the low-pH compartment in lysosomes and macrophages. In NANOPURE, testing is made without pH and atmosphere control (pH free; 25°C or 37°C) to avoid pH hysteresis due to lack of buffer capacity.

The validation studies considered the initial dissolution rates calculated from linear or non-linear statistical regression of the amount dissolved as function of time. The statistical regression functions are also used to estimate the solubility limit (S_o) of the different materials. It was found by testing on γ-Al_2O_3, NM-104 and NM-111 that the uncertainty of the results could be reduced by extending the test duration from 24-hours to 72 hours and this test duration is finally recommended as a minimum for standard testing.



Graphs show dissolution curves for NM-111 ZnO dissolved in the NANOPURE (n=3 per time-point); Gambles solution (n=2 per time-point); Phagolysosomal fluid (n=2 per time-point) and along the gastro-intestinal tract with illustration of the pH in each phase.

A final configuration of the system was established and demonstrated for sequential batch dissolution for simulating passage through the gastro-intestinal tract (GIT): saliva (5 min; pH 6.3; 37°C), stomach (120 min; pH 2; 37°C) and intestine (120 min; pH 7.5; 37°C)) with temperature and pH control.

In the shown example from testing NM-111, considerable different dissolution results were obtained for 72-hour dissolution of NM-111 in NANOPURE, Gambles solution and Phagolysosomal fluid as well as in the three dissolution steps along the gastro-intestinal tract. We further demonstrate the results obtained with the methods and discuss their relevance in regards to testing and biological fate.

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

IN NANOTOOL: A CONTROL BANDING BASED APPROACH TO MANAGE THE RISK OF OCCUPATIONAL EXPOSURE TO INCIDENTAL NANOMATERIALS IN METAL ADDITIVE MANUFACTURING

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During 3D metal printing, incidental metal nanomaterials are emitted, raising concerns regarding occupational health and safety. In the last few years, different approaches have been used to manage the occupational risk of exposure to these incidental nanomaterials (NM), but the definition of standardized methods still remains crucial. The purpose of this work was to explore different approaches to manage this risk on a case study conducted in a Portuguese organization using Selective Laser Melting (SLM) technology, and to develop and test a new control banding based approach.

The case study first consisted of a monitoring campaign using a condensation particle counter (CPC), a scanning mobility particle sizer (SMPS) and air sampling for later scanning electron microscopy (SEM) and energy dispersive X-ray (EDS) analysis, attesting the emission of nano-scale particles and providing insights on number particle concentration, size, shape and chemical composition of airborne particles. Simultaneously, two existing risk assessment methods designed for NM and based in control banding were applied to this case study: Control Banding Nanotool v2.0 and Stoffenmanager Nano v1.0, which showed significant limitations for these incidental NM. Finally, a new approach was developed and tested in this case study: the IN Nanotool. This control banding based method was designed specifically to manage the risk of exposure to incidental NM emitted during metal additive manufacturing. It aims to help ensuring the safety and health conditions of exposed workers, allowing non-experts to manage this risk. In spite of its limitations, using IN Nanotool in this case study showed reliable results in line with the state-of-the-art, revealing its potential to fill the lack of methods for incidental NM.

TOPIC N°8 – MICRO AND NANOPLASTIC POLLUTION

TOXICITY EVALUATION OF MICRO- AND NANO PLASTIC PARTICULES, WITH CO-EXPOSURE TO METAL IONS ON HUMAN INTESTINAL MODELS

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Plastics are polymers that are widely used in a large range of industrial sectors for their attractive properties and their low cost. Because plastics are highly resistant to degradation, they accumulate and pollute the oceans and soils. It is acknowledged that in the environment, plastics are subjected to physical, chemical, and biological stresses such as photodegradation, mechanical stress or heat, which are responsible for plastic fragmentation and the impairment of physicochemical features. As it ages, plastic degrades into smaller fragments called micro and nanoparticles (MNPs). Aged plastic particles have been reported to show a higher specific surface area and a higher adsorption rate for pollutants than pristine particles. Hence, they are more likely to adsorb at their surface persistent organic pollutants (dioxins, pesticides), metal ions, additives or pathogens present in the environment. We are exposed daily to plastic fragments mostly via ingestion, through the consumption of seafood, fish, tap and bottled water. Studies on biological effects of plastic nanoparticles are still insufficient, especially on their intestinal toxicity.

In this context, our objective was to evaluate, in realistic environmental conditions, the biological effects of representative secondary polyethylene terephthalate (PET) particles, derived from plastic water bottles, and of commercial polylactic acid (PLA) particles, which is a biosourced and biodegradable plastic used for medical purposes (sutures) and food industry (teabags, packaging). These particles were used both in their pristine state and after artificial weathering in a Q-SUN test chamber. The biological effects of PET and PLA were evaluated on human intestinal epithelial cell lines representative of healthy individuals and of people suffering from inflammatory bowel disease. To this, co-cultures of wild-type Caco-2 cells or Caco2-LV-Nod2^{1007fs} cells, which is a mutation frequently observed in Crohn's disease patients, and HT29-MTX were used. These cells were exposed to 120, 200 and 450 nm PLA particles or to 200 nm PET particles. Particles were characterized by Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and their degradation after artificial weathering was investigated via liquid chromatography coupled to mass spectrometry (HPLC-MS/MS). Cytotoxicity, DNA damage, and intracellular ROS levels were assessed after 24 h of exposure to realistic concentration of nanoparticles.

The results of the study do not show any toxicity of PET and PLA nanoparticles on Caco-2 and HT29-MTX cell lines. These results are consistent with the literature, suggesting that the toxicity of MNPs may rather lay in their role of pollutant carrier than in intrinsic hazard, but further work is needed to confirm this hypothesis.

This work received funding by the Agence Nationale de la Recherche (ANR), under the grant ANR-21-CE34-0028 (PLASTOX) and from the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES) under the grant EST-21-077 (EXaMINA).

TOPIC N° 8- MICRO AND NANOPLASTICS POLLUTION

LESSONS LEARNED FROM NANO-EHS APPLIED TO ASSESS THE FATE OF PLASTIC PARTICLES IN THE ENVIRONMENT

Mark R. Wiesner[a], J. Sipe[a], L. Ferguson[a], A. Lewis[a], K. Santizo[c], P. M. Pfohl[c], R. Cross[b], G. Adediran[b], A. Praetorius[d], W. Wohlleben[c], Sam Harrison[b] and C. Svendsen[b]

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The fate of plastic waste in the oceans and surface water has garnered much attention, with images of plastic gyres such as the North Pacific Subtropical Gyre. However, there is increasing appreciation that these floating plastic islands represent a small fraction of the plastic present in the world's oceans and freshwaters. Of the approximately 4.8 to 12.7 million metric tons of plastic annually emitted to the ocean, larger plastic materials are likely to fragment into smaller millimeter-sized particles or even smaller colloidal fractions that are not entirely accounted for based on oceanic circulation models. The plastic pollution on land and in freshwaters can be many times greater than the ocean inputs and little is known regarding the levels of colloidal plastics in these environmental compartments. Nanoplastics are the smaller nano-scale fraction of these colloids and are most likely incidentally produced from the fragmentation of larger plastic debris. Although complete breakdown of larger plastic debris can take up to hundreds of years, it is likely that mechanical wear, heat, UV degradation and, in some cases, biological factors, lead to relatively rapid fragmentation of plastic debris down to micron- and potentially nano-scales.

Plastics often contain a wide variety of chemical additives as well as non-intentionally added substances such as degradation products, reaction by-products and/or impurities. Since these other chemicals are not generally covalently bound to the polymer matrix, they may leach out of the plastic. Additives are specific to certain types of plastics and uses. Some are contentious, such as the case of PVC with plasticizers such as phthalates, or flame retardants in automotive parts and insulation and some are subject to testing and registration, including migration properties. Nanomaterials are sometimes incorporated as nanofillers into polymer formulations to enhance existing properties or to add new properties of interest in the products made from these plastic composites. For example, multiwalled carbon nanotubes (MWCNTs) are known for their unique and diverse properties (mechanical, electrical, thermal, electrochemical, optical and hydrophobic properties) and have many applications in the polymer industry, in improving a polymer's mechanical properties, reducing static electricity, or imparting electrical conductivity to polymer matrices. Even though there are many advantages to using MWCNTs in polymers, the potential release of manufactured nanomaterials (MNMs), such as MWCNTs during the product life cycle and resultant probabilities of exposure for manufacturing workers, product users, and the environment, has raised concerns.

This presentation reviews some recent work examining the release of nanomaterials from plastic composites, and methods from the study of nanoparticle fate and transport applied to an evaluation of plastic fragmentation and additive release. This work is supported with funding from the Long Range Research Program under the European Chemical Industry Council (CEFIC- LRI).

TOPIC N° 8 - MICRO AND NANOPLASTICS POLLUTION

TOXICITY OF POLYCAPROLACTONE AND POLYSTYRENE NANOPARTICLES, AGED IN ENVIRONMENTAL CONDITIONS, TOWARDS INTESTINAL CELLS.

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Plastics, which are synthetic polymers containing chemical additives, are an indispensable material in our society because of its excellent properties (low cost, resistance, versatility). Its production has grown considerably and has resulted in significant environmental pollution due to inappropriate waste disposal management. During their passage through the environment, plastics undergo physical, chemical and biological alterations, which lead to their fractionation into smaller particles, called micro and nanoplastics (MNPs). Today, toxicity data on MNPs are insufficient to conclude on their overall impact. This study aimed to characterize the toxic effects of Polystyrene (PS), a petroleum-based plastic, and Polycaprolactone (PCL), a biodegradable plastic, on humans upon ingestion, under conditions simulating their passage through the environment. We used in vitro
models of human intestinal epithelium, i.e., co-cultures of Caco-2 enterocytes and HT29-MTX mucus-secreting cells, representative of healthy people (wild type) or of people with susceptibility to Crohn's disease (Caco-2-NOD2^{1007fs}). They were exposed to PS or PCL NPs of varying primary diameter, and either pristine or artificially weathered. After characterization, their impact on cell metabolism, reactive oxygen species content, DNA integrity and inflammatory response were evaluated. Our results showed that PS and PCL NPs agglomerated and their qhape changed during artificial weathering, and PCL NPs released some monomers. No overt toxic effects of pristine PS and PCL particles were observed in both these cell models, and with both pristine and aged NPs, although NPs accumulated inside the cells (Figure 1). These results are in agreement with the current literature, showing that pristine plastic particles only show mild toxicity to intestinal cells, in vitro. The cell models and exposure conditions should be refined in order to highlight more subtle effects of these particles.

This work received funding by the Agence Nationale de la Recherche (ANR), under the grant ANR-21-CE34-0028 (PLASTOX), from the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES) under the grant EST-21-077 (EXaMINA), and from the Agence de l'environnement et de la maîtrise de l'énergie (ADEME).

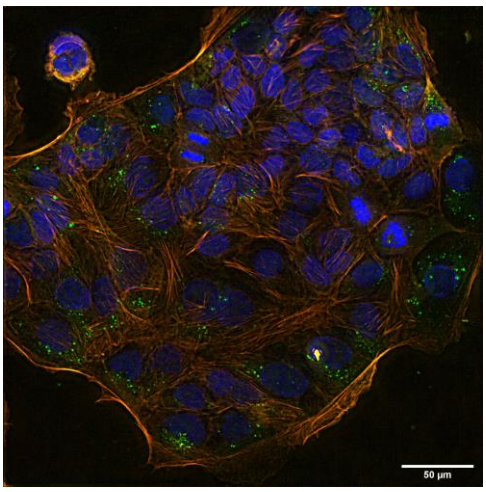

Figure 1. Fluorescence microscopy image of a Caco 2/HT29 MTX co-culture after 24 hours of exposure to fluorescent 200 nm polystyrene nanoparticles (green). Cell nuclei were stained using Hoechst 33342 (blue) and actine filament using Phalloidine-Atto 550 (orange). Scale Bar: 50 µm

TOPIC N° 3 – TRANSFERABILITY AND ACCEPTABILITY OF THE CONCEPT OF SSBD

FACILITATION OF SAFE-BY-DESIGN UNDERSTANTING AND UPTAKE

Joséphine Steck[1], Apostolos Salmatonidis[2], Camilla Delpivo[2], William Brown[3], Polly McLean[3], James Hanlon[3], Richard Seddon[4], Hasnae Ben Jeddi[5], Henk Goede[5], Sébastien Artous[1], Simon Clavaguera[1]

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Safe-by-design (SbD) concept aims to integrate safety preoccupations at the early R&D stages. Publications on SbD applied to nanomaterials have increased sharply since 2017 indicating a growing interest for the scientific communities. However, raising the awareness and understanding of SMEs and industries regarding SbD is still a main priority. In this frame, two similar approaches were developed in both the SAbyNA and SbD4Nano projects, two H2020 sister projects willing to create SbD platform and infrastructure. These approaches aim to offer a selection of resources on one hand and risk management measures (RMM) on the other hand, based on users' needs and providing them resources (e.g., documents, standards, literature paper, etc.) and information (effectiveness, cost … of RMM) that are summarized in documents named 'cards'.

Usability cards of SbD resources (SAbyNA): The development of usability card aims to summarize the content of a specific resource, identifies the main outcome, the type of document and creates a link towards the complete document improving the usability for users. Fifty-two resources were assessed applying three criteria: "applicability", "user-friendliness" and "robustness". Twenty resources (38%) are highly applicable, user friendly and robust. Most of these were standards that are not open-source. Thus, additional resources were selected based on three other criteria ("relevance", "cost effectiveness" and "nano-specificity"). Thirty-two resources were finally selected and as many cards were prepared to facilitate their identification and use through the SAbyNA guidance platform. The users will find the appropriate resource(s) thanks to a sorting system and based on their own expertise. Once the users identify the most relevant resources, it will be possible to find potential outcomes of the resources in the cards including links with other resources, in order to create a tailor-made library. This gathering of pre-selected resources relevant for users facing safe-by-design process complexity in one platform will deeply improve their accessibility and their interoperability. This work will greatly contribute to a practical implementation of the SbD by the final users.

Ecards of RMM (SbD4Nano): The ecards offered by SbD4Nano project are focusing on RMM from two steps of the hierarchy of control that reduce worker exposure to nanomaterials: engineering and administrative controls. Nine ecards provide contextual information of RMMs intended for users of the SbD4Nano tool. Furthermore, ten additional sub-ecards are developed to detail some RMM specifications. The ecards provide a short description on the RMM, advices to ensure the maximum effectiveness and the level in the hierarchy of control. To go even further, resources are listed and scored with five stars corresponding to the following indicators (high, medium or low) used to evaluate them: relevance, applicability, user-friendliness, nano-specificity and robustness. Besides, five criteria were defined to underline the RMM particularities: effectiveness, target group, cost, lifetime and complexity of implementation and maintenance. The e-cards will be included in a new developed nano exposure quantifier (NEQ) tool which will be integrated in the SbD4Nano e-Infrastructure.

Beyond SbD, methods and tools that support the concept of Safe and Sustainable-by-Design (SSbD) are now developed. Facilitating the understanding of this new concept to increase its acceptance by companies opens a new challenge for the next years.

Acknowledgement:
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TOPIC N° 4. COMMONALITIES OF THE SSBD PARADIGM IN INNOVATION

ENVIRONMENTAL LIFE CYCLE ASSESSMENT APPLIED TO MEDICAL DEVICES

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The European Green Deal aims at transforming the EU's economy to a more sustainable one with policies focusing on climate, biodiversity, circularity, human health and environmental protection (European Commission, 2019). This includes an ambitious plan to tackle pollution from all sources and progress towards a zero-pollution economy for a toxic-free environment. With the European Green Deal, the EU commits to reducing net greenhouse gas emission by at least 55% by 2030, and to reach no net emissions by 2050, and the health and care systems are not exempt from assessing, mitigating, and improving the environmental impacts across all the health and care value chain. Indeed, for example, the health care sector is responsible for 4-5% of global total carbon emissions, and generates significant demands for energy and materials, as well as dangerous waste streams that may cause air, soil and water pollution.
These impacts can be assessed by applying widely established tools for quantitatively determining the environmental (e.g., Life Cycle Assessment, LCA), economic (e.g., Life Cycle Costing, LCC), and social (e.g., Social Life Cycle Assessment, s-LCA) impacts of products, processes, and services across the entire life cycle. The importance of employing a life cycle perspective in assessing sustainability of production and consumption systems has been increasingly acknowledged in the EU policies since the early 1990ies (Sala et al., 2021). The European Green Deal (EC, 2019a), for instance, includes several policy initiatives which explicitly cite and mention LC thinking and methods. In the Chemical Strategy for Sustainability (EC, 2020b) a life cycle perspective is required in the identification and minimization of potential negative impacts linked to chemicals and material.
These assessments are needed also for nanomaterials which are increasingly applied in many sectors including construction, textile, food, cosmetics and medicine. Specifically, the application of nanomaterials in medicine has led to novel pharmaceuticals and medical devices that have demonstrated a strong potential for increasing the efficacy/performance and safety of therapeutic and diagnostic procedures to address a wide range of diseases.
One of the objectives of the SAFE-N-MEDTECH project (https://safenmt.com) is to assess the sustainability of nano-based medical technologies along the entire life cycle of the product by means of LCA techniques.
The aim of SAFE-N-MEDTECH project is to build an innovative open access platform to offer companies and reference laboratories, the capabilities, knowhow, networks and services required for the development, testing, assessment, upscaling and market exploitation of nanotechnology-based Medical and Diagnosis Devices.
The aim of this contribution is to present a LCA study for a nanotechnology-based medical device which is a coating developed by TECNAN and evaluated within SAFE-N-MEDTECH. Therefore, this study shows the results of life cycle environmental sustainability assessment of this innovative product to prevent biological/microbial contamination to enhance MDs reprocessing.

CORRELATION OF IMMUNE RESPONSE WITH TEMPORAL EVOLUTION OF BAND STRUCTURE OF METAL OXIDE NANOPARTICLES DUE TO REACTIVITY MEDIATED CHEMICAL TRANSFORMATION

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Cellular immune responses are triggered on recognition of foreign agents to identify and eliminate cellular damage (like debris or degraded/aggregating proteins). Oxidative stress induction due to metal oxide nanostructures is a multi-tier process. The processes include generation of ROS and activation of antioxidant cellular machinery followed by activation of proinflammatory cytokine response through redox sensitive pathways eventually culminating into initiation of stress at an organelle level leading to cytotoxic response. Upon cellular interaction with nanostructures, the induction of stress is a function of physico-chemical properties, dissolution propensity in addition to surface active catalytic properties of nanostructures in its native state. However, with respect to extrinsic conditions (pH, ionic strength and temperature) and change in the reactivity of metal oxide nanoparticles, their electronic band structure also evolve. The aim of our study is to deconvolute the cytokine response of the particles vs dissolved species over a period of time as a result of extrinsic factors.

In this study, four types of metal oxide nanoparticles (CuO, NiO, ZnO & Mn_2O_3) with different hydration enthalpies were synthesized by wet chemical route. The dissolution profile of the NPs in relevant biological media was evaluated where the percentage dissolution in cell culture media was: CuO~ZnO NPs>NiO NPs> Mn_2O_3 NPs quantified by ICP-OES/MS. The evolved structure-property relationship of the NPs over the period of time were evaluated on RAW 264.7 macrophage cell line. Temporally, the optical band gap and the valance band maxima of the remnant NPs (undissolved fragments) were evaluated by Tauc's model and Ultraviolet photoelectron spectroscopy analysis respectively over the period of 24 hours. The flat band potentials were mathematically validated to furnish heat maps that mapped with the redox potential of prevalent biological molecules (-4.12 to -4.80 eV; corroborated with electrochemical measurements) and corresponding cytokine release profile of the cell line on treatment with remnant NPs (post dissolution). Increased reactivity translated in increase of cellular uptake of the remanent NPs leading to higher ROS production for CuO (13 fold) & ZnO (15 fold) followed by NiO (6 fold) and least in the case of Mn_2O_3 (4 fold). It was observed that the band structure evolution in the initial 6hours for NPs with higher degree of dissolution prompted early apoptosis and necrosis whereas beyond 12 hours and for slow dissolving nanomaterials late apoptosis dominated.

Elevation of inflammatory response markers like IL-1β (6-8 fold), IL-6 (10-40 fold) and TNF-α (5-6 fold) was observed within 2 hours of NPs separation duration. Suppression of anti-inflammatory cytokines like IL-10 (2-3.5 fold) with depolarization of mitochondrial membrane potential (upto 0.66 times) for CuO and ZnO NPs established a strong correlation between reactivity mediated chemical and band structure transformation on inflammatory response of the macrophages. This study offers an avenue to foster our understanding towards predictive paradigms that account for immune response as a result of band structure evolution of NPs under dynamic conditions. These paradigms would assist in crafting efficient and safe-by-design nanocarriers, biosensing and theranostic agents.

Keywords: Nanoparticles, Inflammation, Cytokines, Mitochondrial membrane potential

TOPIC N° 4 – COMMONALITIES OF THE SSBD PARADIGM IN INNOVATION

SURFACE ENGINEERING STRATEGY TO REACH A SAFER AND MORE PERMORMING PROFILE OF TiO_2-NPs AS SUNSCREEN UV FILTERS

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TiO_2 nanoparticles (TiO_2-NPs) are used in many applications such as in biomedicine and cosmetics due to its chemical inertness properties, high refractive index, low cost and advantageous surface properties. With their widespread use, TiO_2-NPs toxicity has assumed increasing relevance and concerns about both environment and human exposure through ingestion, dermal penetration, or inhalation route. Their biological effects and the cellular response mechanisms are still not completely elucidated and thus a deep understanding of the toxicological profile is required.
In this study, we have focused on the most significant route of exposure: skin contact, with sunscreen containing TiO_2-NPs as UV filters.
Because the main mechanism underlying the toxicity potentially triggered by TiO_2-NPs seems to involve the reactive oxygen species (ROS) production, the extent and type of cell damage strongly depend on chemical and physical characteristics of NPs, including size, surface composition and photo-activation. Following a "Safe and Sustainability by Design" (SSbD) approach to mitigate their photo-toxicity by preserving their UV filtering capacity, we have designed a surface modification on TiO_2-NPs with a suitable and eco-friendly molecule obtained by a biotechnology approach involving fermentation of by-products from the food industry. After the optimization procedures, the characterization of obtained species was conducted by using spectroscopic, thermal and images techniques, as well as the evaluation of photocatalytic effects and cytotoxicity. A dependence of in vitro and ex vivo responses by the molecular structures used to coat their surface was found.
The new coated UV filters have shown different properties and benefits: a higher SPF (ISO 24443:2012), a better cell compatibility (LDH assay) and a strong reduction of photocatalytic activity (Acid blue 9 test). In conclusion, the implementation of SSbD approach on TiO_2-NPs surface results to be fruitful in optimizing UV protection capacity, obtaining more sustainable and safer UV filters.

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TOPIC N° 8 – MICRO AND NANOPLASTICS POLLUTION

GASTROINTESTINAL DIGESTION AND COLONIC FERMENTATION OF POLYLACTIC ACID (PLA) BIODEGRADABLE MICROPLASTICS AND ITS INTERPLAY WITH GUT MICROBIOTA

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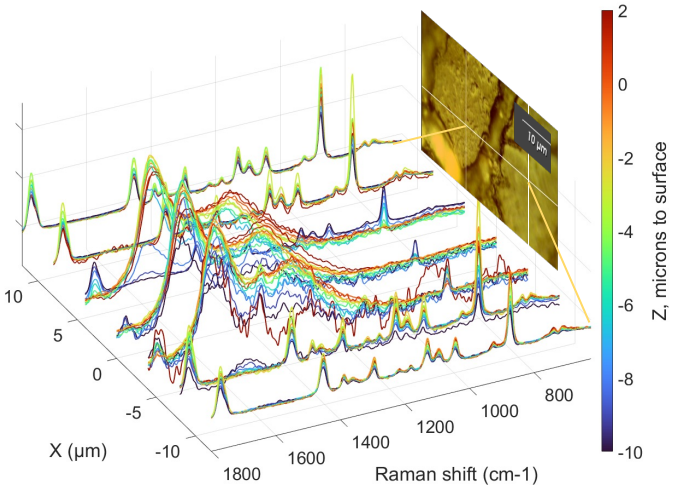
The impact of plastics-derived particles has fostered the implementation of biodegradable polymers. Polylactic acid (PLA), is widely used in food packaging, disposable goods, textiles and biomedical devices. Its biodegradability and biocompatibility are at the core of this, however, some data on aquatic animal uncover the need for a detailed analysis of PLA, which may also impacts intestinal microbial communities of animal intestines. Therefore, despite its GRAS status, the potential effects of PLA microplastics (MPs) ingestion and digestion at gut level need to be assessed. Understanding the impact of MPs on human microbiota and their possible biotransformation in the gastrointestinal tract is critical to evaluate the potential risks of micro(nano)plastics at the digestive level.



We provide scientific evidence about the modifications and effects of PLA MPs ingested in a realistic amount during their passage through the human digestive tract. Upper digestion was simulated in a standardized *in vitro* model and colonic-microbial fermentation in the Dynamic SIMulator of the GastroIntestinal tract (simgi®)[1]. Changes in gut microbiota composition (microbial counts and 16S rRNA gene-based metagenomic analysis), microbial metabolic activity (lactate and short chain fatty acid production), and MPs morphology as well as microbial MPs aggregation/colonization were evaluated in the different digestion phases (oral, gastric and colonic). PLA was used as pellets and milled by blade milling. Extensive characterization is made by field emission scanning electron microscopy and detailed Raman confocal mapping at different positions and depths during different digestion stages of both series (oral, gastric, intestine and colonic fermentation). The results will also be compared with the evolution and impact of polyethylene terephthalate (PET) pellets and blade-milled particles[2]. The **Figure** illustrates representative Raman spectra at PLA surface and within cracks generated at its surface. The role of milling triggers surface defects and modifications near PLA surface, progressive digestion stages progressively affect the surface and the formation of carbonaceous species is increasingly apparent, in line with FE-SEM analyses that underline the presence of surface bacteria. The results on the microbiota illustrates changes associated with PLA modifications.

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[1] https://www.cial.uam-csic.es/simgi/index_eng.html
[2] Tamargo, et al. (2022) "PET microplastics affect human gut microbiota communities during simulated gastrointestinal digestion, first evidence of plausible polymer biodegradation during human digestion," *Scientific Reports*, 12(1), p. 528. https://doi.org/10.1038/s41598-021-04489-w.

TOPIC N° 8

PARTICLE SHAPE AND INTRINSIC CELLULAR VARIABILITY SHAPE THE RESPONSES OF MACROPHAGES TO POLYSTYRENE NANO AND MICRO PARTICLES

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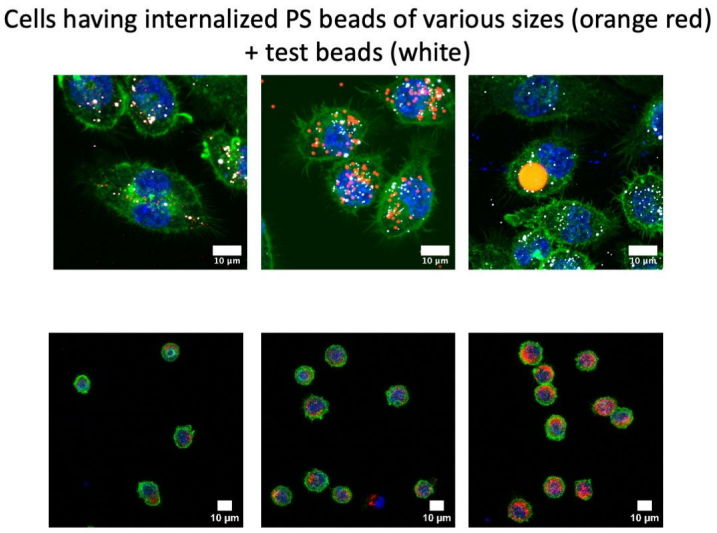
Nanoparticles and among them nanoplastics are generally considered to induce more biological effects than bigger particles because of their greater penetration in bodies and cells. Nevertheless, it is known that particles up to a few microns can translocate through the epithelial barriers and be transported to distal organs. As the particles surface and mass increase with the square and the cube of the size, it may be anticipated that microparticles may induce stronger effects than smaller ones, especially when their size begins to approach the size of the cells.
To investigate this hypothesis, we exposed macrophages (i.e. cells that are able to scavenge particles over a wide size range) to polystyrene particles of 0.1, 1 and 10 µm in diameter, and analyzed their responses by a proteomic approach. This analysis showed an important common core response for the three particles sizes, but also that larger particles induced a more important response than smaller ones at equal mass concentration.
As this suggested a role of the internalized plastic mass in the response, we also investigated an often-overlooked parameter, i.e. the heterogeneity of the cells in their propensity to internalize (plastic) particles. We showed that macrophages vary greatly in their ability to internalize plastic particles. Quite interestingly, "the appetite came with the eating", so that the final population of cells exposed to a single concentration of plastic particles is indeed a mix of cells with very different loads of internalized plastics. Unsurprisingly, heavily-loaded cells show stronger responses than lightly-loaded cells. We also showed that plastic particles persist in macrophages.
When brought together, these data draw a picture where the maintenance of the function of the immune system is assured by the lightly-loaded cells, while the heavily-loaded cells may induce local effects. This balance may change over time in case of continuous exposure, because of particle accumulation.



TOPIC 8
Micro and nanoplastics pollution

TOPIC N° 8 – MICRO AND NANOPLASTICS POLLUTION

EVALUATION OF INTERACTIONS BETWEEN NANO- AND MICRO-PLASTICS WITH NUTRIENTS AND THEIR POTENTIAL EFFECTS ON NUTRIENT HOMEOSTASIS

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The use of plastics has increased exponentially since their invention, raising problems on their accumulation and disposal. The release of plastics pieces smaller than 5 mm are defined microplastics (MPs), while particles in the submicron range are called nanoplastics (NPs). Both MPs and NPs are associated with long-term persistence in the environment, as they are very resistant to degradation. The concern for the potential toxic effects following MPs exposure as well as their accumulation into the human body is increasing: accumulation of MPs into different tissues from healthy volunteers was recently demonstrated, while the side effects after MPs exposure in animal species such as fishes and seabirds are well documented. MPs- and NPs-mediated toxicity could be exerted directly by plastics materials or indirectly through the interaction with other substances such as contaminants or molecules.

Ingestion is one of the most relevant exposure routes due to MP-containing water and food. Depending on their chemical properties, polymer types, weathering and aging processes, MPs and NPs could interact in different ways with molecules, such as nutrients. The interaction of MPs/NPs with food and nutrients along gastro-intestinal tract could lead to adsorption phenomena of essential nutritive molecules on their surface, altering their absorption. The reduced/altered absorption of nutrients such as amino acids, proteins, cholesterol and vitamins may contribute to malabsorption diseases.

In this work, two different MPs types were used, PTFE and HDPE, with the aim to evaluate the interactions between plastics and nutrients during digestion. *In vitro* simulated gastro-intestinal digestion following INFOGEST protocol was used to investigate the interactions between different type of MPs with proteins and cholesterol. After digestion, MPs were analyzed by SDS-PAGE and silver staining or Western Blot for evaluating their ability to adsorb proteins. Although proteins were degraded during *in vitro* digestion, PTFE and HDPE are able to adsorb them and their fragments on the surface. The interaction between MPs and cholesterol were also investigated. Cholesterol is also adsorbed on both HDPE and PTFE surfaces with the subsequent decrease of the fraction available for absorption in humans.

With the aim to evaluate differences in nutrients absorption in the presence or absence of MPs, digests were applied on Caco-2-based *in vitro* intestinal model and absorbed amino acids measured after 3 hours incubation. When proteins were co-digested with MPs, the amount of absorbed amino acids decreases more than 50% with respect to digests obtained in the absence of MPs. No adverse effects on Caco-2 viability and barrier integrity are observed in the presence of MPs, while epithelium permeability slightly increases in the presence of MPs with respect to the physiological conditions.

The preliminary results clearly indicates the role of MPs in interacting with nutrients such as proteins and cholesterol, potentially leading to their malabsorption and nutritional dyshomeostasis. These interactions will be further investigated in order to evaluate the overall impact of MPs on human health.

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

Guidance on Safe-and-Sustainable-and-Recyclable-by-Design plastics: Co-creating towards impact-driven innovations in plastics

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Within SURPASS, we aim to develop Safe-, Sustainable-, Recyclable-by-Design (SSRbD) assessment and guidance dedicated to plastics. SSRbD assessment requires a multidisciplinary approach, starting from the big picture to focus on impact-driven innovation that is practically feasible to perform. To achieve this, the SURPASS team works in multidisciplinary groups to allow co-creation, specifically between companies, scientists and risk assessors.
SURPASS focuses on 3 case studies: 1) Building sector: New recyclable-by-design bio-sourced polyurethane resins with enhanced vitrimer properties to replace Polyvinyl chloride for window frames, 2) Transport sector: New fire resistant recyclable epoxy-vitrimer based composite integrating non releasable fire-retardancy moieties, as alternative to metal for the train structure, 3) Packaging sector: MultiNanoLayered films involving no potentially harmful compatibilizers to replace currently non-recyclable multi-layers films.
For each case study, the SSRbD strategy has been developed and aligned with the proposed framework from the EU Joint Research Centre (JRC). Currently, activities focus on identifying and gathering information on exposure and release, hazard, life-cycle assessment and costs to perform all assessments for the case studies and to feed the information into the digital guiding tool. Specific attention will be paid to additives (IAS) and non-intentionally added substances (NIAS) that might be released from the polymers. An inventory of all materials used will be made, including (N)IAS, their functionality and their toxicity to allow selection of appropriate substances to be used for the next generation of safe-by-design plastics.
Taken together, by co-creating in multidisciplinary teams that start from the big picture and work towards practical solutions, SURPASS aims to contribute to the development of SSRbD polymers that benefit society without harm.

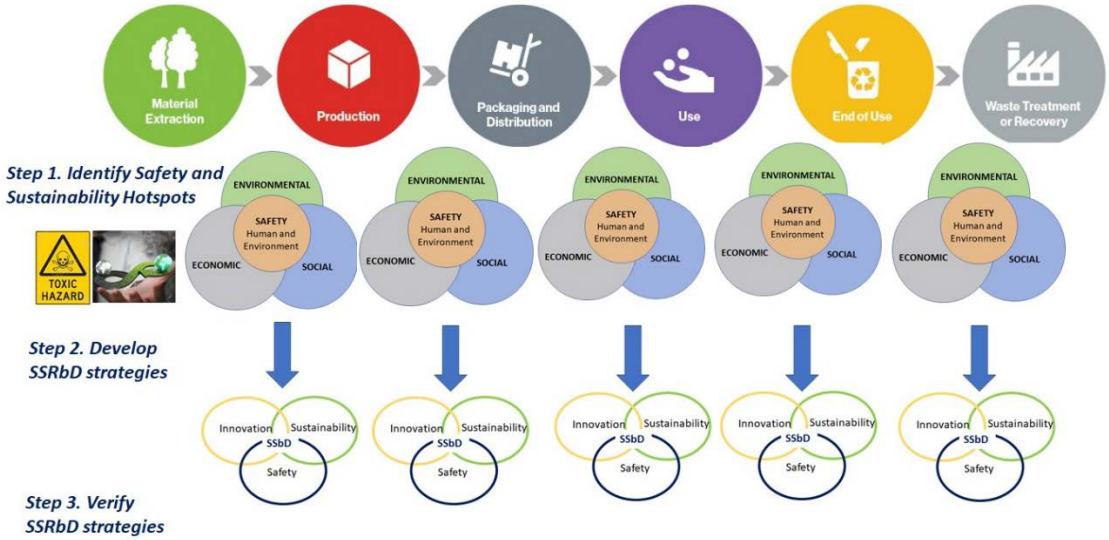

Figure 1. SSRbD approach in SURPASS

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TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

NANOSAFETY MANAGEMENT ON WHOLE VALUE CHAIN OF METAL ADDITIVE MANUFACTURING - LPFB CASE STUDY

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Aeroprint project gave the opportunity to implement an integrative approach of the nano risk management on whole additive manufacturing (AM) value chain. The strength of this study is a combined approach (risk assessment, toxicological assays and measurements) which allows to introduce and address smoothly and strongly nanosafety in AM. The evaluation was carried out on the LPFB (Laser Powder Bed Fusion) process, taking into account not only the printing stage of the parts but also all the other activities such as the manufacture/maintenance of the machines and the manufacture/reuse of the powders. (Figure 1).
This combined approach has rationalized the measurements and confirmed that it is crucial to limit as much as possible the direct contact between the operators and the powder. This is reflected in the implementation of cleaning and sieving systems for powders integrated into the machines, but also trough dedicated procedures during maintenance phases.
In parallel, specific studies were performed on reused powder which are a very common aspect in LPFB process. Powders are re-used from a part printing to another and as it is already known a nanometric fraction could be generated and mixed with the powder during AM process. The introduction of this fraction could impact the initial behavior of the powder. For that powders were collected at different re-use stages (0 – 8 and 12 times) just after sieving process to analyse their dustiness parameter but also their *in-vitro* toxicological response.

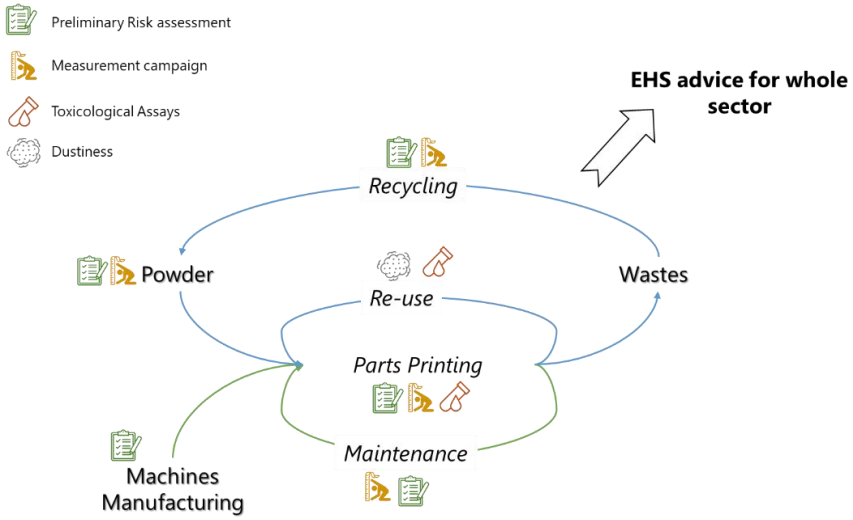

Figure 1 : Nanosafety management on whole value chain of metal additive manufacturing – LPFB case study

Acknowledgement:
Aeroprint project has received funding from the European Union and Auvergne-Rhône-Alpes region.

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

ADVANCES IN EMPLOYING NANOSAFETY STANDARDS IN NANOMATERIAL RESEARCH

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The use of nanomaterials is rapidly expanding in many sectors, such as medicine, manufacturing, energy, etc., however, there is limited research on the potential risks. In many cases, safety aspects are governed by the general health and safety management system of the organization (e.g., ISO 45001:2018 guidelines), based on the hazard statements of the bulk materials. Additionally, control actions can be assessed based on the existing control banding protocols (ISO12901-2:2014) considering the materials' quantities, and the harmonized tiered nanosafety methodologies (ISO 12885:2018).
The current work expounds on the integration of supplementary series of assessments and potential advances in the nanosafety sector, taking into account the risk evaluation of several commonly used nanomaterials, including carbon nanotubes (CNTs) and magnetic nanoparticles. Regarding inhalation exposure, pulmonary deposition models based on the aerodynamic diameter of nanomaterials allow better understanding of the nanomaterial risks due to their accumulation in different parts of the respiratory tract leading to inflammatory and cytotoxic effects. Furthermore, evaluation of dermal exposure is commonly neglected from nanosafety assessments, despite skin hazards.
Therefore, a critical viewpoint is presented on the application of the current nanosafety standards, and the gaps in adapting the standards to the applied materials and processes. Additionally, an approach to circumvent these gaps is outlined (**Figure 1**) considering the nanoparticles' morphological characteristics and physicochemical properties, the dermal penetration potential, use of respiratory deposition models, and documentation of the process's hazards, while maintaining a precautionary approach in any cases of uncertainty. Hence, the proposed framework is complementing the current nanosafety standards which would be beneficial for the safety assessment of scaled-up nanomaterial processes.

Figure 1. Illustration of the current standardisation approach in nanosafety assessment and potential advances.

