Dear participants,

We are delighted to welcome you here in Nancy on the occasion of this "Chemical Risk 2015" conference, the fourth in a round of conferences on occupation health research, organised by the French Research and Safety Institute for occupational accident and disease prevention (INRS) in collaboration with its European occupational health and safety partner (PEROSH).

This fourth conference, similar to the three previous ones, is devoted to chemical risk and will focus on the innovative and original nature of methods for assessing or reducing chemical risk. The first conference addressed a specific group of chemical agents, nanoparticles, the second dealt with mixed exposure situations which are often poorly or under-assessed, and the third focused on occupational allergies which are very problematic due to their high rate of occurrence and the potential consequences on workers.

This new conference on chemical risk will complete the issues previously addressed by dealing specifically with innovative techniques and methods to respond to the challenges involved in the assessment of chemical risk and hazard assessment in a constantly evolving socio-economic environment.

The regulatory developments regarding the consideration of the CMR risk, lowering of occupational exposure limit values (OELs) and exposure checks have led to a growing need for new tools for OSH professionals (industrial hygienists, physicians, experts) related to essential issues such as the feasibility of exposure assessment (cost, representativeness, exposure route), the type of exposure and its short- and long-term consequences on exposed workers (low doses, mechanisms), the ways of minimising chemical concentrations (substitution, collective and personal protective measures, work organisation, etc.).

The "Chemical Risk 2015" conference will provide the opportunity to address most of these issues during the different sessions covering metrology, industrial hygiene, epidemiology, chemistry, toxicology, statistics, digital modelling and prevention. Young researchers and experienced experts will present, over the course of these two and a half days, current reflections and work by the international community on these topics, during seven guest lectures, thirty-four oral presentations and fifty-four posters covering all of the disciplines concerned.

The purpose of this conference, in addition to presenting topics of interest, is also to facilitate exchanges between researchers and engineers and OSH professionals from all throughout the world. The Centre Prouvé in Nancy is an ideal venue and will offer numerous occasions for discussions during breaks and poster sessions associated with the presentation of practical tools. We sincerely hope that you will enjoy this conference.

We warmly thank all the people who have worked to make this conference successful: the members of the organising committee, the international scientific committee, guest speakers and session chairpersons. We wish you an excellent conference and a pleasant stay in Nancy.

Yours sincerely,

The Chairpersons of Chemical Risk 2015

Davy Rousset
Head of the laboratory for inorganic analysis and characterisation of aerosols
Pollutants Metrology Division, INRS

Didier Baptiste
Scientific Director, INRS

SUMMARY

COMMITTEES 5 - 6

PROGRAMME 11 - 20

OPENING SESSION 21

SESSION I: RISK ASSESSMENT, EXPOSURE SCENARIOS & MODELLING 29

Oral presentations *31 - 45*

SESSION 2: AIR AND BIO-MONITORING 47

Oral presentations *49 - 61*

SESSION 3: NEW APPROACHES IN EXPOSURE ASSESSMENT & RISK REDUCTION 63

Oral presentations *65- 72*

SESSION 4: INPUTS FROM NUMERICAL SIMULATION 73

Oral presentations *75 - 80*

SESSION 5: "OMICS" & ALTERNATIVE MODELS IN TOXICOLOGY 81

Oral presentations *83 - 90*

SESSION 6: LOW DOSES AND STRUCTURE-ACTIVITY RELATIONSHIP 91

Oral presentations *93 - 100*

POSTERS 101

Posters *103 - 165*

AUTHOR INDEX 167 - 170

COMMITTEES

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Davy Rousset, Laboratory Chief, Pollutants Metrology Division, INRS

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Professor Goodarz Ahmadi
Goodarz Ahmadi is a **Clarkson Distinguished Professor, and Robert R. Hill Professor of Mechanical and Aeronautical Engineering at Clarkson University. He has been serving as the Dean of Engineering of the Coulter School of Engineering at Clarkson in the last eight years.**
His research interests include multiphase flows, particle transport and deposition, turbulence, granular flows, air pollution, and flow through porous and fractured media. His research has been supported by the National Science Foundation, the Environmental Protection Agency, Department of Energy, NASA, GE, Corning, IBM, Xerox, Dura Pharmaceutical, NYSTAR and AFOSR.
He has authored three books and over 550 publications in archival journals. He also has made more than 1100 presentations at national and international technical conferences and has given more than 160 invited talks and short courses at other institutions. He is serving as a member of editorial board and/or editorial advisor board of eleven international journals.
He is a Fellow of ASME, ISME and ISCE. His bio may be found at:
http://web2.clarkson.edu/projects/fluidflow/ga/ResumeIndex.htm
http://web2.clarkson.edu/projects/fluidflow/

Professor Marc Baril Département santé environnementale et santé au travail - École de santé publique - Université de Montréal -Marc.baril@umontreal.ca

Training

1982	Ph.D. pharmacology/ toxicology - University of Montreal Interrelation between heavy metal (mercury) and drugs
1977	M.Sc. Chemistry University of Québec at Montréal (UQAM) Measure of organochlorine pesticide in northern pike of one sector of the Richelieu river
1973	B.Sc. Chemistry, minor in biology (Biochemistry) - University of Québec at Montréal (UQAM)

Experience

2007	Associate professor Université de Montréal, École de santé publique, Département de santé environmentale et de santé au travail.
1999 - 2011	Occupational and safety research Institut (Robert Sauvé) (IRSST) Scientific advisor
2005	Short term consultant for the Office of Caribbean Coordination of the Pan American Health Organization to help TT University to structure a new BSc in OHS
2002	Short term consultant in occupational health for the Office of Caribbean Coordination and the Guyana office of the Pan American Health Organization
1996-1999	International Programmed on Chemical Safety World Health organization Technical advisor
1980-2011	Commission de la santé et de la sécurité du travail (CSST) (Québec worker compensation board)

International collaboration

2014	Scientific expertise committee ANSES : Danger caracterisation of chemical substances
2014	Scientific expertise committee ANSES :Professional exposure limit(VLEP)
2010-2013	Scientific expertise committee ANSES :Professional exposure limit(VLEP)
2010- 2013	Working group ANSES : Sanitary effects of chemical substances
2006/06	Scientific expertise group AEGL's (Acute exposure guidelines) of EPA
1997	Concise international chemical assessement document (CICAD) - WHO project
1988	CARDS (WHO, ILO and EU project)
1990-1997	Scientific editor for the project CARDS
1989-1996	Collaboration with the Center for scientific information an ILO division
1993	WHO collaboration center in occupational health
1990	Technical advisor for WHO regarding International Harmonization of Legislation for Substances

Membership of corporation or association
Québec Chemist corporation (91-115)
Member of the discipline board 1998-
Canadian society of toxicology
American Industrial Hygiene Association
First President of the UQAM association of graduate student in sciences
Board Air pollution control association Québec (APCA) 1977-82

Daniel Drolet
Daniel holds a Masters in chemistry from the Université du Québec in Montréal. He has worked almost 33 years at IRSST in Montréal. For some fifteen years now, his specialisation has been exposure assessment strategies, in particular, the adjustment of standards for unconventional working hours and the study of toxicological interactions. He has co-developed applications now used in OSH for risk assessment (MIXIE, Saturisk, ProtecPo, tools for thermal constraints, etc.) He is also an active member of the AIHA Exposure Assessment Strategies Committee and has been the initiator of several "cool new tools" (multilingual IHSTAT, IHMOD and IH SkinPerm). In 2012, he received the AIHA's Edward J. Baier prize for his exceptional contribution to the field of occupational hygiene and in 2014 the AQHSST's Antoine-Aumont prize for his contribution to the development of OSH in Québec.

Alain Simonnard
Doctor of Pharmacy, PhD in Toxicology, master in anatomopathology, master in embryology, senior toxicologist

Professionnal experience:
From January 2012 - INRS (Occupational Toxicology)*:* head of toxicology and biometrology department
From February 2011 to December 2011 - THOR Personal Care (Cosmetics): head of toxicology
From October 2008 to December 2010 - Centre International de Toxicologie (CIT – Contract Research Organization): director of toxicology and operations
From March 1995 to october 2008 - Institut de Recherche Perre Fabre (Drugs and Cosmetics): director of toxicology for pierre fabre group
From September 1986 to February 1995 - Centre International de Toxicologie (CIT – Contract Research Organization): director of toxicology

Society membership:
Société Française de Toxicologie (SFT), vice-president
Eurotox,
ESTIV (European Society of Toxicology In Vitro)
Société Française de Toxicologie Pathologique,
Association de Recherche en Toxicologie,
Société de Pharmacologie et de Toxicologie Cellulaire
Société Française de Toxicologie Génétique
American Conference of Industrial Hygienists (**ACGIH**)

Expert's fields:
In 2 different organizations: the faculty of Pharmaceutical Sciences University Paris V (1980 and 1981) and the Centre d'Etudes du Bouchet (Ministry of Defence) (from 1981 to 1986), my research activities concerned the study of series of molecules with oxime group used as antidotes against the intoxications with organophosphate compounds.
In a Contract Research Organization (CIT), I had in charge to perform toxicological studies with potential drugs, agrochemicals and chemicals and to advise clients for development strategy.
At the Institut de Recherche Pierre Fabre and then at CIT, I increased my experience in the drug development, specially as adviser for the project teams or as adviser to establish development plans and to analyze scientific results for drug candidates among them biologics. From strictly scientific point of view, my experience in toxicology and safety pharmacology increased in the field of anticancer (cytotoxic and monoclonal antibodies), cardiovascular and psychotrope drugs but also in New Chemical Entities.
I participated in the implementation of REACH regulation, in the tox evaluation of cosmetic raw material and ingredients as well as in preparation of toxicology dossiers for cosmetics. I was also responsible for the classification, labeling of substances and the unit for the preparation of safety data sheets and I have contributed to the establishment of the Global Harmonization System.
Currently at Institut National de Recherche et de Sécurité (INRS), I am very involved in all areas relating to occupational toxicology, prevention and protection of employees and especially in determining the occupational exposure limits for chemicals in the workplace.

Yngvar Thomassen

Yngvar Thomassen was born in Porsgrunn, Norway, on March 25, 1947. Thomassen's academic training (cand real) was obtained in the Department of Analytical Chemistry at the University of Oslo from where he graduated in 1973. He spent one year at the Norwegian Defense Institute before taking a post research associate position at the Department of Analytical Chemistry at the University of Oslo, where he conducted research for 2 1/2 years. In 1978 he visited The University of Toronto, Department of Environmental Studies and Geology, for one year with a Royal Norwegian grant (visiting scientist).

He is currently Research Director, **Department of Chemical and Biological Work Environment, National Institute of Occupational Health in Oslo** where he has spent 39 years of his professional life.

He is also appointed from 2004 as a professor in environmental chemistry, **Department of Plant and Environmental Sciences, Norwegian University of Life Sciences** outside Oslo.

Thomassen is the author or co-author of 190 scientific publications, the majority focusing on atomic spectrometry and other spectrometric methods for the determination of essential and toxic elements with special emphasis on electrothermal atomic absorption spectrometry. In recent years more focus has been given to other spectrometric methods as inductively coupled plasma atomic emission and mass spectrometry. His main scientific activity during the last ten years has been in the field of environmental and occupational exposure characterisation and assessment in human health studies. His research focuses on clinical aspects of analytical chemistry and the application of atomic spectroscopy within the context of environmental and occupational health.

Thomassen has served on the Editorial Boards of *The Analyst, Analytical Communication and Scandinavian Journal of Work, Environment and Health, Journal of Environmental Monitoring, Journal of Trace Elements in Medicine and Biology* and serves on the Board of
Environmental Science: Processes & Impacts.

He has given over 275 presentations on various aspects of his research, of which 105 were invited lectures at major conferences and symposia. In the period 1983-91 he was a member of the Commission on Toxicology, International Union of Pure and Applied Chemistry (titular) member from 1986, where he initiated the development of human body fluids as quality assurance materials for the measurement of minor, trace and ultra-trace elements and organic metabolites. From 2011-2014 he served as a titular member in the Analytical Chemistry Division.

Thomassen has organised a number of national, nordic and international conferences on topics dealing with analytical chemistry, atomic spectroscopy and environmental and biological issues. His present research focuses on clinical aspects of analytical chemistry and the application of atomic spectroscopy within the context of environmental and occupational health.

David Vernez, Associate Professor

A chemical engineer by training, David Vernez is an industrial hygiene expert. His specialty is first and foremost the assessment and prediction of exposure to pollutants. An associate professor at the *Université de Lausanne* (2014), he was named director of the *Institut universitaire romand de santé au travail* (IST) in Lausanne as from 1 January 2015.

Through modelling and experimenting, David Vernez's research work aims to assess the permeation and metabolism of chemical agents by dermal exposure, to understand the relationship between skin exposure to the sun's ultraviolet rays and the appearance of skin cancers, and to develop predictive models for exposure to atmospheric pollutants.

He teaches undergraduate and graduate programmes and is in charge of the training of Swiss occupational hygienists. He also gives science of exposure classes to students in environmental engineering at the EPFL school, and students in occupational hygiene within the framework of the Masters in toxicology of the Swiss Centre for Applied Human Toxicology.

He is also a member of several scientific committees including that of the French national research and safety institute for occupational accident and disease prevention (INRS, France), the expert committee in charge of limit values for chemicals in occupational environments (ANSES, France), the Swiss Focal Point of EU-OSHA and of the MAK Kommission of SUVA in charge of elaborating occupational exposure limit values.

Håkan Wallin
Erik Håkan Richard Wallin was born in Swedish

University education:
Chemistry and biology - Ph.D. program: University of Lund 1979-1985. Doctor of Philosophy February 1986.

Teaching experience:
Supervisor of pregraduate and Ph.D. students, Copenhagen and Roskilde Universities 1996-2002.

Research appointments:
Research scientist at the National Institute of Public Health, Dep. Toxicology, Oslo, Norway, 1986-1990. Associated senior scientist and acting head of Dept. Environmental Carcinogenesis, the Fibiger Institute, Danish Cancer Society, Copenhagen, Denmark, 1990-93. Docent, Dept. Occup. Env. Med. University of Lund, Lund, Sweden, 1993-95.

Memberships:
Member of the editorial board of Particle and Fibre Toxicology (2008-) and Chemistry Central Journal (2006-); Member of European Association for Cancer Research, Nordic Environmental Mutagen Society, Nordic Mutagen Soc., Working Group, Toksikologi og Nanoteknologi, Danish Board of Technology, 2005-2006;

Present employment:
Senior Scientist (from June 1, 1995), and Research professor 2003-2011, National Res. Ctr. Occupational Environ, Copenhagen, Denmark. Adjunct professor, Institute of Public Health, Copenhagen University, 2009

Publications
Publications in peer-reviewed scientific journals 176 papers have registered in ISI Web of Science were cited 4566 times on August, 2014, h-index 41.

I am coauthor of reports for governments and international organisations and a few book chapters.

PROGRAMME

Wednesday 8 April

8:30	***Registration and coffee***	
10:00	***Opening session*** **Stéphane Pimbert**, INRS Director General **Didier Baptiste**, INRS Scientific Director **Davy Rousset**, Laboratory Chief, Pollutants Metrology Division, INRS	
10:30	Keynote speaker: **Marc Baril**, Montreal University, School of public health, Montreal, CA *Toxicology from a different point of view*	
11:15	Keynote speaker: **David Vernez**, Institute for work and health (IST), Lausanne, CH *Scope and limits of IT models in chemical risk assessment*	
12:00	***Lunch***	
Session 1: RISK ASSESSMENT, EXPOSURE SCENARIOS & MODELLING *Chairpersons: **David Vernez**, IST, Lausanne, CH & **Dorothea Koppisch**, IFA, Sankt-Augustin, DE*		
ORAL PRESENTATIONS		***Page***
13:20	Keynote speaker: **Daniel Drolet**, American Industrial Hygiene Association (AIHA), Montreal, CA *Panorama of existing and upcoming tools to improve the identification and management of chemical risk*	31
14:00	*General rules for a unified hazard banding in accordance with the new European chemistry regulation* **M. Arnone** et al., Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA), Sankt Augustin, DE	33
14:20	*SEIRICH: a computer-based information and support tool for chemical risk assessment in the work environment* **R. Vincent** et al., INRS, Vandœuvre-lès-Nancy, FR	34
14:40	*Stoffenmanager® Implementation Evolutionary Ladder* **K.J.M. Verbist** et al., Cosanta (previously Arbo Unie), Amstelveen, NL	35
15:00	*Assessment of toxic risks during the use of weapons systems* **C. Maisonneuve** et al., Direction Générale de l'Armement (DGA), Bagneux, FR	37
15:20	***Coffee break***	

SESSION 1: RISK ASSESSMENT, EXPOSURE SCENARIOS & MODELLING *Chairpersons:* ***David Vernez****, IST, Lausanne, CH &* ***Dorothea Koppisch****, IFA, Sankt-Augustin, DE*		
15:50	*Modelling exposure to hazardous substances: how conservative is conservative enough?* **D. Koppisch** et al., Institute for Occupational Safety	38
16:10	*An evaluation of the validity and reliability of the Tier 1 exposure assessment tools used under REACH* **M. Van Tongeren** et al., Institute of Occupational Medicine (IOM), Edinburgh, UK	39
16:30	*"TREXMO" Tool: a new tool to support user of occupational exposure models* **N. Savic** et al., Institute for work and health (IST), Lausanne, CH	41
16:50	*Assessment of chemical risk: simplified implementation of physical modelling* **F. Ezanno** et al., GLASSOLUTIONS France, Courbevoie, FR	42
17:10	*Scale-Up of oxygen Carrier for Chemical-looping combustion using Environmentally SuStainable materials (SUCCESS) – Occupational exposure assessment* **L. Geerts** et al., VITO – Institute for Technological Research, Mol, BE	44
17:30	***Poster Session & Exhibition of INRS tools and products*** ***Icebreaker party***	
19:30	***End of the day***	

Thursday 9 April

8:00	***Registration***	
SESSION 2: AIR AND BIO- MONITORING *Chairpersons:* ***Kevin Ashley****, NIOSH, Cincinnati, USA &* ***Florence Pillière****, INRS, Paris, FR*		
ORAL PRESENTATIONS		***Page***
8:30	Keynote speaker: **Yngvar Thomassen**, National Institute of Occupational Health (NIOH), Oslo, NO *Innovative and analytical approaches in exposomic for chemical and morphological characterisation of work-room aerosols metrology*	49
9:10	*New NIOSH sampling and analytical methods for occupational exposure assessment* **K. Ashley**, National Institute for Occupational Safety and Health (NIOSH), Cincinnati, USA	51
9:30	*Appropriate evaluation of 4,4' methylene diphenyl diisocyanate (4,4'-MDI) aerosols using a CIP10 individual dust sampler* **S. Gagné** et al., Institut de recherche Robert-Sauvé en santé et sécurité au travail (IRSST), Montreal, CA	52

9:50	*Feed, food … and then workers' safety: innovative analytical tools for the measurement of mycotoxins* **D. Jargot** et al., INRS, Vandœuvre-lès-Nancy, FR	53
10:10	*Beryllium exposure assessment: review of sampling and analytical developments and impending U.S. regulatory changes* **K. Ashley**, National Institute for Occupational Safety and Health (NIOSH), Cincinnati, USA	54
10:30	***Coffee break***	
11:00	*Occupational exposure to bisphenol A in Finland* **S.P. Porras**, Finnish Institute of Occupational Health (FIOH), Helsinki, FI	56
11:20	*Occupational exposure to Bisphenol A via thermal paper. Urinary biomonitoring study* **S. N'Daw** et al., INRS, Vandœuvre-lès-Nancy, FR	57
11:40	*Reducing inhalation and dermal exposures to polycyclic aromatic compounds and their metabolites in the urine of hot-mix asphalt paving workers* **J. Snawder** et al., National Institute for Occupational Safety and Health (NIOSH), Cincinnati, USA	58
12:00	*Detection of tetrahydroxylated-benzo[a]pyrene isomers in hair as biomarkers of exposure to benzo[a]pyrene and signature of DNA-adduct levels* **N. Grova** et al., Public Health Research Centre (CPR-Santé), Luxembourg, LU	60
12:20	***Lunch***	

Session 3: NEW APPROACHES IN EXPOSURE ASSESSMENT & RISK REDUCTION

*Chairpersons: **Olivier Le Bihan**, INERIS, Verneuil-en-Halatte, FR & **Dominique Thomas**, ENSIC, Nancy, FR*

ORAL PRESENTATIONS		***Page***
13:40	*Monitoring occupational exposure using real-time detection* **J.S. Barbotin**, Service inter-entreprises de santé au travail (SIST) Arve Mont-Blanc (AMB), Scionzier, FR	65
14:00	*Development and validation of a tool for mapping operator exposure at workstations DACTARI: trajectography acquisition device for individual risk analysis* **P. Martin** et al., INRS, Vandœuvre-lès-Nancy, FR	66
14:20	*Risk analysis and reorganisation of a workplace dedicated to a nano-ZrO2 process* **O. Le Bihan** et al., Institut National de l'Environnement Industriel et des Risques (INERIS), Verneuil-en-Halatte, FR	67
14:40	*Skin exposure to bitumen: mutual contributions to ergonomics and metrology* **N. Judon** et al., INRS, Vandœuvre-lès-Nancy, FR	69
15:00	***Coffee break***	