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TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

EXPLORING OXIDATIVE STRESS AND DNA DAMAGE IN PROFESSIONAL WELDERS EXPOSED TO WELDING FUMES

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Background and aim: Occupational exposure to welding fumes could result in various health endpoints in professional welders, including cardiovascular diseases, pulmonary impairments, and cancers. Given the importance of oxidative stress and DNA damage in the development of various diseases, this systematic review aimed to evaluate the associations between welding fumes exposure and changes in oxidative stress [superoxide dismutase (SOD) and malondialdehyde (MDA)] and DNA damage [8-hydroxy-2′-deoxyguanosine (8-OHdG) and DNA-protein crosslink (DPC)] markers in professional welders.
Method: This systematic review was conducted using the Cochrane guidelines and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, and the review protocol was registered on the PROSPERO database (CRD42022298115). Six electronic bibliographic databases, including Medline (Ovid), Scopus, Web of Science Core Collection, Embase, CINAHL, and Google Scholar were searched from inception through September 2021 to identify observational epidemiological studies that fit the study's aim. Two reviewers independently assessed the risk of bias and certainty of the evidence using the National Toxicology Program's Office of Health Assessment and Translation tool and the Grading of Recommendations, Assessment, Development, and Evaluation framework, respectively. The Synthesis Without Meta-analysis (SWiM) method was used to perform narrative synthesis of results. Pooled mean differences with 95% confidence intervals were calculated in a random-effects meta-analysis for the outcomes of interest in the review.
Results: From 450 studies identified through the search strategy, 14 observational epidemiological studies met the inclusion criteria and were included in the review. Most studies reported significantly higher welding fumes levels in welders than in controls. The narrative synthesis results of SOD showed a significant difference between welders and controls, while the meta-analysis results of MDA did not show a significant difference between the studied groups (MD = 0.26; 95% CI, -0.03, 0.55). The meta-analysis results of 8-OHdG (MD = 9.38; 95% CI, 0.55–18.21) and DPC (MD = 1.07; 95% CI, 0.14–2) revealed significant differences between the studied groups. The included studies were at high risk of exclusion and confounding bias. The certainty of the evidence for oxidative stress and DNA damage results were very low and moderate, respectively.
Conclusion: Exposure to welding fumes is associated with DNA damage in professional welders, and 8-OHdG and DPC might be considered reliable markers to assess DNA damage caused by welding fumes exposure. We recommend, however, that the assessment of oxidative stress attributable to welding fumes exposure not be solely based on MDA and SOD.
Keywords: Biomonitoring, DNA damage, Metal particle, Welding fumes, Oxidative stress.

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

A PORTAL SUPPORTING RISK GOVERNANCE OF NANO- AND ADVANCED MATERIALS

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Nanomaterials may be viewed as a key enabling technology with potentially large benefits to society – either by improving existing functionality of currently available materials or by adding completely new material functionality in nano-enabled materials. There are, however, potential risks to human health and environment associated with nanomaterials that go beyond the risks commonly associated with chemicals. Stakeholders have difficulty navigating the complicated landscape of risks to human health and environment associated with nanomaterials and there is a need for a directory of the trustworthy information, guidance, data, methods, and tools. In the past two decades, a significant corpus of nano-related research has been developed in order to refine and complete risk assessment, evaluation, and risk management frameworks. However, governance information and strategy, data, models, tools, and guidance relevant to nanomaterials is still scattered and in continuous development across numerous organizations. This is reflective of the research project-based development trajectory and use of outcomes is at best fragmented and on a case-by-case basis. Better findability and access to these key elements of risk governance could improve their use.
We present a web-site offering a Nano Risk Governance Portal (NRGP) as gateway to support the risk governance of manufactured nano- and advanced-materials and nano-enabled products. The site is built in in collaboration between three research projects funded under the NMBP-13 Horizon 2020 Programme of the European Union (Gov4Nano, NANORIGO, RISKGONE). We describe several elements of this digital portal, which serves as a single entry for stakeholders in need of, or interested in, nano-risk governance aspects. The NRGP allows users to be guided towards information about nanomaterials, risk governance concepts, guidance documents, harmonized methods for risk assessment, publicly accessible data, information and knowledge, as well as a comprehensive directory of risk governance tools for risk identification, risk assessment, and safe-and-sustainable-by-design assessment of manufactured nano- and advanced-materials. We present a technical implementation and content of the first version of the NRGP. We discuss our vision for the future and further plans for developing, implementing, hosting and maintaining the NRGP aimed at ensuring its sustainability. This contains also a procedure to link to, or include, currently available and future (nano)material-related (cloud) platforms, decision support systems, tools, guidance, and databases in line with good governance objectives. Finally, we reflect on the sustainability of the NRGP and its interaction with other digital infrastructures.

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TOPIC 2 – IMPLEMENTATION OF THE SAFE BY DESIGN CONCEPT IN CASE STUDIES

THE BENEFITS OF COORDINATING INTERDISCIPLINARY CASE STUDIES AND MOVING TOWARDS A PRODUCT ORIENTED SDB APPROACH

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The French project SERENADE examined for nearly a decade the Safe(r) by Design of next generation nanomaterials. The main conceptual legacy of this project is a coordinated, yet diverse approach to investigate the safe-by-design development of nanomaterials in a variety of application fields, using a targeted set of inter-disciplinary case studies. The originality of the approach was to cover as many multiple technology readiness levels (TRLs) and life cycle stages as possible, combined with shared hazard and end- of-life assessments in an effort towards a (more) comprehensive and resource driven research.

Safe by Design, in a "traditional" and still prevalent sense, usually results in a structured and (most often) sequential approach deliberately putting the focus on hazard and exposure issues regarding the nanomaterial itself in a bottom-up progression of material development. However, this general strategy lacks flexibility. Within the SERENADE project, a case study examining photocatalytic paint failed to validate the generally accepted Safe by Design scheme through the stage gate. This example examined the product (paint in this case) rather than the nanomaterials it contains. It was found that the essential parameters, namely product specification and functionality, failed to fit into a rigid bottom-up approach and indicated the need for alternative Safe by Design strategies.

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TOPIC N° 2. IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

PRACTICAL GUIDANCE TO A HOLISTIC SAFE AND SUSTAINABLE BY DESIGN (SSBD) APPROACH FOR ADVANCED MATERIALS

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The European Green Deal policy ambitions for a toxic-free environment set out in the Chemicals Strategy for Sustainability and the Zero Pollution Action Plan identify the need for a Safe and Sustainable by Design (SSbD) approach to chemicals and novel materials. This need is addressed by the H2020 SUNSHINE project, which has developed an overarching SSbD approach for advanced materials and has progressed towards its practical operationalisation by applying it to specific multi-component nanomaterials (MCNMs). The SUNSHINE approach enables assessment of safety and sustainability at each stage of product development from a lifecycle perspective (Figure 1). This is achieved via a tiered approach that uses screening-level qualitative (Tier 1) and semi-quantitative (Tier 2) methodologies in the early stages of innovation and quantitative (Tier 3) assessment methods for the later stages. The focus of this contribution is on the qualitative Tier 1 self-assessment methodology designed to evaluate the safety, functionality and sustainability in the early R&D stages of developing advanced materials. This approach was developed to be easily implementable by industries, especially SMEs, which often do not have sufficient time and/or expertise to engage in resource-intensive safety and sustainability evaluations. This early screening self-assessment approach was tested and refined using a real industrial case study in the SUNSHINE project, i.e., nano-enabled PFAS (Polyfluoroalkyl substances)-free anti-sticking coating for bakery molds. The results have demonstrated that this innovative coating material has superior performance in terms of safety, functionality and sustainability as compared to an alternative conventional benchmark material (Teflon).

Figure 1 Life cycle thinking applied to the SSbD approach with the safety, sustainability and functionality dimensions taken into considerat

TOPIC N° 2 – IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

"CHALENGES IN IMPLEMENTATION OF THE SSBD CONCEPT IN NEW LIGHTWEIGHT NANO-ENABLED MATERIALS – CELLULAR LIGHTWEIGHT CONCRETE MATERIALS CASE STUDY"

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The increasing competitiveness of the global market, along with European Commission strategies and policies, in particular the European Green Deal, have challenged the industry to reinvent itself and innovate to search for more sustainable solutions, safe for both human health and the environment, and social-economically viable. This pressure to create differentiated products has often led to research based on uncertainties regarding the health and environmental safety of the solutions developed. Nanotechnology, more specifically the use of engineered nanomaterials (ENMs), is an example of this. The speed at which nano-enabled products appear on the market is not accompanied by regulation adapted to the new challenges, either because the information available is unreliable or because or insufficient.

Lightweight multifunctional materials with increased strength, self-cleaning and/or self-sensing properties are an emerging field of materials development(1), which require becoming competitive or reasonably priced to increase their market penetration, and their market value. Many of these properties are achieved through the use of ENMs, due to their extraordinary physicochemical properties. However, there are significant concerns regarding the effects of ENMs on human health and the environment, thus requiring careful evaluation and control in their management throughout their life cycle, namely in the workplace (2). The implementation of a Safe and Sustainable-by-Design (SSbD) framework aims an integrated assessment of the environmental, economic, social, technical and safety aspects of the material/product and associated processes throughout the entire life cycle (3)(4).

The EU-funded LightCoce project (No 814632) ,which aims offering an open access Ecosystem for upscaling and testing multifunctional lightweight concrete and ceramic materials, sought to apply the SSbD framework, in the applications developed, namely in the production of cellular lightweight concrete material. Several guidelines and standards from CEN(5,6), ISO(7–9) and OECD(10,11) were taken into account to achieve this goal, and the SSbD framework published by JRC (10,11). With this aim, qualitative information on the production process was collected enabling the definition of different potential exposure scenarios. A qualitative assessment of the chemicals with regard to their hazards was also performed on the basis of safety data sheets and literature. The number of engineered nanoparticles (ENPs) and mass concentration were determined with the aim of evaluating the emission sources of ENPs, incidental and background NPs, and the effectiveness of the controls implemented, through the use of direct reading equipment.

Complementary, Life Cycle Assessment methodology has been applied to estimate the potential environmental impacts the new developed concrete and ceramic solutions, helping to identify hotspots for further environmental improvement and impact reduction. In addition to support value creation, a life cycle costing analysis has been caried including both capital costs and operational cost to idnetify the main cost drivers for each product prior to the fure scale up. Sensitivity analysis regarding future energy and labor prices variation was also included.

All the studies carried out aim to support stakeholders in assessing the benefits of a given innovation and in deciding whether or not to adopt it consciously based on available and current knowledge.

Keywords: SSbD framework; engineered nanomaterials; lightweight materials, Life Cycle Assessment.

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TOPIC N° 2 – IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

IMPLEMENTATION OF DESIGN STRATEGIES TO REACH THE DESIRED SSBD PERFORMANCE ATTRIBUTES: FUNCTIONAL TEXTILE COATINGS CASE STUDY

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Coupling textile with nanoparticles-based coating is a design strategy successfully exploited to improve the textile technical and functional performances and, at the same time, to stabilize over a versatile, flexible, low-cost supporting material, active nano-phases that can positively impact in healthcare and environmental remediation applications. Nevertheless, the increasing use of nano-enabled products (NEPs) in everyday life applications raises questions about their safety. To answer, it is necessary to promote and implement a safe-and-sustainable-by-design (SSbD) approach through the whole products life cycle: nanomaterials synthesis, substrate functionalization, use phase, and end of life. At this purpose, a careful data collection and FAIRification strategy need as well as decisional computational tools able to identify design solutions that meet the SSbD key performance indicators (KPIs) in all dimensions: functionality, safety, life-cycle environmental impact and life-cycle costs. In the collaborative research project ASINA we tested different design strategies, across two main life-cycle value chains. In this work we present the results of Value Chain 1 case studies for the production of textile with antimicrobial and depolluting activity, in particular, antimicrobial textiles functionalized with silver NPs as personal protective equipment and photocatalytic filters based on titania NPs deposited on textile or polymeric substrate, for air purification treatment.
The starting materials, identified as Tier 1, were: AgHEC, a sustainable synthesis of AgNPs nucleated at room temperature on hydroxyethylcellulose (patented by CNR-ISSMC); TiO_2@SiO_2, a nanophase of titania coupled with silica (optimized by CNR-ISSMC); and TiO_2-N, a nitrogen-doped titania commercial benchmark nanophase provided by Colorobbia. An extensive p-chem and functional characterization of Tier 1 materials allowed us to define some key decision factors (KDFs) such as synthesis parameters and nanophase coatings and link them to KPIs such as antimicrobial and photocatalytic activity. We so developed a new set of Tier 2 materials, exploring the material design space (KDF values) and evaluating how and if the design alternatives impacted on KPIs in the performance space to attain SSbD solutions. In this work, we focused on the workflow and results surrounding the preparation and characterization of Tier 1 and Tier 2 material and their incorporation into products (antimicrobial and photocatalytic textile coating).
Once the design hypothesis referred to the nano-phases synthesis were investigated to obtain the addressed SSbD solutions (first NMs life cycle phase), we proceeded investigating the second life cycle phase (incorporation) with the integration of nanoforms (NFs) into NEPs.Thus the integration of second and third level SSbD case study was implemented by defining a further design space with selection of appropriate KDFs referred to the type of incorporation process and related and KDFs values range referred to the specific NFs deposition parameters. The second (KPIs) performance space has also been defined accordingly addressing the product functional activity, the worker exposure, and the NFs release during the use phase. The data collected at each stage of the SSbD case studies provided inputs for the ASINA ES (the ASINA multi-objective-optimisation tool) for the identification of the most promising SSbD alternatives.
This research received support from the EU H2020 ASINA (Anticipating Safety Issues at the Design Stage of NAno Product Development) n° 862444.

TOPIC N° 2 – IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

DESIGN STRATEGIES SUPPORTING THE DEVELOPMENT OF ANTIVIRAL NANO-AG-BASED MATERIALS UNDER A SSBD APPROACH

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Safe and sustainable by design (SSbD) is an approach to materials and products design that should match functionality, safety and overall sustainability criteria throughout their lifecycle. To achieve the goal two main areas of expertise are involved: on one side, design strategies, methodologies and techniques, inspired by green chemistry and eco-design principles, and on the other side a robust framework to assess the SSbD performance attributes, in a life-cycle perspective. This challenge is particularly tricky when the materials are nanometric and designed to be bio-active, like the case of antimicrobial solutions, which should be selective for their intended targets (microorganisms) but have minimal effects on off-target human cells or ecosystems. To compare design solutions and get a quick idea of their potential efficacy, drug-discovery research provides us an indicator, the selectivity index, defined as the ratio of the concentration of a drug required to produce toxicity in 50% of cells (CC50) to the concentration of the drug required to produce a desired pharmacological effect in 50% of cells (EC50). Silver nanoparticles (nano-Ag) have been known for their antimicrobial properties for a long time, but the COVID-19 pandemic has generated renewed interest in their potential applications as well as new concerns for their use in some biocidal products. Unfortunately, this interest has also led to the proliferation of fraudulent and uncertified products in the market, which may be unsafe for use. So developing design solutions and guiding principles [1] that match SSbD criteria is of technological and methodological extreme importance.
In this work, we present some Ag-based design alternatives developed to promote their SSbD use in antiviral applications. In agreement with eco-design and green chemistry principles, AgNPs were nucleated in water, at room temperature, using biogenic reducing/capping agents (curcumin, sodium surfactin, hydroxyethylcellulose). To explore the design space and identify the best SSbD solutions, we modified the main synthesis parameters and the type of capping agent in line with a design of experiment (DoE) approach.
A widespread characterization was performed (DLS/ELS, TEM, XRD, UV-VIS, XPS) to assess the physicochemical properties (design variables space) affecting technical and functional performances (design performance attributes), to make some mechanistic hypothesis and derive SSbD driving principles applicable to AgNPs synthesis. The calculation of the selectivity index by assessing the cytotoxicity (Vero cells) and antiviral properties (SARs CoV 2 virus) allowed us to make a first screening for the selection of materials most promising under an SSbD approach. The results pointed out an actual enhanced risk/benefit profile of the proposed Ag-based solutions, particularly for curcumin-capped AgNPs, with respect to commercial alternatives or even with respect to antiviral drugs considered at the beginning of Covid pandemia such as chloroquine.

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

Hazard strategy for nanomaterials and nano-enabled products as part of a safe-and-sustainable-by-design approach

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Within the EU-project SAbyNA, we support implementation of safe-and-sustainable-by-design (SSbD) nanoforms (NF) and nano-enabled products (NEPs) and processes. To this end, we are developing a user-friendly e-infrastructure that assists developers and innovators (from industry) in a tiered approach to identify and implement SSbD interventions for their material, product or process. An important part of SSbD principles involves hazard assessment. We developed a hazard assessment strategy that can be used at an early stage of product innovation.
The hazard assessment strategy consists of 2 parts. Part 1 gathers basic information on the NF or NEPs, including physicochemical (PC) properties and intended application, and aligns this with potential exposure routes and environmental compartments. Clear hazard concerns are identified as red flags. These can already lead to suggestions for specific SSbD interventions, e.g. changing NF properties. In many cases, the level of hazards might be uncertain – as there is little data on novel NFs and NEPs – in this case the user will be directed to Part 2.
In Part 2 the user will be guided through a step-wise approach to gather information from data sources and/or to generate new hazard data specific for the exposure routes/environmental compartments of concern. A testing strategy is suggested to help the user to generate missing data. For human health, data on dissolution rate in physiological fluids, cytotoxicity, genotoxicity, oxidative potential and inflammation are gathered. For environmental safety, data on biodegradation, bioaccumulation, acute and chronic toxicity to aquatic and terrestrial organisms is gathered. Guidance is provided on which assays to use, including standard operation procedures (SOPs) for testing NEPs and how to interpret data for SSbD. Finally, SSbD interventions/design changes are proposed to reduce potential hazard. Overall, the hazard assessment strategy is a practical, user-friendly tool to identify the hazard of NFs and NEPs at an early stage of product innovation.

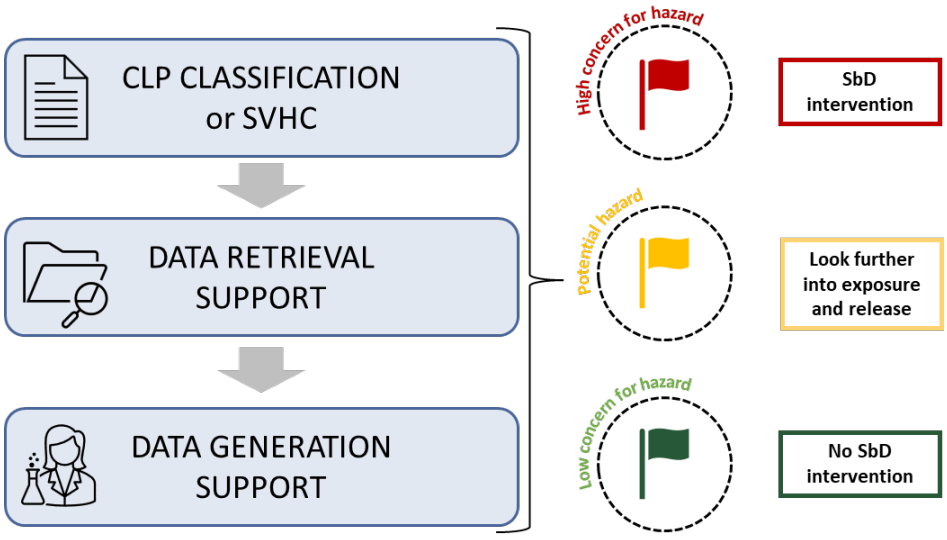

Figure 1. Hazard assessment strategy for NFs and NEPs as part of the SAbyNA SSbD platform.

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

DATA-DRIVEN QUANTITATIVE INTRINSIC HAZARD CRITERIA FOR NANOPRODUCT DEVELOPMENT IN A SAFE-BY-DESIGN PARADIGM: A CASE STUDY OF SILVER NANOFORMS.

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A theoretical safe and sustainable by design (SSbD) framework has been established from EU collaborative efforts towards the definition of quantitative criteria in each SSbD dimension, namely the human and environmental safety dimension, and the environmental, social and economic sustainability dimensions. In this study, we target the safety dimension and we demonstrate the journey towards quantitative intrinsic hazard criteria derived from Findable, Accessible, Interoperable, and Reusable (FAIR) data. Data were curated and merged for the development of New Approach Methodologies (NAMs) i.e. Quantitative Structure-Activity Relationship (QSARs) models based on regression and classification machine learning algorithms, with the intent to predict a hazard class.

The models utilize system (i.e. hydrodynamic size and polydispersity index) and non-system (i.e. elemental composition and core size) dependent nanoscale features in combination with biological in vitro attributes and experimental conditions for various silver NFs, for functional antimicrobial textiles and cosmetics applications. In a second step, interpretable rules (criteria) followed by a certainty factor were obtained by exploiting a Bayesian Network structure crafted by expert reasoning. The probabilistic model shows a predictive capability of ≈78% (average accuracy across all hazard classes). In this work we show how we shifted from the conceptualization of the SSbD framework towards the realistic implementation with pragmatic instances.

This study reveals i) quantitative intrinsic hazard criteria to be considered in the safety aspects during synthesis stage, ii) the challenges within and iii) the future directions for the generation and distillation of such criteria that can feed SSbD paradigms. Specifically, the criteria can guide material engineers to synthesize NFs that are inherently safer from alternative nano-formulations, at the earliest stages of innovation, while the models enable a fast and cost-efficient *in silico* toxicological screening of previously synthesized and hypothetical scenarios of yet-to be synthesized NFs.

TOPIC N° 2 – IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

PREVENTION-TROUGH-DESIGN IN GRAPHENE PRODUCTION PROCESSES

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The growing development of new and advanced nanomaterials (NMs) call for a responsible approach to evaluate and prevent health and safety risks for workers, who could be exposed in the whole life cycle of NMs. Since many uncertainties still remain about health effects and as long as occupational exposure limits will not be enforced, Prevention-through-Design (PtD) approach has been proposed as a framework aimed at preventing risks, taking into account health and safety aspects starting from the design stages of innovation production processes. PtD principles could be applied to NMs, including the design of materials and strategies to eliminate exposures and minimize risks related to the manufacturing processes. In this framework, this approach has been successfully applied to different case studies of graphene based NMs production in Research and Development laboratories with promising applications at the industrial scale. The methodology includes the integration of ISO Control Banding (CB) tool and OECD multi-metric and tiered approach to assess exposure by inhalation improving reliability and effectiveness of the risk analysis framework. In the present study three different graphene-based materials production processes have been compared: 1. chemical vapour deposition (CVD) graphene; 2. graphene nanoplatelets (GNPs); 3. few-layer graphene (FLG). As summarized in Table 1, CB levels have been identified for each production phase in a scale from 1=very low to 5=very high. Far-field (FF) and near-field (NF) average background and standard deviation values of real-time particle number concentration (PNC) have been measured in order to calculate the significant levels (PNC_{SL}, i.e. the average background plus three times the related standard deviation). The ratio ($\gamma > 1$) between the PNC level during the production phase and the significant level may correspond to NMs emission. Evidences obtained by off-line morphological and elemental characterization (SEM-EDS and/or TEM) of airborne NMs sampled in the workplace during each phase are used to complete the assessment. Based on such results and data analysis, control bands have been revised and primary risk management measures are proposed to mitigate workers' exposure. In conclusion, our methodology supports the complementary use of qualitative models and quantitative data to prevent risks in parallel with the industrial scale up of NMs production processes, by identifying tailored control measures giving also the opportunity to evaluate their effectiveness. As companies adopt the PtD approach, early by implementing the hierarchy of control measures, the possibility to minimize the potential for worker injuries and illnesses allows to mitigate the related costs and to improve safe and sustainable innovation processes.

Table 1. Summary of results of graphene case studies: 1. CVD graphene; 2. GNPs; 3. FLG.

Process phase	PNC_{SL} (part/cm^3)	γ	Off-line airborne samples	CB	Risk management
1a. Preparation	3740 (NF)	<1.0	No airborne graphene structures (SEM-EDS)	2	AH, SOPs, ST
1b. CVD growth		<1.0		2	CS, SOPs, ST
1c. Reactor cleaning		~2.0		3	VB, PPE, SOPs, ST
1d. Furnace Cleaning		<1.0		2	CS, SOPs, ST
2a. Thermal expansion	8186 (NF)	~10.0	Some nanostructures with the same morphology of the produced graphene (SEM-EDS)	5	CS, PPE, SOPs, ST
2b. Liquid exfoliation	13,443 (NF)	<1.0	No airborne graphene structures (SEM-EDS)	3	AH, SOPs, ST
3a. Wet Jet Mill	1767 (FF)	~1.2	Rare nanostructures with the same morphology of FLG (SEM-EDS). Rare FLG (TEM)	5	CS, PPE, SOPs, ST
3b. Rotovapor		~3.2	n.a.	4	CS, SOPs, ST
3c. Freeze Drying		n.a.	n.a.	4	CS, SOPs, ST
3d. Storage and Cleaning		~1.2	Rare nanostructures with the same morphology of FLG (SEM-EDS). Rare FLG (TEM)	5	CS, PPE, SOPs, ST

AH=aspiration hood; CS=closed system; FF=far field; NF=near field; PPE=personal protective equipment; SOP_S=standard operating procedures; ST=specific training; VB=ventilated booth; n.a.=not available.

TOPIC N° 1 – YYY

SAFER BY DESIGN APPROACH TO SUPPORT INNOVATION: A PRACTICAL CASE STUDY WITH MICROPLASTICS

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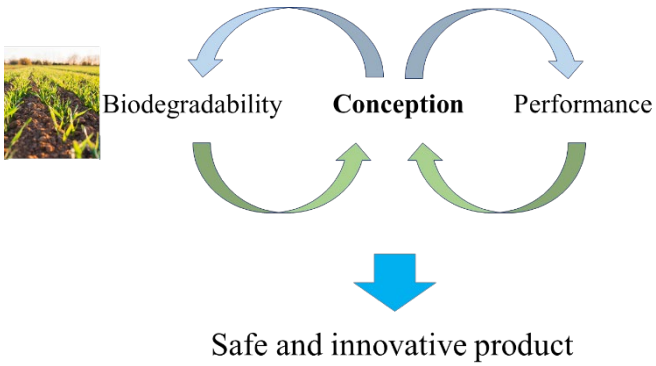
Solvay aims to develop different activities to assess the potential impact of the substances during their manufacturing, their use or their degradation/release. A safer-by-design approach allows the selection of the best candidates from application performance tests, (eco-)toxicological assays and biodegradability results, and is employed during the development of a new range of safe and sustainable products by our research and innovation function.

One of the safer by design approaches for which we gather information at an early stage of development is biodegradability evaluation. According to the application, the substances (coating of seeds, additives in shampoo…) can be released in the environment or in wastewater treatment plants. In this context, the research lab aims to investigate the biodegradability of the product, by following the degradation *via* respirometric analyses from micro-organisms.

Among the substances which have gone through this process during the past years, some microplastics case studies are presented here. Polymers are used in a wide range of applications such as packaging and coating, consumer goods, medical equipment or agriculture. Polymers with a low solubility below 2g.L-1 can be suspected to be microplastics according to the new incoming regulation in Europe. To answer to the needs of their registration and classification several factors such as their ready and/ultimate biodegradability can be highlighted in conventional OECD tests to ensure their environmental safety and to confirm that they can be considered or not as microplastics. Among those tests, our internal facilities allow Solvay to screen both ready biodegradability traits with or without BIMs (bioavailability improvement methods) and notably (emulsion) to highlight the potential of biodegradability of the substance. If these first tests are not conclusive, OECD & regulations propose to investigate both compartmental biodegradability and potential of persistence of these substances.

Associating to these biodegradation results, the impact of the native substance and of its biodegradation byproducts in terms of toxicity and ecotoxicity, will allow in the future to secure our safer by design approach.



TOPIC N° 2 - IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

ALGORITHMIC SELF-OPTIMISATION OF NON-TOXIC SPHERICAL SILVER NANOPARTICLES

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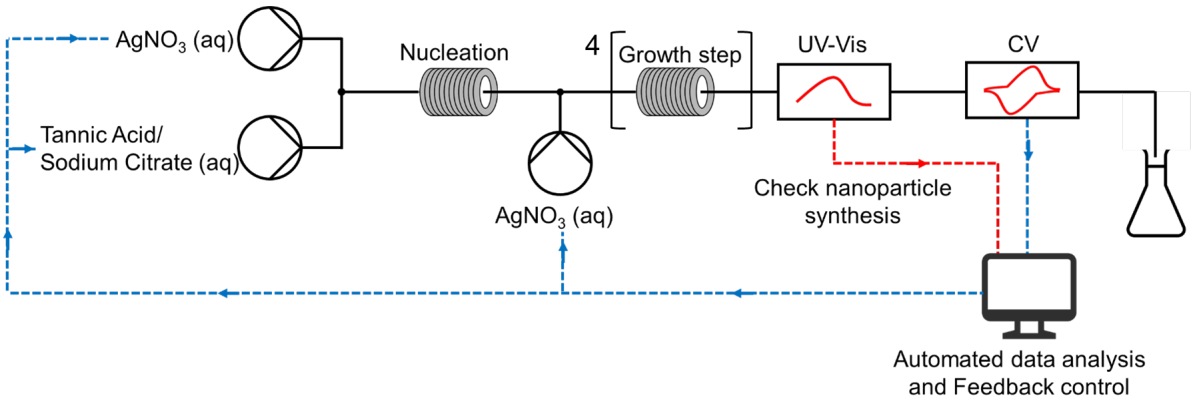
A continuous flow reactor platform and HISENTS biosensor platform have been developed and coupled to initiate a single-objective optimization algorithm for the autonomous, self-optimization of spherical silver nanoparticles (AgNPs) for toxicity. The continuous flow platform was designed under safety and sustainability measures by using multiple stage coiled flow inverted reactors which allow for the selective growth of AgNPs. Continuous flow processing allows for controlled synthesis with scalability and the reduction of solvent waste, which increases the level of safety of the experiment and allows for a 'plug and play' system to which inline reactors and real time analysis of the resulting product can be incorporated. Another advantage of continuous flow production is the reproducibility of nanomaterials, batch synthesis has a significant problem in reproducibility of size, shape, and composition from reaction to reaction. The precise control of flow rate, residence time, temperature, concentration and mixing of nanoparticles offered by flow creates precise control over size, shape, and composition of the nanoparticles. The scalability of flow processes means that optimised conditions for the desired product at lab scale, can be easily transferred to large scale production without compromising product quality, all the while increasing green chemistry metrics. In our study the chemical space was initially explored to determine conditional effects on the size of spherical AgNPs through a combination of in-line UV-Vis spectroscopy, and offline TEM analysis. Subsequently, the continuous flow reactor was coupled to the HISENTS biomembrane sensor which allows for high throughput screening of nanoparticles for biomembrane activity related to toxicity.[1] The chemical space was explored through a data driven feedback loop to determine the lowest biomembrane activity by adjusting the various parameters of the system. Through the combination of these two platforms, the ability to fabricate other metal/metal oxide nanoparticles and test their toxicity is significantly accelerated.



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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

ANTIMICROBIAL ACTIVITY OF ECO-FRIENDLY CuO NANOPARTICLES: PROMISING APPLICATIONS AGAINST MULTIDRUG-RESISTANT *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is classified by the World Health Organization (WHO) a 'high-priority' pathogen for research prompting the discovery and development of new antibiotics, since this bacterium is among the most multidrug-resistant bacteria in circulation nowadays [1]. Bactericidal effects of copper oxide nanoparticles (CuO NPs) have been reported, but the chemical synthesis can generate toxic waste and contaminate the environment. In this sense, the biogenic route is an eco-friendly alternative and more economically viable [2,3]. Thus, in this work, CuO NPs were synthesized using green tea extract (*Camellia sinensis)* as a reducing and capping agent and tested these NPs against different strains of *N. gonorrhoeae*. In addition, comparisons with ceftriaxone, an antibiotic of reference, were made, as well as cytotoxicity assay against Chang cells. The hydrodynamic size, PDI and ζ potential of the CuO NPs were found to be 250 ± 64.22 nm, 0.382 ± 0.05 and -14.53 ± 0.47 mV, respectively. PDI indicates that the NPs have a stable dispersion, and their negative surface charge (ζ potential) is due to the presence of polyphenols on their surface derived from green tea extract. Scanning electron microscopy showed a solid-state size of 75.72 ± 0.82 nm and spherical shape for the CuO NPs. The minimum bactericidal concentration (MBC) for CuO NPs and ceftriaxone against P9-17 strain were found to be 3.125 ug/mL and 30 ug/mL, respectively. For the multidrug resistant strains AR-165 and AR-190, the MBC of CuO NPs was 15.625 ug/mL. The time-kill curve revealed that CuO NPs takes 30 minutes to kill P9-17 and the MBC concentration can kill up to 10^7 cfu/mL without losing effect. Furthermore, the cytotoxicity assay against Chang cells showed that the CuO NPs do not have toxicity up to 100 ug/mL. These results indicate that CuO NPs have promising antibiotic effects, being almost 10 fold more letal to *N. gonorrhoeae* than ceftriaxone. Moreover, the cytotoxicity studies revealed a good biocompatibility, which can enable the use of these NPs as new antibiotics to overcome the increasing drug-resistance problem.

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TOPIC N° 1 – YYY

SAFER BY DESIGN APPROACH TO SUPPORT INNOVATION: A PRACTICAL CASE STUDY WITH NANOMATERIALS

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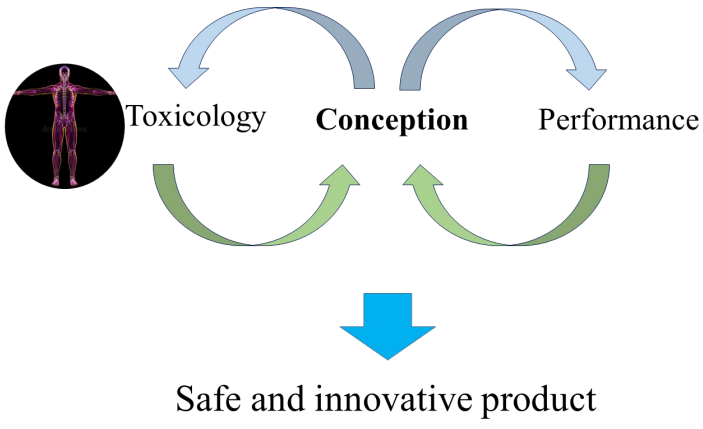
Solvay aims to develop different activities to assess the potential impact of the substances during their manufacturing, their use or their degradation/release. A safer-by-design approach allows the selection of the best candidates from application performance tests, (eco-)toxicological assays and biodegradability results. These approaches are employed during the development of a new range of safe and sustainable products by our Research and innovation function.

One of the safer by design approaches for which we gather information at an early stage of development is toxicological evaluation. In the context of the minimization of animal experimentation with the 3R strategy (Reduce, Reuse and Replace), the development of *in vitro* systems is attractive, at least for the initial phases of toxicological assessments for new substances. Therefore, Solvay uses and develops *in vitro* models relevant to assess typical exposure routes (*i.e* pulmonary, cutaneous, intestinal systems) associated to expected final end-markets of the substance.

Among the materials which have gone through this process during the past years, some nanomaterials case studies are presented here. Nanomaterials are widely used in various applications such as electronics, medical devices, antimicrobial agents, food additives, cosmetics, etc. increasing human exposure and thus the potential risks related to short- and long-term nanomaterial toxicity for both consumers and workers. From January 2020, the REACH regulation has been amended with specific additional requirements tailored for nanomaterials, or nanoforms under this legal framework. Considering these uses and processes of production, innovative methods able to predict and assess effects at environmental release and exposure level are required.

The battery of tox evaluations presented here include a catalog of cell lines mimicking different organs and an exposure up to 100h using real time monitoring of cell proliferation allowing a direct comparison between the candidates under development but also to compare observed effects toward a control (non exposed condition).



TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

ASINA-ES: A QUANTITATIVE BASED DECISION SUPPORT TOOL FOR MULTI-OPTIMAL SAFE AND SUSTAINABLE BY DESIGN SOLUTIONS.

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Important efforts have been made to pave the way towards the safety and sustainability of chemicals and materials by clarifying frameworks, concepts and methodologies and ultimately providing reference guidelines and criteria on how to tackle such ambitious challenges [1]. The industrial need of a quantitative methodology and of associated supporting tools, applicable to specific design case studies to nanomaterials (NMs) and nano enabled products (NEPs), led to the development of a specific management methodology developed within the ASINA project [2,3,4] as well as to the ASINA-ES, the multi-objective-optimisation- and decision support tool that enables safe by design solutions (SbD). ASINA-ES addresses the issue of limited amount of available experimental harmonised data in the innovation process starting phases. Indeed, based on a minimum and sufficient set of direct experimental measurements, safety assessments, FAIRified data and through machine learning outcomes, ASINA multi-objective-optimisation tool interconnects a selected set of Key Decision Factors (KDFs) with the set of Key Performance Indicators (KPIs) by capturing non-linearities. By employing Multiple Criteria Decision Algorithms through a step-wise approach, ASINA-ES identifies a subset of values within the allowed range of KDFs options that comply with the multi-optimization of the KPIs, according to design specifications, safety and sustainability criteria related to the four stages of the NMs' life cycle: synthesis, incorporation, use phase and end-of-life. The proposed methodology allows applying the KPIs weighting at the very last decision step, by preventing anticipated discarding of potential applicable and promising design cases. The flexibility and modular architecture of ASINA multi-objective-optimisation tool allows multiple level application to address different set of KPIs according to a stage-gate innovation process. Thus, the ASINA SbD approach is also extended to the Safe and Sustainable by Design (SSbD) framework by integrating multiple environmental KPIs and by applying Life Cycle Assessment and Carbon Footprint methodologies according to the ISO standards [5,6].
In this work, specific case studies related to NMs synthesis and incorporation phases targeted to the development of NEPs are presented. The related applications are referred to design case studies involving different number and types of KDFs an KPIs that supported the development of the solutions within the addressed ASINA Value Chains.

ASINA has received funding from the European Union's Horizon 2020 research and innovation programme, under grant agreements No 862444.. This paper reflects only the authors' view, and the Commission is not responsible for any use that may be made of the information it contains.

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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

SAFE AND SUSTAINABLE NANOMATERIAL DESIGN THROUGH ACCELERATED WEAR TESTING COUPLED WITH HIGH-THROUGHPUT SCREENING

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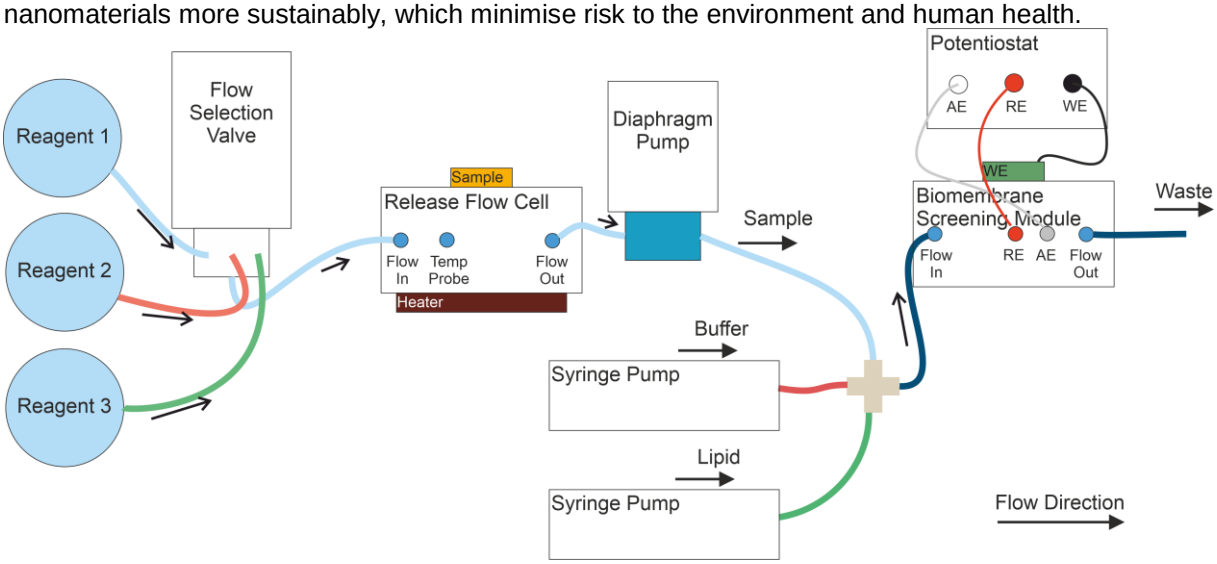
Background: As the use of engineered nanomaterials continues to grow, there is a pressing need to improve the safety and sustainability of these materials through the incorporation of safety-by-design (SbD) and safe- and sustainable-by-design (SSbD) principles. In this field, high-throughput, *in-vitro* screening methods are essential for evaluating toxicity and identifying potential hazards (1). In this study, we accelerate the wear of engineered nanomaterials and perform high-throughput screening on nanomaterial leachate media to understand the toxic properties of nanomaterials at the point of leaching.
Accelerated wear of NMs: The use of customised flow cells has been successful in understanding corrosion behaviour of metals and metal coatings in specific aqueous environments relating to industrial processes (3). The corrosion flow cell technique adopted in this study provides an insight into progressive corrosion behaviour during a sequence of transitions of fluid composition and temperature, with static flow conditions between reagent replacements. This approach enables us to assess the durability of nanomaterials under realistic conditions and identify potential risks associated with their use, supporting the SSbD principle of designing materials that are optimised for performance, sustainability, and safety.
High-throughput screening: To rapidly screen the interaction of nanomaterials with biological membranes by *in-vitro* hazard assessment, a membrane-on-chip sensing technique measures an electrochemical response to detect interaction with a phospholipid monolayer supported on a mercury electrode (2).
Methods: The output from the corrosion flow cell is coupled with the biomembrane sensor cell to report on the biomembrane damaging properties of leachate media. This coupled system provides a high-throughput, automated platform for the assessment of nanomaterial toxicity and enables the evaluation of nanomaterial hazards at the point of leaching. By understanding the toxic properties of nanomaterials at the point of leaching, early in the development process, we can design safer nanomaterials more sustainably, which minimise risk to the environment and human health.