15:30	*Validation of the test bench for N95 filtering face-piece respirators - Comparison of performance measurements with simulated occupational exposure* **C. Brochot** et al., Institut de recherche Robert-Sauvé en santé et sécurité au travail (IRSST), Montreal, CA	71
15:50	*Respiratory protective devices used during removal of asbestos-containing material: method for assessing their performance in work situations* **S. Chazelet** et al., INRS, Vandœuvre-lès-Nancy, FR	72
Session 4: INPUTS FROM NUMERICAL SIMULATION *Chairpersons: **Emmanuel Belut**, INRS, Vandœuvre-lès-Nancy, FR & **Goodarz Ahmadi**, Clarkson University, New–York, USA*		
16:10	Keynote speaker: **Goodarz Ahmadi**, Clarkson University New-York, USA *Computational Modeling of Particle Transport and Deposition in Indoor and Outdoor Environments*	75
16:30	*Estimating emission profiles of a source of particulate matter (transient regime)* **F. Chata** et al., INRS, Vandœuvre-lès-Nancy, FR	77
16:50	*Numerical modelling of transport & deposition of particles in the upper airways* **Y. Hoarau**, Strasbourg University, Strasbourg, FR	79
17:10	***Poster Session & Exhibition of INRS tools and products***	
19:00	***End of the day***	
20:00	***Social event (Beaux-Arts Museum) in Nancy - Buffet included***	

Friday 10 April

8:00	***Registration***	
Session 5: "OMICS" & ALTERNATIVE MODELS IN TOXICOLOGY *Chairpersons: **Marc Baril**, Montreal University, School of public health, Montreal, CA & **Hakan Wallin**, NRCWE, Copenhagen, DK*		
8:30	Keynote speaker: **Hakan Wallin**, National Research Centre for the Working Environment (NRCWE), Copenhagen, DK *Analysis of global gene expression data for risk assessment and revelation of mechanisms of toxicology of nanomaterials*	83
9:10	*Proteome changes in auricular lymph nodes and serum after dermal sensitization to toluene diisocyanate in mice* **P. Hoet** et al., University of Leuven (KU Leuven), Leuven, BE	85

9:30	*Mining brain metabolomic and behavior datasets obtained from adult rats exposed to chemicals like PAHs or a mixture of PCBs, dioxins and furans: a powerful tool to assess the risk for the brain of a chronic exposure to environmental contaminants* **H. Schroeder** et al., University of Lorraine & National Institute of Agronomic Research (INRA), Vandœuvre-lès-Nancy, FR	86
9:50	*Interaction between cells and polymeric nanoparticles: what toxicogenomics can bring* **R. Safar** et al., University of Lorraine, Nancy, FR	88
10:10	*SiO2-NPs translocate through human bronchial barrier reconstituted in vitro* **A. Baeza-Squiban** et al., Paris Diderot University, Paris, FR	90
10:30	***Coffee break***	
Session 6: LOW DOSES AND STRUCTURE-ACTIVITY RELATIONSHIP *Chairpersons:* ***Marc Baril,*** *Montreal University, School of public health, Montréal, CA &* ***Hakan Wallin****, National Research Centre for the Working Environment (NRCWE) , Copenhagen, DK*		
11:00	Keynote speaker: **Alain Simonnard**, INRS, Vandœuvre-lès-Nancy, FR *Chemical risk assessment: toxicology and biometrology innovations*	93
11:40	*Urinary excretion profiles of 42 monohydroxylated metabolites in rats exposed to a mixture of low-dose polycyclic aromatic hydrocarbon for a 90-day period* **N. Grova** et al., Public Health Research Centre (CRP – Santé), Luxembourg, LU	95
12:00	*Dose-related assessment of the neurobehavioral toxicity of a 90-day exposure to a mixture of pesticides in adult Wistar male rats* **H. Schroeder** et al., University of Lorraine & National Institute of Agronomic Research (INRA), Vandœuvre-lès-Nancy, FR	97
12:20	*QSPR models for predicting physico-chemical hazards of substances* **P. Rotureau** et al., Institut National de l'Environnement Industriel et des Risques (INERIS), Verneuil-en-Halatte, FR	98
12:40	*Consideration of physical factors during the development of predictive chemical risk models* **D. Mathieu**, Commissariat à l'énergie atomique et aux énergies alternatives (CEA), DAM, Monts, FR	99
13:00	***Closing session***	
13:15	***End of the Conference***	

POSTERS

	Page
Different methods to determine the oxidative potential of PM2.5 as a predictive marker of their toxicity **Baeza-Squiban A.** et al., Paris Diderot University, UMR CNRS 8251, Paris, FR	103
Assessment of the oxidative potential of nanoparticles: comparison and improvement of methods **Baeza-Squiban A.** et al., Paris Diderot University, Paris, FR	104
Assessment of chemical risk in a petrochemical analysis laboratory (core library), Algeria **Beghdadli B.** et al., Faculty of Medicine, Sidi Bel Abbes, Algeria	105
CHEOPS, a methodological approach to assessing and prioritising chemical risks in the work environment based on toxicity reference values **Berrubé A.** et al., Veolia Recherche et Innovation, Maisons-Laffitte, FR	106
Cytotoxic Drugs: Handling practices and clinical manifestations among hospital staff **Boularas E.A.** et al., Faculty of Medicine-University Djillali Liabes of Sidi Bel Abbes,Algeria	108
Automated generation of reference samples from particles in suspension using the SAGE generation system **Boulet A.** et al., INRS, Vandœuvre-lès-Nancy, FR	109
Prevention techniques of chemical risks in bakery industry **Bulut M.** et al., Candidate at Ege University, Izmir, Turkey, TR	111
Assaying beryllium using molecular fluorescence **Carabin N.** et al., INRS, Vandœuvre-lès-Nancy, FR	112
Comparison of environmental and biological monitoring of exposure to xylenes **Chakroun R.** et al., Institute of Occupational Health and Safety, Tunis, TN	113
Optimisation of the synthesis of molecularly imprinted polymers for the extraction of urinary glycol ether metabolites **Chakroun R.** et al., Institute of Occupational Health and Safety, Tunis, TN	114
Titanium dioxide-induced gene expression profile in rat lung, a sub-acute inhalation study **Chézeau L.** et al., INRS, Vandœuvre-lès-Nancy, FR	115
In a multivariate world is densisty functional theory (DFT) a way to theoretical toxicology? **Correzzola C.** et al., Istituto Nazionale per l'Assicurazione contro gli Infortuni sul lavoro (INAIL), Direzione Regionale Veneto, IT	116

POSTERS

	Page
Co-exposure to toluene (or styrene) and methyl ethyl ketone: impact on biological exposure indicators **Cosnier F.** et al., INRS, Vandœuvre-lès-Nancy, FR	117
Modelling the trend of biomonitoring for occupational exposure in Belgium **Duca R.** et al., Center for Environment and Health, Leuven, BE	118
Experimental model of skin/lung responses to chemical exposure **Duca R., Hoet P.** et al., Center for Environment and Health, Leuven, BE	119
Assessing occupational exposure to multiple volatile organic compounds by biometrology: results of an intervention in companies **Erb A.** et al., INRS, Vandœuvre-lès-Nancy, FR	120
Derivation of cumulative toxicity indicators for indoor semi-volatile organic compounds: the case of reprotoxic and neurotoxic mixtures **Fournier K.** et al., Ecole des Hautes Etudes en Santé Publique (EHESP), Sorbonne Paris Cité, Rennes, FR	121
Assessment of microenvironments contribution on PM2.5 and PAHs exposures of population using integrated models **Gariazzo C.** et al., Istituto Nazionale per l'Assicurazione contro gli Infortuni sul lavoro (INAIL), Monteporzio Catone, IT	122
Biological assessment of exposure to di-2-ethylhexyl phthalate (DEHP) in the soft PVC industry in France **Gaudin R.** et al., INRS, Vandœuvre-lès-Nancy, FR	124
Individual production of reference materials by using a piezoelectric microdosing system - First tests **Giesen Y.** et al., Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA), Sankt Augustin, DE	125
Performance of the µ-Cathia aerosol sampler versus conventional thoracic health-related aerosol fraction **Görner P.** et al., INRS, Vandœuvre-lès-Nancy, FR	126
Setting up of the Bhas 42 in vitro cell transformation assay **Guichard Y.** et al., INRS, Vandœuvre-lès-Nancy, FR	127
Innovative adsorbents for workplace nitrous oxide diffusive sampling **Guillemot M.** et al., INRS, Vandœuvre-lès-Nancy, FR	128

POSTERS

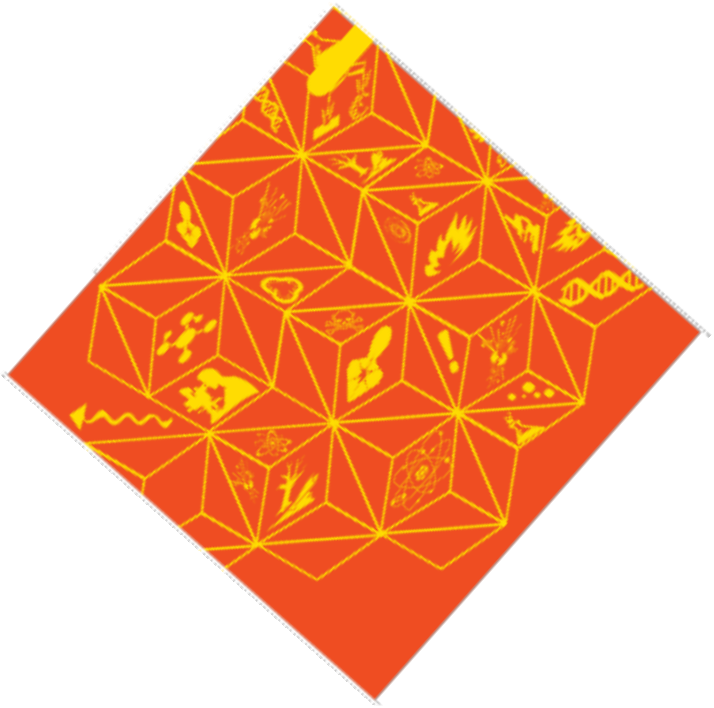
	Page
Internalisation of international regulations on chemical risk in Morocco "Case of transportation of hazardous materials" **Ibnlfassi A.** et al., University Hassan 1, Settat, MA	129
Supercritical CO^2 desorption for air sampling analysis **Langlois E.** et al., INRS, Vandœuvre-lès-Nancy, FR	130
Adjustment of Workplace Exposure Standards for Atmospheric Contaminants for Extended Work Shifts – Models Overview **Laranjeira P.**, Instituto Politécnico do Porto, Felgueiras, PT	132
Chemical risk: a global approach for local solutions **Larnaud H.** et al., Rectorat Académie de Grenoble, Grenoble, FR	133
Draft canister method for sampling and analysis of select volatile organic compounds **Lebouf R.F.** et al., National Institute for Occupational Safety and Health (NIOSH), Morgantown, WV, USA	135
Ventilation assessment and improvement using real time techniques in a slate workshop with high RCS (respirable crystalline silica) concentrations **Madera-Garcia J.** et al., National Silicosis Institute, Oviedo, ES	137
Assessing chemical risks: an overall management tool **Magalhaes-Antoine I.** et al., Bureau Veritas, Nancy, FR	138
Assessment of the toxic effect of the endocrine disruptor lead on workers **Mansouri-Bentayeb O.** et al., Université Badji Mokhtar, Annaba, Algeria	140
The contribution of durum wheat (Triticum durum) to reducing lead toxicity: a study of some physiological indicators in the Wistar rat **Mansouri-Bentayeb O.** et al., Université Badji Mokhtar, Annaba, Algeria	141
Improvement of analytical performance during the use of impactors for characterising nanostructured aerosols **Matéra V.** et al., INRS, Vandœuvre-lès-Nancy, FR	142
Determination of toxicity of an in-housed synthesized PEGylated Nano Graphene using bone marrow mesenchymal stem cells **Mohanan P.V.** et al., Sree Chitra Tirunal Institute for Medical Sciences and Technology, Kerala, India	143

POSTERS

	Page
Occupational exposure to mycotoxins. Biomarkers and airborne contamination measurements **Ndaw S.** et al., INRS, Vandœuvre-lès-Nancy, FR	144
Updating of OELs for complex hydrocarbon solvents **Nies E.** et al., Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA), Sankt Augustin, DE	145
Use of an effective concentration approach to classify alloys in 2015 **Oller A.R.** et al., Nickel Producers Environmental Research Association (NiPERA), University of Real World, Durham, USA	146
Correlation between mass and number concentration of dust at different workplace scenarios with regard to ultrafine particles **Pelzer J.** et al., Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA), Sankt Augustin, DE	148
CFD simulations of air distribution and thermal comfort when using textile air ducts **Peters S.** et al., Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA), Sankt Augustin, DE	149
Development of a monitoring strategy based on UPLC-MS/MS for the assessment of occupational exposure to airborne pharmaceutical compounds **Poels K.** et al., University of Leuven (KU Leuven), Leuven, BE	150
Quantitative cancer risk assessment for occupational exposures to asphalt fumes during built-up roofing asphalt (BURA) operations **Rhomberg L.R.** et al., Gradient, Cambridge, MA, USA	152
The contribution of molecular modelling in assessing product and process safety **Rotureau P.** et al., Institut National de l'Environnement Industriel et des Risques (INERIS), Verneuil-en-Halatte, FR	153
Interaction between cells and polymeric nanoparticles: Contribution of toxicogenomics **Safar R.** et al., University of Lorraine, Nancy, FR	154
Particle-induced cell migration assay (PICMA): rutile TiO2 and SiO2 but not anatase TiO2 and BaSO4 can induce migration of NR8383 alveolar macrophages **Schremmer I.** et al., Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA) - Institute of the Ruhr-University Bochum, Bochum, DE	155
Development of a co-culture model to study the genotoxicity of particulate matter **Sébillaud S.** et al., INRS, Vandœuvre-lès-Nancy, FR	156

POSTERS

	Page
Carbon Nanotubes: A PERSPECTIVE FOR THE FUTURE **Simões H.** et al., Coimbra Health School, Coimbra, PT	157
Development of biotests to ensure quality, safety and improvement of packaging intended for food contact **Souton E.** et al., Institut national de la santé et de la recherche médicale (INSERM), Université de Bourgogne, Dijon, FR	158
Analysis of the effect of para-phenylenediamine on the osmotic stability of human erythrocyte through electrochemical oxidation **Srhayri R.** et al., Université Hassan II - Casablanca, MA	159
Aromatic solvents disturb the stapedial reflex involved in hearing **Wathier L.** et al., INRS, Vandœuvre-lès-Nancy, FR	161
Optimisation of the filtration of ultrafine metallic particles by granular bed **Wingert L.** et al., INRS, Vandœuvre-lès-Nancy, FR et Laboratoire Réactions et Génie des Procédés, UMR CNRS 7274, Nancy, FR	163
ProtecPo: a software for the selection of skin protective materials **Zimmermann F.** et al., INRS, Vandœuvre-lès-Nancy, FR	165



OPENING SESSION

Marc Baril, Montreal University, School of public health, Montreal, CA
Toxicology from a different point of view

David Vernez, Institute for work and health (IST), Lausanne, CH
Scope and limits of IT models in chemical risk assessment

Toxicology from a different point of view

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Based on pharmacology for some or on pharmacy for others, toxicology is in 2015 without doubted a recognise speciality in the field of medical sciences. Actually no one could really predict where this speciality that already evolves at high speed will be in the following 20 or 30 years. The use of modelisation, of new approach in statistics, de in vitro models, new techniques like proteomic, who fifteen years ago was the object of speculation pushes us toward unknown territories.

If that domain who drive us continue to take so much importance it is mostly because of the hard work of its main actors. These actors change thought years. Originally coming from field like biochemistry, pharmacy or even engineering we find at the moment more and more of them coming from the toxicology training curse.

At the moment we could without risk of misleading peoples support the fact that toxicology is a mature science with its implication.

On contrary through years customers of results coming from toxicology research, stay at the same level, gentleman, ladies from general public, risk manager and politician who untimely manage research funds who support the field progression still ask simple response without nuances to complexes toxicology problematic.

We will look together what could limit the development of the field and tools, methods at our disposal to unlock the situation.

Scope and limits of IT models in chemical risk assessment
In silico veritas ?

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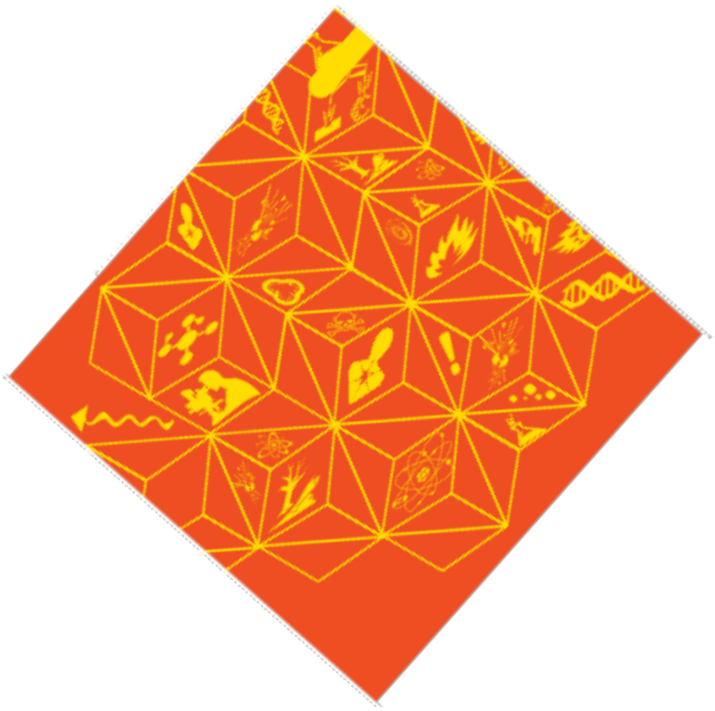
Numerical simulation and modeling tools have become indispensable in the field of chemical risk assessment. The success of these tools is largely explained by their ability to produce huge amounts of data with limited resources, as well as to cope with problems too complex to be solved analytically. Once reserved for specialists, their use has largely democratized and many applications are nowadays available to a broad public.

Due to their short implementation time, modeling tools are often used as a first-line (screening) approach to support and sometimes substitute, expert judgment. Their use to supplement laboratory experiment or field investigation, sometimes out of reach, also tends to develop. For end users that perceive only inputs and outputs, these models are often seen as 'black boxes'. Little is known about their functional limitations, validity or, more generally, their performance.

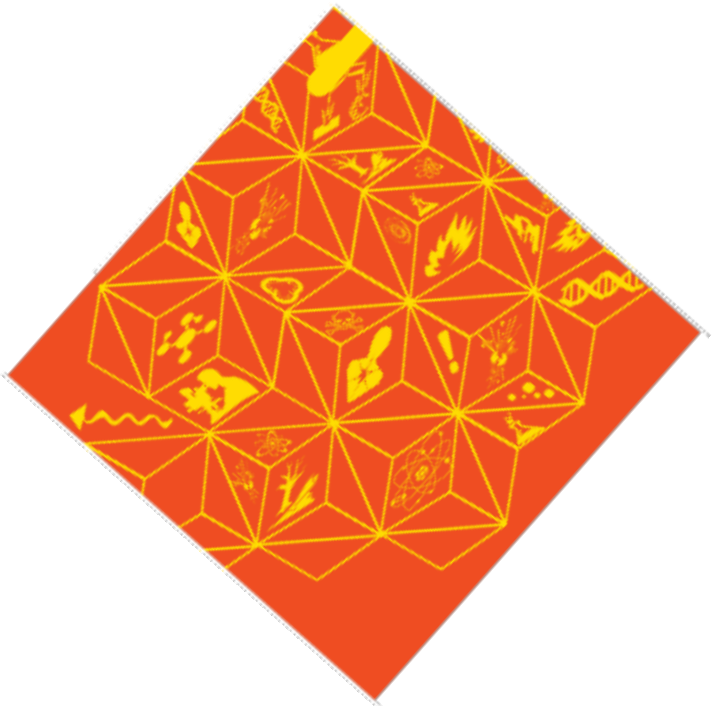
Three examples can be used to illustrate the limitations and uncertainties issues in modeling:

- Toxicokinetic modelling, which allows the prediction of the distribution, metabolism and excretion of the pollutants within the body. Toxicokinetic models are particularly valuable in the identification and quantification of relevant biological exposure indicators.
- The use of QSAR (Quantitative Structure-Activity Relationship) models in the evaluation of the skin permeation of chemical pollutants. With a few straightforward physico-chemical parameters (e.g. molecular weight, octanol-water partition), these models allow to estimate the permeation flux of a substance of interest through the skin. Cutaneous permeation QSARs are typically used to estimate the relative importance of systemic intoxication by skin and respiratory tract while no experimental data is available.
- Predictive models of exposure to chemical airborne pollutants (gas, vapor, dust). Physical, empirical or statistics models are widely used as a screening tool in occupational hygiene. They are currently abundantly used in the REACH regulatory frameworks exposure estimates prior to the marketing of chemical preparations.

The increasing use of modeling, sometimes as an alternative to expert judgment or measurements, raises the question of the scope and limitations of the models. Considerable effort has been invested in understanding the uncertainties and limitations of traditional assessment methods (e.g. validation of analytical methods) and, more generally, in assessing the transparency of health risk assessments. In such a context, similar efforts have to be invested in enlightening the uncertainties of the modeling approaches in order to make it a viable alternative to conventional approaches. Beyond the scientific and technical challenges posed by the development of new models, the real issue lies probably in a better understanding of the existing tools, and in the improvement of their performance.



ORAL PRESENTATIONS



Session 1

RISK ASSESSMENT, EXPOSURE SCENARIOS & MODELLING

Chairpersons:

David Vernez, Institute for work and health (IST), Lausanne

Dorothea Koppisch, Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA), Sankt Augustin

Panorama of existing and upcoming tools to improve the identification and management of chemical risk

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Managing chemical risks in the workplace involves decisions based on the best credible information, with its uncertainty, in order to keep the exposure at an acceptable level and ensure healthy working conditions. For 15 years, researchers, OSH practitioners and organizations, including the *American Industrial Hygiene Association* (AIHA) worked on the production and sharing of knowledge and chemical risk assessment tools to the benefit of the workplace. Those efforts led to an ever-growing number of tools and databases.