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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

DEVELOPING SOIL SPECIES SENSITIVITY DISTRIBUTIONS FOR NANOMATERIALS - CONSIDERING NANOMATERIAL FORMS AND EXPERIMENTAL CONDITIONS

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Safety or safer by design (SbD) engineered nanomaterials (ENMs) may be achieved through interventions to make safer nanoforms (NFs) of the material, safer design of nano-enabled products (NEPs) or through safer processes in the production or use of the NF/NEP. Before any intervention can be taken, it must be understood which strategy is most relevant in achieving this goal. Classification of ENMs according to environmental hazard is used in the SabyNA hazard strategy to identify whether an intervention to improve safety of the NF is a suitable strategy in the early stages of design. In the absence of harmonised classifications of the material, existing data can be used to inform on expected environmental hazard of an ENM. Species sensitivity distribution (SSD) modelling approaches have been applied to a range of ENMs to support hazard thresholds sufficient for protecting aquatic ecosystems. This study proposes an environmental hazard classification for ENMs based on soil SSD-derived HC5 values. To address the knowledge gap in soil SSDs, a soil ecotoxicity database for ENMs was constructed compiling literature derived dose-response data. Using silver (Ag) ENMs as a case study, over 2,400 data points from > 100 test species (soil microbes, invertebrates and plants) were collated from ~140 soil ecotoxicity studies for different NFs of silver. The database also captures information on physico-chemical properties of Ag ENMs, experimental conditions and the fate of Ag NFs within exposure settings.

Previous efforts to generate SSDs for ENMs have usually combined data from multiple nanoforms of the same material, assuming this will be sufficiently protective and not distinguishing for differences in key physico-chemical properties. The database allows us to evaluate whether dissimilar nanoforms require separate SSDs to be established. The variability within Ag ENMs and ionic Ag toxicity across species and their effect endpoints was explored, and results show consistently greater variability in Ag ENM toxicity compared to ionic Ag. SSDs have been developed for Ag ENMs grouped by size, shape and surface coating, to assess whether SSD-derived HC5 values differ across Ag ENM characteristics. Results show significant differences in Ag nanoform toxicity across different coating types, with lower HC5 values for citrate-coated Ag ENMs compared to PVP-coated and uncoated Ag ENMs. This supports design of safer NFs as a relevant intervention that could be considered as part of a wider SSbD strategy for this material. Based on nanoform SSD-derived HC5 values, Ag ENMs with different coating types also fall within different hazard classifications in soil exposures. This work provides novel findings on soil species sensitivities to ENMs, and explores the influence of both particle property and environmental conditions on soil toxicity. Findings from this study further support knowledge for a safety-by-design approach for ENMs for the protection of soil ecosystems.

Acknowledgments – This work was developed under the "Simple, robust and cost-effective approaches to guide industry in the development of safer nanomaterials and nano-enabled products (SAbyNA)" project, financed by the Horizon 2020 EU's research and innovation funding programme.

TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT TO SUPPORT GROUPING OF NANOMATERIALS IN AQUATIC SYSTEMS AND IDENTIFICATION OF RELEVANT NANOFORMS AS A TARGET FOR SAFE(R)-BY-DESIGN

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An Integrated Approach to Testing and Assessment (IATA) is an approach to guide the collection and integration of relevant existing information as well as the targeted generation of new data to identify and characterise the hazards or assess the risk of chemicals. An IATA for aquatic systems provides a framework for the collection and/or generation of new data to identify the exposure relevant form of the nanoform (NF) for hazard assessment. This is an important component for safe(r)-by-design (SbD) strategies for which improvement in the safety of the NF across its life cycle is required.

To facilitate SbD, an IATA for aquatic systems is constructed to identify the most relevant environmental compartment(s) of exposure and the exposure relevant form. This supports prioritisation of interventions to the design of new NFs to reduce their environmental hazard. The IATA consists of decision nodes focused around dissolution, dispersion stability, chemical transformations and the relative contribution to toxicity of the particle and dissolved component of the overall exposure. Tiered testing strategies and associated thresholds for grouping NFs according to these key behaviours are proposed. Worked examples using microbial communities isolated from soils and lettuce plants in hydroponic systems, demonstrates identification of the exposure relevant form of the NF in these case studies and different grouping of NFs through application of the IATA. These worked examples also demonstrate that the IATA is applicable not just for aquatic ecosystems but may be utilised for any test in which the exposure or fate assessment is conducted in an aqueous media.

The implication of grouping NFs according to media dependent behaviours targeted by the decision nodes is discussed in the context of identifying targeted interventions in the design of a NF to improve its safety. It is proposed that grouping strategies such as this IATA for aquatic systems can be a useful tool as part of wider SbD strategies, delivering data that can be used in wider decision support systems to inform on suitable interventions in design to improve nanomaterial safety.

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

METALS IN EXHALED BREATH CONDENSATES OF WELDERS

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Background
Exposure to welding fumes may result in serious adverse health effects such as pneumoconiosis, chronic obstructive pulmonary disease, and pulmonary fibrosis. Since 2017, welding fumes are considered as carcinogenic to human with an increase incidence of lung cancer in welders (International Agency for Research on Cancer). To verify compliance, occupational hygienists collect air samples in the breathing zone of workers. However, exposure is not properly assessed when workers wear respirators. The objective of the project was to verify is we could use metals in exhaled breath condensates (EBC) of welders and use them as biomarkers of exposure.
Method
The project was run in collaboration with the Boilermaker union in Alberta (Boilermaker Lodge 146). Samples were collected during courses provided by the union in Edmonton and Calgary as well as one mining facility in Saskatchewan. Exhaled breath condensate samples were collected in the morning prior to shift and in the afternoon after shift in R-Tubes (Respiratory Research). EBC samples were aliquoted in micro-tubes and frozen at -80ºC prior to analysis. Simultaneously, air samples were collected during shift on a PVC filter using a 3-pieces cassette connected to a personal sampling pump. Filters were weighted prior to and after sampling for gravimetry. Metal analysis in EBC samples and filters were performed at the School of Public Health, Université de Montréal, Québec, Canada.
Results
In total, 33 boilermakers were recruited for this project which included 6 women, 11 smokers, and 20 ex-smokers. In average, welders were 35 years old and had been welding for 9 years. Particle concentrations in air samples ranged from 0.26 to 5.18 mg/m^3 with a geometric mean of 1.5 mg/m^3. Thirteen metals were measured in EBC samples: Al, As, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, Zn. Mn, Ni, and Fe concentrations were similar to levels observed in a previous study (1). Most metals did not show a significant difference between pre-shift and post-shift, although most of them had a lower median for post-shift. Only Be and Pb showed a significant decrease in concentration between pre and post-shift while V increased significantly.
Conclusion
More has to be done to understand metal levels in exhaled breath condensates.

Keywords
Welding fumes, Occupational exposure, Exhaled breath condensates, Metals

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TOPIC 2. IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

SUNSHINE INDUSTRIAL CASE STUDY: SSbD alternatives as anti-stick coatings of aluminium trays and moulds for bakery applications.

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In the production of aluminium trays and moulds for bakery applications, PTFE coatings represent the standard material for anti-stick properties. However, EU Reach regulation is banning PFOS/PFOA and posing severe limits on PTFE quality and composition because they are persistent, bioaccumulating, toxic and widespread in nature. The development of sol-gel coatings based on a core-shell SiC@TiO_2 nanostructure promoted by Laurentia Technologies and investigated within the SUNSHINE EU-project, represents a key-step toward a Safe and Sustainable by Design (SSbD) approach addressed to replace PTFE coating with multicomponent nanomaterials (MCNM).

SiC is a non-oxide inert ceramic material selected for the anti-stick and high thermal conductivity features. While, TiO_2 is added to improve SiC compatibility and prevent its agglomeration once embedded into the silicone paint applied on the aluminium trays. The advantage of applying SiC-based nanoparticles stems from the improved thermal conductivity which contributes to reduce cooking times and temperatures, so promoting a longer durability and ensuring the antistick functionality. On the other hand, the recent EFSA opinion poses some concerns about the use of TiO_2 NPs for food application fields. Therefore, we developed a second SSbD alternative by replacing TiO_2 shell with amorphous SiO_2, characterized by the same hydrophilic character and easily hetero-nucleated on SiC seeds through a sol-gel route.

We performed a widespread characterization on both MCNM and nano-enabled coating, evaluating the main physicochemical properties (size, shape, surface charge, composition, dissolution, crystalline phase) and their evolution once exposed to a set of relevant media according to the identified exposure scenarios. For the SSbD alternatives (SiC@TiO_2 and SiC@SiO_2) the technical function of the coatings (wettability, abrasion resistance, thermal durability) was measured and the life cycle assessment of the process was implemented. Exposure and hazard testing campaigns, related to manufacturing and use stages, contributed to evaluate the (eco)toxicological impact of the MCNM.
The results suggested that both the SSbD alternatives, SiC@TiO_2 and SiC@SiO_2, represent a promising substitute to PTFE coating enabling to preserve the technical functions with a reduced (eco)toxicological and environmental impact.

Acknowledgment: This work has received funding form the European Union's Horizon 2020 Research and Innovation programme under the Grant Agreement No 952924 SUNSHINE project.

TOPIC 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSbD PURPOSES

Emissions Characterization from Different Commercial 3D-printers Using NEP Filaments – A SAbyNA Case Study

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SAbyNA aims to provide simple, robust, and cost-effective approaches to guide industry in the development of safer nanomaterials (NMs) and nano-enabled products (NEPs). One industrial sector studied by SAbyNA is additive manufacturing, which is rapidly developing as the 3D printing process has many diverse applications. Despite the numerous advantages of 3D printing (e.g., cost-effectiveness, versatility), concerns arise from the potential adverse health effects that might occur due to exposure to the generated emissions. These emissions comprise airborne contaminants which may include carcinogens and respiratory irritants (Mendez et al. 2017)[1]. Moreover, NMs used in polymeric filaments to provide advanced functionalities to the final product, may pose another hazard as they could be released to the breathing air during the 3D printing process. In the present work, we are studying the emissions sourcing from commercially available 3D printers in a real-world occupational setting with the aim to identify the relation of emissions with specific process parameters (i.e., nozzle temperature) to support a selection of a SbD strategy.
3D printers based on the Fused Deposition Modeling (FDM) technology, used different filaments that were developed within SAbyNA: a conventional polypropylene (PP), a nano-enabled PP filament loaded with Ag nanoparticles (PP-nanoAg), a conventional polycarbonate (PC) and a nano-enabled PC filament loaded with carbon nanotubes (PC-CNTs). For the online monitoring of the emissions in terms of particle number concentration and size distribution during the 3D printing process a Nanoscan-SMPS (TSI 3910; 10-420 nm) was used. Size segregated samples were collected using SKC Sioutas cascade impactor which housed polycarbonate filters for offline chemical analysis and samples were also collected for electron microscopy analysis.
The PC-based filaments (PC, PC-CNTs) showed similar emissions patterns that were depended on the nozzle temperature, higher temperature resulted in higher emission in terms of particle number concentration. Moreover, a minor impact on the emissions in terms of particle number concentrations is observed when NEP filaments are used (Figure 1). The PP filament showed a similar dependance on the nozzle temperature (higher temperature led to higher emissions), but no clear effect of the Ag nanofillers was observed in that case. These results will support the SAbyNA platform development to offer SbD solutions based on the optimization of process parameters.

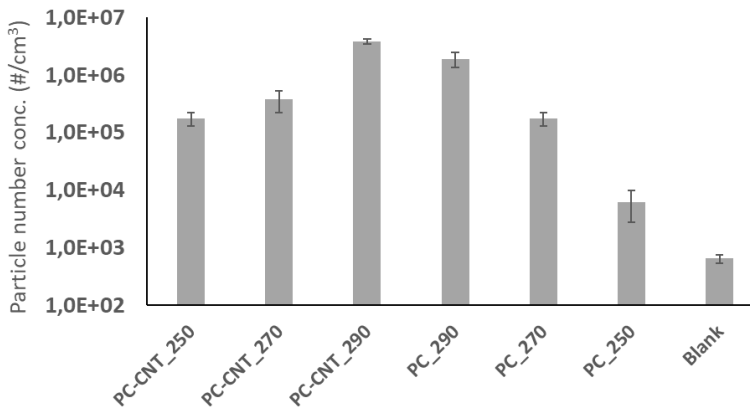

Figure 1 Averaged particle number emissions for the different filaments and in function with nozzle temperature

Funding from the SAbyNA project (EU Horizon 2020, grant agreement No 862419) is acknowledged.

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TOPIC N°2 – IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

Combining physicochemical characteristics, ecotoxicity and functionality data to select safe(r) nanoforms in paint – SAbyNA project

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When applied to engineered nanomaterials, safe by design (SbD) approach evaluates available data related to functionality, hazard, and cost for the entire life cycle of a nano-enabled product (NEP) to select the overall cost-efficient and safe(r) materials and processes. Such approach is developed in the SAbyNA platform, which is being validated using case studies of two industrial sectors: additive manufacturing and paint. At the early stage of the design of an outdoor paint, mixed metal oxide (MMO) nanoparticles were considered by CNRS/CEREGE as efficient, valuable, and safer alternative to titanium oxides-based materials, currently incorporated in paints to enhance their solar reflectance. Mixed-metal oxide nanoparticles were synthesized using different annealing temperatures (from 600°C to 1000°C) and coated with organic ligands. The coated nano-MMO synthesized at 1000°C were preferred for their highest solar reflectance when the functionality assessment was conducted alone. SbD must also consider relevant safety and sustainability aspects, and so the environmental hazard profile of the nanoparticles has also been evaluated at this stage of innovation to inform and support decisions around the selection of this nano-MMO design into the outdoor paint product. Bacteria, algae, and daphnia toxicity assays were selected to produce an ecotoxicity screening for each nano-MMO in soil and freshwater environments. Assays were optimised to reduce material needs, essential in early stages of design and innovation where there is often limited material availability. Assay sensibility to highlight significant differences in the ecotoxicity profiles of the different coated nano-MMO will be presented. The environmental hazard profiles obtained will be discussed in the context of SAbyNA platform developed in part to go beyond physicochemical properties toward environmental hazard endpoints to evaluate SbD interventions.

Funding information
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TOPIC N° 2 – IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

APPLICATION OF SAFE-BY-DESIGN APPROACHES IN NANOTECHNOLOGY SUPPLY CHAINS – CASES STUDIES EXPERIMENTS AND FIELD MEASUREMENTS

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A major challenge for the global nanotechnology sector is the development of safe and functional engineered nanomaterials (ENMs) and nano-enabled products (NEPs). The application of the Safe-by-Design (SbD) concept has been adopted as a means to reduce human health and environmental risks, applying preventive safety measures during the design stage of a facility, process, material or product. However and despite its importance, SbD prescriptions are still in their infancy, and are hampered among other things by the lack of comprehensive data about the performance, hazard and release potential of the great variety of NEPs in use. To operationalise and implement the SbD approach on industrial case studies, experiments and field measurements have been conducted. The present paper focuses on the main experimental results obtained during the practical implementation of the SbD approach for two case studies.
The first one is the surface modification of sulfur doped-TiO_2 (nano)particles with SiO_2 in a photocatalytic paint for the degradation of indoor air pollutants and the second one focuses on modified microcapsules of SiO_2 in personal and beauty care creams to provide perfume, antimicrobial and antioxidant properties. The use along the life cycle of characterisation instruments and methods, the realisation of field measurements along the formulation process of products containing (nano)particles and the implementation of scenarios that mimic the use and the end-of-life of the final product, in strong collaboration between academic and industrial actors (figure) lead to the development of safer products and process with added value for companies (table).

Life cycle steps	Methods	Type of information	SbD characterisation
Particle modification	Microscopy/EDS	Size, morphology (nm)	Product function – nano range verification
	BET	Specific surface area (m^2/g)	Product function - Size
	Bulk density	Density (-)	Product function
	Dustiness	Index in number or in mass (#/mg, mg/kg)	Safety product - define the propensity to emit aerosol particles during powder handling-
Formulation	Field measurements	Aerosol characterisation -Size, number concentration (nm, $\#/cm^3$)	Safety process and product - Assessment of exposure by inhalation of nano-objects and their aggregates and agglomerates
Use phase	Mechanical stress Accelerated ageing	Release rate((#/s, #/g)	Safety product - reduce the emissivity of the product as much as possible, taking into account the function of the product.
End of life	Incineration	Emissions ($\#/cm^3$)	

Thus, to illustrate this Sbd evolution, the effect of the surface modification on SiO2 shows a decrease of 39 % in the mass dustiness index, may be due to a change, in, the hydrophilic or hydrophobic character of the surface, leading to an evolution of emissions recorded during the field measurement resulting in a shift of aerosol emissions from the submicronic to the micronic size range.

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TOPIC N° 2 – IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

SURPASS: DEMONSTRATION OF INNOVATIVE TECHNOLOGIES TOWARDS MORE SAFE, SUSTAINABLE AND RECYCLABLE POLYMERIC MATERIALS

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The European Project SURPASS aims to develop Safe, Sustainable and Recyclable by Design (SSRbD) novel materials, by addressing three different industry-relevant cases in the building, transportation, and packaging sectors, respectively.
The first case study, namely the building sector, proposes to develop biobased polyurethane (PU) formulations to be used as window insulating frames. Indresmat (IND), leading this case study, has already developed a market-ready PU formulation, adequate for this application. The scope of the case study will be to increase the biocontent of the PU formulation and later-on investigating further the use of vitrimer chemistry to enhance recyclability of the PU and enable upcycling. Within the project, the bio-based PU structure developed by Indresmat will be converted in a vitrimer, which will be reprocessable through compression molding at low temperature, via the introduction of dynamic chemistry (oxime-carbamate) directly in the PU backbone.
The second case study targets the transportation sector and specifically the railway one. Composites have a high potential in replacing metal and enable weight reduction, which immediately translates into energy saving. However, composites have mainly been used in secondary structures or in interior application since their use in primary structures, such as rolling stocks, is ruled by Fire, Smoke and Toxicity (FST) requirements according EN45545. As glass- or carbon-based fibre reinforcements have good fire properties, it is mainly the resin that has to be improved regarding fire resistance. The scope of SURPASS is to provide reprocessable, repairable and recyclable thermoset composites, which have the potential to improve the circularity of composites and ensure their sustainability. Disulfide-based epoxy vitrimer matrices are under development to meet the specifications of key sectors (transport, wind energy, etc.). The manufacture of reinforced composites by different techniques (infusion, prepregs, etc.) has been validated by CIDETEC, which is leading this case study. The incorporation of new functionalities, such as fire resistance, and the validation of recycling are now on the table. In short, SURPASS targets the development of epoxy vitrimers that fulfil all railway FST requirements, while meeting the required mechanical performance and being intrinsically recyclable at end-of-life.
The third case study, focusing on the packaging sector, aims for the realization of multinanocomponent (MNL) films with good barrier, mechanical and optical properties in order to reduce material costs and improve the recyclability of the final product. MNL is a continuous process which allows in a single step to combine one or several materials in a film composed of up to several thousands of alternating layers in the nanometric range. Films produced by MNL can theoretically be indefinitely recycled through extensional compounding and reprocessing into a new MNL film.
This and the search for decontaminating agents for allowing upcycling are the object of this task.

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

EXPOSURE RISK ASSESSMENT CASE STUDY - ADVANCED (NANO)MATERIALS IN DIRECT CHILL CASTING LABORATORY AND INDUSTRIAL LINES

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The European Commission prioritized the advanced materials as a Key Enabling Technology to compete with the technological developments from other countries (USA, China). In this context, two initiatives detach from a broad range in the European ecosystem for research and innovation, i.e. the framework for Safe- and Sustainable-by-Design (SSbD) chemicals and materials, and the Advanced Materials 2030 Initiative (AMI2030). Both initiatives are based on an interdisciplinary cooperation between stakeholders to accelerate and widespread the impact of green and/or digital transitions. Transdisciplinary research and innovation teams are required to link the numerous knowledge fields and data need to be shared to enable the successful implementation of these initiatives.
The H2020 FLAMINGo project (GA nº 101007011) aims to develop a high efficient production advanced (nano)material and introduce them in the conventional metallurgical and forming industrial technologies for production of electric vehicle components. Different potential solutions of multimaterials with a nano-component are being combined with casting and extrusion processes to find a tailored solution to the specific performance profiles. The qualitative hazard assessment of the advanced (nano)materials selected, together with the exposure assessment to nanomaterials in the occupational environments, have been allowing to perform a risk assessment for the range of potential solutions considered. Further, the environmental impact has also been analyzed.
In this case study, the data from workers exposure monitorization to advanced (nano)materials performed throughout the activities of the direct chill casting process in laboratory and industrial lines are discussed. For the monitoring campaigns, a DISCmini 2.0 from Testo was used to measure the particle number concentration, the mean particle size diameter, and the lung deposited surface area. In the weighing and furnace activities of the direct chill casting process, an innovative aluminum metal-matrix composite (Al - 45 wt % TiC, in powder form constituted by coarse particles previously produced through high energy ball milling for encapsulation of the TiC nanoparticles in the aluminum matrix) was used together with an aluminum alloy to produce reinforced billets of aluminum metal-matrix composite (Al - 1 wt % TiC) in the direct chill casting and cutting activities. The relationship between the potential emission sources related with the manufacturing process and the background (nanomaterials emission from other sources than the case study process) was evaluated through a combined approach using temporal and spatial analysis for both laboratory and industrial lines.
The application of a criteria proposed by OECD [1] and CEN [2] to evaluate workers exposure to airborne advanced (nano)materials suggested that workers exposure seems to be significant in the weighing, furnace and direct chill casting activities of the laboratory line, and in the weighing and cutting activities of the industrial line. Although, samples should have been collected to characterize the materials detected by the DISCmini 2.0 monitoring equipment to possibly distinguish between natural, incidental and/or engineered nanomaterials. Finally, the safe and the environmental life cycle assessments from the innovative advanced (nano)material and processing solutions analyzed in the FLAMINGo project should be integrated as early as possible, to allow for the testing of the SSbD framework proposed by the Joint Research Centre.

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TOPIC 2
TOPIC 2. IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

THE ROLE OF NANOMATERIAL SURFACE CHEMISTRY IN SAFE-BY-DESIGN APPROACH

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There is now a general consensus that size, shape, chemical nature, and surface chemistry of nanomaterials (NMs) are major players defining their adverse effects in biological systems. For safer and functional engineered nanomaterials (ENMs) and nano-enabled products (NEPs), their safety should be carefully evaluated starting from the design stage. Thus, Safe-by-Design (SbD) concept in their applications has increasingly been considered. In this presentation, the SbD concept for NMs safety by emphasizing the NMs surface chemistry is presented and discussed. In the context of SbD4Nano project, a platform of NMs supplied by the industrial partners were evaluated through the surface chemistry to understand its role in the SbD effort. The evaluated NMs were graphene, carbon nanofibers (CNFs), SiO_2, and TiO_2. The core-shell NMs formed by combining SiO_2 and lipids, and TiO_2/SiO_2 NMs doped with sulphur (S) were also included in the platform. By using a range of small molecules with biological origin such as glycine, phenylalanine, glucose and maltose, the surfaces of the NMs in the platform were modified. First, they were evaluated for their cytotoxicity and genotoxicity. Then, they are tested for their ecotoxicity, dustiness and performance in their industrial applications. For example, Figure 1 shows the SEM, TEM images and the influence of surface chemistry of CNFs on three different cell lines in dose and time dependent manner. As can be seen from the SEM and TEM images, CNFs are very polydisperse with variety of sizes and shapes. It is almost impossible to control size and shape of these materials possibly to control their toxicity. However, when their surface chemistry is controlled, a significant reduction in their toxic behavior has been observed. A noteworthy reduction in their ecotoxicity and dustiness has also been found. Although some variation in the results is observed, the study suggests that surface chemistry could have a significant player in the SbD approach.

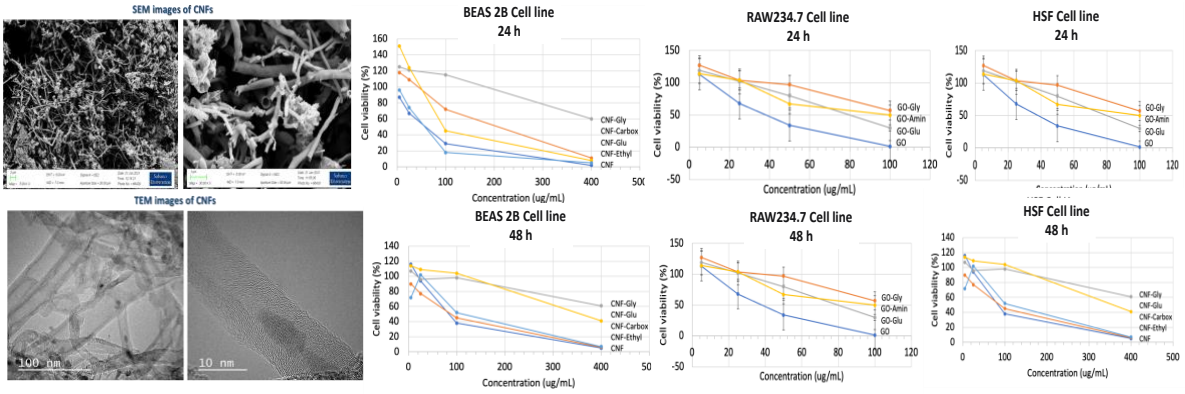

Figure 1. SEM, TEM and surface chemistry dependent cytotoxicity trends of CNFs.

Keywords: Safe-by-Design, surface chemistry, cytotoxicity, ecotoxicity

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

A MULTIMODAL APPROACH TO QUANTIFY SURFACE FUNCTIONAL GROUPS ON NANOMATERIALS FOR SAFE AND SUSTAINABLE BY DESIGN APPROACHES

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Engineered nanomaterials (NM) with their large surface-to-volume ratios and their for some materials observed size-dependent functional properties are of increasing relevance for current and future developments in various fields such as medical and pharmaceutical industry, computing and electronics or food and consumer products. The performance and safety of NM are determined by the sum of their intrinsic physicochemical properties.[1] Especially, the particle surface chemistry, which is largely controlled by the chemical nature and density of functional groups (FG) and ligands, is an important key driver for NM performance, stability, and processibility as well as the interaction of NM with the environment. Thus, methods for FG quantification can foster the sustainable development of functional and safe(r) NM.
Aiming at the development of simple, versatile, and multimodal tools for the quantification of many bioanalytically relevant FG and ligands, we investigated and compared various analytical methods commonly used for FG quantification (Figure 1).[2,3] This includes electrochemical titration methods, dye-based optical assays, and other instrumental analytical techniques such as nuclear magnetic resonance and thermal analysis methods.
The potential of our multimodal approach for FG quantification was demonstrated for commercial and custom-made polymeric and silica particles of varying FG, used as optical pH sensors.[4] In the future, our strategy can contribute to establish multi-method characterization strategies to provide a more detailed picture of the structure-properties relationship.

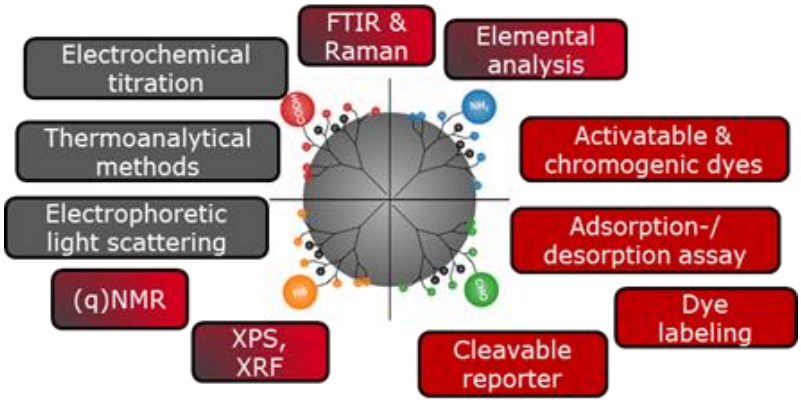

Figure 1. Overview of analytical methods commonly used to quantify the total number of FG (grey) and accessible number of FG (red) present on the NP surface.

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TOPIC N° 1. METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

SAFE-BY-DESIGN STRATEGIES FOR NANOFORMS AND NANO-ENABLED PRODUCTS TO BE INTEGRATED IN THE SABYNA GUIDANCE PLATFORM

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In the last years, the increasing use and production of nano-enabled products (NEPs) containing nanoforms (NFs) have raised concerns about the environmental and human safety of these innovative materials. For this reason, the Safe-By-Design (SbD) concept has been used by a broad spectrum of stakeholders, from manufacturers, researchers, and regulators, to support the development of methods and strategies to control and minimize the risks of nanomaterials at the early stage of product development and across the whole NEP life cycle. Amongst the EU-financed projects within H2020, the SAbyNA project (G.A. 862419) is developing an integrative user-friendly Guidance Platform that will support manufacturers in the decision process about the production of SbD NFs and NEPs along their life cycle.

To this goal, in the context of the SAbyNA project, a thorough analysis of state-of-the-art SbD strategies applied to NFs and NEPs considering scientific papers, projects deliverables, reports, and industrial know-how was conducted.

This information was then categorized using a structured framework following the European Chemicals Agency (ECHA) guidelines considering the connections of SbD strategies with i) the intended use of NF/NEP, ii) the technical functions of the NF incorporated in the NEP, iii) the physico-chemical properties that determine the technical function of the NF in the NEP, and iv) the physico-chemical properties of NF/NEP that determine the hazardous mode of action and/or release. In this context, the SAbyNA Guidance Platform will help the final user to find the most appropriate SbD strategies not only to minimize the risk but also to preserve the technical function of the NF and the NEP.

The SAbyNA Platform is going to be used to guide the identification and application of SbD approaches for NFs/NEPs representative of two important industrial sectors: 3D printing and paint sectors.

Acknowledgement

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

A LOCAL OPTIMIZATION APPROACH TO DISCOVER NOVEL SAFE-BY-DESIGN NANOMATERIALS

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Nanomaterial (NM) risk assessment is challenging because of the diversity in their physical and chemical composition, their surface functionalization and structural properties, and the variety of alternative configurations. Significant public and private investment over the past decades enabled the collection of nanotoxicity data, and the development of nanoinformatics tools for data management / processing and property prediction to enable in silico risk assessment of NMs [1].

Recently, we suggested new guidelines for computation-friendly nanomaterial representations [2]. Based on these guidelines we demonstrated proof-of-concept Nano-InChI [3] extensions that significantly streamline similarity assessments and enable more accurate calculations of nanomaterial affinities.

Based on the above representation proposal, we herein develop an optimization framework where known nanomaterials are used to design novel nanomaterials featuring an optimized safety profile. Descriptor information is taken from available datasets, linking composition, structure and experimental conditions information to the nanomaterial toxicity profile. A tree graph structure is used here to parse and encode the available data. Through this graph-encoding we calculate nanomaterial similarities, and develop and train a Bayesian analysis tool and models for toxicity predictions. A screening tool is built on top of these models, to guide a local search towards safer nanomaterial configurations.

A simplified version of the tool will be integrated into the NanoInformaTIX project platform [4], along with data sources and other models to enable risk assessment and property predictions, and to support the safer design of quality products.

Keywords: nanomaterial, toxicity, safe-by-design, Bayesian analysis, nanoinformatics

Funding
This work was funded via the EU H2020 project "NanoInformaTIX: Development and Implementation of a Sustainable Modelling Platform for NanoInformatics" (grant agreement No. 814426).

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TOPIC 1 - METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

ACCOMPLISHING SSBD AT PRODUCTION THROUGH THE SAFETY BY PROCESS CONTROL CONCEPT

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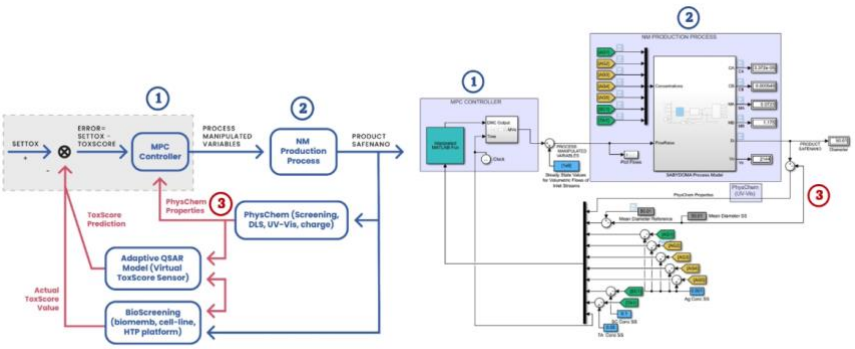
The increasing importance of nanomaterials (NMs) for a variety of applications, their rising production volumes and the need to comply with strict specifications for their functionality and safety, raise the need for higher effectiveness at the production stage. At the same time, requirements for safe and sustainable by design (SSbD) chemicals and materials include minimising the production of substances of concern and reducing waste. All the above goals emphasise the need for advanced control methodologies capable of simultaneously achieving continuous processes' targets for efficacy, functionality and safety. In the H2020 SABYDOMA project, we have introduced the *Safety by Process Control* (SbPC) concept, which provides a viable methodology to address this problem, by intervening at the point of production and guiding delicate NM production processes with multiple inputs/outputs and sets of constraints, to ensure maximum adherence to specifications and minimal human and environmental implications. The *SbPC* approach is based on the model predictive control (MPC) methodology adapted to the NM production problem, and combined use of experimental and virtual characterisation methods. Based on a dynamic predictive model of the process, an optimisation problem is solved periodically that provides the optimal process actions and operating conditions over a series of steps, but only the first one is implemented. A characterisation fusing scheme has been generated to overcome the challenging task of combining data from methods with multiple time frames: some physicochemical characterisation methods produce outputs every 30 sec, while detailed toxicological assays require more time. This fused scheme serves as a surrogate for methods with an impractical time frame.
The proposed control methodology has been tested on a series of plug flow reactors that synthesise Ag nanoparticles, where nucleation occurs in the first reactor and growth in the other reactors. It has been demonstrated that the proposed methodology produces effective control schemes for the NM production plant that overcome disturbances and achieve the desired set point values with speed and robustness. The method is easily transferable to other NM production processes through its generalised main concept and fine-tuning of the control scheme.



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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

SOCIO-ECONOMIC LIFE CYCLE-BASED FRAMEWORK FOR SAFE AND SUSTAINABLE BY DESIGN OF ADVANCED MATERIALS

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This study describes an innovative approach to socio-economic assessment of (advanced) engineered nanomaterials and nano-enabled products (NEPs) to support safe-and-sustainable-by-design (SSbD) decision making by industries in the early stages of product development. This semi-quantitative methodology is developed based on a sound conceptual framework grounded in the combination of social life cycle analysis and multi-criteria decision analysis methods and supports decision making based upon socio-economic impacts assessed over the full life cycle of a product. To facilitate its application by industries, the methodology was developed as an excel-based tool and is currently being transferred to a web-based self-assessment tool. This easy-to-use, cost- and time-efficient tool can guide users through their SSbD decision making regarding newly developed nanomaterials and NEPs and can also be applied to re-evaluate existing materials and NEPs in order to improve their sustainability from a socio-economic perspective. The relatively low requirements of this tool regarding the level of efforts and expert knowledge needed for its application make it a good starting point for initial assessment to highlight socio-economic issues in the value chain. As a stepping stone for a more holistic assessment, the S-LCA self-assessment tool is now being integrated as a mid-level (Tier 2) sustainability assessment in the H2020 SUNSHINE project e-infrastructure for SSbD of MCNMs. Currently, testing and refinement of the tool in real case studies is being conducted including but not limited to photocatalytic ZnO/Silica complexes used in scratch and abrasion-resistant coatings for the construction sector, core–shell silicon carbide (SiC)–titania (TiO_2) anti-stick coatings for use in consumer products and graphene oxide-based materials for electrodes and energy storage (batteries).

This work has been carried under the H2020 Gov4Nano and SUNSHINE projects.
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TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

AUTOMATED SAMPLING DEVICE TO STUDY THE DYNAMIC SOLUBILITY OF INORGANIC NANOMATERIALS

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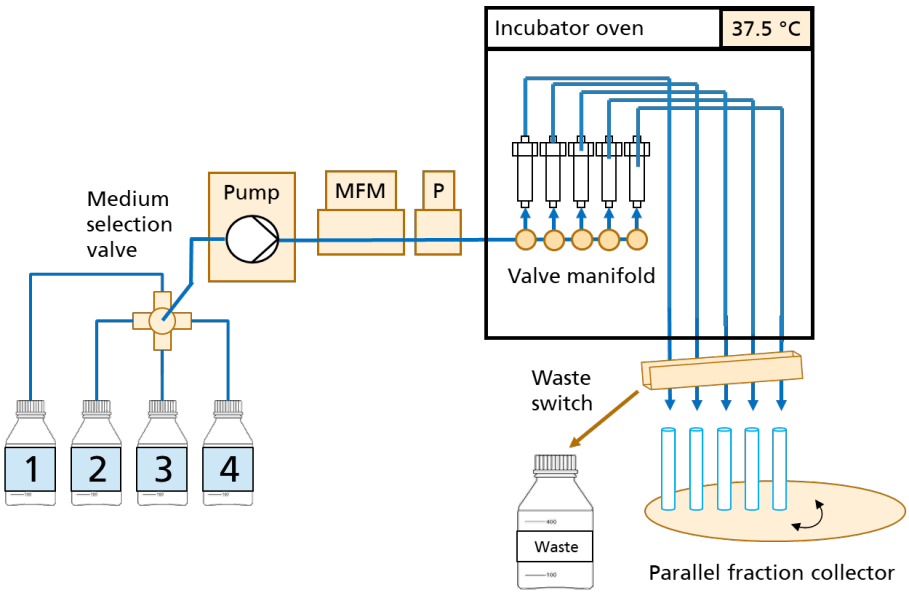
The investigation of the solubility of a material in water or aqueous media is one of the crucial tasks in the risk assessment of a chemical compound. The solubility of a material is depending mainly on the chemical identity, but also on other factors, e.g. on the size of the investigated structures. In solubility studies carried out under static conditions (following OECD Test Guideline 105: Water Solubility), the maximum solubility of such materials is determined. However, to better mimic real-life situations, it is of advantage to expose the material under investigation to a slow, but constant stream of fresh media. One example from the field of toxicology is the situation in the lung, where lung fluid is continuously produced and exchanged. Another example from the field of ecotoxicology is the investigation of the fate of a material dumped in running water. In such situations, the constant delivery of fresh medium affects the dissolution process and the solubility cannot be described adequately by an investigation under static conditions. To gain a more complete insight into the dissolution properties and to acquire more relevant material data for the assessment of the (eco-)toxicological properties of materials, new techniques and tools are required.
To fulfill this need, Fraunhofer IMM developed, tested and validated a fully automated sampling system for studies of the long-term dynamic solubility of powdered materials, i.e. inorganic (nano-)materials, in close collaboration with Grace GmbH. In the developed system, the material under investigation is loaded into a column and exposed to a stream of fluid, similar to an extraction experiment. Tests with different biologically relevant media were conducted (model fluids for human perspiration, lung fluid and gastro-intestinal system). By using a valve manifold, up to five columns can be processed in parallel, and with a parallelized fraction collector, the eluate fractions of all samples can be collected over the course of the experiment, i.e. for several hours or days. Afterwards, the eluate fractions can be analyzed with state-of-the-art offline techniques such as ICP-OES or ICP-MS. The system is a flexible and user-friendly tool to perform dynamic solubility studies for a wide range of materials and media, in an automated and reproducible fashion.
The system enables systematic studies on the dynamic solubility of materials and therefore allows to gain a clearer view on the fate of a material in biologically or environmentally relevant media.