For ten years, the *Exposure Assessment Strategies Committee* (EASC) AIHA has contributed to this trend by making available to industrial hygienists tools like IHStat, IHMod, IHSkinPerm, and the Dermal Assessment Control banding Tool. These tools have been built to be easily translated. As examples, IHSTAT a tool for statistical analysis of workplace exposure is available in 15 languages and IHMOD, a modeling tool to chemical exposures in the workplace is translated in 4 languages. And that's not all, with the collaborations around the world, we hope to spread the use of these tools. These tools are in constant evolution and others are currently under development (IHEST, the "Checklist").

The next evolution of the tools is to integrate the probabilistic approach (Monte Carlo simulation) with the same models already used. It will then be possible for the user not only to have an answer to his question, but more importantly, to estimate the confidence limits around this answer. The manager will thus have a much better view of the level of risk for a given scenario. In addition, the use of sensitivity analysis, that is to say, the estimated weight of each variable in the model for a given scenario, can allow him to guide the prevention strategies. For example, in a scenario of a chemical spill in a room, is it better to wait until the ventilation system extracts the airborne contaminants? If not, how should emergency responders be protected given the expected concentrations?

This presentation will show examples in real time. The aim of the EASC is to produce tools that will help to understand the influence of the determinants on the level of risk and the likelihood of its occurrence. The Monte Carlo simulation allows one to see what happens in the distribution tails. This is important because it is often in these extremes that occupational diseases and the work accidents may occur. This approach, used for the analysis of chemical risks, could also be extended for physical, ergonomic, safety and psychosocial risk factors.

The EASC welcomes any person or organization who is willing to join this team of volunteers to improve, translate the tools, or proposes new ones.

General rules for a unified hazard banding in accordance with the new European chemistry regulation

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Hazard banding is the classification of hazards arising from handling hazardous substances into hazard categories, which are also referred to as "hazard bands". In control banding approaches these hazard bands can be assigned using the "risk phrases" (R-phrases) of the substances (67/548/EEC Dangerous Substances Directive; DSD) or the H(azard) statements from the Globally Harmonised System (GHS) implemented throughout the European Union by the Classification Labelling & Packaging Regulation (EC) No 1272/2008 (CLP Regulation). In addition to the new H statements CLP leads also to changes in the dilution rules for the classification of mixtures. From the first of June 2015 all chemical products (pure substances and mixtures) have to be labelled according to the new legislation. Due to this all control banding tools that use the R-phrases from the DSD and the concentration limits from the 1999/45/EC Dangerous Preparations Directive (DPD) for their assignment of hazards have to be adapted to the new rules.

A first approach to adapt the assignment was to translate the R-phrases to H statements and use the same assignment to hazard bands. However not all R-phrases can be translated directly to H statements, and some of the H statements have no corresponding R-phrases. We therefore propose to derive general rules for the assignment of hazards to hazard bands.

As a first rule we use the H statements and the concentration limits for mixtures from the CLP regulation as basis for the assignment of hazard bands. All health-related H statements (H3xx) were assigned to six hazard bands with respect to the severity of the underlying health hazard. These hazard bands range from **n.a.** = "not applicable" through **A** = "low hazard", **B** = "moderate hazard", **C** = "high hazard" and **D** = "very high hazard" to **E** = "extremely high hazard". The dilution rules from the CLP regulation are applied for the assignment on tasks performed with self-diluted mixtures of hazardous substances. Hereby the generic concentration limits for labelling from the CLP regulation were chosen as the concentration limits for the assignment of hazard band **n.a.** The second rule is that the H statements are assigned specifically to the route of exposure concerned in the respective H statement. For an exposure route not concerned in the H statement the hazard band **n.a.** is assigned. This is done to enable the user to adjust risk management measures to the specific uptake route. Rule three finally claims that the hazard banding has to support the important principle of substitution. To ensure this proven carcinogens or mutagens are assigned to hazard band **E**, while all less severe hazards are assigned at worst to hazard band **D**. In addition, the graduated assignment of acute toxic substances to hazard bands from **D** to **B** with respect to their severity is also proposed in order to encourage substitution.

The chosen principles for assigning hazard bands with respect to the severity of the hazard, the exposure route and the possibility of substitution are in line with the requirements for qualitative risk characterisation described in the REACH Guidance Part E: "*Risk characterisation*". The safety data sheets (SDS) of the products as source of the required hazard information and the CLP regulation as basis for the assignment of hazard bands ensures that the proposed new GHS hazard banding is in conformity with the new European legislation on the handling of hazardous substances (CLP and REACH). This presentation should raise the question if the implementation of this GHS hazard banding in the various control banding tools in the European Union can result in a unified classification of health hazards from the handling of hazardous substances.

SEIRICH: a computer-based information and support tool for chemical risk assessment in the work environment

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Chemicals placed on the European market within the framework of the REACH regulation require the end user, regardless of their activity, to assess the risks according to the provisions of the French Labour Code.
For assessing chemical risks in the work environment, taking into account risks to health, fire/explosion and environmental risks, numerous methods exist (Triolet & Héry, 2009) which should be harmonised (Vincent *et al.*, 2011) in order to ensure consistency among prevention actions.
INRS has developed a computer application SEIRICH, aimed at simplifying chemical risk assessment (Vincent *et al.*, 2005) and informing companies about prevention and their statutory obligations.
This work is part of a national agreement on prevention of chemical risk, and involves numerous partners, including the Ministry of Labour, the Occupational Risk Directorate of Social Security and several trade organisations. This tool includes the changes due to the classification and labelling of substances and mixtures according to the CLP regulation (EU regulation EC 1272/2008).
SEIRICH is designed to be used both by novices to chemical risk assessment and experts alike. This tool will have several functionalities aimed at simplifying chemical risk assessment for companies:

- inventorying of products and emissive processes through easy input of necessary data from the SDS or the label;
- prioritisation of products and emissive processes according to their risk level;
- chemical risk assessment based on three procedures adapted to the user's degree of expertise;
- technical and legal advice adapted to the context;
- management of documents for assessing and describing workstations;
- follow-up for prevention actions, etc.

After a test phase currently in progress involving companies, which will serve to better adapt the application to the different target audiences (micro, small & medium enterprises, occupational health services, etc.), SEIRICH will be made available for download free of charge, via a special website (www.seirich.fr) by 1 June 2015 at the latest.

Triolet J. & Héry M. (2009). *Les méthodes d'évaluation des risques chimiques. Une analyse critique. INRS, Cahier de notes documentaires, ND 2312.*

Vincent R., Héry M., Bonthoux F., Certin J.F., Guichard C., Haberer M., Maitre D., Levasseur C., Leroy M.H., Gaches G. & Barat C. (2011). *Evaluation des risques chimiques. Cahier des charges. Préconisations pour le développement d'applications informatiques. INRS, Hygiène et Sécurité du Travail (HST), PR 48.*

Vincent R., Bonthoux F., Mallet G., Iparraguire J.F. & Rio S. (2005*). Méthodologie d'évaluation simplifiée du risque chimique : un outil d'aide à la décision. INRS, Cahier de notes documentaires, ND 2233.*

EU (2008). *EC Regulation No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures.*

Stoffenmanager® Implementation Evolutionary Ladder

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Workers handling hazardous substances might be at risk of developing acute or chronic adverse health effects. Companies are often insufficiently aware of the various risks involved, have difficulties in understanding the complex chemical substance legislation and lack expertise in performing a systematic risk assessment of the chemicals that are being used. Over the years different tools have been developed to assist and empower companies to perform a qualitative or quantitative risk assessment. Although intended to be self-explaining, in practice this is not always the case. Therefore a project was started by TNO, Arbo Unie and EY, funded by The Netherlands Organisation for Health Research and Development (ZonMw), to actively assist companies with the risk assessment of chemical substances using Stoffenmanager® and with sustainable chemicals management in general.

In total 45 companies were invited to join the project. Companies were selected via the consortium parties network and with active support from the Paint and printing ink manufacturing industry and the Rubber and Plastics (-composites) manufacturing industry. A 7-steps implementation evolutionary ladder was developed, starting with substance registration and moving further towards complete risk assessment and increasing and maintaining a high level of safety culture within companies. Participation was free of charge, only investment of time was required. The project consisted of different phases:

1) Pre-implementation phase using questionnaires and interviews to discern at what point on the implementation evolutionary ladder companies at the start were;
2) Implementation phase where a mix of active individual and collective support was offered providing joint training and exchange meetings, site-visits by a personal coaches and an online platform with background information and a LinkedIn community;
3) Post-implementation phase using questionnaires and interviews to discern what progress companies had made on the implementation evolutionary ladder.

During the implementation phase 4 joint meetings were organized. Approximately 40 companies were visited by the personal coaches. Most of the companies moved one or more steps up the implementation ladder (Figure 1). Intrinsically motivated OSH-professionals proved to be of vital importance for a company to improve. Also (active) management support, the acceptance of Stoffenmanager® under REACH and by the Dutch Labour Inspectorate and external incentives like audits or visits by authorities were identified as factors for a successful implementation of chemical substance management. Identified barriers for a successful implementation were the significant time investment needed, lack of a tool manual and the unavailability of data (in the MSDS) needed to perform the risk assessment.

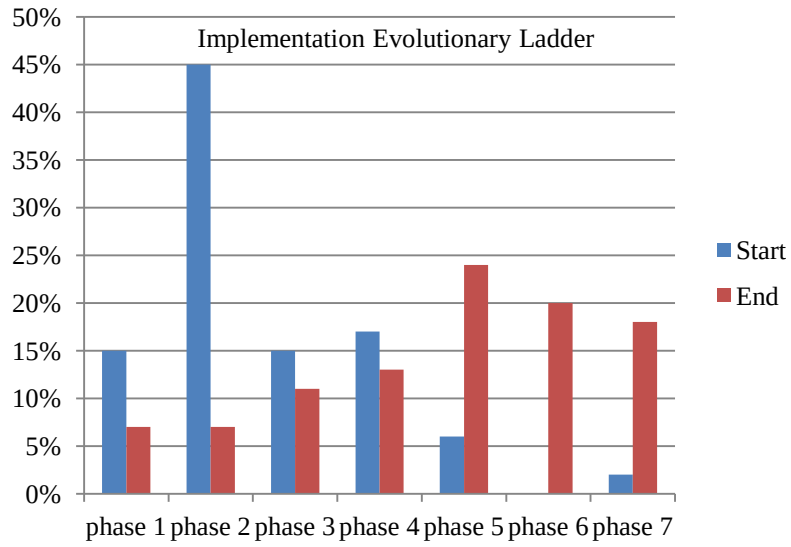

Figure 1. Figure illustrating progress of companies moving steps up on the evolutionary ladder.