TOPIC 6. CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

CATALYTICALLY GROUPING REACTIVITY TO FUNDAMENTALLY UNDERSTAND TOXICITY OF NANOMATERIALS

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Quantitative characterization of nanomaterials' surface reactivity is essential for grouping and read across in risk assessment; it may, in turn, enable more relevant dose metrics. We propose a catalytic approach for quantification and classification of reactive sites on nanomaterials' surface by methanol chemisorption and temperature-programmed surface reaction (TPSR) analyses, respectively. The latter can group nanomaterials according to their reactivity profile (acidic, basic, redox or combination thereof), while the former delivers the number of reactive sites at the nanomaterial' surface; the surface density of reactive sites can be calculated by combination of the number of reactive sites and the specific surface area values.

The number of active sites can be used to normalize reactivity values of probe reactions such as dithiothreitol (DTT) oxidation. This can be measured as its conversion, or normalized using a positive control: the Normalized Index of Oxidant Generation and toxicity (NIOG). *Per se,* these methods *do not allow quantitative comparison of reactivity between NMs*. Thus, we propose to normalize DDT conversion rate per active site number, as determined by methanol chemisorption*, i.e.*, to obtain an Oxidative Turnover Frequency (OxTOF), which would allow comparing the oxidative activity of materials in a quantitative manner

This acellular approach for generation of normalized reactivity data would enable a more meaningful grouping of engineered nanomaterials, reducing the need for cellular assays. The quantitative reactive characterization of surfaces may be a convenient and simple strategy to group and predict the risks of advanced nanomaterials as well as for safe-and-sustainable-by-design developments.

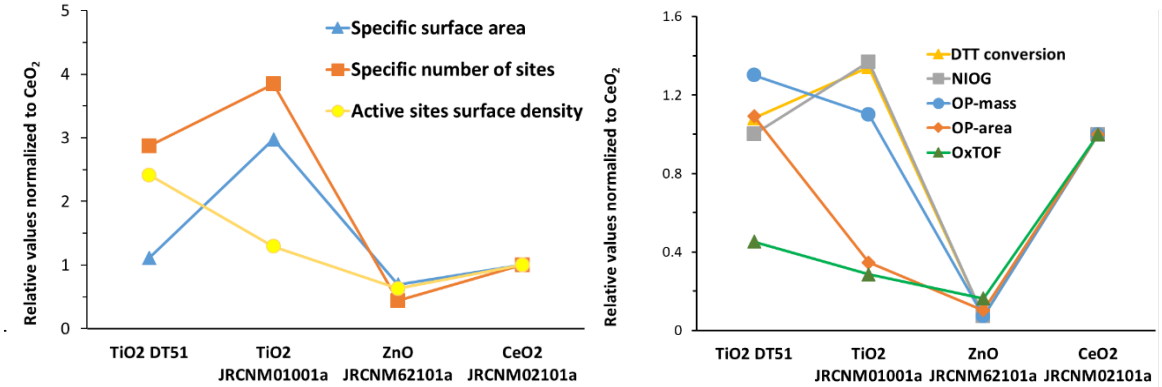

Figure. **Left**: Surface analysis: Specific surface area obtained by N_2 adsorption isotherm, Specific number of active sites obtained by methanol chemisorption, and Active sites surface density obtained by combination thereof (values normalized to CeO_2 JRCNM02101a). **Right:** Oxidative potential evaluated by DTT catalytic oxidation and expressed based on five different descriptors: conversion, normalised index of oxidant generation and toxicity, reaction rate per mass, reaction rate per surface unit, and oxidative turnover frequency (reaction rate per active site). The five oxidative potential descriptors normalized to CeO_2 JRCNM02101a.

European Commission h2020 project NanoInformaTIX (GA 81446). JRC reference materials were supplied by Dr. Juan Riego at JRC-Ispra

TOPIC 6. CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

COMBINATION OF ZNO-CISPLATIN NPS AND NITRIC OXIDE FOR CHEMOTHERAPEUTIC EFFICACY IMPROVEMENT

Joana Claudio PIERETTI[1]; Giovana Marchini ARMENTANO[1]; Bruna Moreira FREIRE[1]; Bianca de Melo SANTANA[1]; Bruno Lemos BATISTA[1]; Marcela Sorelli CARNEIRO-RAMOS[1]; Amedea Barozzi SEABRA[1]

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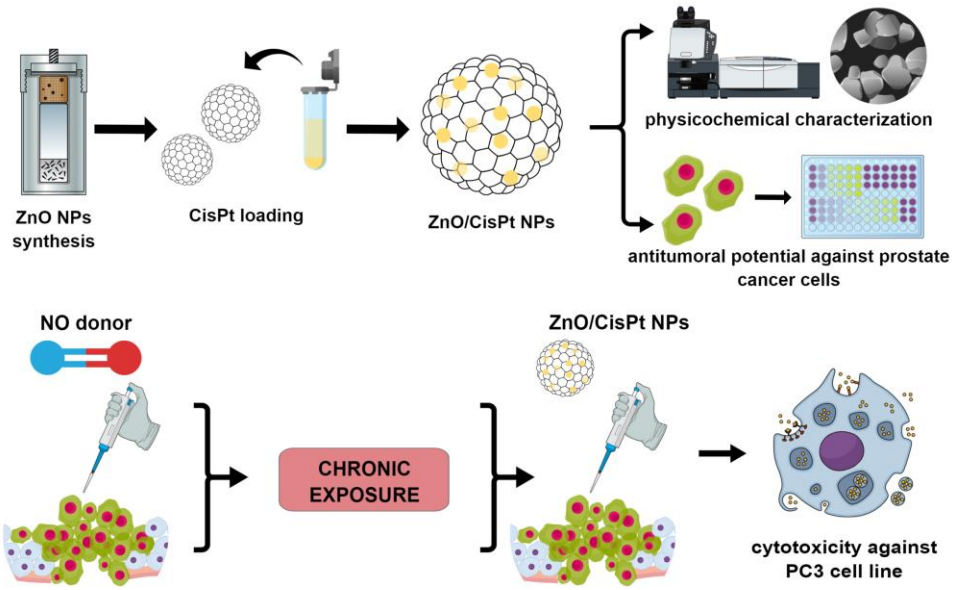
Cancer care is one of the main global health challenges, in which main difficulties are related to late diagnosis and inefficient therapy. Common chemotherapeutics still demonstrate low efficacy and/or targetability, promotion of undesirable side effects, besides being impacted by drug resistance mechanisms. Therefore, novel strategies employing nanoparticulated systems and co-therapies have been developed. Nanoparticulated systems can delivery several drug classes, promoting a targeted treatment combined with a sustained drug release, along with unique mechanisms that may corroborate to overcoming resistance to commonly used drugs. Porous nanoparticles (NPs), such as zinc oxide (ZnO NPs), can be synthesized and carried with specific drugs, being an excellent candidate for carrying chemotherapeutics and improving their biodistribution and specificity. Hence, we developed cisplatin (CisPt)-loaded ZnO NPs, which demonstrated a targeted CisPt releasing in tumour microenvironment conditions and higher cytotoxicity against prostate cancer cells (PC3) when compared to free CisPt.
To improve CisPt-ZnO NPs, the nanomaterial was combined with a chronic exposure to nitric oxide (NO), in non-cytotoxic concentrations. NO is an endogenous biological signalling molecule that plays key roles in physiological and pathological pathways. In cancer, NO may promote either cell proliferation and infiltration, when present at low concentrations (pM-nM), or it may sensitize or directly kill cancer cells, at high concentrations (µM-mM). Our results showed that PC3 cells chronically exposed to NO were more susceptible to ZnO/CisPt NPs treatment, indicated 30 % increase in the reduction of viable cells, compared to cells that were not treated with NO. Moreover, the chronic exposure to NO enhanced the selectivity index (SI), calculated by the IC50 ratio of PC3 and normal human fibroblast cells (FN-1). We hypothesize that S-nitrosylation and transnitrosylation pathways are key pathways for sensitizing cancer cells, promoting an improved potential of nanoparticulated formulations.



TOPIC N° 3 – Characterization of Advanced Materials, Including Nano Materials

Synthesis and Characterisation of a Graphene Oxide-Gold Nanohybrid for Use as Test Material

Taiwo Hassan Akere [1,2], Aline M. Z. de Medeiros [3,4], Diego Stéfani T. Martinez [3,4], Bashiru Ibrahim [1,2], Hanene Ali-Boucetta [2] and Eugenia Valsami-Jones [1*]

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This paper reports the synthesis and characterization of a graphene oxide–gold nanohybrid (GO-Au) and evaluates its suitability as a test material, e.g., in nano(eco)toxicological studies. In this study, we synthesised graphene oxide (GO) and used it as a substrate for the growth of nano-Au decorations, via the chemical reduction of gold (III) using sodium citrate. The GO-Au nanohybrid synthesis was successful, producing AuNPs (~17.09 ± 4.6 nm) that were homogenously distributed on the GO sheets. They exhibited reproducible characteristics when characterised using UV-Vis, TGA, TEM, FTIR, AFM, XPS and Raman spectroscopy. The nanohybrid also showed good stability in different environmental media and its physicochemical characteristics did not deteriorate over a period of months. The amount of Au in each of the GO-Au nanohybrid samples was highly comparable, suggesting a potential for use as chemical label. The outcome of this research represents a crucial step forward in the development of a standard protocol for the synthesis of GO-Au nanohybrids. It also paves the way towards a better understanding of the nanotoxicity of GO-Au nanohybrid in biological and environmental systems.

TOPIC N° 6. CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

CHARACTERIZING ENVIRONMENTAL BEHAVIOR OF NANOMATERIALS USING RADIOLABELING APPROACHES

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In order to characterize the potential hazards of anthropogenic nanomaterials to humanity and the environment, as well as the successful implementation of SSbD approaches, it is imperative to have access to analytic tools that provide sensitive detection at low concentrations in complex media such as surface and waste water, sewage sludge, soil, biota, etc. against same element and particle backgrounds. The radiolabeling of nanomaterials provides these features for laboratory studies. We present an overview of our radiolabelling efforts with examples of their applications.

We have developed a library of radiolabelling methods for the most common anthropogenic nanomaterials, including nanoplastics, that allow us to track nanomaterials in release, mobility and uptake studies including such complex systems as waste water treatment plants, plants, water organisms and soil. The labeling techniques are the synthesis of the nanoparticles using radioactive starting materials, the binding of the radiotracer to the nanoparticles, the activation of the nanoparticles using proton/neutron irradiation, the recoil labeling utilizing the recoil of a nuclear reaction to implant a radiotracer into the nanoparticle, and the in-diffusion of radiotracers into the nanoparticles at elevated temperatures. Using these methods we have produced [$^{105/110m}$Ag]Ag, [$^{124/125/131}$I]CNTs, [^{48}V]TiO_2, [$^{139/141}$Ce]CeO_2, [^{7}Be]MWCNT, [^{64}Cu]SiO_2, [^{64}Cu]PS, etc. for accurate quantification in complex media at an environmentally relevant low concentrations range even with a background of the same element and without complicated sample preparations necessary.

Using these approaches, we can go beyond mere quantification and gain mechanistic insights into nanomaterial behavior in the environment. For example, we have tracked the dissolution and internalization of CeO_2 NP in freshwater shrimp, the dissolution of CdSe/ZnS quantum dots in waste water treatment or the size-dependent uptake of TiO_2 in plants.

TOPIC N° 8 – MICRO AND NANOPLASTICS POLLUTION

ECOTOXICOLOGICAL ASSESSMENT OF BIOBASED AND BIODEGRADABLE ANTIFOULING PAINTS FOR MARINE APPLICATIONS (PROJECT - NAUTILUS)

Patricia Solorzano[1], Melissa Faria[1], Verónica Gonzalez[1], Elena Cerro-Gálvez[1], Rubén Martínez[1], Marinella Farré[2], Sandra Pérez[2], Marta Llorca[2], Socorro Vázquez[1]

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Abstract

Biofouling is a problematic phenomenon to human aquatic activity, especially marine, due to the damage it causes to both dynamic and static infrastructure leading to serious economic costs. However, more problematic are the amount of biodegradable resistant plastics being continuously released into the aquatic environment from multiple sources including antifouling paints. This is because modern antifouling paints consist of polymer matrix coatings containing various types of biocides, and therefore present a double impact on aquatic ecosystems, one as biocide release, and the other as a source of microplastics. As society has become stricter with the incorporation of environmentally safe formulations in compounds, and regulations are pushing towards the design of safe and sustainable products, developing safe antifouling paints is compulsory and the challenge can only be met with a close and balanced synergy between industry and scientists. The project NAUTILUS brings together industrial and academic partners compromised in the eco-design of new biodegradable antifouling paints towards minimizing their impact on the environment. Strategies include multi-monitoring of biodegradability capacity (compound life cycle) under laboratory (micro and mesocosms) and open sea scenarios, identification, and quantification of released and transformation products, and conducting multi-species ecotoxicity assessments.

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

DEVELOPMENT OF A SIMPLE 2D DERMAL TOXICITY MODEL FOR SCREENING NANOMATERIALS

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Driven by the updates to cosmetic regulation (Regulation (EC) No 1223/2009), dermal hazard assessment is now primarily assessed using validated *in vitro* test methods[1,2,3,4] rather than the *in vivo* tests used historically. However, these methods can still be expensive, particularly if screening hazard of numerous particles, as is typically done if following safe-by-design (SbD) principles. Furthermore, the available methods only assess short-term effects, which may not reflect some of the applications relevant to nanomaterials (NM) (e.g. in cosmetics), nor the relevant mode of toxicity of NM that can persist on the skin and in hair follicles for a longer period of time. Limited data is available for the assessment of NM with these validated methods, and any available data shows inconsistency in the dosing of 3D tissues to NM. Typically, NM are applied as a liquid dispersion of particles at concentrations up to 1 mg/mL. As these methods require only 30 µL of applied sample, very low masses will be deposited onto the tissues, which is far less than the 25 mg suggested in the guidance documents for dry powders.

With these considerations in mind, we aimed to develop a testing strategy using a simple 2D *in vitro* dermal toxicity model that is suitable for use in SbD of NM and has the capacity to widen the scope of current dermal hazard assessment methods. To do this, the widely used immortalized keratinocyte cell line HaCaT was utilised as it benefits from being easy to culture and has previously been used to screen chemicals for dermal irritation and sensitization[5,6,7]. We investigated the cytotoxic and inflammatory effects induced in HaCaT cells following exposure to a range of benchmark NM (SiO_2, TiO_2, ZnO, Ag, and CuO). Based on those indicated in regulatory accepted guidance methods, variations in the exposure and recovery times were included in our method development to determine the optimum conditions for screening NM.

We compared the results obtained in our simple 2D model with those achieved following the validated OECD method for dermal irritation (OECD TG 439) using a 3D reconstituted tissue model. These comparisons also included different methods of dosing the 3D tissues to allow comments to be made on current practices for nanomaterial dermal hazard assessment.

Acknowledgement
This work has received funding from the European Union's H2020 Research and Innovation programme under the Grant Agreement No 862419 SAbyNA project.

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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

USING EXISTING RESOURCES FOR SILICATE NANO MATERIALS ENVIRONMENTAL HAZARD ASSESSMENT - SPECIES SENSITIVITY DISTRIBUTION (SSD) MODELLING, IN THE FRAME OF SABYNA PROJECT

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Implementation of nanotechnology in new products is occurring at a rapid pace, contributing to new and improved products in many sectors including energy, health, safety, etc. Ensuring that these innovative nano-enabled products are safe and sustainable for human and environmental health has become a priority for the European Commission and a challenge for industries to comply with the recommended frameworks. In this sense, the SAbyNA project (H2020-NMBP-15-2020) was developed with the main aim to produce a Guidance Platform for the industries to eventually implement safe-by-design more efficiently and cost-effectively. Species sensitivity distributions (SSDs) are commonly used in ecotoxicology as an important tool to predict and classify hazards of substances and chemicals by estimating their ecological risk from existing data. SSDs use the toxicity data from several species to provide a probabilistic estimation of the toxicity threshold, ranking each species-derived threshold concentration and considering the 5th percentile of this distribution to be the safe threshold concentration (the HC_5). The SSD modeling was proposed to be used as stage 1 of the SAbyNA guide platform, as a categorizing system for environmental impact assessment of targeted nano forms (NFs). In this work SSD models for aquatic and terrestrial species of synthetic amorphous silicon dioxide (SiO_2) nanomaterials (SiO_2 NM) were developed as currently it is not provided by the European Chemical Agency. Search for data regarding SiO_2 NM environmental hazard assessment was limited when compared to other nanomaterials such as AgNM and TiO_2 NM, and yet more following specific refinement criteria such as testing >3 concentrations. Out of a total of 121 studies 17 and 3 for freshwater and soil, respectively, fit the established criteria for SSD modeling. A database of 161 toxicity data points (acute and chronic exposures) from 18 different species, 5 terrestrial (2 phylum) and 13 freshwater (for 7 phylum) species were attained. The extrapolated HC_5 value for freshwater species was 1.312mg/L, where the algae species *Chlorella sp*. was found to be the most sensitive while the least sensitive was the gammaproteobacteria *Vibro fisheri*. The HC_5 value obtained for soil species was 4.12mg/kg, with the red worm *Eisenia fetida* plotted as the most sensitive species and the mealworm *Tenebrio molitor* as the least.

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

EVALUATION OF CYTO-GENOTOXICITY IN BRONCHIAL EPITHELIAL CELLS EXPOSED TO FLG NANOFLAKES

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Numerous companies and research laboratories produce Graphene-based nanomaterials (GBNs). Thanks to the widespread diffusion of these materials in applications intended for the public (in clothes, in accessories and in sensors on the skin), the interaction with man is no longer limited to their production and handling, but also to their more or less intensive use. This makes it necessary to implement new study protocols for these materials in order to achieve a safe-by-design production.
Few-layer graphene (FLG) has attracted much interest due to applications in hydrogen storage and reinforced nanocomposites. As a result, these nanomaterials are in growing demand, increasing occupational exposure.
The genotoxicity of FLG materials is poorly investigated in *in vitro* models of the lung.
Here we evaluated the cyto-genotoxic and oxidative effects induced in vitro in the human bronchial epithelial cell line BEAS-2B exposed for 24 h to 2, 10 and 40 µg/ml of FLG nanoflakes in the appropriate complete culture medium (BEGM).
FLG nanoflakes used in this study were produced in a research lab using a liquid phase exfoliation (LPE) from pristine graphite by wet-jet mill. The flakes have an average lateral size of about 1.15 µm and thickness of 1.6 nm.
The FLG material in culture medium was dimensionally characterized (hydrodynamic diameter) by Dynamic Light Scattering (DLS) at the beginning of exposure (t0) and after 24 h (t24), to evaluate suspension stability.
Cell viability and apoptosis were evaluated by the Guava ViaCount cytofluorimetric assay, using the Guava EasyCyte Flow Cytometer.
To determine the membrane damage, the lactate dehydrogenase (LDH) assay was performed using a spectrophotometric microplate reader.
The genotoxic/oxidative DNA damage was assessed by Fpg (Formamido-pyrimidine DNA glycosylase) modified comet assay. Direct and oxidative DNA damage, comets and apoptotic cells percentage were evaluated.
Before proceeding to dilute FLG, we tested different media: complete culture medium, water, alcohol+BSA (Bovine Serum Albumine). The best turned out to be the BEGM: the hydrodynamic diameter is the smallest, indicating a better dispersion of the agglomerates/aggregates.
After 24 hours, all the agglomerates have precipitated resulting at DLS analysis smaller hydrodynamic diameters and it can be assumed that the powder is in contact with the cells at the bottom of the wells.
Regarding cytotoxicity, a slight decrease in cell viability at the highest concentrations and a slight increase in cell apoptosis and membrane damage only at the highest concentration were observed.
Finally, a slight increase in direct DNA damage, % of comets and apoptotic cells is observed at the highest concentrations; the same trend is observed for oxidative damage to DNA, but only at the highest concentration.
In summary, the FLG nanoflakes, under our experimental conditions, cause in BEAS-2B cells a slight dose-dependent cyto-genotoxic and oxidative effect, but statistically significant only at the highest concentrations.
Given the huge amount of new graphene-based products and the speed with which they are produced, the scientific community will have to be involved in advance in evaluating the potential negative effects on human health and the environment, even before these substances are placed on the market, in order to guarantee safe-by-design production.

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

POTENTIAL TOXICITY OF ZINC OXIDE NANOPARTICLES (ZNO NPS) IN SALINE ENVIRONMENT

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Zinc oxide nanoparticles (ZnO NPs) have antimicrobial and regenerative properties and are generally recognized as safe - GRA by the Food and Drug Administration - FDA. However, the assessment of the toxicity of NPs under strict safety parameters is necessary before extrapolating their use to the general population. Ecotoxicological studies are essential to evaluate the impact of NPs on the aquatic environment, mainly with representative biological organisms. The aim of this study was to evaluate the potential toxicity by the exposure of *Artemia franciscana's* cysts and nauplii to ZnO NPs with different sizes: 50 nm (Sigma Aldrich®), 100 nm (Sigma Aldrich®) and 118 nm synthesized by our research group. Inductively coupled plasma mass spectrometry (ICP-MS) was used to quantify the concentration of zinc (Zn) absorbed by cysts and nauplii of *A. franciscana,* from ZnO NPs of three different sizes. ICP-MS showed total zinc content of 93.6%; 72.7% and 62.4% for ZnO NPs of 118 nm, 100 nm, and 50 nm, respectively. Moreover, the exposure experiment revealed that the ZnO NPs (118 nm) have the lowest amount of ionic zinc release, compared to commercial ZnO NPs. Each size of NPs at 2 mg L^{-1} was able to release the following amounts of ionic zinc: 21.5%, 68.1%, and 83.5% considering the sizes of 118 nm, 100 nm, and 50 nm, respectively. Figure 1 shows the total zinc uptake by cysts and nauplii forms of *A. franciscana* incubated with ZnO NPs (118, 100 and 50 nm). *A. franciscana* can absorb Zn from ZnO NPs and, therefore, may be a suitable biological model for nanotoxicity assessments. In addition, cysts absorbed less zinc compared to nauplii, possibly due to the presence of a chorion that covers and protects the cystic form. Currently, studies to verify the assessment of the hatching rate and mortality of *Artemia* even as the locomotor activity essay for nauplii will be carried out for comparison.

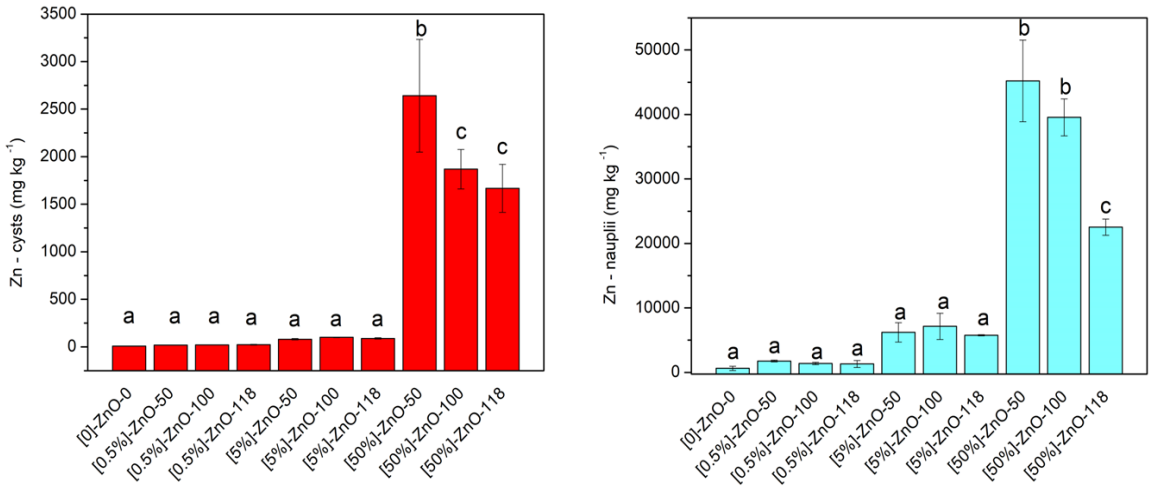

Figure 1: Total zinc uptake by cysts and nauplii of *A. franciscana* incubated with ZnO NPs (118, 100 and 50 nm) at different concentrations being 0.5% (2 mg/L), 5% (20 mg/L) and 50% (200 mg/L) for 56 h. Statistical differences are within the 95% confidence interval.

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

ECOTOXICOLOGY AS A TOOL FOR BIOMONITORING AQUATIC ENVIRONMENTS: STUDIES WITH ARTEMIA FRANCISCANA AND ZINC OXIDE NANOPARTICLES

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Ecotoxicological studies are essential to assess the impact of contamination in differents environments. The aim of this study was to evaluate the acute toxicity of an hydrothermal synthesized ZnO NPs (118 nm), at different concentrations (2 mg/L, 20 mg/L and 200 mg/L), in cysts and nauplii of *Artemia franciscana*. Artificial sea water (ASW) was used as blank. *A. franciscana* cysts were placed in flat-bottomed ELISA plate wells (n=10, in quintuplicate) with 200 µL of each treatment. To obtain free-swimming nauplii the cysts were hydrated in glass containers with 3% ASW solution with constant aeration, temperature of 28ºC, pH 8.0, and 12 hours light/dark cycle. Twenty-eight hours after initial hydration, nauplii were collected and immediately placed in flat-bottomed ELISA plate wells (n=5, in quintuplicate) with 200 µL of each tested suspension. Cysts hatching rate and nauplii mortality was assessed at the 28 and 56 h mark after exposition. Figure 1 shows the hatched and unhatched and highlights percentages of the umbrella stage at 28 h and 56 h endpoints. Importantly, for 2 mg/L and considering the percentage of individuals in the umbrella stage after 28 h (38% of the initial number of nauplii), a total of 21% of these developed (8% of the initial number of nauplii) while for 20 mg/L, the number in the umbrella stage after 28 h (22% of the initial amount of nauplii), a total of 18% of these developed (4% of the initial amount of nauplii) and for 200 mg/L, the number of umbrella stage after 28 h (34% of the initial amount of nauplii), a total of 12% of these developed (4% of the initial amount of nauplii). 21% (2 mg/L), 18% (20 mg/L) and 12% (200 mg/L) are values that indicate that the hatching percentage decreases as the concentration increases. Due to the fact that not all cysts managed to reach the subsequent stage of development as expected, we hypothesize that the action of ZnO NPs suspensions may be associated with a delay or total development impediment of the *A. franciscana* life cycle. The nauplii mortality essay only shows toxicity levels (32%) at 56 h in a 200 mg/L concentration. We conclude that Zn NPs at 2 mg/L, 20 mg/L and 200 mg/L concentration affect the hatching rate and survivability of *A. franciscana* nauplii, as well as reinforce the use this species as a viable model to investigate toxic effects of nanoparticles.

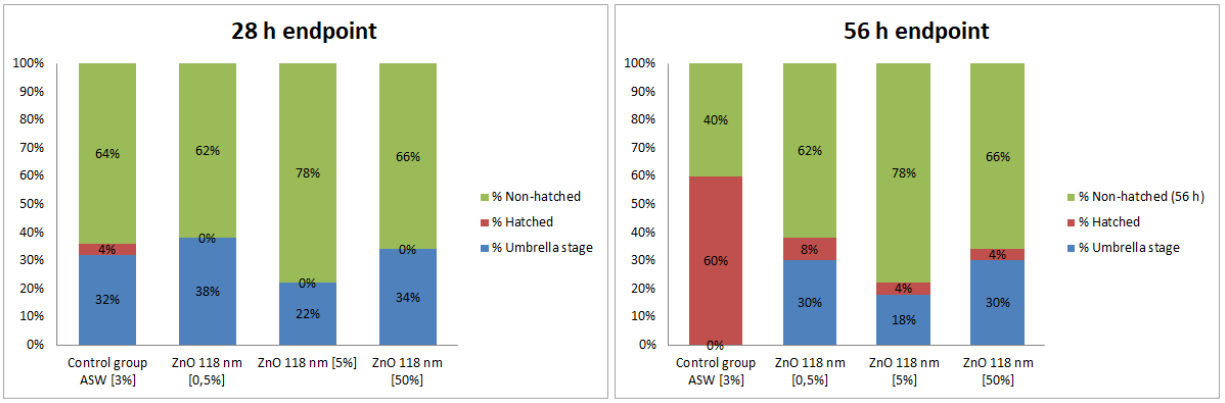

Figure 1: Hatching and non-hatching analyzes at 28 h and 56 h endpoint evince percentages of umbrella stage and complete hatched cysts exposed to ZnO NPs (118 nm) at different concentrations being 0.5% (2 mg/L), 5% (20 mg/L) and 50% (200 mg/L).

TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

ESTABLISHMENT OF A SIMPLE AND COST-EFFECTIVE *IN VITRO* MODEL FOR THE HAZARD ASSESSMENT OF NANOMATERIALS IN PULMONARY SYSTEM

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Fast, simple and high-throughput methods are highly needed to perform rapid screening of nanomaterials (NMs) toxicity. *In vitro* systems represent an efficient tool for it, allowing the classification and grouping according to their toxicity, especially in the context of Safe and Sustainable by Design (SSbD). Conventional submerged monoculture *in vitro* models have been widely used to study the toxic effects of NMs, however, these models do not reflect the complexity of tissue. Co-culture models move closer to this complexity and as such warrant investigation. In this work, we compared the sensitivity and reliability of monotype cultures (Calu3 cell line and THP-1 differentiated macrophages, separately) with co-cultures combining these two cell models, in exposure to SiO_2 (NM203), Ag (NM300K) and ZnO (NM110) nanoparticles (NPs). Monotype cultures and co-cultures were exposed to 25, 50, 100 µg/mL of SiO_2 particles, 2.5, 5, 25 and 50 µg/mL of Ag particles, 1.25, 2.5, 12.5 and 25 µg/mL of ZnO particles and to 25, 50, 100 µg/mL of LPS for 24 hours, and after that, inflammatory responses (TNFα, IL-6 and IL-8) were assessed. Then, monotypic cell cultures and co-cultures were compared to identify the most suitable *in vitro* model for screening studies. The two models showed different outcomes in exposure to the four NPs tested. For instance, based on the inflammatory responses in monocultures and co-cultures exposed to NM203, only a slight increase in the production of the pro-inflammatory cytokine TNFα was induced in monotype Calu3 and THP-1 cultures, whereas the co-culture was more sensitive to the exposure of SiO_2 NPs. In contrast, IL-6 levels increased in Calu3 and THP-1 monocultures and IL-8 increased in THP-1 monotype culture exposed to SiO_2 NPs. The obtained results show significative differences in monoculture and coculture. This highlights the challenge of establishing an ideal *in vitro* model for the hazard assessment of nanomaterials in pulmonary system. This work has received funding from the European Union's H2020 Research and Innovation programme under the Grant Agreement No 862419 SAbyNA project.

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

Multiple endpoint approach to asses toxicity of ENMs and their components in nano-enabled products

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The exposure of engineered nanomaterials (ENMs) to soil organisms can occur through anthropogenic activities such as the application of sewage sludge to agricultural soils. Development of nano-enabled products (NEPs) using a safe-by-design approach requires the consideration of the ecotoxicological effects of the ENMs used in an NEP across the product life cycle, from manufacture to end of life, while retaining the overall design and functionality of the product.
In this case study, we consider silver (Ag) ENMs that are coated on textiles, designed to provide an anti-bacterial function, developed as part of a safe-by-design approach for NEPs. These Ag ENMs are synthesised with two different chelating agents, quaternized hydroxyethyl cellulose and curcumin. We consider the toxicity of both the Ag ENM and the constituent chelating agents to the *in vivo* model nematode *Caenorhabditis elegans.* Exposures were performed in simulated soil porewater and growth and reproduction were measured as sensitive non-lethal adverse outcomes of toxicity.
To further explore toxicity at specific ECs, we measure molecular-level events and compare the underlying Ag ENM toxicity to that of the chelating agents. Accumulation of ROS in an organism can lead to oxidative stress and in turn causing DNA damage that instigates apoptosis. We measure reactive oxygen species (ROS) production and downstream damage and repair responses across the juvenile to adult exposure period. endpoints to obtain more information about molecular level events underlying toxicity. Using multiple toxicity endpoints through organism life stages across ENM components will better inform safe-by-design approaches.

TOPIC 1 - METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

ADAPTED METHODS AND WORKFLOW FOR NANOMATERIALS RISK ASSESSMENT IN AQUATIC ENVIRONMENT

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Owning to their unique qualities and the almost infinite tailoring possibilities, engineered nanomaterials (ENMs) have been exponentially explored for many applications and the medium-term forecasts predict that ENMs manufacturing will constantly grow during the next decades. Consequently, the volume and variety of ENMs released into the environment during manufacture, transport, use and disposal will increase accordingly. However, so far, experimental environmental risk assessment studies have lacked behind the studies on applications or human toxicology risks. Two of the major bottlenecks in the study of the impact of nanomaterials in the environment are: i) lack of fit-for-purpose analytical techniques for their identification and quantification in real matrix and ii) lack of fit-for-purpose ecotoxicity tests that take into account the specific nature of the ENMs such as specific surface or colloidal stability, which highly differentiate them from bulky materials and chemicals. In this work we will show our recent developments in econanosafety related to:
- Development of analytical technologies for the identification and quantification of the presence of ENMs in water environment and organisms (biota); lab-bench technologies were tailored using state-of-the-art equipment currently being used mainly for nanomaterials applications such as scanning electon microscopy (SEM), nanoparticle tracking analysis (NTA) and lab-on-a-chip systems were developed for *in situ* monitoring based on microfluidics, surface enhanced Raman scattering and electrochemical sensors;
- Development of new tests and models for environmental risk assessment; an ecotoxicity test was be developed based on the OECD TG 236 for the acute toxicity testing of nanomaterials based on sub-lethal endpoints register, ensuring reproducibility and stability of the tested nanomaterials. Additionally, an adapted test for bioaccumulation of nanomaterials was developed and used with marine mussels as models of great importance in the marine ecosystem, food webs and food safety due to their relevance in aquaculture for human consumption.

Acknowledgements

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TOPIC 1 - METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

ZEBRAFISH EMBRYOTOXICITY AND MARINE MUSSELS BIOACCUMULATION REDUCTION ASSESSMENT OF MODIFIED CARBON NANOFIBERS

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Carbon nanofibers (CNFs) have many interesting applications in the chemical industry, material science, and energy storage fields [1]. However, these nanomaterials have raised increased concern due to their high specific surface area that could confere them high interaction potential with organisms or cells. Aquatic environment is one of the sinks for nanomaterials included in commercial products at their end-of-life.
In this work, we studied pristine and surface modified CNFs behaviour in fresh and marine waters. Also, their toxicity and bioaccumulation were assessed with the main aim of understanding the environmental impact of those modifications on the NMs.
Initially, CNFs had to be dispersed in aqueous solution with the help of a stabilizer, humic acid. Then, the former and CNFs modified with glucose or glycine were dispersed in artificial fresh and marine waters. Their stability and kinetics were studied during the maximum test period for the zebrafish embryotoxicity and bioaccumulation assays. Then, zebrafish embrytoxicity tests were conducted using a modified version of the OECD TG 236 that includes quantification of NMs during the assay and sub-lethal endpoints. Finally, bioaccumulation assays by feed exposure using marine mussels in a modification of the OECD TG 305 were carried out with the NMs.
According to the results, the formulation of the CNFs has a significant impact in their stability in the media, zebrafish embryotoxicity and bioaccumulation. Despite of the fact that none of the materials exerted acute lethal toxicity to the zebrafish embryos, CNFs dispersed with humic acid triggered some sublethal toxicity in particular, related to the neuromotor system (spontaneous movements). While surface modification with glycine completed eliminated this sub-lethal effect, the modification with glucose seems to add cardiotoxicity. On the other hand, CNFs sugest no accumulation in mussel tissues.
The present study shows how surface modification can help to overcome some of the possible hazards related to the release of NMs such as CNFs into the aquatic environment.

Acknowledgements

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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

INVESTIGATING ZEBRAFISH (*Danio rerio*) EMBRYOS DEVELOPMENT AND LARVAE BEHAVIOR TO ASSESS THE HAZARD OF ANTIMICROBIAL CuO NANOPARTICLES

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Nanoparticles (NPs) and nano-enabled products (NEPs) emerged as novel antimicrobial agents with proven efficacy against antimicrobial resistant (AMR) bacteria, which can be found in water bodies associated to fish and animal farming [1]. Nevertheless, in line with the concept of Safe and Sustainable by Design, their safety and sustainability must be evaluated at an early phase of the innovation process [2]. In fact, the increase in nanomaterials (NMs) manufacture and use may lead to inappropriate disposal into the aquatic environment, representing a potential risk to non-target species [3]. In this context, the EU project AMROCE aims at reducing the spread of AMR bacteria in the aquatic environments through a platform of novel nano-antimicrobial products. Among the several NMs with antimicrobial properties, CuO NPs have been extensively used as bactericidal agent [4], yet their potential toxicity to cells and organisms is recognized [3]. In order to contribute to a safe development of the new nano-biocidals, this work aims at evaluating the nanosafety of CuO-based NMs in exploitation scenarios by using zebrafish (*D. rerio*), a promising model organism for high-throughput developmental and behavioral screening [4].
In this study, sonochemically synthesised water-based CuO NPs (wCuO) in the framework of the ERA-NET project AMROCE were investigated, in comparison with sonochemical Zn-doped CuO (ZnCuO) NPs, already developed under the EU-H2020 project PROTECT for coating water depuration membranes. This comparison was conducted to investigate any differences in response based on the NPs physico-chemical structures and identify CuO-induced adverse outcomes, to be used to predict the hazards from antimicrobial NEPs that will be further developed.
NPs suspensions were characterized by TEM, DLS and ICP-OES, while the aquatic toxicity potential was assessed by the Fish Embryo acute Toxicity (FET) test (OECD n. 236). Zebrafish embryos were exposed to NPs at increasing concentrations (0.1, 1, 10, 100 mg/L) for 96 hours and were screened every 24 hours for lethal and sub-lethal endpoints. Acute toxicity was analyzed by calculating the LC50 and EC50. No significant lethal effects were found. The morphometric analyses revealed significant differences in the NPs-treated embryos' length, eyes size and distance and yolk area. A complete lack of hatching was already evident at the lower concentrations for wCuO, while this effect decreased in ZnCuO-treated embryos, as testified by the higher effect concentrations, suggesting that the modulation of the NPs physico-chemical structure, including metal doping, may contribute to the safety profile of such effective antimicrobial NPs. According to these results, in addition to the behavioral assessment in zebrafish larvae at the end of the FET test through the EthoVision XT Software (Noldus Information Technology©), the spontaneous tail coiling is also being analysed in pre-hatching embryos, by the use of the DanioScope Software (Noldus Information Technology©). This will allow the definition of additional hazard response functions for sublethal effects, including hatching efficiency and neurological dysfunctions, at low exposure concentrations to be implemented in the Life Cycle Assessment studies performed in parallel.

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TOPIC 1 - METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

ZEBRAFISH EMBRYOTOXICITY AND MARINE MUSSELS BIOACCUMULATION REDUCTION ASSESSMENT OF MODIFIED GRAPHENE OXIDES

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Due to their interesting properties graphene oxides are currently used in numerous applications to improve final products specifications such as strenght, conductivity, antibacterial effect or adsorption properties among others [1-3]. However, these nanomaterials have raised increased concern due to their high specific surface area that could confere them high interaction potential with organisms or cells. Aquatic environment is one of the sinks for nanomaterials included in commercial products at their end-of-life.
In this work, we studied graphene oxides in different forms as well as surface modified graphene oxides behaviour in fresh and marine waters. Additionally, their toxicity and bioaccumulation were studied with the main aim of understanding the environmental impact as a consequence of those modifications in the NMs..
Graphene oxides in powder, dispersed in aqueous solution and modified with glucose or ethylenediamine were dispersed in artificial fresh and marine waters. Their stability and kinetics were studied during the maximum test period for the zebrafish embryotoxicity and bioaccumulation assays. Then, zebrafish embrytoxicity tests were conducted using a modified version of the OECD TG 236 that includes quantification of NMs during the assay and sub-lethal endpoints. Finally, bioaccumulation assays by feed exposure using marine mussels in a modification of the OECD TG 305 were carried out with the NMs.
According to the results, the formulation of the graphene oxide has a significant impact in their stability in the media, zebrafish embryotoxicity and bioaccumulation. Despite of the fact that none of the materials exerted acute lethal toxicity to the zebrafish embryos, graphene oxide in powder triggered some sublethal toxicity in particular, related to the lipid metabolism. Both surface modifications with glucose and ethylenediamine reduced or completed eliminated these sub-lethal effects. On the other hand, graphene oxide powder that showed significant accumulation in the mussels (up to 366 µg/g) while graphene oxide dispersed in liquid did not.
The present study shows how surface modification can help to overcome some of the possible hazards related to the release of NMs such as graphene oxides into the aquatic environment.

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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

EVALUATION OF THE BIOLOGICAL EFFECTS OF METAL NANOPARTICLES IN AN *IN VITRO* LUNG SYSTEM AT DOSES REPRESENTATIVE OF ENVIRONMENTAL CONCENTRATIONS

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This work is part of the H2020 European project ASINA which aims to promote Safe and Sustainable-by-Design (SSbD) solutions of new nanomaterials (NMs) across all life cycle stages. The implementation of SSbD novel approaches for new NMs is of pivotal importance in the framework of the European Commission's Green Deal. Nowadays, NMs are used in a wide variety of commercial products, such as creams, facemasks, protective clothing, and biomedical devices[1]. Here we report a novel framework to identify and assess the hazard of the new silver (Ag) and titania (TiO_2) based NMs designed with different coatings according to a SSbD approach, and considering the potential human exposure during NMs production and use. Ag and TiO_2 nanoparticles (NPs) are heavily used due to the antimicrobial properties of the first and the photocatalytic activity of the latter. In order to assess the potential hazard of these NPs it was defined, as preliminary information, the most likely internal dose of a person exposed during the production of Ag and TiO_2 nano-enabled products starting from monitoring campaign in a manufacturing site[2]. The selection of relevant doses of exposure to assess the hazard is becoming increasingly[3] important in the risk assessment framework. Starting from the monitoring campaign data and applying the MPPD model to determine the lung retained dose of NPs, it was possible to estimate the doses representative of a chronic human exposure. The average alveolar retention dose was calculated considering a working week of 8 hours for 5 days and the exposure doses used in this study (in the order of ng/cm^2) are representative of a chronic exposure of 1 month, 6 month and a year. As *in vitro* model, we used a human cell contact co-cultures (A549 and THP-1 cells differentiated in macrophages) representative of the alveolar space, cultured and exposed at the air-liquid-interface (ALI). Exposure to aerosolized NPs was performed by the Vitrocell® Cloud Alpha 12 system. This approach allows for more reliable results than by submerged culture systems due to a closer replication of the human physiology[4]. Before exposing the cells to the doses, we determined the deposition efficiency of each NP by using the quartz crystal microbalance available with the Vitrocell® Cloud system. Our results show that different nanoparticles have a different deposition efficiency depending on their characteristic and that this step is critical for properly define the concentration of NPs to be nebulized in order to obtain the final dose of exposure. The preliminary biological evaluation was done relying on an Adverse Outcome Pathways (AOPs)-oriented strategy in order to connect NPs physico-chemical (p-chem) properties and the mechanistic aspects of their biological reactivity to the potential health effects in humans[5]. The p-chem properties of the NPs were evaluated by TEM and DLS and the effects on cell viability and cytokine release were evaluated 24 hours after exposure. The preliminary results suggest the lack of hazard for the doses representative of a chronic inhalation exposure and confirm the safety of the NPs developed in the project.

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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

THE EFFECT OF SERUM-SUPPLEMENTED MEDIUM ON THE QUALITY OF NANOMATERIAL SUSPENSIONS AND OUTCOMES OF THE *IN VITRO* MICRONUCLEUS ASSAY

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The micronucleus (MN) assay is a validated method that allows assessing the genotoxicity of nanomaterials. As micronuclei formation requires cells to divide, cells are grown in a serum-supplemented medium. However, serum can compromise the stability of the nanomaterial (NM) suspension. In the present work, we analyse the effect of serum on the outcomes of the MN assay using three well-known NMs: Ag-NP, SiO_2-NP, and ZnO-NP.

First, a stock suspension of each material was prepared in distilled water by sonication, until the agglomerate size was reduced approximately by half. Then, the NM was suspended in either serum-supplemented or serum-free medium, and the tested concentrations were prepared by serial dilutions. The stability of the suspension was determined by dynamic light scattering (DLS), evaluating the particle size and quality of the suspensions, before and after the treatment. Human B lymphoblastoid TK6 cells were exposed for 21 h (1.5 cell cycles) to five concentrations of each reference NM. After treatment, cells were allowed to recover for 21 h (1.5 cell cycles) in the presence of serum, after which the induction of MN was analysed by flow cytometry. Each experiment also included cell-free wells to evaluate potential interferences with the NMs when using flow cytometer.

The supplementation of the medium with serum led to a decrease in the quality of the material suspension. Serum seemed to cause agglomeration of the NMs, causing differences in polydispersity between the serum-supplemented and serum-free conditions. This was observed especially after the 21 h treatment period. On the other hand, the absence of serum affected the sensitivity of the cells. According to the OECD genotoxicity test guidelines, the concentrations to be tested should not exceed 55±5% cytotoxicity. Higher toxicity of the material suspension without serum was evidenced in all the reference materials, especially in the case of Ag-NP and ZnO-NP. Hence, the range of concentrations used in the MN assay was lower in the serum-free cultures compared with that used with the serum-supplemented cultures. Both Ag and ZnO showed a significant increase of the frequency of MN in higher concentrations. Only SiO_2 showed slightly lower increase.

In conclusion, the use of serum-supplemented medium during treatment with NMs must be evaluated depending on the aim of the research. When the minimum differences in the polydispersity of the nanomaterial suspension is essential during the treatment period, exposing the cells in the absence of serum might be an option, but it should be always accomplished by a thorough evaluation of the genotoxicity results.

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Keywords – Human B lymphoblastoid TK6 cells, Ag-NP, SiO_2-NP, ZnO-NP, Genotoxicity, Serum-supplemented medium

TOPIC 1. METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

DEVELOPMENT OF AN APPROACHE TO CHARACTERIZE NANOPARTICLES TOXICITY IN THE ENVIRONMENT THROUGH TOXICOKINETICS AND TOXICODYNAMICS ANALYSIS IN BIOFILMS OF PSEUDOMONAS PUTIDA

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Due to their exceptional characteristics, nanomaterials are applied in multiple fields, including automotive, textiles or biomedical sectors, being Nanotechnology conceived as a Key Enabling Technology (KET). For this reason, the risks to health and environment posed by the special particularities of nanomaterial enabled products are critical to identify and control their associated hazards. Environmental research has focussed on elucidating and modelling of toxicokinetics and toxicodynamics of different types of nanomaterials in soil and aquatic organisms. In this regard, biofilms are considered as good bioindicators of contamination due to their ability to accumulate and react quickly to xenobiotics. Despite this, currently there is not any OECD validated protocol to study toxicokinetics and toxicodynamics of xenobiotics, and there is only one available study in the current literature where both parameters are analysed in these structures (Chaumet *et al*., 2019)

The aim of this work was to set up the appropriate conditions to develop a protocol to study Multi-Component NanoMaterials (MCNMs) and High Aspect Ratio Nanoparticles (HARNs) tokicokinetics and toxicodynamics in biofilms. *Pseudomonas putida*, a Gram-negative bacterium that can be found in soils being involved in several relevant functions, was selected as model organism. Different conditions of temperature, surface material, incubation time etc. were tested in order to obtain significant biofilm biomass to perform toxicokinetic studies. Results showed that the incubation of bacterial cultures during 96 hours at 37°C, in polystyrene well plates coated with a polyelectrolyte lead to the highest amount of biofilm. For toxicodynamics studies, ROS production was selected as a parameter to be analysed using the CM-H2DCFDA dye. The appropriate experimental conditions were first set up with planktonic bacteria exposed to H_2O_2, and then applied in biofilms.

This study has received funding from the European Union's Horizon 2020 program (DIAGONAL, Grant Agreement no 953152). The DIAGONAL project aims to address existing gaps at risk assessment, risk management and risk governance levels providing new knowledge on multicomponent nanomaterials and high aspect ratio nanomaterials.

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TOPIC 1 - METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

ZEBRAFISH EMBRYOTOXICITY ASSESSMENT OF FLUORESCENT SiO_2 NPs

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Nowadays, Silica nanoparticles (SiO_2 NPs) have been extensively used in different fields, such as biotechnology. Its porous structure, high surface area, easy functionalization and biocompatibility are all great features that make this type of NPs very atractive for biomedical (diagnostic and clinical application), agriculture and environment applications [1,2].
In this work, different types of SiO_2 NPs functionalized with fluorescent probes were analyzed: fluorescent Core-shell SiO_2 NPs (from ACZON, NFg530-NH_2) and 50 nm Red Fluorescent SiO_2 NPs (from HiQ-Nano, non-modified and modified with -COOH). The ecotoxicity of these NPs were assessed by zebrafish embryo toxicity (ZET) test, using a modified version of the OECD TG 236 that includes quantification of NPs during the assay and sub-lethal endpoints. Additionally, the NPs were previously dispersed in artificial freshwater and characterized in terms of fluorescence and size, to test NPs stability in the media.
Fluorescent Core-shell SiO_2 NPs did not induce any lethal toxicity to the zebrafish embryos. However, some sub-lethal effects related with neuromuscular coordination were triggered, namely in spontaneous movements and hatching rate.
Neither 50 nm Red Fluorescent SiO_2 NPs exerted any lethal effect. However, both non-modified and –COOH modified NPs induced a sub-lethal effect in neuro-motor coordination altering the rate of spontaneous movements. Interestingly, only non-modified NPs altered free swimming of larvae. In this case, NPs modification with –COOH groups seem to decrease the toxicity of the SiO_2 NPs, which could be related to their modified surface charge that will affect the interaction between the embryo and the NPs.

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TOPIC 1 - METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

DEVELOPMENT OF A NOVEL ZEBRAFISH-ON-A-CHIP DEVICE FOR FLOW-THROUGH MONITORING OF NANOPARTICLES TOXICITY

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Abstract:

Engineered nanoparticles (NPs) are commercially produced materials, being used in a variety of sectors, and their use is increasing. Human exposure to NPs is inevitable. Because of their small size, NPs find their way easily to enter the human body and cross the various biological barriers and may reach the most sensitive organs [1]. Toxicological studies provide a base for the protection of both human and environment. An established model organism for toxicology is Zebrafish (*Danio rerio)*. The advantages of embryonic and larval stages of zebrafish include body transparency, small size, low cost of cultivation and high genetic homology with humans [2]. However, the manual experimental methods used for handling and investigating this organism are limited due to their low throughput, labor intensiveness and inaccuracy in delivering external stimuli to the zebrafish while quantifying various neuronal and behavioral responses. Microfluidic and lab-on-a-chip devices have emerged to overcome these technical challenges, providing new platforms that can sort, track and investigate animals in their different developmental stages in a reliable and more automatic manner [3]. In this work, we developed a zebrafish-on-a-chip system for *in situ* assessment of nanoparticles effects. The device will allow a continuous circulation of the NPs solution to understand the impact of the constant presence of the NPs in suspension. The microfluidic device was designed by Autocad and made up of poly(dimethylsiloxane) (PDMS) and Poly(methyl methacrylate) (PMMA). The design of the chip and the flow conditions were characterized and optimized in order to achieve a stable environmentally relevant concentration of NPs in the zebrafish embryos chambers. Specific developmental features (chorion, yolk, eye, pupil), respective per hpf (hours post-fertilization) were photographed using an inverted microscope and the toxic effects on zebrafish development were characterized by the elemental morphological, functional and behavioral transformations at different hpf. The zebrafish-on–a-chip was validated and results obtained were compared with the standard assays according the OECD TG 236.

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

ROLE OF SURFACE CHEMISTRY IN AMORPHOUS SILICA NANOMATERIALS EFFECTS ON HUMAN DENDRITIC CELLS MATURATION

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Approximately 30% of the European population suffers from respiratory allergies and this prevalence is increasing worldwide. Like air pollutants, nanomaterials could induce airway inflammation and may have an adjuvant effect on allergic response development. Amorphous silica nanomaterials (SAS-NMs) are widely used in pharmaceuticals, cosmetics, and food industries. Their presence in everyday products requires a better evaluation towards their immunotoxic potential.
Dendritic cells (DCs) have a key role in triggering the immune response. They capture and present antigens in an immunogenic or tolerogenic way. In the presence of "danger signals", they undergo maturation, resulting in the expression of co-stimulation and activation markers. This leads to their migration to draining lymph nodes, where they activate naive T-cells. Our previous work has shown that SAS-NMs could act as an immunogenic danger signal by increasing DCs maturation and T-cell response, the first steps of the adaptative immune response.
The surface of amorphous silica nanomaterials contains silanol groups (Si-OH) whose reactivity will depend on whether they are close enough to establish hydrogen bonds. Surface silanol groups, so far identified as critical for cytotoxicity, could play a determining role in the activation of dendritic cells. To explore this hypothesis, we compared the effects of two fumed and two precipitated synthetic amorphous silicas, with different physicochemical properties, on the activation of a surrogate model of human dendritic cells, the monocytic THP-1 cell line.
THP-1 cells were exposed for 16 hours to the SAS-NMs, and the expressions of the activation marker CD54 and the co-stimulation marker CD86 were measured for each condition. Cytotoxicity, measured by propidium iodide labelling, was of the same range for all the nanomaterials tested, although slightly lower for the precipitated NMs with the lowest specific surface area. Our results showed that both SAS-NMs increased the expression of the surface marker CD54, but to a greater extent for the pyrogenic than for the precipitated. Furthermore, activation was found with two precipitated silicas of different specific surface areas (200 vs 36 m^2/g). It was also observed after post-treatment aiming at reducing silanol density for a specific form. These results suggested that specific surface area or silanol density could play a role but are insufficient to explain the observed pattern of cell activation.
We hypothesize that surface chemistry is at the core of the interactions between SAS-NMs and DCs. A more detailed understanding of these interactions could lead to a safer production of SAS-NMs through a safer-by-design approach.

TOPIC 1. METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES 2023

SUPERVISED MACHINE-LEARNING PREDICTIONS OF NANOMATERIALS FUNCTIONALITIES.

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Nanomaterials (NMs) are widely employed in fields such as medicine, pharmaceutical, cosmeceutical, textiles, automotive and aviation industries. In view of their widespread use, the focus has been on safety assessments; however, it is also necessary to ensure their functionalities. Therefore, we aimed to develop machine learning (ML) tools capable of predicting the functionality (i.e., antimicrobial and antioxidant capacity) of NMs and nano-enabled products (NEPs) while revealing attributes of importance. We manually crafted three comprehensive datasets derived from the literature and split them into three studies targeting different outcomes:

1. Study I: Predicting the antimicrobial efficiency of metal oxide NMs expressed as the Zone of Inhibition (ZOI). DOI: 10.5281/zenodo.7640446.
2. Study II: Predicting the antimicrobial properties of textiles coated with NMs expressed as a percentage reduction. DOI: 10.5281/zenodo.7638994.
3. Study III: Predicting the antioxidant efficiency of NM expressed as DPPH assay measurements. DOI: 10.5281/zenodo.6584826.

Following data pre-processing, several regression models were trained, validated, and evaluated with various performance metrics in order to determine the algorithm's performance. The Random Forest models showed the highest predictive performance in all three cases, with more than 70% accuracy (R2=0.78, R2=0.70, and R2=0.83 respectively). Performing important attribute evaluation, we ranked the attributes by their importance. The analysis revealed that P-chem properties are the most important attributes influencing the prediction. Our findings are consistent with the prior research landscape regarding the importance of p-chem characteristics.

This study expands the application of ML in nano-domains beyond safety-related outcomes by capturing the functional performance. In silico approaches are in high demand as data in the field of nanotechnologies grows. The great advantage of these methods is overcoming the limitations of conventional approach by reducing the cost and time involved in assessing the functionalities of NMs. This concept also aids the safe-by-design concept and framework proposed by the JRC by incorporating tools in the functionality dimension.

TOPIC Nº 1 - METHODS, TOOLS, AND TECHNOLOGIES FOR SSbD PURPOSES

IDENTIFYING AND QUANTIFYING RELEASE FROM ASINA ANTIBACTERIAL NANO-ENABLED PRODUCTS TOWARDS SAFE-BY-DESIGN STRATEGIES ALONG THE WHOLE PRODUCTS'S LIFE CYCLE

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The increasing presence of nano-enabled products (NEPs) into the market is rising concerns about potential risks during their use along different stages of their life cycle. Providing solutions by following the Safe-by-Design (SbD) principles is crucial for a better approach to the reducing the risks from early industrial development stages. In line with research responsible innovation (RRI) policy, the H2020 ASINA project (G.A. 862444) has the ambition to promote consistent, applicable and scientifically sound SbD nano-practices, by considering all the NEPs design 'dimensions'. To this end, NEPs with antimicrobial properties are considered in the project as part of one of the representative categories or Value Chains. Understanding the factors and mechanisms that influence the release and the transformations of nanoforms (NFs) is of high relevance when applying SbD strategies. Besides to the release of NFs, synthetic textiles are reported to be a likely source of microfibres release, which is the most reported form of microplastics.
Within ASINA, the NFs release pathway analysis along and beyond the product value chain of the different NEPs was performed. Then, from the identified relevant release scenarios, specific simulation release experiments were designed. In detail, textiles coated with Ag nanoparticles (NPs) were investigate. For the use phase, washing and wearing simulation experiments were conducted simulating sequential washing cycles together with wearing cycles (soft-abrasion by crock-meter simulating dermal contact) and landfilling disposal at the end of life of the NEP. For the end-of-life, leaching tests were employed by simulating the landfilling under acidic conditions. The release of total Ag was quantified by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and characterized by electron microscopy techniques coupled with energy-dispersive X-ray spectroscopy (EDX).
The results confirmed the release of Ag after the experiments of simulating sequential activities of washing, wearing and landfilling, showing the importance of closely simulate the use and end-of-life stages of NEPs.

Funding information
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TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

NANO EXPOSURE QUANTIFIER (NEQ) – A QUANTITATIVE TOOL FOR ASSESSING EXPOSURE IN THE WORKPLACE

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The EU "safe-by-design for Nano" project aims to develop a tool to assist users in the SBD process to reduce risk as a result from working with- or using new products containing manufactured nanomaterials (MNs). Therefore, a quantitative exposure assessment tool that helps users with different levels of expertise to reduce the exposure from MNs following an integrated Tiered safe-by-design approach was developed.

A set of model determinants and corresponding calculations were integrated into a conceptual model. Also, an exposure database was created containing a large set of exposure measurements collected from the literature. This data was used to validate and refine the model. Lastly, a SBD module was added to assist users modify their material / process design to reduce exposure.

The NEQ (available on https://diamonds.tno.nl/#neq) offers an approach by smartly adapting the questions based on the depth of information the user can provide allowing anywhere between a Tier-1 and a Tier-2 approach. After answering questions, the user is presented an exposure estimate including uncertainty. The user is guided to enter a new design that reduces the exposure.

TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

SIMPLE AND ROBUST METHODOLOGIES TO PROMOTE SBD APPROACHES FOR NANOFORMS AND NANO-ENABLED PRODUCTS – SABYNA PAINT SECTOR CASE STUDY

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One crucial point to support innovation and development of safer nanoforms and nano-enabled products is to offer companies simple and robust methodologies to characterize them. To respond to this need, the SAbyNA project proposes compiling and simplifying existing methods tailoring them to specific sectors for safe by design, such as the paint sector.
For this purpose, a set of versatile nano SiO_2-based paint were produced to determine the release of SiO_2 nanoforms during its lifetime. The experimental designs were focused on two use scenarios: indoor use (i.e., painted surfaces simulating wet and dirty conditions such as the bathroom, kitchen, children's room) and outdoor use (i.e., painted facades). To comply with these use scenarios, two cascades activities were proposed and tested: for indoor use, the painted panels were firstly washed and then subjected to abrasion simulating skin contact. For outdoor conditions, the painted panels were exposed to weathering and then abrasion to simulate paint abrasion.
As shown in the table below, this provides a cross-sectional view between the test methods (used to mimic the indoor and outdoor use scenario) and the main expected results. Based on this, manufacturers can then compare different paint formulations and validate the most effective one for safe by design approach.

Investigated Use Scenario	Experimental activities	Testing method	Expected outputs
Indoor Use	Paint washing	Wet-scrub resistance	Evaluation of the low gloss, mass loss and thickness removal layer
	Dermal contact	Crockmeter	Amount of nanoforms released
Outdoor Use	Weathering	Weathering/aging	Evaluation of emissions to environmental compartment
	Abrasion	Taber abrasion	Mass loss and determination of the NEP release rates
		Cryomilling	Milled product to be used for further testing (e.g. measurements of the particles size and Si mass content)

Acknowledgements:
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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

RAMAN SCATTERING IMAGING FOR DETECTION AND QUANTIFICATION OF NANOMATERIALS DURING BIOACCUMULATION TESTS

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The fast developing of the nanotechnology field has allowed the introduction of the nanomaterials in many consumer products. To better understanding about their potential human health risk, data about their bioaccumulation in products for human consumption is necessary, but determining a dose-response for this is still a challenge. We demonstrate for the first time, to the best of our knowledge, an analytical approach to accurately determine the nominal concentration in water as well as the bioaccumulation in mussels of different nanomaterials, e.g. carbon-based material and titanium dioxide, by Raman scattering imaging (Figure 1). We have shown that the optimized protocol is independent of the colloidal stability of the nanomaterials, thereby making it applicable to nanomaterials ranging from well-dispersed to poorly defined and very heterogeneous dispersions derived from nanopowders. Furthermore, neither extensive physicochemical characterization of nanomaterials nor modelling are required, thus minimizing the potential for systematic errors. Moreover, this approach could also be adapted for different nanomaterials that have a characteristic Raman fingerprint or other mollusks (e.g., clams).

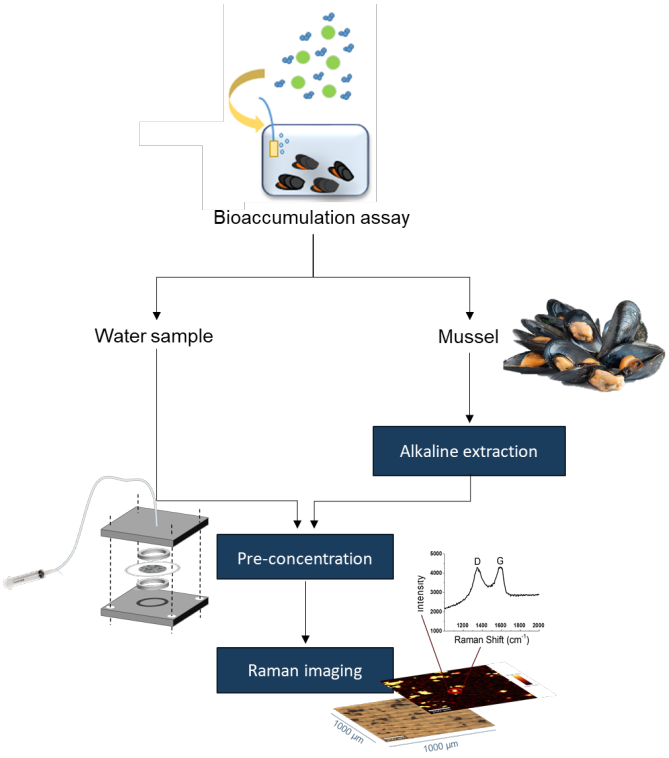

Figure 1. A schematic illustration of the experimental steps to determine the concentration of nanomaterials in water and mussels during a bioaccumulation assay including (1) Alkaline digestion of mussel to extract the nanomaterilas, (2) nanomaterial' pre-concentration by filtrating artificial seawater or mussel' extract, and (3) Raman spectroscopy analysis to determine the remaining dispersed nanomaterial in water after initial dose and the bioaccumulation in mussel.

Acknowledgements
This work was funded by the project Horizon2020 SbD4Nano (862195) and SbDToolBox NORTE-01-0145-FEDER-000047, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). L.R.-L. acknowledges funding to FCT (Fundação para a Ciência e Technologia) for the Scientific Employment Stimulus Program (2020.04021.CEECIND).

TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

ASSESSMENT OF PRIMARY AND SECONDARY GENOTOXICITY OF REDUCED GRAPHENE OXIDE USING AN *IN VITRO* LUNG CO-CULTURE MODEL

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Nanomaterials' potential to inflict a genotoxic response in the lung has been traditionally assessed *in vitro* with monocultures due to their simplicity, good reproducibility, and compatibility with high throughput screening. However, monocultures are not a good representation of *in vivo* tissues as they lack the complex multicellular structure and cellular communications that typically influence cell behaviour. Notably, secondary genotoxicity, mediated by inflammatory cells, cannot be detected in monocultures of lung bronchial epithelial cells. Therefore, co-culture systems that consist of two or more distinct cell types are another option for modelling *in vivo* tissues when more complex models are needed.

The aim of this study was to utilize an *in vitro* lung co-culture model to examine the primary and secondary genotoxic effects of reduced graphene oxides (rGO) with different physicochemical properties. In order to mimic actual nanomaterial exposure via inhalation and the cell–particle interactions occurring in the lung, human bronchial epithelial 16HBE14o- cells were cultured either alone, or together with differentiated monocytic THP-1 cells. Assessment of genotoxicity was performed using the cytokinesis-block micronucleus assay that detects unrepairable chromosome damage. Taking advantage of the graphene materials' own reflective properties, cellular internalization was assessed by confocal laser scanning microscopy, using Hoechst 33342 and CellMask™ staining for identifying the nuclei and membranes, respectively.

To obtain a monoculture, human bronchial epithelial 16HBE14o- cells were seeded on 6-well plates on d0. Human leukemia monocytic THP-1 cells, differentiated into macrophage-like cells (dTHP-1) with 50 nM Phorbol 12-myristate 13-acetate (PMA), were added to half of the wells on top of a confluent 16HBE14o- cell layer on d4, and the co-culture system was allowed to stabilize for 24 h before the rGO exposure. rGOs were dispersed in 0.05% w/v BSA-water solution by probe sonication and the dispersion was further diluted with culture medium. Both mono- and cocultures were exposed to the rGO (100–6.26 µg/ml) for 24 h and afterwards they were treated with 3.0 µg/ml cytochalasin B for 44 h, fixed and dropped onto microscope slides. Monocultures were stained with acridine orange and DAPI. Cocultures were stained with CD14 and CD324 to differentiate the dTHP-1 and 16HBE14o- cells, respectively. In both cases, the micronuclei were scored in 16HBE14o-. Manual scoring of the micronuclei frequencies is currently in progress. Then, results from the mono- and cocultures will be compared to evaluate the potential mechanisms of genotoxic action of rGO.

Funding information
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Keywords: genotoxicity, co-culture, graphene-based nanomaterials, reduced graphene oxide, cytokinesis-block micronucleus assay, lung bronchial epithelial cells

TOPIC 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSbD PURPOSES

Investigation of the VOC and particles emissions from 3D-printing polymeric filaments at the heating process temperature

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Among 3D printing techniques, the fused deposition modelling method using polymeric filaments is extensively used today both by hobbyists up to industry. Numerous polymers including materials with nanoparticles charges are processed targeting varieties of properties (color, density, conductivity…) to the final objects. This increasing use generates concerns about the potential risks to human health due to exposure to the released emissions. Indeed, numerous studies reports that 3D printers emit volatile organic compounds (VOCs) as well nanoparticles into the air[1]. These airborne particles would be mainly formed from nucleation of low volatile organic compounds emitted from the heated filament during printing and which grow due to condensation of emitted vapors and coagulation[2]. Furthermore, the use of nanomaterial components in polymer filaments put a specific release hazard.
In this present work, we have studied both the VOCs and particles emissions from polymeric filaments at their printing temperatures. In this goal, a specific experimental set-up was implemented where a filament sample (~50-100 mg) is introduced in a tubular furnace stabilized at a defined temperature and swept by a controlled clean dry airflow. At the furnace outlet, different emissions characterizations are performed. VOCs are collected on a 25mm quartz filter and an adsorbent tube to discriminate the condensable and volatile fractions respectively, and samples are then analyzed by TD-GCMS. In-line monitoring of the particles number concentration and size distribution are measured using a FMPS (TSI, Model 3091; 5,6-560 nm); particles were also collected on PC filters for SEM observation. By this approach, the aim is to characterize the generated emissions in simulated controlled conditions without significant cross-contamination and interferences brought by environmental background as it might occur in real world environment. This method have to allow the comparison of the emission rates between polymers and an easier chemical characterization (VOC and particles).
Two nano-enabled filaments were studied, a PP added with Ag nanoparticles (PP-nAg) at 230°C and a PC with carbon nanotubes (PC-CNT) at 300°C. Results shows that the mass concentrations of the total VOC are higher than the particles ones and the condensable fraction represents about 25% (Figure 1). Main VOC have been identified (~80% of the total VOC) giving a release signature of the materials and a knowledge of potential hazardous VOC. Furthermore, SEM results have showed no release of CNT or Ag. These results suggest that lower volatile (semi-volatile) organics compounds are emitted in significant amounts leading to particle formation and growth. Finally, these measurements highlight that PC-CNT emissions are higher than from PP-nAg.

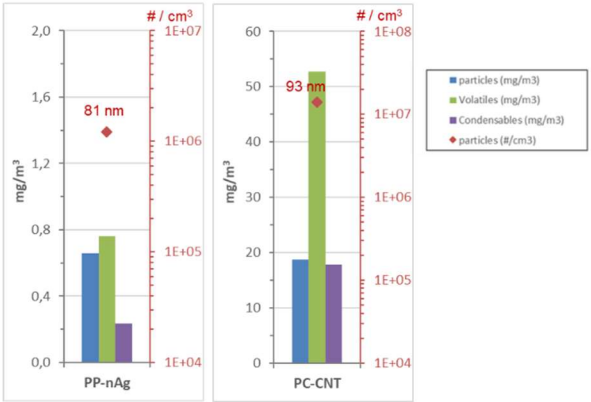

Figure 1: Emissions from PP-nAg and PC-CNT filaments at 3D-printing temperature

Funding from the SAbyNA project (EU Horizon 2020, grant agreement No 862419) is acknowledged.

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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

CHALLENGING LOW-COST PM SENSORS FOR MEASURING PARTICLE SIZE DISTRIBUTION OF AIRBORNE NOAA

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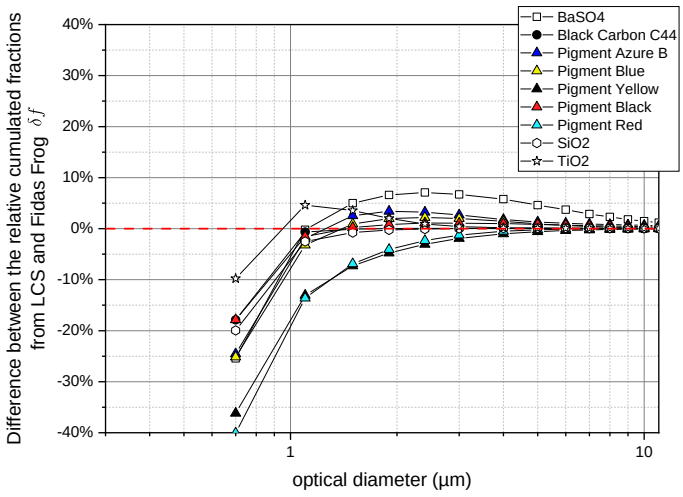
So far, the characterization of the airborne particles that are emitted by a process in the workplace air was carried out by high or medium cost instruments. High-cost instruments are typically used by to experts who work in scientific laboratories They can be transportable but not necessarily wearable, and many of these instruments cost more than 35 k€. Instruments that can be worn by workers in their breathing aera and can easily be deployed in workplaces by occupational hygienists are typically in the medium-cost range, between 8 k€ and 20 k€. Due to their costs, those instruments are generally not present in multiple sampling points in workplaces, which greatly limits the representativeness of the measurements. Since this last decade, low-cost particulate matter sensors (LCS) have emerged on the commercial market and can measure one or more PM mass concentrations and/or number particulate concentrations, and for certain sensors, a basic particulate size classification. Those sensors in their OEM (original equipment manufacturer) version are much more compact than the medium and high-cost instruments. They are able to provide measurements at a high frequency (1 Hz) for a cost mostly between 10 and 100 €. Therefore, one can imagine using a multitude of those distributed LCS in work-places to measure the workplace dust concentrations with high spatio-temporal resolution.
As part of the European mandate M/461, the usability of LCS for measuring airborne Nano-Objects, their Aggregates and Agglomerates (NOAA) that could occur in certain workplaces has been evaluated. Because NOAA cover a large range of sizes, it is possible to get some data from LCS, which generally start to detect particles from an optical diameter of 0,3 µm. Thus, we evaluated three replicates of six different type of LCS (referenced as OPC-R1, Next-PM, Next-PM CR, SPS30, PMS7003, SDS011) regarding their counting efficiency and their capacity to estimate the number size distribution of particles, in comparison with an optical spectrometer such as the Palas Fidas Frog (a medium cost instrument), for nine different aerosolized NOAA powders.
Results show that below 1 µm the counting efficiency of the LCS is lower than the one of the Fidas Frog, resulting in an under estimation of the relative particle size distribution as seen in the figure. Above 1 µm, the counting efficiency of the LCSs is consistently within ~±10% with the Fidas Frog. Depending on the nature of the NOAA powder generated for those tests (refractive index, morphology, density, etc.), the results' variation demonstrated the sensors sensitivity to these parameters.
Detailed results on counting efficiency and particle size distributions will be presented and discussed to establish the possibilities and the limitation of the use of LCS for characterizing NOAA aerosols in workplace air.



Examples of deviations between the relative cumulated particles size distributions measured by LCS (OPC-R1, Alphasense) and Fidas Frog (Palas)

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

ENHANCING COATINGS FOR SAFE AND SUSTAINABLE ALTERNATIVES FOR PFAS

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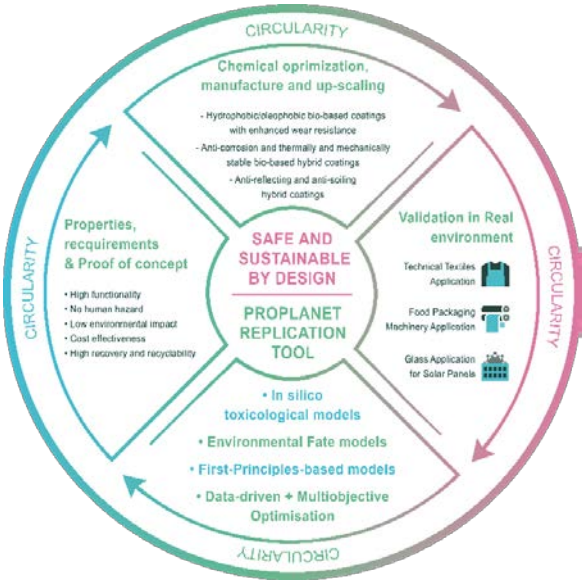
PFAS (per- and poly-fluoroalkyl substances) are extensively used in various products and applications. This includes oil and water repellent coatings for textiles, anti-stick coatings for food packaging and equipment, anti-soiling and anti-reflection coatings for glass, cosmetics, and construction materials (just to name a few). However, these so-called "forever chemicals" are hazardous to human health as they do not decompose under normal conditions, leading to their accumulation in the environment. Via the intake of contaminated water and food these substances eventually end up in humans and other organisms. In fact, PFAS have been detected in human blood and are associated with organ damage, weakened immunity, high cholesterol levels and cancer. In response, PFAS will soon be banned [1], and alternatives must be identified urgently. The EU-funded project PROPLANET aims to address this issue by developing novel materials that can replace PFAS without sacrificing functionality or performance. The international and interdisciplinary project consortium consisting of 13 institutions and companies, experts across relevant domains (modelling and simulation, safety and sustainability, circularity-by-design, as well as manufacturers and end-users), is striving to replace PFAS by viable and sustainable alternatives. The project focuses on minimizing hazards to human health and the environment, while also considering economic, recyclability, and circularity aspects. PROPLANET follows the safety and sustainability by design (SSbD) concept [2] and will investigate and validate materials for coatings on (1) textiles, (2) food packaging equipment, and (3) low-maintenance glass. The experimental investigations will be supported by mathematical and computational models (multi-objective optimization and data-driven algorithms aided by artificial intelligence) that will be combined in an open-source replication tool. This tool will allow the end-user to reduce waste, explore new applications for materials and support their route to market while following an eco-design path. Through this approach, PROPLANET intends to bridge the gap between environmental protection, safety, chemical improvements, and a circular economy.



PROPLANET's scope.

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[2] C. Patinha Caldeira *et al.*, Safe and Sustainable by Design chemicals and materials Review of safety and sustainability dimensions, aspects, methods, indicators, and tools, EUR 30991 EN, Publications Office of the European Union, Luxembourg, 2022, ISBN 978-92-76-47609-2, doi:10.2760/68587, JRC127109.

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

INTERACTIONS OF BISPYRIDINIUM COMPOUNDS WITH THE DOPC MEMBRANE AS REVEALED BY CONSTRAINT DYNAMICS SIMULATIONS AND RAPID CYCLIC VOLTAMMETRY

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Bispyridinium compounds (BPCs) have important applications as potential pharmaceuticals and antidotes for nerve agent poisoning. These compounds consist of two pyridinium groups linked via the nitrogen atom by an alkyl chain of varying length, while t-butyl groups can substitute the hydrogen atoms of the pyridinium groups. The length of the alkyl chain and the presence or absence of t-butyl groups influence the interactions of BPCs with membranes. These selective interactions of the BPCs with membranes allow us to use the membranes as sensors to trace specific BPCs in blood, water, or other solutions. To confirm the selectivity of membranes to BPCs, we studied the interaction of six BPCs (i.e., having alkyl chain lengths of 1, 5, and 10 with or without t-butyl groups) with the 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) bilayer membrane when both are in water. We applied Rapid Cyclic Voltammetry (RCV) and Constraint Dynamics Simulations to create rapid cyclic voltammograms and to find the free energy profiles, respectively. Since BPCs have a positive charge of +2e, two negative anions (in our study, iodine anions I-) coexist in the solution. Different BPCs led to differences in the RCV voltammograms (e.g., depression of the capacitance peaks). These differences were further studied by Atomistic Simulations under the influence of an external electric field. Atomistic Simulations revealed a transition from the initial DOPC bilayer orientation to a new one, perpendicular to the first. In the absence of an external electric field, the free energy barriers were larger than 30 kcal/mol for all the examined systems, indicating that it is extremely rare for a BPC to pass through the DOPC membrane. Additionally, the free energy profiles reveal that BPCs prefer to reside in water, followed by the polar part of the DOPC membrane, while BPCs do not prefer the hydrophobic part of the DOPC membrane.

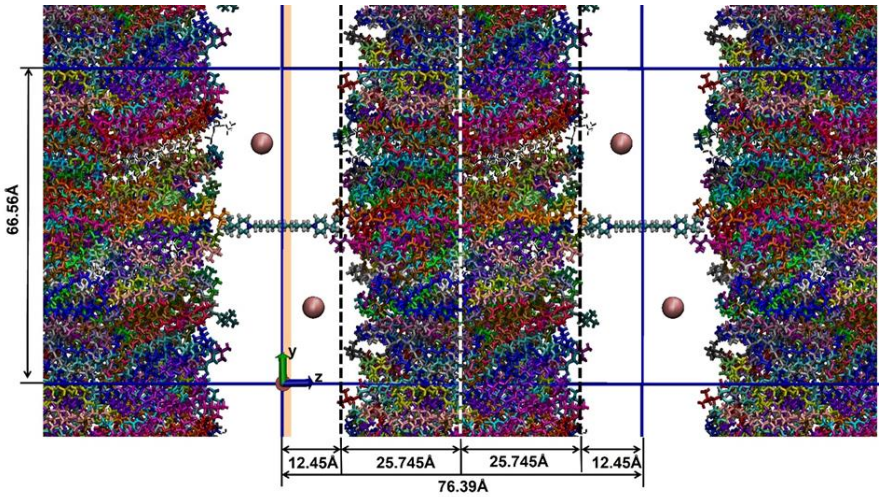

Figure 1. Simulation box (central box) of Bispyrdinium compound-DOPC membrane system. Iodine counter anions are illustrated with pink spheres. Each DOPC molecule is illustrated with a different color. Water molecules have been omitted for clarity.