Using both a general (joint meetings) and individual approach (site visits) most participating companies clearly improved on the implementation ladder and in correctly understanding and applying Stoffenmanager®. It can therefore be concluded that active training and coaching helps companies to improve their chemical risk management and also helps to avoid making mistakes when using and applying Stoffenmanager®. Use of validated tools embedded in a community platform support companies to organize and structure their chemical risk management in a business-wise way. However, much depends upon motivated OHS-professionals, management support and willingness to invest time.

Acknowledgement: This work was supported by The Netherlands Organisation for Health Research and Development under project number 208031004.

Assessment of toxic risks during the use of weapons systems

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Soldiers, and more widely, defence personnel, may be exposed to numerous chemical compounds during the use of weapons systems and possibly during the cleaning and maintenance of these systems. Among the specific risks to personnel, chemical risks associated with the use of pyrotechnic materials (smoke produced from firing) must be assessed taking into account numerous parameters specific to the military context, such as specific emissions, unusual lengths and conditions of exposure. The goal of this assessment is to deduce the means of controlling the risks that are adapted to the command and/or designers of the system. To date, the scientific and technical literature specify mainly the risks associated with firing small artillery in military or civil (recreational shooting) environments due to the presence of lead and/or other metal substitutes, but larger weapons systems are still insufficiently documented.

During this presentation, we will describe a chemical risk assessment conducted during the qualification of a weapon system using large projectiles moved by a solid fuel (propellant). The goal of this assessment was to identify hazardous exposure for future users of the system, and to propose and justify protective equipment or even limits on the use of the system. This goal falls within a broader objective to control toxic risks related to exposure of soldiers in operational situations and also during training.

At the end of a phase consisting in describing the chemical compounds potentially emitted by the system, combining bibliographic research, modelling and sampling, different exposure scenarios related to the use of the weapon system were adopted for the firing phase and then the maintenance phase. During several qualification firing tests, air samples and also samples from the surface of the system were taken, in order to document, for example, exposure during maintenance phases after firing. These analytical stages that are relatively classic in other contexts in fact combine several difficulties, including the short period of exposure and the complexity of the mixture, and were specifically validated (*Saurat et al.*, 2014).

These exposures were compared with occupational exposure limit values adapted to the exposure conditions if they existed or otherwise interpreted by a pluridisciplinary group (mainly for surface contaminations). Lastly, for each scenario, the level of risk was assessed and where necessary, recommendations for controlling the risks were made.

Saurat, D., Cordat, M.F., Guillard, A.C., & Mullot, J.U. (2014). *Detection and quantification of short-time chemical exposures in some military activities* dans les *Actes de la conférence sur la surveillance de l'air (AIRMON 2014)*, Marseille (France), 54.

Modelling exposure to hazardous substances: How conservative is conservative enough?

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For the assessment of workplace health risks due to inhalation, the exposure of the workers to hazardous substances must be known. If not enough or no measurement values are available for the worker under consideration, the exposure level can be modelled.

Two main reasons for exposure estimation exist: One reason is the risk assessment for a specific workplace. This risk assessment can be executed for future, current or historic exposure scenarios. Examples are the development of exposure scenarios for the REACH registration process, compliance testing or investigations on occupational diseases. The second main reason is an exposure assessment within epidemiological studies for the approval of the relation between exposure and disease or for the derivation of occupational exposure limits (OELs).

Model developers in the field of occupational safety and health see themselves often confronted with the demand that the exposure estimation should be conservative. This means, that if a working place is classified as safe, it should really be safe. This demand is often translated into: overestimation by a model is better than underestimation. On the other hand model results should correlate with measured exposure.

In the case of compliance testing it is important to protect all workers, so the maximum of the exposure, (called worst case) or the reasonable worst case (often defined as the 95. percentile of the exposure distribution) should be modelled. In the case of epidemiological studies it is on one hand important to get information about the exposure of an average worker, described by the mean or the median of the exposure distribution. On the other hand for the derivation of OELs it is important to know the lowest exposure that can cause a disease, which means that the lower percentiles are relevant.

This considerations show that exposure modelling in the field of occupational safety and health should include not only a point-estimate for the exposure level. In contrast it would be better to provide estimates for the mean or median of the exposure distribution as well as for the variation so that higher and lower percentiles can be estimated.

During the talk this considerations will be underpinned with examples as well from exposure measurements as from exposure modelling. Finally implications for model developers and for the validation of models will be derived.

An evaluation of the validity and reliability of the Tier 1 exposure assessment tools used under REACH

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Generic tools are widely used for chemical safety exposure assessments under REACH. Risk assessment under REACH follows a tiered approach in which the first tier should provide a conservative (i.e., protective) system that can discriminate between substances in scenarios of concern and those which are considered safe. Several 1st tier assessment tools such as ECETOC TRA, MEASE, EMKG-EXPO-TOOL, STOFFENMANAGER and RISKOFDERM are recommended by the European Chemicals Agency (ECHA) for estimating occupational exposure. To date only limited validations of the Tier 1 tool estimates against external measurement data have been done. Carried out by the Institute of Occupational Medicine (IOM), Edinburgh UK and Fraunhofer ITEM, Hannover, Germany, the Evaluation of the Tier 1 Exposure Assessment Models (ETEAM) project, evaluated the conceptual and external validity, scope of application and between-user reliability of the different REACH Tier 1 exposure assessment tools.

In the external validation exercise, descriptions of exposure situations and related personal exposure measurements (individual and aggregated data) were obtained from providers in Europe and the US. IOM exposure scientists entered the situation information into the tools, to generate exposure estimates for comparison with the measurement data. Several comparisons were carried out to examine the level of conservatism, including determination of the proportion of measurement values which exceeded the tool estimates and calculation of the ratio of the measurement value to the tool estimate. "High", "medium" and "low" conservatism were defined as ≤10%; 11≤25% and >25% of the measurements exceeding the tool estimate respectively. A geometric mean of the ratio of the measurement value to the tool estimate of below 1 was taken as an indication of a degree of conservatism. The impacts of various exposure determinants as implemented in the tools were investigated using linear mixed effects statistical models. Differences in the level of conservatism for all of the tools were observed between exposure category, PROC codes, data providers and the presence/ absence of local exhaust ventilation. Correlations between the measurement results and tool predictions were generally stronger for powders and non-volatile liquids than for the other exposure categories. Despite a concerted effort to develop a comprehensive exposure measurement database for the comparison exercise, there remained important gaps, with relatively few comparator measurements available for exposure to non-volatile liquids, metal dust and metal fume.

When applying the Tier 1 tools to an exposure situation, users must select from several possible input parameters. Previous studies have suggested that results from exposure assessments of very similar situations using expert judgement and exposure tools can vary considerably between assessors. The between-user reliability for the Tier 1 tools was therefore investigated using a remote-completion exercise and focus group. Tool parameters and other factors potentially associated with between-user variability, for example user demographics and previous exposure assessment and tool-use experience, were identified and evaluated.

In the remote-completion exercise, participants (N=146) generated dermal and inhalation exposure estimates (N=4066) from a defined set of exposure situation descriptions/Tier 1 tool combinations over a fixed time period. Qualitative information on decision-making processes associated with tool use was collected during the focus group. The interactions between users, tools and situations were analysed and described. Whilst within user variation was comparatively minor, significant variation was observed between users when selecting task/ activity, dustiness and risk management measures within the tools. Considerable variability in the resultant user-generated exposure estimates for the same situation was observed, which appeared to be unrelated to user characteristics.

The results of the ETEAM project will be of assistance to tool developers in further refinement of the tools' validity and reliability, and will help authorities to evaluate the conservativism of the exposure estimates within registrants' exposure scenarios.

Acknowledgement: This work was supported by the German Federal Institute for Occupational Health and Occupational Medicine (BAuA). The assistance given by colleagues at BAuA, the data owners and the ETEAM Project Advisory Board during the study is gratefully acknowledged, as is the support of IOM colleagues and the participants in the between user reliability exercise.

"TREXMO" Tool: a New Tool to Support User of Occupational Exposure Models

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Occupational exposure models are frequently used in exposure assessment required by the European Union's regulation on the Regulation, Evaluation, Authorization and Restriction of Chemicals (REACH). These models predict exposure concentrations in a given occupational exposure scenario. The occupational exposure models vary significantly in complexity, purpose and required level of expertise from the user, and are strongly influenced by input parameters. Given the same exposure scenario, different users, having slightly different interpretation of input parameters, may get different results.

This paper describes the development of a new exposure assessment tool, which includes the most used exposure models. The goal is to reduce the data required to obtain exposure prediction for the same scenario using different models. The tool, that we named "TREXMO" (Translation of the Exposure Models), produces estimates for each individual model by "translating" the input parameters into other models. A set of established translation rules allows the conversion of input parameters into different exposure models to provide the most appropriate (analog) exposure scenario.

The translation links were developed for commonly used exposure models: Advanced REACH Tool (ART), Stoffenmanager, ECETOC TRA, MEASE, EASE and EMKG-EXPO-Tool. Each model was rebuilt into Data Description and Transformation Language (DDTL, University of Geneva). DDTL is a three-fold descriptive language used to generate complex datasets within forms, to establish workflow of individual, and to enable communication to the other forms. The language is intended to facilitate re-build of exposure models and implementing of previously established translation rules.

"TREXMO" Tool has several advantages: 1) Six of the most commonly used exposure models can be used with one single interface; 2) the software tool can be used to obtain predictions from a single exposure model as well as to produce appropriate input parameters within the other models; 3) the tool allows systematic comparison of the exposure estimates; 4) Finally, in structuring the translation and reducing the required input data, the model is also expected to reduce between-user uncertainty and operating time.

Assessment of chemical risk: simplified implementation of physical modelling

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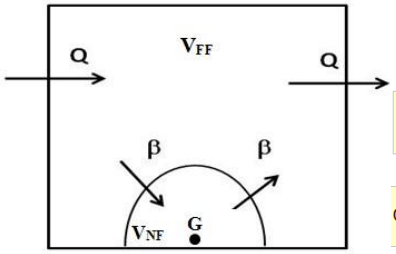
In France, assessment of exposure to chemical agents by inhalation must necessarily be done by air measurement in the event of exposure to a CMR substance or when the level of risk is not low.

An initial portrait of chemical risk at Glassolutions' two sites, carried out according to the INRS ND2233 method (Vincent *et al.*, 2005) (based on the combination of the following parameters: dangerousness, volatility, process, collective protection), showed that 87% of exposure situations were classified as medium- or high-risk. The cost of the subsequent measurement campaign to be carried out, not to mention technical feasibility problems, was estimated at €400 K for the first year.

Since the ND2233 method (Vincent *et al.*, 2005) did not appear to be sufficiently discriminant, producing too many "false positives", the idea was to test at two pilot sites physical modelling proposed by AIHA to characterise exposure situations, before using the simplified method to enable HSE managers onsite to carry out the assessment themselves.