Funding information
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TOPIC N° 1 – METHODS, TOOLS, AND TECNHOLOGIES FOR SSBD PURPOSES

NANO-QSAR TOOLBOX – PLATFORM ENABLING COMPUTER PREDICTION OF PHYSICOCHEMICAL PROPERTIES, TOXICITY AND ECOTOXICITY OF SiO_2 AND ZnO NANOPARTICLES

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Nanoparticles are one of the most innovative and fascinating achievements of modern science. Due to their ultra-small size (< 100nm), they exhibit a range of unique physicochemical/biological properties and find applications in many fields, such as medicine, cosmetics, and even construction materials production. Although nanoparticles possess numerous advantageous properties, their potential impact on the environment and human health is a growing concern. Moreover, in XII 2018, new regulations were announced (EC Regulation 2018/1881), introducing from 2020 the obligation to carry out a risk assessment separately for each so-called nanoform of the substance. This raises the need for entities using nanoparticles, including SiO_2 and ZnO, in their products to carry out a risk assessment for a large number of different nanoforms. Here, it is necessary to mention that required experimental testing is associated with huge costs, increasing the use of laboratory animals, and extending the time to market new nano-based products. Thus, the nano-QSAR Toolbox (nQTb) responds to the need to replace experimental methods with computer methods according to the recommendations of Regulation 1907/2006 on Registration, Evaluation, Authorization and Restriction of Chemical. The nQTb is tailored to the registrants' needs and offers a computational prediction of relevant phys-chem properties, toxicity and ecotoxicity endpoints required during the registration process of new nanoforms in ECHA. In nano-QSAR Toolbox, the prediction of critical endpoints is based on a set of QSAR/QSPR models, where quantitative/qualitative relationships between nanostructure and observed biological activity or phys-chem properties are identified through the application of advanced machine learning methods. The developed nano-QSAR Toolbox allows for the estimation of critical endpoints for the nanoform structure defined by the user, where chemical composition, surface functionalization, size distribution and shape are considered. This eliminates the need for the user to have specialized knowledge e.g., in the calculation of descriptors with quantum chemistry, making the developed tool accessible to a broader range of researchers and manufacturers. Moreover, besides the registration aspects, the presented nano-QSAR Toolbox can be used during the virtual screening of nanoforms considered for the established application in terms of biological activity and phys-chem properties, making the tool consistent with the idea of safe-and-sustainable-by-design (SSbD).

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TOPIC N° 3 – TRANSFERABILITY AND ACCEPTABILITY OF THE CONCEPT OF SSBD

THE SUNSHINE TRUSTED ENVIRONMENT AND FORESIGHT PLATFORM FOR SUSTAINABLE INNOVATION

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Major industrial sectors such as construction, structural and functional materials, active ingredients, food, healthcare, energy, cosmetics and electronics are investing in research and technological development (RTD) of multi-component nanomaterials (MCNMs).
These new materials offer unprecedented technological benefits as the integration of different components in a unique system that can produce new or improved functionalities. However, MCNMs also pose substantial design challenges as well as environmental, health and safety (EHS) concerns. The latter are particularly complex due to the differing rates of degradation and toxicities of the separate and interacting components, and their more complex interactions with biological and environmental systems. These concerns need fundamental research and regulatory guidance to address the unique properties of these advanced materials.
To address these challenges, SUNSHINE, an industry-orientated project, will develop Safe and Sustainable by Design (SSbD) approaches for products incorporating MCNMs with a strong focus on multi-component HARNs, as those are of particular concern.

In particular, SUNSHINE will ensure that safety regulations can keep pace with MCNM innovation. This will be done by extending and applying the Regulatory Preparedness concept to MCNMs, which refers to improving the anticipation capabilities of regulators and (regulatory) risk assessors and to facilitate, where needed, timely adaptation of (safety) legislation, guidance guidelines and standards. Timely clarity on how regulators deal with novel materials such as MCNMs reduces uncertainty for industry about the information needed to comply with a regulation and how testing should be performed.

The creation of Trusted Environment is important for both SSbD and Regulatory Preparedness because when knowledge is openly shared between industry stakeholders and Regulators from the early stages of innovation, the time for MCNM-based materials and products to reach the market can be substantially reduced, while ensuring high levels of health and environmental protection.

This poster illustrates the basis of a working framework to shape a Trusted Environment in particular for industry and regulators which will enable science-based Foresight Studies leading to the development of policy recommendations which will benefit both Society and Industry. More specifically, the poster illustrates twelve essential boundary conditions, needed to develop a Trusted Environment as an essential first step in hosting a Foresight Platform composed of regulators and Industry. It describes the required input and output of the foresight studies and how these can address the common benefit of the platform members working towards new regulations and standardisation. This will be tested in the SUNSHINE project.

The SUNSHINE project is funded under the European Union's Horizon 2020 Research and Innovation programme, Grant Agreement 952924

TOPIC 3. TRANSFERABILITY AND ACCEPTABILITY OF THE CONCEPT OF SSBD

WHY IMPLEMENT THE SAFE-BY-DESIGN PRINCIPLE IN PRODUCT DEVELOPMENT: A PERSPECTIVE FROM NANOTECHNOLOGY

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The adoption of the Safe-by-Design (SbD) approach in the R&D&I ecosystem can be a real gamechanger toward achieving the ambitious goals of the EU Green Deal and the Chemical Strategy for Sustainability. It is hoped that the broad implementation of SbD in the chemical industry will reduce the number of hazardous substances placed on the market in the EU, and that it will improve the safety of products throughout their lifecycle by avoiding unexpected risk to humans and the environment. The SbD4Nano project is examining the effective implementation of the approach to the design and development of nanomaterials and nano-enabled products, which are expected to have an increasing market share in the future, to enable safe and sustainable development of new technologies in a timely and cost-effective manner.

For SbD to be successful, stakeholder industries and downstream users of nanomaterials and nano-enabled products will need to engage with the principle and adopt it throughout their product development process. For widespread adoption of SbD, stakeholders will need to understand why the positive aspects of SbD can justify the resources needed to integrate it into early-stage product development. This poster outlines how the SbD4Nano project has examined what would incentivize industry to adopt such a strategy, including the ethical and monetary advantages manufacturers that apply SbD in product development could gain, potentially giving them a competitive advantage over non-adopters. Next to that, it highlights latest regulatory developments in this field, and how SbD fits into the regulatory framework.

Binary interactions of aluminium oxide and copper oxide nanoparticles in freshwater systems

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ABSTRACT

Commercial production and use of engineered nanoparticles (ENPs) for industrial applications and in consumer products are increasing rapidly due to their novel properties. For example, ENPs such as aluminium oxide (nAl_2O_3) and copper oxide nanoparticles (nCuO) are extensively used in cosmetics, paints, textiles, etc. Hence, they are found to co-exist in the aquatic systems. Data is available on fate and behaviour of nAl_2O_3 and nCuO as on their own in the aquatic systems. However, only limited information is available on how the fate of ENPs mixtures is affected by physicochemical transformations such as aggregation, dissolution, dispersion, adsorption and complexation. Interactions in such mixtures are likely to have implications to aquatic organisms. Herein, we show that the aggregation and dissolution of nAl_2O_3 and nCuO mixtures in freshwater systems are influenced by the resultant interactions among the ENPs. The characteristic composition of the freshwater system affects the binary interactions of ENPs. Hetero-aggregation in the mixture was confirmed by energy dispersive X-ray spectroscopy (EDS) and transmission electron microscopy (TEM). The degree of aggregation of nAl_2O_3/nCuO mixtures increased with decreasing amount of nCuO. This is attributed to adsorption of nCuO onto the surfaces of nAl_2O_3. The resultant aggregate sizes tend to be larger in mixtures than those observed on single ENPs and may possess characteristic effects on aquatic organisms. Increased aggregation may impede dissolution of ENPs leading to a reduction in ionic species. The dissolution was observed to increases with concentration of the mixtures and also influenced by specific water chemistry. This study confirms that the fate of mixtures is unique and that it may not be possible to predict behaviour based on the fate of individual components.

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

INFLUENCE OF PARTICLE SIZE, FACE VELOCITY, AND EXHALED BREATH CONDENSATE ON THE FILTRATION EFFICIENCY OF MASKS AND MASK MATERIALS FOR COVID-19 TRANSMISSION PREVENTION

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Non-medical facemasks such as disposable, commercially produced cloth and homemade masks are not regulated like surgical masks. Their performance, in terms of filtration efficiency and breathability, is variable and unreliable. This research was performed with two objectives: 1) to provide a quantitative evaluation of various non-medical facemasks, assess potential of fabrics for the reduction of transmission of bioaerosols, and compare them to surgical and N95 masks; and 2) to evaluate the effect of face velocity and exhaled breath condensates on filtration efficiency.
Using a testing line with a NaCl challenge aerosol, four types of commercial reusable cloth masks, two types of disposable non-medical masks, three types of surgical masks and seven types of commonly available materials were tested individually and in combinations. The testing line and procedure were adapted from the ASTM F2299 testing method used for testing surgical masks. Filtration efficiencies at 0.15 µm particle diameter at a face velocity of 25 cm/s for commercial cloth masks, disposable non-medical masks, surgical masks, commercial mask combinations and homemade combinations ranged at 16-29%, 39-76%, 91-97%, 51-95%, 45-94% respectively. The pressure drop results for the different masks and material combinations were all under 3 mm H_2O/cm^2 except for one material configuration.
Then, the filtration capability of the masks and filter materials were tested at two different face velocities (10 and 25 cm/s). Four of these masks were tested at two additional face velocities (17.5 and 32.5 cm/s): level 3 surgical masks, two designs of cotton masks and disposable non-medical masks. Seven of these masks were also exposed to aerated simulated exhaled breath condensate (EBC) for 1 to 24 hours and their filtration efficiency was tested immediately following exposure.
The filtration efficiency of non-woven masks decreased by 1-17% as the face velocity of the challenge aerosol stream increased. The extent of decrease in filtration efficiency found for non-woven masks over the face velocity range allowed for ASTM testing could affect the compliance or non-compliance to the filtration efficiency requirements. On the other hand, an increase in the face velocity did not decrease the filtration efficiency of woven and knitted materials and of two of the commercial cotton facemasks. For cloth masks, the filtration efficiency decreased by up to 20% after EBC exposure. There was no correlation between the EBC exposure, and the pressure drop.
With proper layering, household materials can achieve the high filtration efficiency and low pressure drop requirements of surgical masks. The filtration capabilities of disposable and cloth mask fabrics vary considerably meaning that they are not a reliable or consistent facemask option, regardless of fit. Cloth masks should be worn with a filter for a maximum of 8 hours to prevent a decrease in filtration efficiency due to breath condensate accumulation.

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO GOVERNANCE, A JOURNEY THROUGH REGULATION & STANDARDIZATION

COMPARISON OF METAL TOXICITIES COMMONLY USED FOR PHOTOCATALYSIS: REVIEW AND RECOMMENDATIONS

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Metal components and nanoparticles are used to develop new heterogeneous photocatalysts with desired properties both in laboratory and industrial scale. In addition to the appropriate mechanical and physical properties that newly developed materials should present to allow their use in the areas of interest, the potential impact that they represent for the human health and the environment is another critical factor that should be considered before their implementation. We assessed the toxicity of 14 different metals and nanomaterials. Based upon the literature survey, the inert or least toxic metals to human health and environment from the selected materials include Tin, Nickel, Aluminium and Titanium dioxide when used in pure composition. Metals such as Zinc, Iron and Copper becomes toxic after a certain concentration. The only toxic metal from the selected components was Antimony, which exerts toxic effect even at low concentration. It should also be noted that toxicity of metals depends upon duration, dosage of exposure, the chemical combination or susceptibility. The choice of nanoparticles for photocatalyst with less toxicity include gold and iron carbide while toxicity towards human health was observed for ruthenium oxide, titanium dioxide and manganese nanoparticle. It should also be noted that toxicity of nanoparticles has conflicting data since size, shape and functionalization are the main factor in determining the toxicity of nanoparticles rather than the chemical composition. The present review and recommendations will help to design safer photocatalysts.

This study is part of the project NEFERTITI, which has received funding from the European Union's Horizon 2020 program (Grant Agreement no 101022202).

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO GOVERNANCE, A JOURNEY THROUGH REGULATION & STANDARDIZATION

THE EU PROJECT NANOHARMONY – TOWARDS HARMONIZED TEST METHODS FOR NANOMATERIALS

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Test Guidelines (TGs) and standards are needed to be up-to-date for regulation to keep pace with innovation. They are key tools for gaining information on the characteristics and hazards of chemicals. With the developments of nanomaterials (NMs), TGs had and still have to be updated or newly developed for e.g. substance safety assessments. TGs have the great advantage that they are agreed upon by the OECD Member countries and that data based on the use of TGs have to be accepted by these countries through the Mutual Acceptance of Data (MAD). This lowers the barriers for trade worldwide. TGs also contribute to the reduction of animal testing and help reduce the costs of regulatory compliance.

The NanoHarmony project (funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931) supports the development of eight scientifically reliable test methods and good practice documents for NMs, based on the translation of existing scientific knowledge and data into a form that has regulatory relevance. All these NanoHarmony activities are closely connected to projects in the OECD Test Guidelines Programme. Data are gathered, new ones generated when necessary and subsequently used together with other information to lay the scientific foundations for adaptation or development of new OECD TGs and Guidance Documents (GDs).

NanoHarmony also analyses processes in test method development, to establish a framework for seamless and smooth cooperation between all stakeholders for timely developments of test methods ready for regulation. Involvement of all stakeholders in this process is key to NanoHarmony and successfully facilitated through open workshops, interviews and webinars. Methodological gaps and process-related obstacles identified during developing TGs are used to propose an optimized framework to further streamline the development of regulatory relevant test methods. The valuable contributions from stakeholders and researchers donation of existing data to the NanoHarmony tasks were important elements for the overall progress in the TG and GD developments.

To keep pace with new developments in materials and methods, it is necessary to continuously support updates and developments of new OECD TGs. To assist in this process, NanoHarmony has elaborated several legacy items. 1) Future TG developers are supported by developed training material on TG development as well as by the NanoHarmony Process Mentor (an online guidance tool to help developers of future TGs). 2) Continuation of regular online workshops on TG developments. 3) Recommendations on further streamlining the process of TG development and future needs in the form of the NanoHarmony White Paper.

TOPIC 5. FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

REGULATORY QUESTIONS TO ADDRESS WHEN PLACING MULTICOMPONENT NANOMATERIALS ON THE MARKET IN THE EU: CASE STUDIES FROM THE SUNSHINE PROJECT

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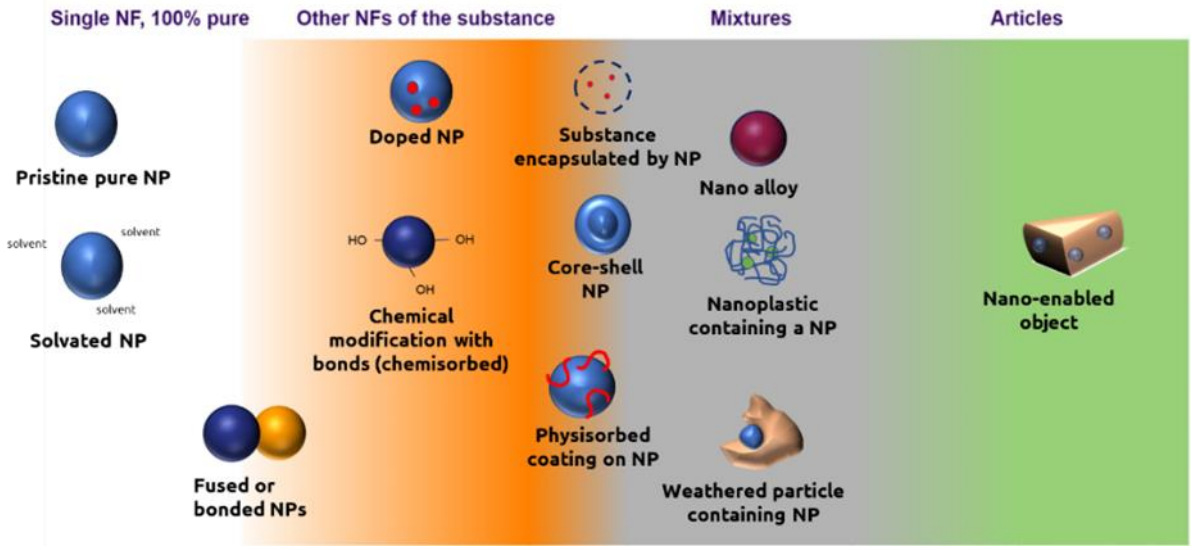
Multicomponent nanomaterials may be regarded as advanced hybrid materials that contain more than one functional component conjugated by chemical bonds or other intermolecular mechanisms, with at least one component meeting the definition of a nanomaterial. These hybrid materials are being examined for use across many major industrial sectors. These new materials offer unprecedented technological benefits as the integration of different components in a unique system can produce synergistic functionalities. However, they may pose substantial design challenges and also might induce specific safety and sustainability concerns due to their complex interactions with biological and environmental systems, which the SUNSHINE project is attempting to better understand. Several case studies from SME industrial organisations are being used to elucidate these issues.



Within the SUNSHINE project, work on regulatory preparedness is also examining whether the safety of the MCNM structures is sufficiently covered in the existing regulatory frameworks in the EU using these case studies as examples. This poster presents the results of this work highlighting several areas where entities placing MCNMs on the market might have difficulties understanding their regulatory obligations and where specific guidance from regulatory authorities could facilitate the expansion of this emerging technology.

TOPIC 5. FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

AN EXAMINATION OF THE IMPACTS OF THE TERMS POTENTIALLY USED IN THE DESCRIPTION OF THE TERM "MULTICOMPONENT NANOMATERIAL"

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Major industrial sectors such as construction, cosmetics, food, energy, electronics, healthcare, active ingredients, structural and functional materials are investing in the development of multi-component nanomaterials (MCNMs). These advanced hybrid materials can be formed by the conjugation of two or more functional components (e.g. nanoparticles, nanocrystals, organic molecules) or by a nanomaterial (NM) with a unique chemical origin modified by coatings.

The SUNSHINE project has attempted to understand how such structures might fit into the existing regulatory framework in the EU and the regulatory preparedness for these novel structures and the products that will contain them (e.g. how the safety of MCNMs is covered in existing regulatory frameworks). In order to do this, it is important to understand which structures would be described as a MCNM. This poster describes some possible parameters that could be used in such a description and how their use would include or exclude structures that might be described as a MCNM.

TOPIC N° 5 - FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

Tuball ™ Single wall Carbon Nanotubes: Recent regulatory developments

Van Kerckhove Gunther

OCSiAl Europe Sarl, Grand Duchy de Luxembourg

The company OCSiAl was founded in 2009 and is the first SWCNT manufacturer who has completed the EU-REACH registration and updated last time since April 2020 OCSiAl, is allowed to commercialize up to 100 tons/y of its substance Tuball™, being additionally compliant to the new REACH regulation for Nanoforms. From December 19, 2017, OCSiAl's substance is regulated as a new chemical substance *by EPA*. Since December 5, 2019, our substance *Tuball*™ has been approved by the US EPA and has no limitations on commercialization at his market. His regulatory status with EPA advanced with the publication of a significant new use rule ("SNUR") in the Federal Register covering OCSiAl's products. A SNUR is the regulatory mechanism that EPA uses to bind second and third tier customers to the terms of EPA's consent order, so as to allow unimpeded sales up and down the supply chain. Since December 2022, obtained a written testing waiver from EPA that it is no longer required to conduct the 90 days rat inhalation toxicity study. The full distribution chain is open to OCSiAl and its products. Since Tuball™ is used and also tested in various applications on an ongoing basis, it is receiving a lot of interests worldwide. That is the reason why it is necessary that OCSiAl establishes the necessary regulatory and quality standards worldwide.The first part of this presentation will be focused on the the morphology vs EHS status of our Tuball™ substance. The second part will be about this registration & compliance status, the third part will involve experiences in exposure assessments and final part will present the outcome of other additional EHS studies we have executed so far. As SWCNT manufacturer, OCSiAl is doing continues investments in improving the understanding of its different (new) Tuball products and the potential hazards through their (entire) life cycle. The company is involved as well in generating additional test data and collaborating with industry associations and networks. This presentation will describe the steps that have been taken by the company's H&S Lead Manager, Gunther Van Kerckhove, in order to successfully introduce OCSAl's carbon nanotubes Tuball™ regulatory status and to outline our future plans regarding the Tuball™ substance and containing products.

TOPIC N° 5 – FROM NANOMATERIALS RISK ASSESSMENT TO RISK GOVERNANCE, A JOURNEY THROUGH REGULATION AND STANDARDIZATION

IN VITRO ASSESSMENT OF SKIN IRRITATION POTENTIAL OF GRAPHENE BASED MATERIALS USING RECONSTRUCTED HUMAN EPIDERMIS (RHE)

Natalia Fernández-Pampín[1], Juan Antonio Tamayo-Ramos[2], Nandita Dasgupta[1], Dalia de la Fuente-Vivas[1], Rocío Barros[1], Sonia Martel Martín[1], Laura Gómez-Cuadrado[1], Carlos Rumbo[1]

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Graphene-based materials (GBMs) have attracted a lot of attention during the last years due to their extraordinary physicochemical properties. These materials are employed in a wide range of fields, including electronic and biomedical applications[1,2,3]. From a safety perspective, the major risk for human health associated to these materials is currently related to occupational exposure[4], being the inhalation and dermal contact the most relevant routes of exposure to GBMs[5]. In fact, around 90 % of skin diseases associated with occupational settings are represented by irritant and allergic contact dermatitis[6]. However, the existing information about cutaneous toxicity of GBMs is scarce. Considering the possible risks in the work environment due to the skin irritation potential of these materials, the present study investigated the cutaneous toxicity of a group of GBMs, including small flakes graphene (SFG), large flakes graphene (LFG) and graphene nanoplatelets (GNplatelets). The inclusion of New Approach Methods (NAMs) for skin sensitization into Organization for Economic Co-operation and Development (OECD) has proved to be a good replacement of animal models. This methodology simulates the human exposure by direct epidermal contact to chemicals. Therefore, to evaluate the skin toxicity, the *in vitro* EpiDerm Skin Irritation Test (EPI-200-SIT) was employed. Following the OECD Test Nº 439 to determine *in vitro* skin irritation on reconstructed human epidermis (RhE), it was observed that none of the GBMs caused damage to the tissues. According to EU and Globally Harmonized System of Classification and Labelling Chemicals, GHS, (R38 / Category 2 or no label), an irritant is predicted if the viability of the tissues exposed to the test substance is reduced below 50 % of the viability of the controls. In the case of these GBMs, these materials did not reduce the tissues viability, so they can be considered as non-irritant in the tested conditions. These results provide new insights about the skin irritation potential of GBMs with different properties, demonstrating that the hazard assessment using human *in vitro* models is a critical aspect to increase the knowledge on their potential impact upon human health.

This study is part of the project DIAGONAL (H2020, NMBP-16-2020, Grant Agreement no 953152), which aims to address existing gaps at risk assessment, risk management and risk governance levels providing new knowledge on multicomponent nanomaterials and high aspect ratio nanomaterials.

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TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

DEVELOPMENT OF A METHOD FOR SYSTEMATIC CLASSIFICATION OF NANOCARRIER SYSTEMS

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According to the EU recommendation, nanomaterials are defined by dimensions, ranging from 1-100nm. Regulators and scientists have been discussing for a long time whether nanocarriers count as a nano-form, although their diameters may often be larger than 100 nm in one dimension. The reason for this ambiguity is that nanocarriers are often based on complex nanoformulations to release active substances into the environment (controlled release), which in turn can be in the nanoscale and the carrier itself not. As these nanoformulations can be very complex, it is a major analytical challenge to determine the particle size of the nanocarrier and the release rates of the ingredients. In addition, it is difficult to assess their mobility and fate in the environment. With regard to environmental risk assessment, we like to present a novel approach to group and characterize nanocarriers through a systematic review and structuring of current technologies. Based on this review, we aim to identify undetected risks and challenges for chemical regulation. On behalf of the German Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection, a working definition and systematic categorisation of nanocarrier systems will be elaborated. For this purpose, a review of the state of development based on literature data and patents as well as expert discussions will be conducted. From the resulting categorisation (e.g., inorganic/organic or hybrid liposomal carrier systems for medical products, cosmetics or agriculture), three representative nanocarrier types will be selected with respect to potential challenges in environmental risk assessment. These "critical" types will be used to elaborate analytical methods for nanomaterial characterization. The guiding question here is whether the current test protocols (e.g., OECD guidelines) are suitable for assessing the environmental behaviour of nanocarrier types, given their specific characteristics. In this way, the influence of nanocarriers on the change in the environmental behaviour of active substances should be determined as an example. At the end, the implications for an appropriate safety assessment should be described in more detail based on the selected types, to adapt the current environmental risk assessment procedure of these advanced materials, if necessary.

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

WORKERS' EXPOSURE TO AIRBORNE HEXAGONAL BORON NITRIDE IN THE WORKPLACE: SEM-EDS CHARACTERIZATION AND PRELIMINARY REAL TIME MEASUREMENTS

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Hexagonal boron nitride (h-BN) is a binary compound consisting of planar layers in which N and B atoms alternate to form regular hexagons. The h-BN formed by few layers (FL h-BN) of nanometer thickness is used to add polymer matrices or solvent mixtures to obtain composite advanced materials with improved thermal, electrical and mechanical properties compared to the original matrices.
In our study, the production of FL h-BN, includes the synthesis phase, which is done by liquid phase exfoliation of h-BN-based ink followed by a solvent replacement phase and subsequent freeze-drying. The final phase consists of unloading the freeze-dryers and transferring the powder into storage containers, followed by equipment cleaning (STO-CLE phase).
The FL h-BN exposure study was based on the OECD harmonized multi-metric tired approach for nanomaterials, already successfully applied in other case studies such as graphene and SiO_2 nanoparticles. Real-time measurements of particle number concentration (PNC), average diameter and Lung Deposition Surface Area were acquired by a multi-metric instrument strategy including CPC (Condensation Particle Counter), OPS (Opticle Particle Sizer), DISCmini (Diffusion Size Classifier) and NSAM (Nanoparticle Surface Area Monitor). Sioutas and Mini-Moudi inertial impactors were used to collect the airborne particulate in the worker's breathing zone, for subsequent off-line analysis by scanning electron microscopy (SEM).
During the STO-CLE phase (i.e. where FL h-BN is in powder form), PNC values show an increase of about one-third if compared to background reference levels. Moreover SEM images show aggregates formed by individual layers which morphology and lateral dimensions are consistent with the material produced (crystalline flakes having average lateral dimensions between 100 and 1000nm).Finally, EDS (Energy Dispersive Spectroscopy) results confirm the presence of B and N atoms on the sampled material (Figure 1).
These results show that the combination of real-time measurements and sampling for off-line analysis represents an useful approach for the characterization of airborne FL h-BN advanced nanomaterials in the workplace. This preliminary study will be integrated with further data collection in order to acquire other necessary parameters to quantify the workers' exposure during the process.

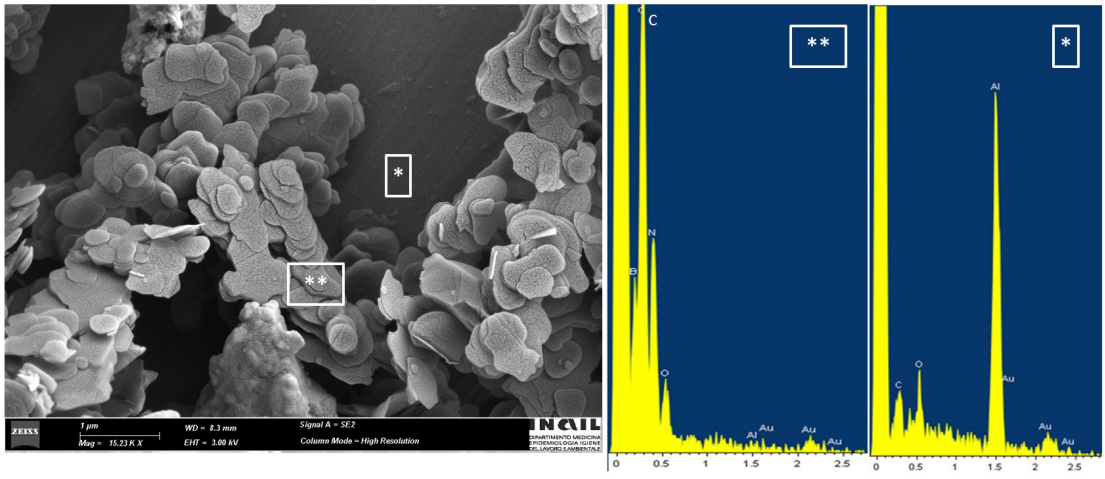

Figure 1. SEM image and EDS spectra on the sampled material (**) and on an Al-filter empty zone (*) during FL h-BN production.

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

EVALUATING THE RELATIVE HYDROPHOBICITY/HYDROPHILICITY OF METAL AND METAL OXIDE NANOPARTICLES

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The hydrophobicity of nanomaterials is a well-established feature that can dictate their toxicity level. The hydrophobicity impacts the uptake rates of nanoparticles by the cells and affects their overall toxicity. Highly hydrophilic materials have low cell uptake rates due to their repulsion from the headgroups of the membrane lipids. Similarly, highly hydrophobic compounds are poorly uptaken due to their low solubility in the water-based cytoplasm. Therefore, nanoparticles must exhibit a certain hydrophilicity/hydrophobicity balance to be able to cross the membrane and remain soluble in the cytoplasm. A proxy for the relative hydrophilicity/hydrophobicity is the enthalpy of immersion which represents the enthalpy change corresponding with the immersion of a solute in a liquid. In this work we describe the relative hydrophilicity/hydrophobicity of a nanomaterial in terms of the relative enthalpies of immersion of that slab of nanomaterial in water and in octanol, respectively. This way the behaviour of the material in aqueous and lipid phases is represented. We evaluated the immersion enthalpy of 7 metal oxides and of 17 metals samples with three different surfaces each in octanol and in water using the molecular dynamics GROMACS package. Furthermore, we employed the force field and unit lattice cell descriptors of each nanomaterial slab as training data for a neural network model to predict the immersion enthalpy values in the two solutions or a robust ranking thereof. We created an ensemble of neural network models that were trained and validated on various samples of the molecular dynamics dataset. With the present method, we were able to predict the rankings of various materials' relative hydrophilicity/hydrophobicity using only molecular interaction and geometric descriptors. Through this work we show that the ranking of materials in terms of their relative hydrophilicity/hydrophobicity can be obtained from first principles using commonly accessible descriptors, therefore eliminating the necessity for expensive molecular dynamics simulations and potential experimental input.

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

Monitoring nanomaterial-induced oxidative stress using cyclic voltammetry

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The expanding field of nanotechnology has provided novel applications in many different areas including medicine and dentistry due to the unique properties exhibited by nanoparticles (NPs). However, various types of NPs have been shown to generate reactive oxygen species (ROS), a known cause of oxidative stress, and consequently toxicity. Conventional colorimetric and fluorescence-based methods used to evaluate oxidative stress often suffer from NP-induced interferences. To address this problem, we hereby propose label-free impedance-based and cyclic voltammetry-based (CV) methods. The nanomaterials (NMs) used in this project are Ag, CeO_2 and TiO_2 of different shapes and sizes, all synthetized by Applied Nanoparticles S. L. The NMs' inherent redox properties were studied by monitoring NM-mediated oxidation of L-ascorbic acid (AA), a low molecular weight antioxidant found in blood and other biofluids, via CV with a multiplexed potentiostat along with disposable screen-printed carbon/carbon/Ag electrodes. Our results show that the sensitivity for detection of AA with screen-printed electrodes is comparable to the sensitivity obtained with a conventional electrolytic cell comprised of bulky electrodes, which require thorough cleaning and polishing after each measurement. In addition, none of the NMs tested exhibited interference with the detection method at the voltage range applied (-1.0 to 1.2 V) and dispersed in Hank's Balance Salt Solution. Notably, we observed size-, concentration- and time-dependent oxidation of AA mediated by the NMs; about 50 % of the AA in the sample was consumed (oxidized) within 3 hours when 3.5 nm CeO_2 or 8 nm TiO_2 were present. On the contrary, 50 nm TiO_2 seemed to preserve AA in the reduced state. The ranking of NMs based on AA consumption from high to low is: 3.5 nm CeO_2, 8 nm TiO_2, 142 nm x 38 nm TiO_2 nanorods, 5-10 um x 50 nm Ag nanowires, 20 nm Ag, 50 nm Ag, 10 nm x 10 nm CeO_2, 50 nm CeO_2 and 50 nm TiO_2. CV analysis of human sera exposed to NMs revealed similar findings. Thus, this method seems sensitive even in complex biological environments.
In addition, a label-based fluorescence assay (CellROX reagent, Thermo Scientific) and label-free bioimpedance analyzer (xCelligence, Agilent) were used to monitor ROS production and proliferation/viability, respectively, of lung cancer cells (A549) exposed to the different NMs in the presence or not of AA and 2-phospho-AA. In agreement with the CV data, analysis of ROS by fluorometry demonstrated significant ROS generation by A549 cells exposed to 3.5 nm CeO_2 when compared to control or to exposure to bigger size CeO_2 NMs ($p< 0,01$). Regarding viability of A549 cells, exposure to AgNW causes an abrupt and significant drop of viability at all concentrations tested and addition of AA can significantly ameliorate the toxic effect of Ag nanowires specially at the highest concentrations ($p< 0,001$). TiO_2 NMs were slightly cytotoxic and addition of AA significantly increased the viability of A549 cells, particularly for cells exposed to 8 nm TiO_2 ($p < 0,012$). AgNPs of size 20 nm and 50 nm and CeO_2 stamps were slightly toxic only at the highest concentration tested (100 µg/ml) irrespective of AA presence in the culture media. Finally, TEM images revealed considerable cytosolic uptake of all NPs, especially for 3.5 nm CeO_2 and CeO_2 stamps. Taken together, we demonstrate that CV is a suitable method for analyzing NM's inherent oxidative potential. This method is not prone to NM interference and has high-throughput potential when used along with disposable screen-printed electrodes.

Funding information
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TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

EVALUATION OF THE POTENTIAL TOXICITY OF ZINC OXIDE NANOPARTICLES (ZNO NPS) AGAINST PC3 CELLS

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Zinc oxide (ZnO) is an emerging material known for its toxicity against cancer cells and antimicrobial properties. Zinc oxide nanoparticles (ZnO NPs) are considered safe by Food and Drug Administration (FDA). The surface of ZnO is rich in -OH groups, which permit ZnO to slowly dissolve in both acidic (tumor cells and tumor microenvironment) and strong basic conditions. Based on this property, ZnO NPs have gained wide interest in biomedical applications. In this work, ZnO NPs were synthesized by a biogenic method using *Salvia rosmarinus* (rosemary) and *Mentha viridis* (mint) leaf extracts. The synthesis of ZnO NPs using green chemistry principles provides an alternative approach to ecological concerns by minimizing the use of toxic chemicals. Plant extract-mediated synthesis is a simple, cost-effective, efficient, and feasible process. The ZnO NPs synthesized by rosemary, and mint extract have an average hydrodynamic size of 266 nm ± 13 and 171 nm ± 5, respectively, indicating difference in size formation upon plant extract. Both NPs presented a similar polydispersity index (PDI) and zeta potential. X-ray diffraction patterns from ZnO synthesized with mint indicated the purity of ZnO nanocrystalline formation. In accordance with the JCPDS 36-1451card, identical to the hexagonal phase with Wurtzite structures. Through Fourier Transform Infrared (FTIR) it was possible to observe differences in the composition and electron distribution of the surface of ZnO NP synthesized with mint compared to ZnO synthesized with rosemary. This difference can be attributed to the presence of a specific capping agent given by the specific plant extract. The cytotoxicity of both ZnO NPs was screened towards human prostatic carcinoma cells (PC3). Figure 1 shows cell viability for 24 h. The results demonstrated a decrease in PC3 viability upon treatment with both NPs in a concentration-dependent manner. Indeed, ZnO NPs synthesized with *S. rosmarinus* at 20 µg. mL^{-1} decreased cell viability of PC3 cells by 75 %, whereas ZnO NPs synthesized with *M. viridis* decreased cell viability by 75 % at 50 µg. mL^{-1}. These results indicate that plant-extract ZnO NPs can be used to decrease the cell viability of PC3 cells, and the selected plant extract used for the NP biosynthesis plays a role in the decrease of cell viability.

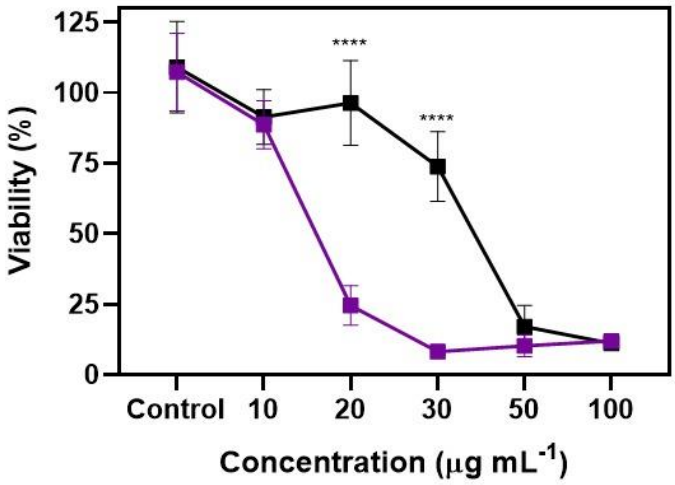

Figure 1 - Cytotoxicity assay of PC3 cells treated with ZnO NP synthesized with mint (black line) and synthesized with rosemary (purple line) at different concentrations.

TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSbD PURPOSES

EVALUATION OF THE POTENTIAL TOXICITY OF SELENIUM NANOPARTICLES (SE NPS) AGAINST PC3 CELLS

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Several works have demonstrated that selenium nanoparticles (Se NPs) are less cytotoxicity than selenite and selenomethionine. The reported anticancer activity of Se NPs is assumed to be their pro-oxidant property in tumor cells triggering the generation of reactive oxygen species leading to mitochondrial and endoplasmic reticulum damage, which in turn leads to DNA damage. In this work, Se NPs were synthesized by a biogenic method using *Salvia rosmarinus (*rosemary*)* leaf extract. Biogenic synthesis provides an option for ecological concerns by minimizing toxic chemicals. Plant extract-mediated synthesis is a simple, cost-effective, efficient, and viable process. Se NPs synthesized at room temperature (Se-RT) and heating at 100 ºC (Se-T100) have a polydispersity index (PDI) of 0.55 ± 0.01 and 0.07 ± 0.02, and a zeta potential of -15.5 mV ± 0.4 and -17.7 mV ± 0.4 at pH 9, respectively. The mean hydrodynamic size of Se-RT and Se-T100 NPs were 412 nm ± 18 and 268 nm ± 8, respectively. Heating the reacting mixture at 100 °C decreased 65 % and 87% of the hydrodynamic size and PDI values, respectively, of Se NPs, compared with Se NPs biosynthesized at room temperature. Through Fourier transform infrared (FTIR) it is possible to observe the presence of molecules derived from plant extract responsible for capping and stabilizing Se NPs. Both NPs presented the same spectrum, indicating that heating the reaction mixture did not influence the composition of the surface. The cytotoxicity of both NPs was screened towards human prostatic carcinoma cells (PC3). The results demonstrated a decrease in PC3 viability upon treatment with Se-T100 in a concentration-dependent manner. Figure 1 shows cell viability for 24 h. Interestingly, Se-RT did not show a significant decrease in PC3 viability compared with Se-T100. This difference might be assigned the lower size of Se NPs obtained by heating. The results highlight the successful biosynthesis of Se NPs and their potential toxicity toward cancer cells. We are currently evaluating the toxicity of these NPs against other tumoral and non-tumoral cell lines.

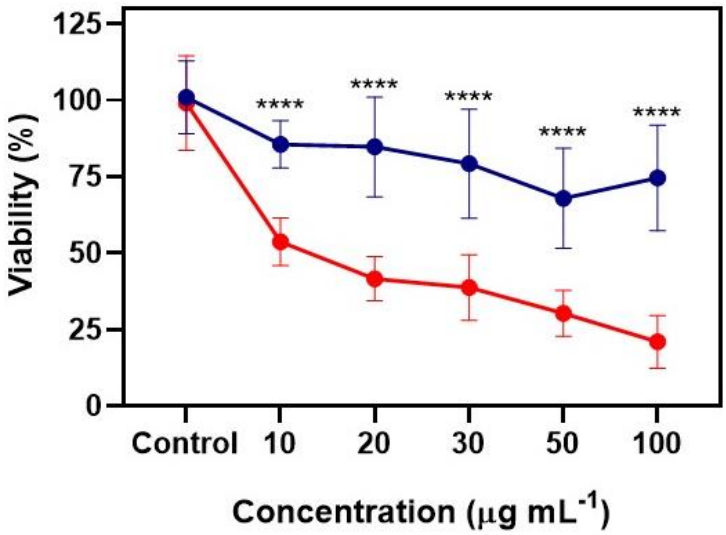

Figure 1 - Cytotoxicity assay of PC3 cells treated with Se NP synthesized at room temperature (blue line) and synthesized at 100 ºC (red line) at different concentrations (from 10 μg. mL^{-1} up to 100 μg. mL^{-1}).