The model best adapted to Glassolutions' work situations is the 2-zone model with continuous emission (Keil *et al.*, 2009). AIHA's tool calculates the pollutant concentrations based on five input data values: the volume of the near-field zone V_{NF} in which the operator is located as well as the pollutant emitted at an emission rate G, the volume of the far-field zone V_{FF}, the interzone flow rate β, and the ventilatory flow rate Q (Figure 1).



$$C_{NF}(t) = \frac{G}{Q} + \frac{G}{\beta} + G\left(\frac{\beta \cdot Q + \lambda_2 \cdot V_{NF}(\beta + Q)}{\beta \cdot Q \cdot V_{NF}(\lambda_1 - \lambda_2)}\right)\exp(\lambda_1 \cdot t) - G\left(\frac{\beta \cdot Q + \lambda_1 \cdot V_{NF}(\beta + Q)}{\beta \cdot Q \cdot V_{NF}(\lambda_1 - \lambda_2)}\right)\exp(\lambda_2 \cdot t)$$

$$C_{FF}(t) = \frac{G}{Q} + G\left(\frac{\lambda_1 \cdot V_N + \beta}{\beta}\right)\left(\frac{\beta \cdot Q + \lambda_2 \cdot V_N(\beta + Q)}{\beta \cdot Q \cdot V_N(\lambda_1 - \lambda_2)}\right)\exp(\lambda_1 t) - G\left(\frac{\lambda_2 \cdot V_N + \beta}{\beta}\right)\left(\frac{\beta \cdot Q + \lambda_1 \cdot V_N(\beta + Q)}{\beta \cdot Q \cdot V_N(\lambda_1 - \lambda_2)}\right)\exp(\lambda_2 t)$$

Figure 1 Representation of the 2-zone model and equations for calculating concentrations

Physical modelling was applied at 58 work situations classified by ND2233 as "NOT LOW RISK". At the same time, measurement campaigns were carried out to test the validity of the modelling. Measurements produced air concentrations of the substance that were lower and also in line with those obtained by modelling. These initial results therefore confirmed the theoretical value of modelling at Glassolutions while highlighting the difficulties involved in its practical implementation.

Simplification hypotheses were therefore constructed for all the parameters of the equation $C=f(V_{FF}, V_{NF}, Q, \beta, G)$ based on real work observations and air speed measurements. The impact of each parameter on the concentration of the substance was analysed. The parameters were thus able to be simplified using the following hypotheses:

-all of the product evaporated, the rate of emission is therefore equal to the quantity used per unit of time,
-the operator is either seated or standing, which determines the possible values for V_{NF} and β,
-the operator works in a volume V_{FF} corresponding to a small room or a big workshop,
-the work space is or is not ventilated, criterion defining the Q values.

For example, the volume of the near-field zone was simplified by observing that, at Glassolutions, operators work facing a work surface, or in a hemisphere. The two possible options are therefore related to the operator's position, either sitting (r=0.5m, or $V_{NF}=0.26m^3$), or standing (r=1m or $V_{NF}=2.09m^3$).

Since the rate of emission G is difficult to determine in practice, the simplification solution consisted in reversing the equation by setting an objective for air concentration of the substance at less than 10% of the OEL.

The final equation G=f(V_{ZR}, V_{ZE}, Q β, 10%OEL) gives the maximum quantity of the product to be used, under the exposure conditions adopted, to not exceed 10% of the OEL. If this quantity is not exceeded, the risk is low and the measuring obligation is no longer imposed for these situations.

All of the concentrations obtained by simplified modelling produced results higher than those obtained by complete modelling. In the end, 57% of exposure situations were classified as "LOW RISK" by physical modelling. The remaining situations were either previously classified as "low risk" by the INRS ND2233 method (13%), or required further analysis by an expert capable of determining a degree of risk on a qualitative level, or required an assessment through measurement.

In conclusion, with the limits to modelling taken into account (model applying to volatile organic compounds with an OEL, for which the total evaporation hypothesis is realistic), simplification reduces the need for measuring and more easily identifies work situations for which improvements are necessary.

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Scale-Up of oxygen Carrier for Chemical-looping combustion using Environmentally SuStainable materials (SUCCESS) – Occupational exposure assessment

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Carbon dioxide (CO_2) is the major greenhouse gas and there is an increasing consensus that CO_2 from combustion of fossil fuels contributes to a global climate change. One way to decrease CO_2 emissions is to capture the CO_2. Chemical Looping Combustion (CLC) is a promising process for CO_2 capturing because the CO_2 produced is pure and ready for re-use without the need of additional equipment/energy for separation. CLC consists of two reactors, a fuel reactor and an air reactor. The fuel is introduced in the fuel reactor which contains an oxygen carrier (OC). The OC is transferred to the air reactor for oxidation and re-transferred to the fuel reactor. The OCs are complex metal-oxides which are fluid-like due to the small particles and spherical shape.

Process safety in terms of exposure and hazards related to workers has highest priority in an operating environment. Therefore it is also of high importance to investigate this at an early stage of process development.

Exposure through inhalation is considered as the most relevant exposure route during CLC. The generation of dust during manipulation of the OCs is simulated for the purpose of 1) physical-chemical characterization of the material released to the workplace atmosphere, 2) quantitative exposure assessment and 3) hazard assessment of particles generated in a simulated 'occupational' exposure setting using alternative methods. The release of OCs at the CLC workplace is simulated by sieving the OCs, simulating the handling of intensely used metal-oxide particles. This simulation is considered a worst case scenario for the real world. Measured values (Table 1) are compared with occupational exposure limit values (if available), and discussed.

		Concentration in µg/m^3							
OC	**Fraction**	**Mg**	**Ca**	**Mn**	**Fe**	**Cu**	**Al**	**Ni**	**Total Dust**
background	PM100	<	0,91	0,64	1,14	0,10	<	<	26,2
C28	PM100	10,35	***3.550***	***224,86***	12,89	0,17	0,38	0,05	1370,2
Fe imp Al2O3	PM100	0,30	1,28	0,78	*42,41*	0,04	*85.700*	<	201,0
Cu imp Al2O3	PM100	0,31	1,23	0,37	4,11	*152,43*	*124.300*	0,38	428,5
background	PM10	<	0,32	0,08	0,34	0,06	<	<	25,3
C28	PM10	0,65	*320,38*	*20,10*	5,57	0,06	0,14	0,02	106,2
Fe imp Al2O3	PM10	0,07	0,36	0,16	*11,61*	<	2,97	<	78,4
Cu imp Al2O3	PM10	0,09	0,39	0,08	1,94	*78,37*	*167,63*	0,19	266,2
OEL	PM100	10.000 (MgO)	2.000 (CaO)	200	5.000 – Fe_2O_3 (PM10)	1.000	1.000 (PM4)	100 (soluble) 200 (insoluble	

Table 1 : Exposure measurements in a simulated exposure scenario for real oxygen carrier use

Metals in the complex OCs have to be evaluated for their intrinsic hazardous properties. A test strategy based on the use of non-animal alternative methods is proposed to assess potential health hazards of selected OCs. These experimental models will allow to measure toxicological endpoints which are relevant to the hazardous properties of the OCs through the inhalation route. The alternative experimental models will be exposed to a) powder of the bulk material and b) PM10 as collected during the simulated workplace exposure.

These exposure and effect measurements, and the development of exposure scenarios will lead to a ‘realistic’ exposure assessment and risk management recommendations for the futur real plant.

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Session 2

AIR AND BIO-MONITORING

Chairpersons:

Kevin Ashley, NIOSH**,** Cincinnati, USA
Florence Pillière, INRS, Paris, France

Innovative and analytical approaches in exposomic for chemical and morphological characterisation of work-room aerosols metrology

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Exposomics is the study of the exposome and relies on the application of internal and external exposure assessment methods. External exposure assessment relies on measuring environmental stressors e.g. airborne particulate matter. Although a number of major advances in both chemical identification and quantitative analysis have been achieved, exposure assessment remains the weakest part of the majority of occupational risk assessments.

Common approaches of exposure assessment strategies in occupational health include personal and stationary air sampling, application of direct reading instruments and laboratory-based analysis. The use of biomarkers to determine exposure, effect of exposure, disease progression, and susceptibility factors adds also to the complexity of exposomics. A key factor in describing the exposome is the ability to accurately measure exposures and effects of exposures.

A number of factors are involved in determining toxicological responses to chemical exposures, thus a major aim of our exposomics studies has been to characterize air particulate matter with respect to particle type, deposited amounts, mass size distribution, solubility, chemical reactivity, surface characteristics, as well as frequency and duration of exposure. The assessment of and accounting for the bioaccessibility and bioavailability should also play an important role in occupational risk assessment. However, until recently information on bioaccessibility is not clearly included in the risk assessment process. These factors will be highlighted with examples from exposure assessment studies developed for and performed in tunnel construction, mining, primary aluminium production and welding.

New NIOSH sampling and analytical methods for occupational exposure assessment

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The NIOSH Manual of Analytical Methods (NMAM: www.cdc.gov/niosh/nmam) is a collection of methods for sampling and analysis of contaminants in workplace air (or surfaces) and in the blood and urine of workers who are occupationally exposed. NIOSH methods are used worldwide for occupational exposure assessment to chemical and biological agents. These methods have been developed or adapted by NIOSH and/or its partners and have been evaluated according to established experimental protocols and performance criteria. NMAM also includes associated chapters on quality assurance, sampling guidance, instrumentation, aerosol measurement, gas and vapor monitoring, portable monitoring devices, and so forth.

Several new NIOSH analytical methods for chemical agents in air samples and in biological specimens have been published recently while numerous others are in development. Examples of newly-promulgated methods for workplace air sampling and analysis include procedures for measurement of inorganic acids, vinyl acetate and metals/metalloids. Newly recommended procedures for gravimetric and elemental analysis of occupational air samples, based on the use of internal sampling capsules, are presently under review. New NIOSH biomonitoring methods include procedures for measurement of toluene in blood and various biomarkers of exposure in urine collected from workers.

Often NIOSH methods are developed in coordination with voluntary consensus standards organizations such as ASTM International, the Comité Europeen du Normalisation (CEN) and the International Organization for Standardization (ISO). NIOSH also has formal Memoranda of Understanding (MOU) with several sister institutes in other countries, e.g., France, Germany, England, Canada and Japan. MOUs can serve as a vehicle for analytical data sharing, methods development and technology transfer.

An overview of the new NIOSH methods along with related research and technology transfer activities will be provided, with selected examples in applications to exposure science. Efforts to provide guidance on direct-reading monitoring devices are also of considerable importance. Included in the discussion are newly approved methods and those under development, as well as needs for new methods, updates and recommendations concerning exposure assessment protocols.