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

MULTISCALE MODELLING OF MILK PROTEIN INTERACTION WITH IRON SURFACES

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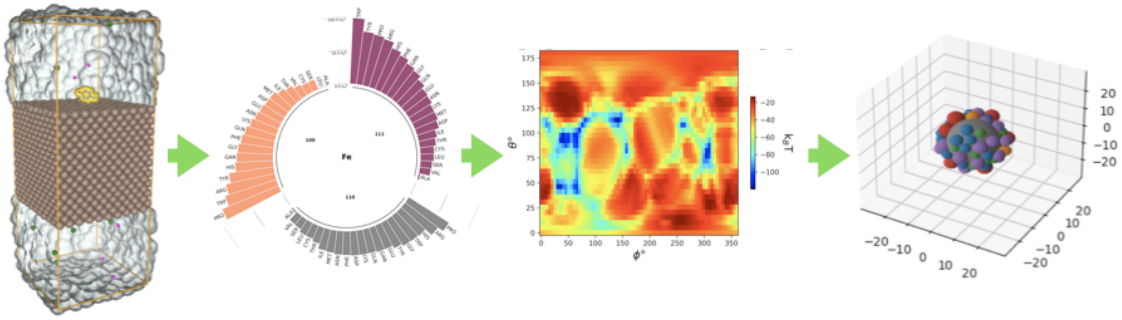
The interactions at the bionano interface, a nanoscale layer where the material comes into touch with biomolecules, regulate the biological activity and biocompatibility of inorganic materials. Understanding these interactions may help us to control fouling, development of biofilms and changes in the functionality of biomolecules. In a variety of contexts foreign inorganic materials may come into contact with biological tissues or liquids. Studying milk fouling and proteins aggregation on heat exchangers and therefore contamination problem is essential to reduce the cost of cleaning and increase the quality of milk products.
Here we study the interaction of bovine milk proteins with iron surface. Iron is one of the most common materials used in the food industry, and milk is one of the most common sources of proteins in the human diet. By calculating the adsorption energies of the milk proteins we quantify the adsorption strength and rank proteins by adsorption affinity.
Protein adsorption energies were predicted by United Atoms (UA) approach, in which protein and the nano particles (NP) are represented in a coarse-grained way. The NP is structured as a bead with the uniform density, while the protein is represented via one- bead-per- amino acid rigid body. The interaction of the whole protein with the NP then can be calculated additively, via individual terms corresponding to each amino acid (AA). The UA method is modeled with different resolutions and requires parameterization of the AA-NP potential term at short distances (< 1nm) via potential mean force (PMF) profiles obtained with all-atom AWT-Metadynamics simulations (GROMACS/Plumed). Obtained PMFs were used to predict adsorption energies of the proteins with the UA method. The coordinates of protein structures were predicted computationally by i-TASSER from corresponding sequences. Kinetic Monte Carlo was used to discuss the competitive adsorption and rank the abundance of the proteins on the surface.



Multiscale method includes all-atomistic model for each AA on NP surface to obtain PMFs,The angle-averaged heat map using CG-UA model and Finlay to rank the proteins based on the HA model of protein corona formation on spherical NP.

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANOMATERIALS

GRAPHENE OCCUPATIONAL EXPOSURE: DUSTINESS TESTING FOR EXPOSURE POTENTIAL STUDIES

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Occupational exposure to nanoscale two-dimensional materials (2DM), such as graphene, must be controlled utilizing the best occupational hygiene practices to maintain acceptable risk levels in the workplaces. The emission (and exposure) potential of these materials in dry, powder form is important factor in the design of safe working environment, and in the evaluation and assessment of current conditions in the 2DM processes.

Dustiness measurement and characterization provide users, such as manufacturers, producers, occupational hygienists, and workers, with information on the potential for dust emissions when the bulk material is handled or processed in workplaces. The particle size distribution of the aerosol as well as the morphology and chemical composition of its particles can be used to further characterize the aerosol and aid users to evaluate and control the health risk of airborne dust.

In this study, graphene nanomaterials were tested according to recent European standard for dustiness testing of materials containing nano-objects and their agglomerates and aggregates NOAA (EN 17199-1), using the rotating drum method (EN 17199-2). The dustiness indices and emission rates were determined for four different graphene materials, including graphene oxides and reduced graphene oxides. The results of this study will be ultimately combined with occupational hygiene assessment in the workplace for safety evaluations and development ideas of the tasks and activities in the graphene process.

This work was part of the Graphene Flagship Core 3 project. The Graphene Flagship is a scientific research initiative funded by the European Commission (Grant agreement ID: 881603).

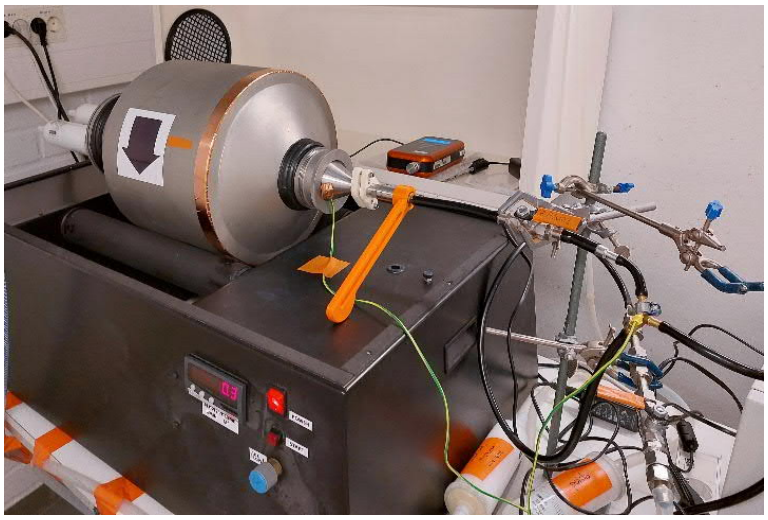

Figure 1. The dustiness test set-up.

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

INTERACTION POTENTIAL-DERIVED NANOMATERIAL DESCRIPTORS FOR MACHINE LEARNING MODELS OF BIOACTIVITY

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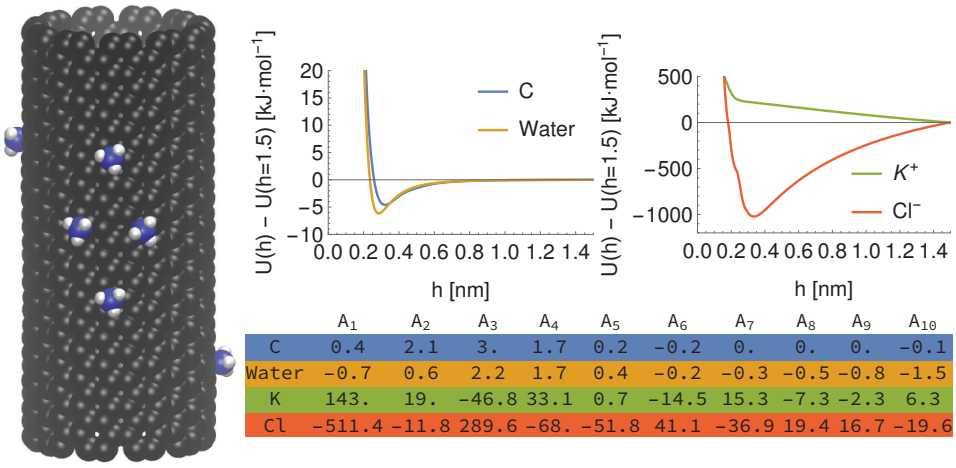
The representation of an arbitrary nanomaterial (NM) in terms of a set of descriptors for machine learning (ML) models of their advanced properties is not a straightforward task, especially if these descriptors are required to be universal and applicable to new NMs. A key challenge is including complex structural details, e.g., different crystal phases, surface modifications or defects, and the geometry and size of the NM. As a consequence of all these factors, two NMs with the same empirical formula may display very different properties, and thus NM descriptors must take into account this wide complexity.

Here, we present a methodology for producing a set of descriptors characterising an NM in terms of its interaction with its environment and its local chemical properties. We use molecular dynamics forcefields, available for a wide variety of NMs, to generate potential energy curves describing the interaction of the NM with molecular probes. By employing a set of different probes, e.g,. neutral carbon atoms, water, charged ions and small organic molecules, we build a representation of the NM including any charge distributions, the surface structure and defects, and the overall geometry. We convert these potential curves into a set of numerical coefficients, producing a compact encoding of the chemical properties of the NM in a form useful for ML models. We demonstrate the applicability of this technique by using the results as input for an ML model for the prediction of the binding affinities of small molecules to the NM, finding good agreement for a range of metal, metal oxide and carbonaceous NMs while differentiating between different crystal phases, surface types, and surface ligands.



	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}
C	0.4	2.1	3.	1.7	0.2	-0.2	0.	0.	0.	-0.1
Water	-0.7	0.6	2.2	1.7	0.4	-0.2	-0.3	-0.5	-0.8	-1.5
K	143.	19.	-46.8	33.1	0.7	-14.5	15.3	-7.3	-2.3	6.3
Cl	-511.4	-11.8	289.6	-68.	-51.8	41.1	-36.9	19.4	16.7	-19.6

Figure 1: A representation of the procedure for parameterising advanced materials. Left: A carbon nanotube with surface modification by NH_3^+ groups. Top right: potential curves for four probe species interacting with the CNT. Bottom left: basis set expansion coefficients of these potentials.

TOPIC N°6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

DUSTINESS ANALYSIS OF FINE INDUSTRIAL AND EVERYDAY-LIFE POWDERS BY VORTEX SHAKER METHOD

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Many industries use or produce materials in the form of granular material with fine grain sizes. These industrial powders are prone to become airborne during handling and processing. In addition to process-related technical issues (such as cross-contamination, product loss, filter clogging…), the uncontrolled aerosolization of powders may also lead to an increased risk of inhalation and dust explosion [1]. Dust emission from granular material is commonly referred as dustiness, which can be defined as its propensity to be dispersed in air when subjected to mechanical stimulus. Since dustiness is not an intrinsic property of a powder and highly depends on parameters such as the energy developed by the mechanical solicitation [2] or the relative humidity [3], studies have mainly focused on developing and standardizing measurement techniques. In addition to existing methods such as the rotating drum and the continuous drop, another standard method, involving a vortex shaker and enabling the emitted respirable fraction to be measured in mass and particle number metrics, has recently been developed [4]. The present study aspires to determine the vortex shaker dustiness indexes of a wide range of commercially available and everyday life powders, together with their associated physicochemical properties, pursuing a dual objective of:

1) Better understanding of the impact of powders properties (such as chemical composition, grain size and distribution or bulk/skeletal densities) on the measured dustiness indexes;
2) Building a large scope dustiness scale intended to feed databases of occupational health decision-making tools.

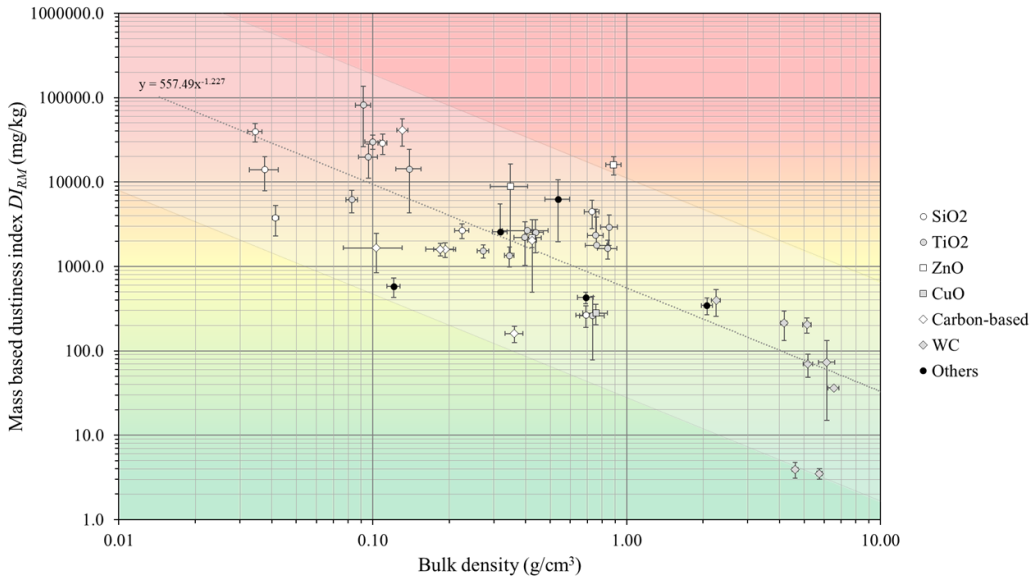

Figure 1 – Mass-based dustiness index as a function of bulk density for various powders

Preliminary results, obtained on a library of more than fifty reference powders, already evidenced a broad range of dustiness indexes, covering more than 4 orders of magnitude (respectively ranging from 3.5 to 81 000 mg/kg for mass-based indexes and from 75 to 1 884 000 p/cm^3 for number-based indexes). Additionally, trends started to emerge from results, with for instance a tendency to measure lower dustiness indexes for powders presenting higher bulk densities (as illustrated in figure 1). Ongoing work, including the screening of additional powders and the experimental determination of their interdependent physical properties, will enable to identify the main drivers responsible for powder aerosolization and to provide a more thorough vision for dustiness level classification.

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TOPIC N° 6. CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

SURFBIO - INNOVATION HUB FOR SURFACE AND COLLOID BIOLOGY SCIENCE

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Centered at the Jožef Stefan Institute, Ljubljana, Slovenia, five top research & innovation partners across Europe are creating an innovation hub to study microbe-colloid–surface interactions using high-tech methodologies and equipment. The SURFBIO Innovation Hub aims to provide biotechnology researchers, academic institutions, industry and policy makers with training services and assessments to optimize novel materials for a variety of applications and will offer new, industry-oriented, research services opened to industry and institutions, covering all the needs in only one Hub, and collecting the activities together. This will lead to the founding of a SURFBIO professional society to act as a network center with the goal of fostering advanced microbial materials applications throughout Europe.

Understanding the interactions of colloids (microorganisms, nanoparticles and biomolecules) with surfaces and between themselves is a key factor that can lead to improvements of advanced materials. As such the emerging field of Colloid Biology is positioned on the intersection between material science and molecular microbiology, dealing with artificial multispecies bio-aggregates, bio-films and bio-nano-constructs of bacteria and nanoparticles, to create novel advanced materials. The colloid-biological interactions can be studied and analyzed by applying different tools and techniques. Impacts of the networking activities will be:

- high-impact research results on surface and colloid biology
- improved knowledge transfer
- increased patenting
- increased peer-reviewed publications on the topic
- expanded range of testable samples
- contract research for industry
- boosted interest on surface and colloid biology
- standardization of methodologies
- new possibilities in analytical testing

So far, two public handbooks, two webinars and two MOOCs have been prepared and are available freely via the SURFBIO website (https://surfbio.eu/) providing information and training on the colloid biological preparation and analysis of novel advanced materials for industrial application.

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

OPTIMIZATION OF ULTRAFINE PARTICLES SAMPLING USING CASCADE IMPACTORS

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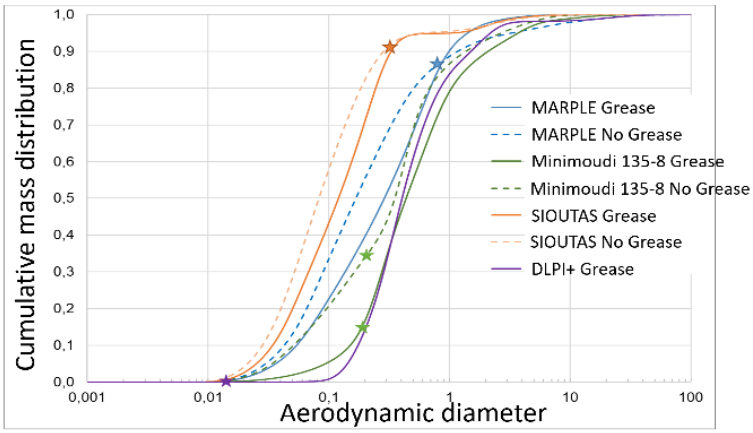
To provide characterization methods suited for occupational exposure situations, this study focused on optimizing ultrafine particles (UFP) sampling using cascade impactors. Indeed, various industrial processes may generate UFP, which are characterized by particle diameter lower than 100 nm and refer to nanosized particles not intentionally produced (welding, thermal spraying, additive manufacturing ...) (Matera *et al*, 2019). Both the size distribution of the aerosol and its chemical characterization can be obtained on the samples collected with cascade impactors.
A test bench able to produce welding fumes was used to generate a highly reproducible test aerosol of metallic ultrafine particles (mass median aerodynamic diameter MMAD = 430 nm, $\rho_{eff} = 0.55$ g.cm^{-3}). Three different individual cascade impactors were studied (Marple, Sioutas and Mini-MOUDI 135-8). The size distributions were compared to those obtained from the DLPI$^+$, a static low-pressure impactor, used as a reference (particles collected according to 14 size fractions in the range 16 nm – 10 µm). In particular, our investigations aimed at optimizing preparation of the collection substrates (PVC membranes) by determining the nature and the quantity of grease to be used. The nominal amount of grease deposited on the impaction surface corresponds to a height of 9 µm as suggested by Pak *et al* (1992). The cumulative mass distributions for the different impactors used under optimized lubrificating conditions (compared to non-greased supports) were further processed by data inversion (Bau and Witschger, 2013).



(★: *Lowest cutoff diameter of the device considered)*

For the same aerosol, the MMAD varies from 80 to 450 nm, depending on the type of device used and the way the substrates are prepared. The results (i) confirm that the collection substrates have to be prepared beforehand by lubricating them whatever the device considered, and show that (ii) the nature and the quantity of the grease has an influence on the resulting mass size distribution, and (iii) this optimization allows the phenomena of particles bouncing and re-entrainment to be significantly reduced (Schumann *et al*, 1988, Chen *et al*, 2011). Furthermore, the Mini-MOUDI 135-8 leads to results very close to those of DLPI$^+$, thanks to its high resolution in terms of number of stages (8) and low cutoff diameter (180 nm) (Matera *et al* 2022).

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TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

DEVELOPMENT OF ANTIMICROBIAL NANOCOATINGS FOR EVERYDAY SURFACES: OVERVIEW OF THE PROJECT MIRIA

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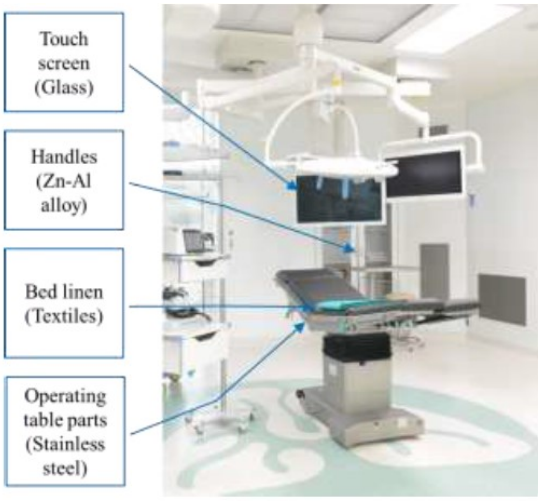
Since the end of 2019, the spread of COVID has deeply changed our lifestyle, resulting in historical events and decisions affecting the whole EU society economically and psychologically. In the wake of the COVID outbreak, there has been large concern about the infection spread of pathogens via high-traffic surfaces (e.g. door handles, handrails, and medical equipment). However, advanced and commercial product coatings that target both mixtures of pathogens (i.e. bacteria, fungi, viruses, and specifically SARS-CoV-2) and many types of surfaces (e.g., glass, metal, polymers, and textiles) are rare.

In this economic, societal and clinical context, the project MIRIA aims to fill this void by developing coatings:

- with a 99.99% effectiveness against a wide range of pathogens, namely Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus, Escherichia coli, Candida auris, Aspergillus niger, Adenovirus (BSL-2), Influenza virus, and SARS-CoV-2 (BSL3);
- that could be implemented in hospitals or other environments where cross-contamination and contagion risk are significant issues;
- which toxicity to human cells is sufficiently low and which show no sustained pro-inflammatory effects.

This will be achieved by designing different types of active molecules (i.e., nanoparticles and light-activated molecules), embedding them in coatings via different technologies (sol-gel, suspension plasma spray, bio-based coatings), and by controlling the durability and functionality of these technological solutions to select the most promising ones. As the initial project target, MIRIA case study consists of a demanding hospital environment. Pilot scale facilities will be integrated to obtain biocidal nanocoatings demonstrated at TRL6 in a simulated operating theatre environment.

MIRIA has received funding from the European Commission under the grant 101058751 from the call HORIZON-CL4-2021-RESILIENCE-01-20. Its consortium includes 15 partners from 8 EU countries.



TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

EMISSION CHARACTERIZATION OF AEROSOLS RELEASED BY HIGH TEMPERATURE METALLURGICAL PROCESSES USING METALLIC POWDERS

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High temperature metallurgical processes use metals most of the time in powder form in the manufacturing process. However, it is well known that these generate fine particle production. The French regulation tend to enforce particle and gas emission reduction in the workplace and the environment. In spite of this requirement, the nature of these emissions is not well known yet. In this context, many stakeholders of this sector of which the members of this project (CaRPE) underline the necessity to master the effluent flow (powders, particles, gas) originating from the manufacturing processes.
The main objective of this project was to focus on three processes (Thermal spraying, additive manufacturing, atomization) and to characterize the waste stream to better control it. Thus, different kind of emissions were broached in the project. Channeled emissions were characterized upstream and downstream the filtering system and filtration efficiency was assessed. Diffuse emissions were dealt with in the vicinity of the production processes. Operator exposure was also considered.
In the framework of the CaRPE project, several sites were selected to characterize different kind of emissions as indicated above. Three measurement campaigns resorting to a full panel of instruments dedicated to particle and gas detection were carried out on these sites.
The results of this presentation are only focused on thermal spraying.
Results evidence in some cases production of nanostructured particles. Despite a rather high filtering efficiency, some processes release nanoparticles with high number concentrations in the environment. Diffuse emissions were observed in certain cases. Lastly, some of the operators are possibly exposed to nanoparticles at different stages of the production.
In conclusion, the improvement of the filtration system could help controlling release at stack emission. Good manufacturing practices and use of protective equipment both collective and individual could reduce diffuse emissions and worker exposure.
In view of future developments, knowledge of the morphologies, sizes, and structures of the particles should help the manufacturer dimensioning the filtration systems and avoiding pollution. Work remains to be done to avoid as much as possible particle release to the environment, diffuse emissions, and operator exposure.

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

INNOVATIVE MASS SPECTROMETRY PLATFORM FOR NANOPARTICLE CHARACTERIZATION IN AEROSOLS

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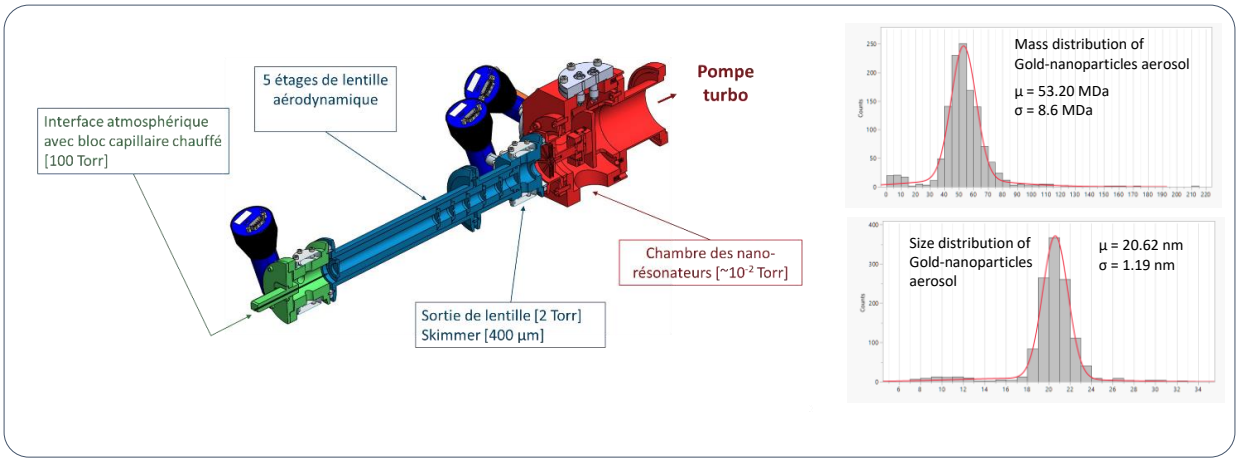
Thanks to their tunable physical, chemical and biological properties, nanoparticles (NPs) have attracted tremendous interest in industrial as well as biomedical applications. Consequently, synthetic NP production has grown with technological advances, accompanied by inevitable issues related to environmental contamination and human toxicity concerns. As a conservative estimation, the global nanomaterials market was valued at about 4.1 billion in 2015 and is expected to reach USD 11.3 billion by 2020. Therefore, scientific approaches to characterize NPs of synthetic origin are particularly important. Of particular concern are aerosolized nano-particles as they are easily displaced over long distances and therefore particularly difficult to guard against. Although NPs are generally characterized based on the size or morphological analysis, the mass of whole particles can be of interest as it represents the total amount of material in the particle regardless of shape, density, or elemental composition. In addition, the shape of nonspherical NPs presents a conceptual challenge, making them difficult to characterize in terms of size or morphological characteristics.

Recently, our group demonstrated the suitability of NEMS-MS technology to characterize the particle mass distribution of synthetic and viral NPs nebulized from a condensed phase to the gas phase. We developed a novel nano-electromechanical sensor mass spectrometry (NEMS-MS) technology to characterize the mass distributions of various NPs. NEMS resonators were mounted as a detector in a home-made NEMS-based MS prototype, which consists of a surface acoustic wave nebulizer (SAWN) coupled to three chambers with decreasing pressure: a capillary chamber (100 Torr), an aerodynamic lens chamber (2 Torr), and a NEMS-sensor chamber (7.10^{-2} Torr). The analyte was nebulized under atmospheric pressure, aspirated through the heated capillary, and collimated by an aerodynamic lens onto the NEMS sensor. The NEMS-based MS system was validated using mass detection measurements on a calibrated sample of polystyrene nanoparticles. We hypothesize that this technology could contribute significantly to our understanding of NP's fate in aerosols.



The mini NEMS-MS prototype and results from mass sensing of a 20 nm gold nanoparticles aerosol

TOPIC 6
Characterization of advanced materials, including nano materials

TOPIC 6. CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS
Topic 8. Micro and nanoplastics pollution

SILVER NANOCUBES AND THEIR PLASMONIC FEATURES AS AN EFFICIENT SERS SUBSTRATE

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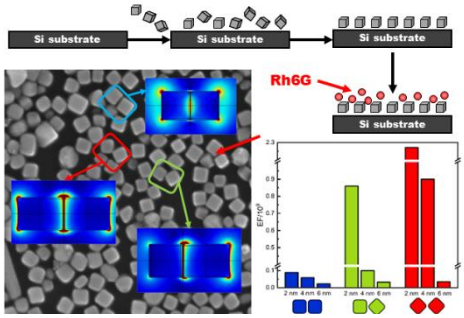
During the last years, the fate of plastics pollutants have become one of the major concerns of the research community, due to their toxic effects might have towards the environment, especially the aquatic ecosystem.

One of the most sensitive tools used to detect various species is the Surface-enhanced Raman spectroscopy (SERS), which provide the way to improve the limit of detection, enhancing the signal due to the increased electromagnetic field localization on controllable fabricated substrate features or nanoparticles assemblies.

We synthesized silver nanocubes (Ag NCs) having the edge length of 60 nm and used as efficient substrate for Rhodamine 6G detection at low concentrations based on SERS phenomenon. A high EF was 1.78×10^8, while the LOD was 4.16×10^{-12} M. From the SEM micrographs, three distinct particle arrangements were identified such as: face-to-face, edge-to-face, and edge-to-edge. The Finite Element Method simulations were employed to gain further insight in which particle arrangement contribute the most to the overall EF calculated from the experimental data. Even though, more particles arrangements substrate could be considered, the one with two AgNCs (dimer) gives more information about the local electric field with just 4 possible configurations, whilst for the rest of them, the number of unique combinations increases with each particle added to the system, thus further complicating the model with no substantial benefits. The highest calculated EF was $\sim 10^9$ for the edge-to-edge configuration followed by the edge-to-face and face-face arrangement, which is in good agreement with experimental calculated value, $\sim 10^8$, considering the frequency of appearance of each configuration.

Our studies related to the use of smaller AgNCs on Si surface, with no nanostructuration, as SERS based substrates are very encouraging, giving the chance to fabricate sustainable SERS substrates towards low concentration detection of different molecules.



[1] Q. Trung Lê et al. J. of Hazardous Materials 402, 2021, 123499. https://doi.org/10.1016/j.jhazmat.2020.123499.

TOPIC N° 6 – CHARACTERIZATION OF ADVANCED MATERIALS, INCLUDING NANO MATERIALS

INFRARED SPECTROSCOPY-BASED METHOD FOR EVALUATING CHANGES IN THE SECONDARY STRUCTURE OF PROTEINS INTERACTING WITH CERIUM OXIDE NANOPARTICLES

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Cerium oxide (CeO_2) nanoparticles (NPs) are expected to have applications in the biomedical field because of their antioxidative properties. Inorganic NPs interact with proteins at the NP surface and change their conformation[1,2]; however, the principle underlying this interaction is still unclear. This study aimed to investigate the secondary structural changes occurring in bovine serum albumin (BSA) mixed with surface-modified CeO_2 NPs using Fourier transform infrared spectroscopy. CeO_2 NPs (diameter: 240 nm) were synthesized from an aqueous cerium (III) nitrate solution using a homogeneous precipitation method. The surfaces of the NPs were modified by the catechol compounds dopamine and 3,4-dihydroxyhydrocinnamic acid (DHCA). In the presence of these CeO_2 NPs (0.11–0.43 mg/mL), β-sheet formation of BSA (30 mg/mL) was promoted especially on the amine-modified (positively charged) NPs. The local concentration of BSA on the surface of the positively charged NPs may have resulted in structural changes due to electrostatic and other interactions with BSA. Since the increase rate of β-sheet was <1.5 in BSA, which natively did not have many β-sheets, this change is likely caused by the interaction of not one but multiple molecules of BSA with the NPs. β-sheet formation may promote BSA stacking in the presence of NPs. Further investigations of the interaction mechanism between NPs and proteins are expected to lead to the safe biomedical applications of inorganic NPs.

[1] N. Sakaguchi and M. Umezawa, et al., Changes in the secondary structure and assembly of proteins on fluoride ceramic (CeF_3) nanoparticle surfaces. *ACS Appl. Bio Mater.*, 5(6): 2843-2850 (2022)
[2] S. Kaumbekova and M. Umezawa M, et al., Effect of carbonaceous ultrafine particles on the structure and oligomerization of Aβ42 peptide. *Environ. Poll.*, 323: 121273 (2023)

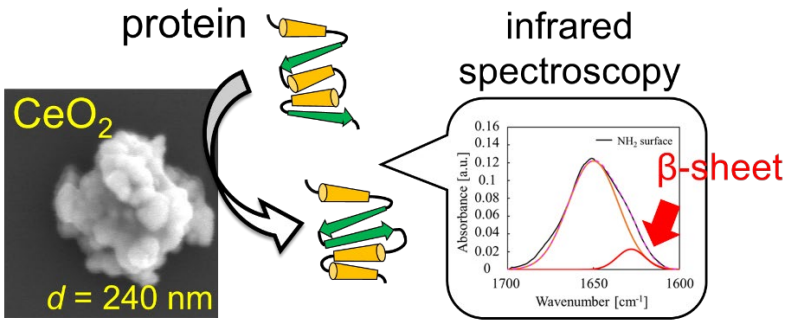

Fig. Schematic Illustration of Conformation Visualized by Infrared (FT-IR) Spectroscopy for Proteins Interacted with NPs

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

OCCUPATIONAL EXPOSURE TO GRAPHENE IN FOUR WORK ENVIRONMENTS

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Research and development of graphene materials and their applications have been gaining wide interest after it was first isolated in 2004. As a material, it shows outstanding properties that make it an attractive for many applications. Considerations of the occupational safety when studying, handling, or utilizing graphene or graphene containing materials are important due to the nanoscale nature of these materials with only little knowledge of their health effects at the moment.

The aim of this study was to assess the exposure and risk of graphene work in occupational settings. The study is a part of Graphene Flagship Core 3, EU's Graphene Flagship project, with the aim of promoting and exploring the use of graphene materials and technologies to create new technologies, products, and materials.

Exposure assessment measurements were carried out in four graphene handling environments: 1) a research laboratory, where measurements were carried out in a cleanroom and a laser laboratory; 2) a research laboratory, where measurements were done during conductive plastic granule (including 10% graphene) production; 3) a research institute, where measurement were carried out during small-scale graphene handling (weighing, pouring, dustiness testing); and 4) a company that manufactures graphene.

The measurement strategy followed EN17058 standard. In each setting, the particle number concentration and size distribution were measured with ELPI+ (Dekati Ltd.), personal exposure to nanoparticles was measured with DISCmini (Testo SE & Co. KGaA), TEM samples were collected for particle composition and morphology analysis, and filter samples were collected for elemental carbon analysis. Stationary samplings were carried out as activity based static measurements in selected locations in the nearfield and farfield areas, with the background particle concentration taken into account.

The measurements demonstrated that the nanoparticle concentrations varied between work environments. The concentration and dispersion of nanoparticles into surroundings were related to processes and the technical control means used. For example, the nanoparticle concentration was clearly higher during high temperature process compared to clean room environment. More detailed results will be presented in the conference.

TOPIC N°2 – IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

ADAPTED METHODOLOGIES FOR SSBD ENVIRONMENTAL HAZARD EVALUATION OF NANOFORMS EMBEDDED IN PAINT FRAGMENTS – SABYNA PROJECT

Patricia Solórzano Vives[1], Melanie Auffan[2], Andrea Carboni[2], Rubén Martínez[1], Melissa Faria[1], Jerome Rose[2], Armand Masion[2], Gregory Bochard[3], Socorro Vázquez[1].

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The aim of the EU-project SAbyNA is to develop tools and methodologies for Safe by Design (SbD) purposes, enhancing the SbD framework to be followed by industrial companies. Within this project, several case studies from different industrial sectors have been evaluated, such as the paint industry, in which different nanoforms (NFs) have been included in their formulations during recent years. The specific structural characteristics of the NFs such as their size, shape, and greater surface area allow modifying the properties of the newly generated paints (NEPs: nano-enabled products) which can enhance their quality, durability, functionalization, etc.
As one of the paint case studies of this project, mixed metal oxide (MMO) nanoparticles (NPs) were considered by CNRS/CEREGE as efficient, valuable, and safer alternative to titanium oxides-based materials, currently incorporated in paints to enhance their solar reflectance. These MMO NPs embedded in polymeric paint matrixes developed by CNRS/CEREGE in collaboration with ALLIOS SAS, will be the focus of environmental toxicity evaluation. We have adapted different standardized methodologies to evaluate environmental toxicity in aquatic compartments for this specific NF and NEP. The implemented methodologies include i) Microbial respiration inhibition: based on the OECD 209 merged with the *OECD 301F* and the *ISO 14851:2019* ii) acute aquatic toxicity assays before and after biodegradation, such as *OECD 202*, *OECD 201* and *Daphnia magna Feeding rate assay* based on Barata et al. 2006.

Acknowledgements – This work was developed under the "Simple, robust and cost-effective approaches to guide industry in the development of safer nanomaterials and nano-enabled products (SAbyNA)" project, financed by the Horizon 2020 EU's research and innovation funding programme.

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TOPIC N° 1. METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

CONSIDERING MATRIX EFFECTS IN THE ASSESSMENT OF ENVIRONMENTAL SAFETY FOR ENGINEERED NANOMATERIALS AND NANO-ENABLED PRODUCTS.

Emily Eagles[1], Amaia Green Etxabe[1], Fie Rubner-Petersen[1], Claus Svendsen[1], David Spurgeon[1], Sarah Thacker[2], Jasmine Pullen[2], Magda Blosi[3], Anna Costa[3], David Burrueco Subira[4], Elma Lahive[1]

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Abstract 3000 characters (Arial 10) without picture or 2000 characters (Arial 10) with picture

Engineered nanomaterials (ENMs) are often manufactured with a chelating agent to enhance the stability or functionality of the ENMs. This ENM functionality is important to the overall design and manufacture of nano-enabled products (NEPs). When considering the NEP safety, the potential ecotoxicological effects of an ENM and NEPs need to be addressed, considering firstly the relative toxicity of the ENMs; secondly, whether toxicity originates from the core particle, the chelating agent or the NEP matrix; and finally, whether there are interactions which may alter toxicity compared to that predicted from the toxicity of the individual components. Occurrence of ENMs in soil can be a result of direct addition or as constituents of industrial and domestic waste materials and sewage sludge – which are applied to soil as a conditioner. The aim of this study was to compare the fate and toxicity of silver (Ag) ENMs developed as part of a safe-by-design approach for NEPs. The Ag ENMs are synthesised with two different chelating agents (quaternized hydroxyethyl cellulose and curcumin) and provide an anti-bacterial function to the NEP. We consider the fate and effects of the ENM together with the chelating agents (alone and in combination) on a model soil species, the annelid Enchytraeus crypticus, using acute toxicity tests. Survival is monitored through time during a 96h test period, the exposures were performed in media representative of soil pore water (both synthetic and extracted soil pore water). Finally, the overall NEP will be considered by performing tests with coated textile fibres to establish whether differences in the ENMs will be important to the overall safety of the NEP.

Acknowledgements: This work was supported by the EU-H2020 project ASINA (Anticipating Safety Issues at the Design Stage of NAno Product Development), GA n.862444

TOPIC N° 2 – IMPLEMENTATION OF THE SSBD CONCEPT IN CASE STUDIES

Safe-, sUstainable- and Recyclable-by design Polymeric systems A guidance towardS next generation of plasticS (SURPASS)

Andrea Tummino[1], Clara Hoyas[2], Simon Clavaguera[1] on behalf of the SURPASS consortium

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Plastic waste outlive us on this planet as they take centuries to break down. The hazard of endocrine disruption by leached substances, fast growing and pollution of land, air and water as well as greenhouse gas emissions are only some of the adverse effects of plastic waste on public and environmental health. Still, 70% of plastic waste collected in Europe is landfilled or incinerated.
The overall objective of SURPASS project is to lead by example the transition towards more Safe, Sustainable and Recyclable by Design (SSRbD) polymeric materials. The SURPASS consortium of 13 partners consisting of research and technology organizations and industries will:

1. Develop SSRbD alternatives with no potentially hazardous additives through industrially relevant case-studies (TRL3-5) targeting the three sectors representing 70% of the European plastic demand:
   - Building: bio-sourced polyurethane resins with enhanced vitrimer properties to replace PVC for window frames (≥ 40% carbon footprint reduction)
   - Transport: lightweight, therefore less energy-consuming epoxy-vitrimer (≥ 30% carbon footprint reduction), as alternative to metal for the train structure, anticipating emerging use of non-recyclable composites.
   - Packaging: MultiNanoLayered films involving no compatibilizers to replace currently non-recyclable multi-layers films (≥ 60% carbon footprint reduction).
2. Optimize reprocessing technologies adapted to the new SSRbD systems to support achievement of ambitious recyclability targets.
3. Develop a scoring-based assessment that will guide material designers, formulators and recyclers to design SSRbD polymeric materials, operating over the plastic's entire life cycle, including hazard, health, environmental and economic assessment.
4. Merge all data and relevant methodologies in a digital infrastructure, offering an open-access user-friendly interface for innovators.

SURPASS will in particular address its results to SMEs, representing more than 99% of enterprises, and therefore has an outstanding potential to contribute to the transition towards green economy.

Involved partners:

1. **CEA**: Commissariat à l'Energie Atomique et aux Energies Alternatives, France
2. **IPC**: Centre Technique Industriel de la Plasturgie et des Composites, France
3. **CIDETEC**: Fundacion CIDETEC, Spain
4. **LEITAT**: Acondicionamiento Tarrasense Associacion, Spain
5. **INDRESMAT**: Indresmat SL, Spain
6. **UGA:** Universite Grenoble Alpes, France
7. **GEONARDO LTD:** Geonardo Environmental Technologies Ltd, Hungary
8. **BASF SE**: BASF SE, Germany
9. **RIVM**: Rijksinstituut voor Volksgezondheid en Milieu, Netherlands,
10. **UNE**: Asociacion Espanola de Normalizacion, Spain,
11. **FhG ICT**: Fraunhofer Gesellschaft zur Forderung der Angewandten Forschung EV, Germany,
12. **WIPAK**: WIPAK Gryspeert SAS, France,
13. **WFO**: Waste Free Oceans Europe, Belgium,

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TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

INTERACTIVE AND DYNAMIC SCOPING REVIEW ON THE RELEVANCE OF EXTRACELLULAR VESICLES IN NANOTOXICOLOGY

João Meneses[1], Thais S. M. Lima[2], Eli Fernández-de Gortari[1], Ernesto Alfaro-Moreno[1], Ana Ribeiro[1]

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The widespread use of nanomaterials (NMs) has been revolutionizing our society, demanding new approaches to evaluate their nanosafety and potential toxic effects on human health. Emerging studies suggest that extracellular vesicles (EVs), which play a critical role in intercellular communication, can be affected by NMs exposure. However, there is still limited knowledge of the influence of NMs on EVs and the mechanisms underlying these effects. Thus, this work aims to identify and map the available evidence on the outcome of NMs on cellular responses mediated by EVs.
We conducted a systematic search on databases (e.g., PubMed) following the PECO model (Population = EVs, Exposure = NMs, Comparator = EVs without exposure to NMs, Outcome = Cellular responses in EVs) to help methodologically assess the association between exposure and outcome. We screened 2944 articles and selected 18 for the final review. To analyse the effects of NMs exposure on EVs secretion and consequent biological outcomes, we developed a dynamic and interactive co-occurrence network of the most relevant keywords in the selected studies (see Fig. 1).
Results showed that NMs exposure affects EVs biogenesis, miRNA cargo, and protein expression, which are all involved in cell communication. The most frequently referred biological activities in the 18 articles were angiogenesis, biogenesis, and cell communication. In 15 out of the 18 selected studies, NMs were able to stimulate EVs biogenesis. Additionally, we found that the presence of gold nanoparticles, MIONs, and single-walled carbon nanotubes influenced NMs' elimination.
Overall, our findings contribute to understanding EVs in nanotoxicology, emphasize the importance of considering EVs in the risk assessment of NMs, and highlight the need for further research to elucidate the underlying mechanisms.

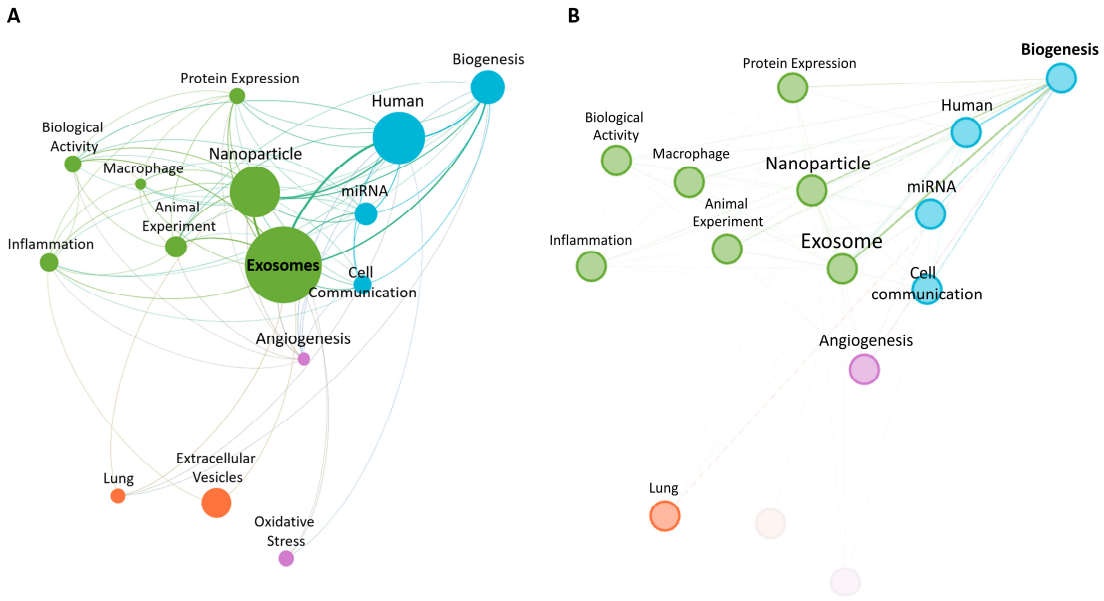

Fig. 1 *Co-occurrence network of the most frequently used keywords in the 18 selected studies. (A) Full network; (B) Highlight of Biogenesis connections. Each colour suggests a set of keywords shared among the studies.*

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

IN VITRO-BASED ENVIRONMENTAL RISK ASSESSMENT OF NANOMATERIALS

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Nanomaterials (NMs) have unique properties at the nanoscale that have revolutionized various industrial sectors, including energy, food, and medicine. However, the potential adverse effects of these materials on human health and the environment are of increasing concern. Risk Assessment (RA) can be adapted to identify and quantify the risks resulting from releasing NMs into the environment and their resulting exposure to humans and ecosystems. However, the ability of RA to quantify risks associated with NMs heavily relies on the availability of in vivo studies, which are becoming less common due to the push for humane animal experimentation. In the field of nanotoxicology, demanding requirements for in vivo studies pose a challenge for RA. As new NMs are rapidly developed, it becomes increasingly tricky to promptly calculate the corresponding dose-response assessment.
In vitro tests, with reduced costs and ethical concerns, offer a promising alternative source for evaluating the risk assessment of NMs, but their integration into RA methodology has not been sufficiently explored. Previous work (Romeo, 2022) explored an extrapolation strategy from in vitro to animal data focused on effect factors in life cycle assessment (LCA). Combining in vitro dosimetry and lung dosimetry, the Combined Dosimetry model (CoDo) was developed as a promising strategy for inhaled spherical particles. In addition to CoDo, a parallelogram approach was chosen to extrapolate from cellular to whole-organism responses (as an in vitro-to-in vivo extrapolation factor).
Drawing from previous research, we propose adapting the approach used in LCA studies to explore the feasibility of incorporating in vitro data into the RA methodology for NMs. Building upon the extrapolation strategy for the effect factors, we can utilize the same method to assess the potential risks of identified hazards with the help of in vitro data.
Furthermore, the extrapolation of in vivo results based on in vitro information has revealed the need for fit-for-purpose data to ensure thorough validation and generalization. The extrapolation process may fail due to missing data in the extrapolation chain. Advanced techniques such as supervised learning and deep learning can be investigated to maximize the coverage and usage of available in vitro and in vivo datasets. By inputting appropriate nanomaterial physicochemical descriptors and experimental conditions, the resulting model can accurately capture the structure-activity relationship, supporting the prediction of nanomaterial activities and accelerating the integration of engineered nanomaterials into advanced applications while enhancing their safety during use.

Reference:
Romeo, D. (2022). *In vitro-based Life Cycle Impact Assessment: Developing human effect factors for inhaled nanoparticles* [PhD Thesis]. ETH Zurich.

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

AUTOMATIC WORKFLOW FOR IN VITRO HIGH-THROUGHPUT SCREENING DATA FAIRIFICATION, PREPROCESSING AND SCORING

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The development of new chemical substances and advanced materials, including nanomaterials (NMs), pose complex challenges to ensure safety for humans and environment. Regulatory agencies are interested in adopting safety data generated under the umbrella term "New Approach Methodologies" (NAM) encompassing technologies for high-throughput, efficient integration of experimental data, QSAR and read across reports during innovation, from an idea to a chemical substance/NM product launch. Data management based on FAIR (Findability, Accessibility, Interoperability, and Reuse) guiding principles supports consistent curation and reuse of the accumulated data by the nanosafety, cheminformatics and bioinformatics communities. In vitro high-throughput screening (HTS) hazard data is used for efficient clustering, ranking, prioritization of NMs and read across. A previously developed in-vitro toxicity scoring and ranking concept, the Tox5Score [1] is applied in two stages: (i) normalization of the HTS metrics, separately in the range [0,1], for each time point and endpoint; (ii) combination of the normalized metric values to obtain final Tox5 endpoint scores. However, the usage of Excel based data preprocessing with the application of the software ToxPi (the US-EPA Toxicological Prioritization Index) requires time consuming manual processing, which is hard to scale up for larger NM datasets and occasionally prone to errors.

Here we present an automated workflow for data FAIRification, preprocessing and calculation of the Tox5Score from raw HTS data. A new Python module for collection and annotation of raw data, consequent normalization and calculation of dose-response metrics was developed. The module can be used independently or as a part of developed by us Orange [2] workflow with custom widgets for fine tuning of the data processing. The Orange (open source system for visual programming and machine learning [2]) workflow includes separate widgets for data normalization, dose-response calculation, Tox5 in-vitro toxicity scoring, ranking for specific cell, visualization of ToxScore for endpoint- and time-point-specific toxicity, ranks, and combined toxicity scores for each material. The widget's table output can be exported in convenient file formats (e.g. CSV).
In addition, the new Python module and Orange workflow extends the eNanoMapper FAIRification workflow [3] by facilitating FAIRification of HTS data. The resulting FAIR data includes both raw and interpreted data (scores) in machine readable format and can be distributed as data archive and/or be integrated into the eNanoMapper database and Nanosafety Data Interface [4].

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TOPIC MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

OPEN-SOURCE FOR RAMAN SPECTROSCOPY HARMONISATION

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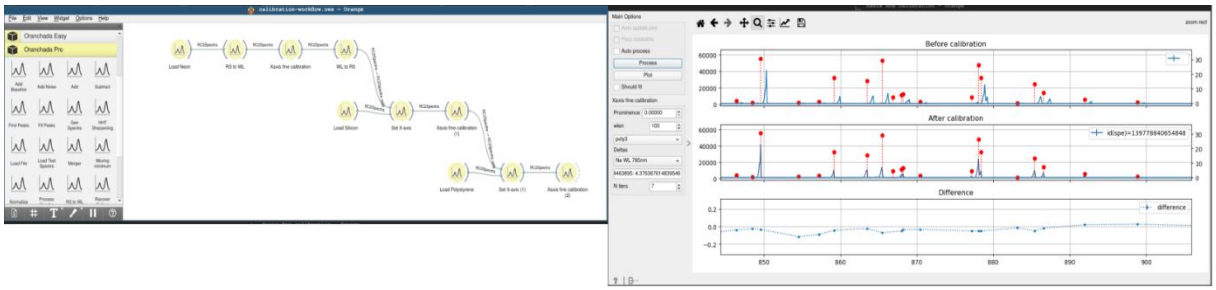
Raman spectroscopy is becoming a key technology used in the research and development for characterization of materials. Ideally, spectra should be comparable and linked to specific material properties. However, in real life, Raman spectra differ between instruments and depend on the spectrometer, optical path, or sample environment, among others, and only a limited number of calibration standards for Raman spectroscopy are available to date 10.1177/00037028221090988. The CHARISMA EU's Horizon 2020 project (No. 952921) aims to harmonize and standardize characterization by Raman spectroscopy, including hardware, measurement protocols, and in silico methods, enabling end users to share digital spectral data through a FAIR database across domains and across the entire life cycle of diverse products.

We present **https://github.com/h2020charisma/ramanchada2**, an open-source, MIT-licensed, Python package that collects existing and novel state-of-the-art algorithms allowing the users to process Raman spectra, perform energy and efficiency calibrations and generate synthetic spectra based on user specification and/or data from DFT simulations. Installation with pip is available.ramanchada2 is used as a core dependency of a FAIR Raman database, implemented as a Python API with a cloud backend and HDF5 storage for Raman spectra and metadata. The metadata query and spectra search API is compatible with the eNanoMapper database and the NanoSafety Data Interface 10.1038/s41565-021-00911-6.
To facilitate Raman spectra analysis by end users, we are also developing **oranchada**, an user friendly wrapper of all **ramanchada2** functionality. It is available as an add-on for the popular data mining software Orange.
For the harmonisation of Raman spectroscopy terminology, a collaboratively edited knowledge base is established at https://wiki.charisma.ideaconsult.net/. It is implemented with Wikibase, an extension for MediaWiki.



Screenshots of **Oranchada** https://github.com/h2020charisma/oranchada

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

NANOPASS – BRIDGING THE GAPS IN NANOSAFETY FOR ANIMAL-FREE PREDICTION OF ADVERSE OUTCOMES

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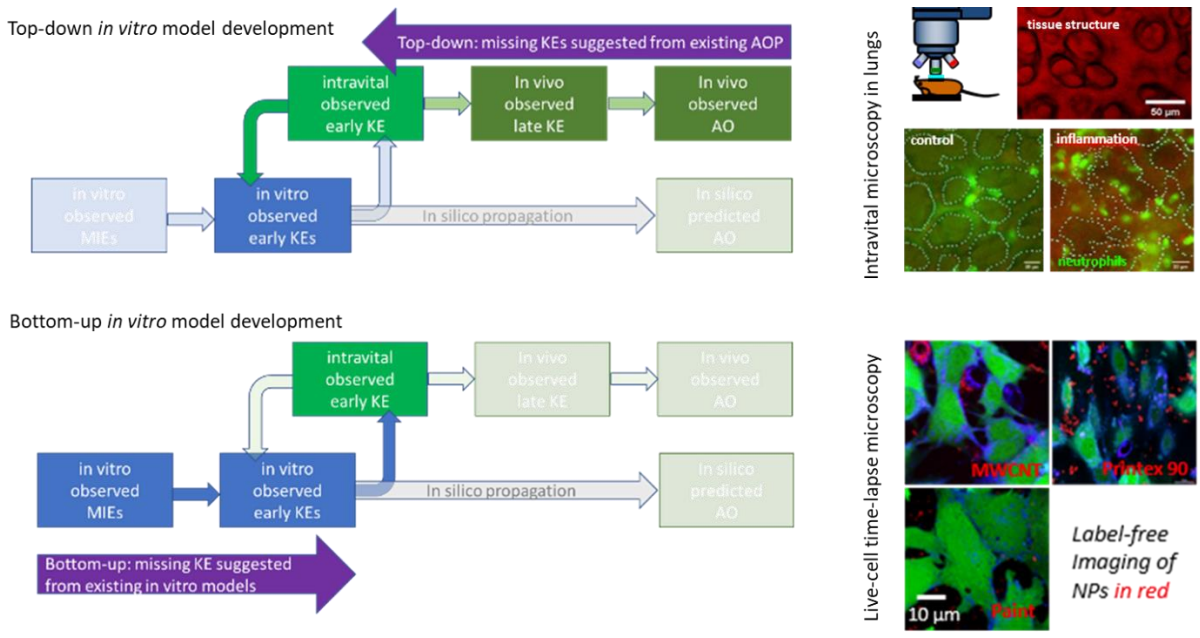
The current animal-based testing of materials' short- and long-term health effects is slow, expensive, and has limited capacity, which stifles the development of new (nano)materials and hinders efficient regulation of the market. To enable cost-efficient high-throughput screening required for industry and regulation, the recently started EU-funded nanoPASS project proposes to shift the focus of nanosafety testing from late endpoints to early key events (KEs) leading to adverse outcomes (AOs). As such tests can only be based on mechanistic understanding, we need to close the knowledge gaps and match KEs *in vitro* and *in vivo*. To this end, the *nanoPASS* consortium will apply key bridging methods: *intravital in vivo* microscopy, quantitative time-lapse *in vitro* microscopies, and automated identification of the modes of action (i.e. KE relationships) with proprietary *in silico* algorithms, supported by datamining of the worlds' largest in vivo database and single-cell omics data, and computational modelling of structure-function relationships.
With this toolset, we aim to 1) develop new *in vitro* systems that can replicate early KEs leading to AOs related to inhalation of NMs, 2) identify methods to track the dynamics of these KEs, 3) develop quantitative *in silico* models to predict AOs, and 4) calibrate the *in vitro/in silico* AO predictions against *in vivo* data for 40+ well-characterized benchmark materials. Finally, we will 5) validate the AO predictions on several families of industrial materials sampled at different stages of their life cycle (including construction materials, new advanced nanomaterials, electronic waste, plastics, and medical materials), and then propose reliable testing protocols to ECVAM and guidelines to OECD. With the consortium of 6 complementary research laboratories, an SME as the developer and provider of the testing technology, a material producing company as potential end-user, and an industrial association to facilitate dissemination, *nanoPASS* covers the whole value chain of the new animal-free safety testing technology, and thus paves the way towards safe adoption of new nanotechnologies.



Key methods and concepts of the new EU-funded project nanoPASS

TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

SCREENING LEVEL ENVIRONMENTAL EXPOSURE ASSESSMENT THROUGH THE SIMPLEBOX4NANO WEB APPLICATION

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The ability to predict the exposure of chemicals in the environment is crucial for assessing their potential risks. However, conducting exposure assessments for chemicals can be expensive and time-consuming, especially when dealing with large numbers of chemicals. Screening level environmental exposure models provide a cost-effective and efficient solution to this problem. They are designed to provide conservative estimates of exposure without the need for complex data requirements, making them a valuable tool in regulatory environmental risk assessment. The increasing use of nanomaterials and nanoforms in various industries has led to concerns about their potential risks to the environment and human health. Traditional exposure assessment models have had to be adjusted to account for the unique properties of nanomaterials and predict their exposure accurately. This has led to significant investment in the development of nanomaterial exposure models [1-3], including the SimpleBox4Nano (SB4N) model [3]. However, the end-users of these models, including industry professionals, regulators, and consultants, are not typically experts in modeling or software development. Therefore, it is essential that these screening level exposure models have intuitive and user-friendly interfaces. To address this issue, a web application based on the SB4N Excel version was developed. The web application (http://www.enaloscloud.novamechanics.com/beta/simplebox4nano/) provides a user-friendly environment without compromising the capabilities of the original Excel-based version. In addition to the web application, SB4N is also available as an open-source project on GitHub (https://github.com/NovaMechanicsOpenSource/SimpleBox4Nano), making it accessible to anyone interested in using or developing the model further. The Proplanet project has already expressed interest in further extending it with new materials. The development of user-friendly screening level exposure models like SB4N and its web application is a critical step in facilitating the responsible use and regulation of nanomaterials and other chemicals, helping to ensure their safety and minimize their potential risks to the environment and human health.

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TOPIC N°7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

ENALOS CLOUD PLATFORM: DEMOCRATIZING ACCESS TO DATA DRIVEN, MACHINE LEARNING & ARTIFICIAL INTELLIGENCE CHEM/NANO INFORMATICS MODELS

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Enalos Cloud Platform, developed by NovaMechanics Ltd, is a cutting-edge cloud platform that provides an innovative solution for cheminformatics and nanoinformatics modeling. The platform is available online and free of charge, making it accessible to researchers and scientists globally. It is powered by a broad range of predictive models that are provided as web services. These models are built on reliable open-source software, such as KNIME platform, WEKA, ImageJ, and R, as well as proprietary software (Enalos+ nodes). Next, through Enalos Cloud Platform, they can be made available under the technology of Software as a Service (SaaS), making them highly accessible and easy to use for researchers and scientists alike. This combination of software and resources enables the platform to provide scientists with flexible and powerful cloud computing resources that reduce the barriers to entry for complex scientific calculations. Enalos Cloud Platform also offers a user-friendly environment that is specifically designed for non-informatics experts. More specifically, the platform has been designed to reduce the time and resources spent on experimental activities and to provide researchers with a highly efficient and effective way of predicting toxicity and property calculations for chemical structures or nanomaterials. The platform is thus an invaluable tool for researchers in the fields of drug design, materials science, and patient stratification decision-making processes. Enalos Cloud Platform is actively involved in supporting several H2020 European projects, including NanoCommons, Scenarios, EthnoHERBS, Capstone, RiskGONE, Sabydoma, DIAGONAL, NanoSolveIT, and CompSafeNano. By collaborating with these projects, Enalos Cloud Platform is dedicated to democratizing access to data-driven, machine learning, and artificial intelligence cheminformatics and nanoinformatics models. Through these partnerships, Enalos Cloud Platform is expanding its capabilities and providing scientists with state-of-the-art tools and resources for addressing complex scientific challenges in drug discovery, materials design, and risk assessment.

Funding information
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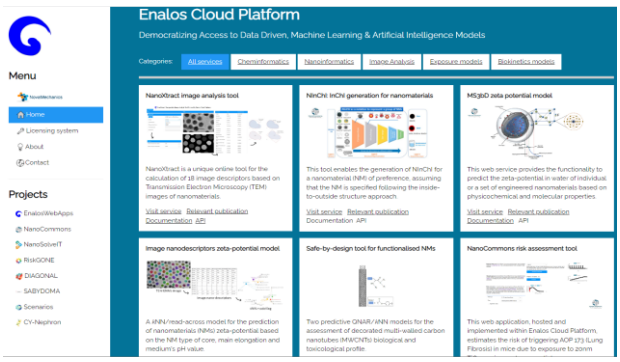

Enalos Cloud Platform landing page, http://www.enaloscloud.novamechanics.com/

TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

NANOCONSTRUCT: A TOOLBOX FOR THE DIGITAL RECONSTRUCTION OF ENERGY MINIMIZED NANOPARTICLES, NANOSHEETS AND NANOTUBES POWERED BY ENALOS CLOUD PLATFORM

Panagiotis D. Kolokathis[1,2,3], Nikolaos K. Sidiropoulos[1], Andreas Tsoumanis[1,2,3],Anastasios G. Papadiamantis[2,3], Iseult Lynch[3,4], Antreas Afantitis[1,2,3]

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Computational tools have been widely used for predicting material properties in the past. Nowadays, the rapid development of computers has increased interest in these tools due to their speed and low cost compared to physical experiments. Furthermore, mixing nanomaterials with other materials to create sols, emulsions, gels, or foams can lead to improved material properties. Efficient computational screening techniques are needed to quickly discover the most promising combinations. Atomistic simulations can be used for the screening process after digitally reconstructing the systems to be investigated. However, digitally reconstructing systems containing secondary phases, such as Nanoparticles (NPs), Nanosheets (NSs), and/or Nanotubes (NTs), is not a straightforward task. NanoConstruct, a toolbox powered by the Enalos Cloud Platform (enaloscloud.novamechanics.com), has been developed to overcome this barrier. NanoConstruct uses Crystallographic Information Files available on crystallographic databases as input to geometrically reconstruct crystalline NPs, while maintaining stoichiometry by removing excess atoms on the surface. Additionally, NanoConstruct searches the OPENKIM database and selects the Force-Field (FF) that is less generic and simultaneously contains every chemical element of the NP. The option to select a different OPENKIM FF than the suggested one is also available. After the FF selection, energy minimization is applied to investigate the NP's stability, while several descriptors are calculated for subsequent Machine Learning analysis. A similar procedure is applied for the NS and NT construction.

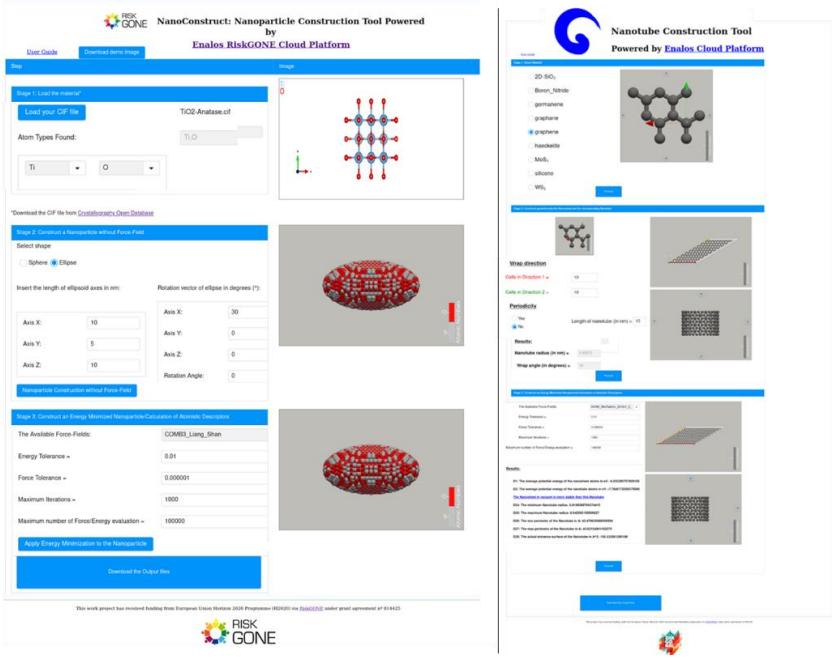

Figure 1. NanoConstruct Graphical User Interface

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TOPIC N° 1 – METHODS, TOOLS AND TECHNOLOGIES FOR SSBD PURPOSES

ZETA PREDICT: AN ENALOS CLOUD PLATFORM TOOLBOX FOR THE ZETA POTENTIAL CALCULATION OF COATED SPHERICAL NANOPARTICLES BASED ON SEM/TEM IMAGES, DLVO THEORY AND ATOMISTIC FORCE FIELDS

Panagiotis D. Kolokathis[1,2], Nikolaos K. Sidiropoulos[1], Konstantinos Kotsis[3], Vladimir Lobaskin[3], Antreas Afantitis[1,2]

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Zeta (ζ) potential is a widely used metric to describe the stability of colloidal dispersions. Zeta potential is the value of the electric potential at the slipping plane (e.g. at the distance of a Debye length from the surface which is considered to be the thickness of the double layer). Several experimental techniques are used to calculate the zeta potential (e.g. electrophoresis, streaming potential, colloid vibration current etc.). The Zeta-Predict toolbox powered by Enalos Cloud Platform suggests an alternative method for the zeta potential calculation based on SEM/TEM images of colloidal dispersions, the DLVO theory, and atomistic force-fields. In this method, special treatment has been applied for the van der Waals interactions by applying the Hamaker equations for coated spherical nanoparticles. The method is limited to colloidal dispersions that experience coagulation after large time intervals where the colloidal particle distances remain unchanged. The equilibrium distance among these colloidal particles is calculated using SEM images imported through the Zeta-Predict Toolbox by the user. Zeta-Predict toolbox provides the option to users to calculate the particle radius (core and shell) and the interparticle distance by selecting points in the SEM image using their computer mouse. Moreover, users insert as input the temperature, the dielectric constant of the solvent, the chemical formulas, and the densities of the nanoparticle (core and shell) and the solvent. In addition, the concentration, and the valence of the anions/cations inside the solvent are selected too. After running the Zeta-Predict toolbox, the zeta-potential, the surface electric potential and the surface charge density are calculated and provided as output at the bottom of the Graphical User Interface.

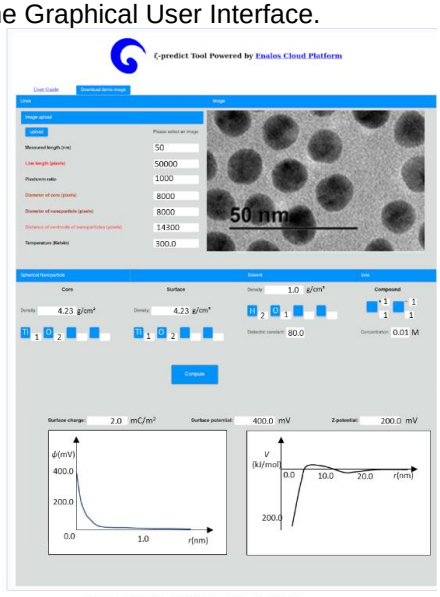

Figure 1. ζ-predict Graphical User Interface

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TOPIC N° 7 – MODELLING, DIGITALIZATION OF NANOSAFETY AND DATA MANAGEMENT

META-MODELLING FOR PREDICTION OF INTERACTION POTENTIALS BETWEEN BIOMOLECULES AND THE SURFACE OF NANOMATERIALS

Ewelina Wyrzykowska[1], Mateusz Balicki[1], Ian Rouse[2], Julia Subbotina[2], Parinaz Mosadeghi[2], Iwona Anusiewicz[1,3], Piotr Skurski[1,3], Vladimir Lobaskin[2], Tomasz Puzyn[1,3]

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Potential interaction between nanomaterials' surface and biomolecules inside human body are relevant for the safety and hazard assessment from the perspective of protein or bio-corona formation and surface reactivity of nanomaterials. Although experimental testing requires substantial workload, financial and time resources, surveys can be provided by combining diversified computational approaches. New nanoinformatics methods and approaches are developed in numerous projects focused on safety of nanomaterials to delivery tools enabling the increase of knowledge about toxicity mechanisms and estimation of potential hazard before synthesis and marketing. Thus, computational approaches are critical for the *safe-and-sustainable-by-design* (SSbD) approach.
Here, we present an approach of meta-modelling, which integrate molecular modelling with machine learning modelling, where the first approach allows for simulation of interactions between nanosurface with biomolecules, while the second approach affords identification of quantitative relationships and prediction of interaction potentials for new nanomaterials and biomolecules previously not considered. The developed quantitative structure-property relationships model (QSPR model) estimates the adsorption energy of biomolecules on the surface of nanomaterials taking into account characteristics of both kind of considered individuals expressed numerically by so-called descriptors. Here, biomolecules are represented by simple constitutional and topological descriptors. However, nanomaterials required development of more sophisticated descriptors facing the unsolved so far issue of appropriate molecular model of nanoparticle/nanosurface/nanotube for quantum-mechanical calculations or simplified descriptors encoding their physico-chemical characteristics. Thus, next to the main goal of the development of meta-model for prediction adsorption energy, the indirect goal of our study was to delivery new methodology for nanomaterials' descriptors calculation. In result, we provided a set of original descriptors reflecting e.g., molecular roughness and likely interaction energy, which are essential for the electrostatic and exchange interactions between biomolecules and the surface of nanomaterials. The training set of developed meta-model includes metals, metal oxides and C-based nanomaterials with diversified structure e.g., clusters, surfaces, tubes; and amino acids being simple representants of biomolecules. Considering the fact, that the meta-model combines independent descriptors for nanomaterials and biomolecules, it can be applied for the estimations of diversified combinations of new nanomaterials and biomolecules. The obtained statistical parameters confirm its credibility and potential benefits in the application for SSbD of new nanomaterials.

Acknowlegements: The research was supported by the European Commission Horizon 2020 programme, grant No. 814572 (NanoSolveIT) and No. 101008099 (CompSafeNano).

TOPIC N° 8 – MICRO AND NANOPLASTICS POLLUTION

STUDY OF THE VOC/UFP COEXPOSURES INDUCED EFFECTS: PREREQUISITES AND EXAMPLE OF POLYMER 3D PRINTING

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The exposure to different chemicals, in various physical forms, is a reality at workplace. Yet few toxicology studies have considered the hazard associated with the inhalation of complex mixtures, as is the case in additive manufacturing.
Additive manufacturing is rapidly expanding in recent years and has applications in many occupational sectors. One of the processes used is the technique of fused filament fabrication. It consists in manufacturing an object by laying heated polymer through a nozzle. This process can produce fumes composed of volatile organic compounds (VOCs) and ultrafine particles (UFPs) aerosol.
For example, the acrylonitrile-butadiene-styrene (ABS) polymer, which is highly emissive, generates PUFs, VOCs and other substances included in the composition of filaments (flame-retardants, metals, plasticizers, dyes, etc.). Even if the atmospheric concentrations of each substance do not reach their occupational exposure limit value, some questions remain about the health effects of these vapours and particles mixtures. Some studies in humans or rats have shown effects on the respiratory tract and on the arteriolar blood pressure; however, these studies need to be strengthened, by inhalation toxicology studies.
For this purpose, a generation device must enable the production of a steady atmosphere for at least 6 hours and reproducible over days with controlled PUF and VOC concentration levels. Initial results obtained with a commercial 3D printer have shown that PUF emissions from an ABS filament are neither constant over a day nor reproducible between prints. The use of a test bench reproducing fumes released during the thermo-degradation of polymers used in 3D printing is therefore required to overcome the constraints of commercial printers (limited temperature ramp, insufficient quantity of aerosol, unsteady emissions, untight enclosure). After the characterization of the fumes, the device will be integrated into the rat oro-nasal exposure system. During this second step, care will be taken to ensure the transport of the fumes to the inhalation towers while preserving their physico-chemical properties. This test bench will also be used to assess the toxic properties of other types of polymers used in additive manufacturing.

TOPIC N° 8– MICRO AND NANOPLASTICS POLLUTION

AGRICULTURAL PLASTIC POLLUTION : WASTE MANAGEMENT STRATEGIES FOR ENVIRONMENTAL SUSTAINABILITY

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The need for agricultural plastics has expanded as a result of the development of agricultural activities and the shift to precision/smart agriculture. At least 28 million farmers in the EU employed plastics to increase crop yields, control insects and weeds, and keep the soil moist. According to the established research, synthetic plastics are the preferred material for mulching films, protective nets, greenhouse covering materials, agrochemical containers, protection nets, seed trays, spray tanks, and insect traps. Due to their tendency to degrade into tiny particles like microplastics and nanoplastics as well as the release of certain chemicals due to ultraviolet radiation from the sun that are added during the manufacturing process (additives) to improve the properties of the plastic, plastic materials are one of the main pollutants in ecosystems (such as biophenol).

Sustainable approaches to managing plastic waste, however, did not keep up with the rising demand for plastics. 10% of agricultural plastics, according to recent studies, were recycled properly. The vast majority of the used polymers were dumped in landfills. In the treatment of plastic waste, a number of interventions were investigated, including liquefaction and physical, chemical, and mechanical recycling. In other instances, scientists and industrial farms had invested in the progressive replacement of plastics derived from fossil fuels with biodegradable materials. Every waste management plan has particular advantages and drawbacks.

The purpose of this work is to present alternative waste management strategies (Figure 1) and evaluate their environmental consequences given that all methods have advantages and drawbacks (eg Nanoplastics vs LCA performance) including latest developments and state of the art technologies.

Figure 1. Agricultural Plastic Waste Management Methods.

TOPIC N°8 - MICRO AND NANOPLASTICS POLLUTION

EFFECT OF SIMULATED GASTROINTESTINAL DIGESTION ON THE TOXICITY OF BACTERIAL BIOFILMS COLONIZING HDPE AND PET MICROPLASTICS

Giulia Antonello[1,2,3], Alberto Katsumiti[4], Itziar Polanco[4], Francesco Barbero[3], Vanesa Benito[4], Ibai Nafarrate[4], Ainhoa Bilbao[4], Isabel Rodríguez-Llopis[4], Dmitri Ciorni[5], Korinna Altmann[5], Ivana Fenoglio[3], Chiara Riganti[2], Enrico Bergamaschi[1] and Felipe Goñi de Cerio[4]

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High density polyethylene (HDPE) and polyethylene terephthalate (PET) are the two most popular thermoplastic polymers used in food packaging worldwide. Recent studies have found HDPE and PET microplastics (MPs) in a wide range of foods and beverages, raising questions about the potential health impact on human health following ingestion of these materials. Microorganisms quickly colonize synthetic polymers weakly biodegradable like HDPE and PET. HDPE and PET MPs may thus act as carriers for the spread of human pathogen such as the foodborne *Vibrio parahaemolyticus* that can cause acute gastroenteritis associated with the consumption of seafood products surviving the low gastric pH and maintaining its pathogenicity. Moreover, MPs interaction with digestive fluids can result in a biocorona formation that can change their biological identity as well as their interaction with cells and tissues. In this study we evaluated the possible effects on intestinal cells cultures of 5 µm HDPE and 130 µm PET MPs with and without *V. parahaemolyticus* biofilm (HDPE, PET, HDPE+V and PET+V) applying an *in vitro* simulated human digestion system (SHDS) to reach as closer as possible the physiological conditions. Undifferentiated Caco-2 cells were exposed for 24 h to 1-100 µg/ml range concentration of SHDS-treated or not treated HDPE, PET, HDPE+V and PET+V; the cell viability (Alamar blue) and inflammatory responses (TNF-α, IL-6 and IL-8) were investigated. Analysis of *V. parahaemolyticus* colonization on MPs using scanning electron microscopy (SEM) demonstrated that 3 days of *V. parahaemolyticus* incubation led to the formation of a dense biofilm on MPs. In parallel, findings from the *in vitro* investigations showed that the presence of *V. parahaemolyticus* biofilm MPs increased the inflammatory responses and the loss in cell viability in a dose-dependent manner. Obtained data suggest that HDPE and PET MPs may act as carriers of *V. parahaemolyticus* and cause significant cellular reactions, which can trigger off a chain of events that can lead to serious cell and tissue damage. This work was supported by the EU H2020 Project "Plastics Fate and Effects in the human body" (PlasticsFatE) under Grant Agreement no. 965367.

TOPIC N°8 – MICRO AND NANOPLASTICS POLLUTION

DEVELOPMENT OF A METHOD FOR COLLECTING AND ANALYZING AIRBORNE MICRO- AND NANO-PLASTICS

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In recent years, micro-plastics (MPs) and nano-plastics (NPs) have become particularly due to their worrying presence in the marine and terrestrial environmental compartments, and more rarely in the atmosphere. The detection of MPs and NPs in the air and the dynamics of atmospheric emission sources remain poorly documented in the literature despite the fact that these contaminants could have an impact on the environment and on human health. The identification of MPs and NPs emission sources in the indoor areas (ventilation protocols, occupation scenarios and localized sources) is difficult and shows limited temporal resolution.

Recently, an original gas/solid separation process based on selective electrostatic precipitation according to the shape factor was developed and tested by the LITEN laboratory of the CEA in Grenoble (Clavaguera et al., 2020). Various prototypes (unipolar diffusion chargers, fibre selectors) have been built and were tested separately. The electrostatic precipitator was adapted to allow a direct sampling of MPs and NPs from indoor air and to facilitate the characterization of the collected MPs and NPs without specific sample preparation. The prototype was tested through a multiparametric study using controlled aerosols (atomised monodispersed polystyrene latex beads and abraded polydispersed polymeric fibers).

Experimental results related to the evaluation of the collection efficiency and the selectivity of this prototype with respect to MPs and NPs generated in a controlled manner in the laboratory will be shared. The investigated analytical methods (µFTIR and µRaman spectroscopy) for the identification and quantification of the MPs and NPs collected will be described along with the assessment of their limitations.

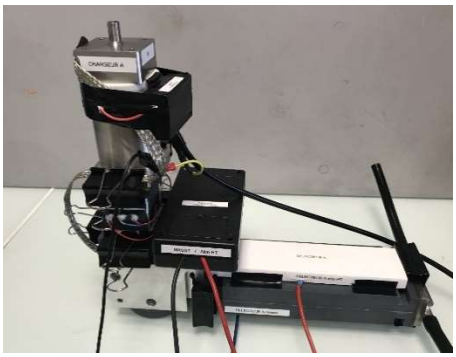

Figure 1. Prototype composed of a unipolar diffusion charger and a fiber selector.

Clavaguera, S., Guiot, A., Brouard, C., Berne, P., Pourprix, M., Ritoux, S., Motzkus, C., 2020. Airborne fiber separation, an application to real-time detection. Presented at the European Aerosol Conference 2020.

TOPIC N° 8 – MICRO AND NANOPLASTICS POLLUTION

ASSESSMENT OF MICROPLASTICS IN MUSSELS FROM NORTHERN ADRIATIC SEA

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Purpose: Due to improper management and poor biodegradability, plastic waste has become one of the most serious environmental problems, especially microplastics (MPs), defined as particles between 0.1 and 5 mm in size, are now ubiquitous. Marine habitats are particularly sensitive to this pollution, which accounts for 60-95% of marine litter.
A strong campaign against microplastics has also been conducted in the cosmetics field, resulting in the recent ban on their use as ingredients.
The aim of this project funded by the Ministry of Health (Department of Veterinary Public Health, Food Safety and Collegiate Bodies for Health Protection) was to analyze the presence of MPs in mussels (*Mytilus galloprovincialis*) reared in Emilia-Romagna (Northern Adriatic Sea).
The northern and central Adriatic coasts are characterized by intense human activity, with heavy marine traffic, intensive mussel aquaculture, fish farming and seasonal tourism. For this reason the Northern Adriatic Sea represents a site at high risk of plastic contamination.
Mussels are perfect bioindicators to detect MPs, as they are filter feeders of MPs that can be accumulated very easily. In fact, several MPs have been found in these mussels, which can have a significant impact on both humans and marine life. There is ample evidence that MPs are bioaccumulated and biomagnified within the marine food chain.
Although much research has been conducted, the overall knowledge of these issues in the Adriatic Sea is still limited and fragmented.
Methods: More than 100 samples were collected from different rearing sites. Each sample was processed and homogenized. A 10% potassium hydroxide (KOH) solution was added to digest the tissues in the shortest time possible without damaging the main plastic polymers. Afterwards, the samples were filtered on glass fiber filters (1 µm pores). Finally, the membranes with retained materials were microscopically observed. As a control to confirm and identify the chemical composition of polymers, particles were analyzed by Raman spectroscopy.
Results: The analyses detected the presence of MPs in 11% of the samples. The most frequent MPs types were fragments (63.6%) followed by fibers (36.3%), whose sizes ranged from 32 to 581 µm. In addition, the colors identified were blue (45.4%), green (45.4%) and transparent (9.0%). The final Raman analyses associated different specters with the most probable chemical composition of those MPs, and it found polymers as Polystyrene (PS), Polypropylene (PP), and Polyvinylchloride (PVC).

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AUTHOR INDEX

Scroll to the title and select a **Blue** link to open a paper. After viewing the paper, use the bookmarks to the left to return to the Author Index.

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z