Appropriate evaluation of 4,4’ methylene diphenyl diisocyanate (4,4’-MDI) aerosols using a CIP10 individual dust sampler

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4,4’ methylene diphenyl diisocyanate (4,4’-MDI) is recognised as an irritant and a respiratory and skin sensitiser. The main occupational disease related to overexposure to MDI monomer and oligomer is occupational asthma. When assessing such exposure, all of the isocyanate functions should be measured because the health problems are related to exposure to them. Therefore, the oligomers should be measured as well as the monomer. MDI is used in many industrial applications involving polyurethane. An example of an application in which MDI is used is spraying on quick-setting insulation foam. It is known that sampling MDI while insulation foam is being applied is a challenge when existing sampling techniques are used.

The reference method used here for evaluating exposure to MDI during spraying on of insulation foam is the bubbler. A bubbler collects the aerosol by using a flow of air that bubbles through a solvent containing a derivatising agent. The MDI aerosol is thus solubilised instantly by the solvent and stabilised immediately by the derivatising agent. Since the collection and the reaction take place immediately in the liquid medium, the effectiveness of the bubbler is considered to be optimum. However, the bubbler approach has serious limitations such as the risks of explosion, of leakage, and of the container being punctured or bursting. For several decades now, studies have been focusing on replacement sampling techniques that would make it possible to avoid the limitations related to the use of bubblers, but that would offer the same sampling effectiveness. Sampling devices such as filters, strippers, and other devices have been proposed, but so far those techniques are of limited effectiveness or are not yet characterised for MDI sampling during spraying on of insulation foam.

A new alternative using an individual dust sampler (CIP10) in its microbiological configuration (CIP10M) was investigated for sampling MDI while applying insulation foam. The aqueous medium normally used with the CIP10M was replaced with a non-volatile co-solvent to which the derivatising agent 1-(2-methoxy-phenyl) piperazine (MOPIP) was added. When the CIP10M is in operation, the air containing the MDI aerosol is sucked in through the inlet of the CIP10M and directed towards the rotary cup containing the co-solvent. Once the aerosols reach the co-solvent by being driven by the centrifugal force, the MDI aerosols are dissolved in the co-solvent and are stabilised immediately by the MOPIP. The aim of this presentation is to describe the laboratory optimisation of the CIP10M for sampling MDI effectively and to compare the performance of a CIP10M for sampling MDI aerosols in real operations in the field while insulation foam is being applied, in parallel with the reference bubbler technique and with the stripper.

The linearity domain of the method in air extends from 0.04 ug/m^3 to 1.3 ug/m^3, assuming sampling of 60 min at 10 L/min. The comparative sampling was conducted in a real environment of spraying on MDI foam, with CIP10Ms, bubblers containing toluene/MOPIP (reference method) and strippers. The results obtained show that the CIP10M procures levels of MDI monomer that are of the same order of magnitude as the levels obtained with the bubblers, and levels of MDI oligomers that are higher. The mean negative bias observed for MDI monomer was 14% whereas the positive bias observed for MDI oligomers led to twice the concentration of the bubblers, the two biases being calculated with a confidence level of 95%. For its part, the stripper significantly underestimated the levels of MDI present. The CIP10M appears to be a promising approach for assessing exposure of MDI aerosols in insulation foam applications.

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Feed, food … and then workers' safety: Innovative analytical tools for the measurement of mycotoxins

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The occurrence of mycotoxins in crops affects feed and food safety and international trade. The effects on animal and human health are widely recognized but the risk to workers remains poorly documented. The dangers posed by these substances justify an assessment of occupational exposure when handling contaminated materials. A method has been experimentally validated for the most frequently occurring mycotoxins and is now proposed with a specific air-sampling strategy and a detailed analytical protocol. (INRS, 2013) It meets the criteria required for reproducible and reliable methods and, being developed for health and safety laboratories to directly measure one or more of 7 airborne mycotoxins, was intentionally kept simple.

A number of new devices for sample preparation have been tested:
- a molecularly imprinted polymer (Mip);
- an oligosorbent (OS) based on grafted aptamers,
- and some recently proposed multi-mycotoxin immunoaffinity cartridges.

We assessed whether molecular recognition based-sorbent could constitute a cost-effective alternative to the antibody-based sorbents that are currently used. (Mycodiag project, 2014) Furthermore, these potentially reusable tools could form part of a complete on-line and automated pre-column extraction; the advantages would be fewer manual steps, reduced risk of loss and improved reproducibility and quality assurance. OS and Mip miniaturization, and on-line coupling of these sorbents with nano liquid chromatography, was undertaken within the framework of the Mycodiag project. Multi-mycotoxin immunoaffinity columns would allow for a screening method to be conducted simultaneously with quantitative measurements. By April 2015, the quantification limits of the updated methods will have been estimated and compared to the sensitivity required for real measurements.

Mycotoxins are chemicals produced by biological organisms and require measurement methods which combine traditionally isolated domains: chemical and biological risk; dust sampling and measurement of organics; food/feed/environmental safety and worker safety. Long before the innovative tools can be adopted for feed and food control, their potential for extracting mycotoxins from contaminated airborne dust is thus determined. Our own requirements are technical rather than legal, and are aimed at increasing the quantification limit. Further improvements to their user-friendliness should make it possible to perform more and easier measurements. Biological monitoring will also be assessed in the near-future as a complementary action to air sampling. This should respond to research needs related to the newly identified mycotoxin exposure pathways: inhalation or skin contact.

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Beryllium Exposure Assessment: Review of Sampling and Analytical Developments and Impending U.S. Regulatory Changes

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Beryllium is used in a variety of industries worldwide due to its combination of low density and high tensile strength. It is also a good neutron reflector, making it desirable for nuclear applications. However, inhalation of sub-microgram quantities can result in sensitization and, in sensitized individuals, chronic beryllium disease (CBD), which leads to gradual degradation of pulmonary function and has no known cure (Kreiss et al., 2007). In recent years, as the toxicity of beryllium (metal, alloys, and oxide) has become better known, occupational exposure limit values (OELVs) have been lowered. In 2009, ACGIH lowered its Threshold Limit Value to 0.05 $\mu g/m^3$ based on the ISO 7708 inhalable fraction (ACGIH, 2014). Some jurisdictions have lower limits, and others, such as Germany (Nies, 2012), are considering reductions. Although less well established, studies have indicated a potential risk from dermal exposure to beryllium (Tinkle et al. 2003; Day et al, 2006). The low OELVs make exposure assessment for beryllium particularly challenging from both sampling and analytical standpoints.

This presentation will review and update developments in sampling and analytical technologies for beryllium exposure assessment. Recent efforts to improve air sampling technologies have focused in two areas. Current samplers designed to collect the inhalable fraction are not disposable and can be difficult to clean to sub-nanogram levels between uses. For this reason, a disposable inhalable sampler is under development (Volckens, 2014). Also, some particulate matter deposits on the inner walls of most samplers (Harper and Demange, 2007). Sampler inserts have been developed that can alleviate this problem (Lee et al, 2014).

During the decade of 2000-2009, considerable analytical advancements took place, such as development of a sensitive, specific, field-deployable fluorescence method (Ashley et al., 2007) and studies showing the optimal methods for solubilizing beryllium (Oatts et al., 2012; Goldcamp et al., 2009). In recent years, advancements have been more limited. Direct-measurement techniques such as laser induced breakdown spectroscopy (LIBS) have shown promise but continue to have limitations. Also, due to differences in toxicity among the various forms of beryllium, methods to differentiate among those forms analytically are desirable, but no demonstrated methods are currently available that are within the reach of a typical environmental or industrial hygiene laboratory.

A brief discussion will be provided on potential benefits and pitfalls of dermal sampling (Whitney, 2014). As an example, hands contaminated with beryllium particulate may be a vector for inhalation exposure (Nicas and Best, 2008) that would not be measured by air sampling alone.

In the United States, changes to both the Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) and the Department of Energy's (DOE) Chronic Beryllium Disease Prevention Program (CBDPP), 10 CFR 850, have been under consideration for several years and are expected to be promulgated in early 2015. The CBDPP is the only U.S. regulation requiring Be surface sampling, but changes to housekeeping standards in the OSHA regulation may result in an increased need for surface sampling. In addition, changes to one or both of these regulations may cause new sampling or analytical challenges.

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Occupational exposure to bisphenol A in Finland

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The aim of this research project was to assess occupational exposure to bisphenol A (BPA) in Finland using biomonitoring and industrial hygienic measurements. Exposure was evaluated in paint manufacturing, in composite product and thermal paper manufacturing, and in a tractor factory. In addition, exposure to BPA in the handling of thermal paper receipts was evaluated by simulating work as a cashier.

The results revealed specific work tasks in which significant occupational exposure to BPA may occur. In the manufacturing of liquid paint hardener, urine samples collected after the working day showed BPA levels of up to 100–170 µg/l. Workers in thermal paper manufacturing were also exposed to BPA, especially those working in the manufacture of coating material and operating coating machines. Average end of shift and evening urine BPA concentrations were over 300 µg/l in coating machine workers. The highest BPA concentrations were in the range of 1000–1500 µg/l. BPA concentrations in air samples were typically low except in some short-term duties related to the handling of solid BPA. The results suggest that the exposure mainly take place via contaminated skin (including skin-mouth contact) and clothes. In the rest of the companies and work tasks, urine BPA levels were typically in the range of the general population.

Possible occupational BPA exposure via thermal paper receipts was simulated using three volunteers. The simulation experiments were conducted under conditions representing reasonable worst case exposure as a cashier in a supermarket. The volunteers' urine BPA concentrations were followed for two days. The concentrations remained at the level of the general population and no significant increases above normal background daily variation were seen. The results suggest that normal handling of BPA-containing thermal paper as a cashier does not lead to significant additional BPA exposure. Estimated maximum daily intakes of BPA during thermal paper simulation experiments remained below 0.2 µg/kg. Diet seemed to be the major cause of daily intra-individual variation in urinary BPA levels (seen, e.g., as peaks before the simulation experiments and in simulation experiment with BPA-free paper).

In addition, background urinary levels of BPA were investigated in occupationally non-exposed volunteers working at FIOH (n=121). The 95. percentile of the urine BPA concentration was 8 µg/l, which was chosen as the reference limit for the non-occupationally exposed population. A urinary BPA concentration of 250 µg/l was recommended as a target level of occupational exposure in industrial workplaces using BPA. This level can be calculated to roughly correspond to the recent EFSA proposal for temporary TDI for BPA (5 µg/kg/day). Target levels are meant to help workplaces improve their industrial hygienic practices. It should be, however, noted that there are currently several uncertainties related to the dose-response relationships of the health hazards of BPA.

According to the results, exposure to BPA may occur especially in work tasks in which pure BPA is used. In these work tasks, special attention should be paid to wearing suitable personal protective equipment. The skin should be carefully protected and respiratory protective devices with dust filters should be used in dusty working conditions. Biomonitoring is recommended as the primary method for the assessment of occupational BPA exposure.

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Occupational exposure to Bisphenol A via thermal paper. Urinary biomonitoring study

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As an essential component of polycarbonate plastics and epoxy resins, Bisphenol A (BPA) is found in numerous industrial and consumer products, including electronic goods, household equipment, metallic food and beverage cans, bottles and dental devices. BPA is also used in the production of thermal paper. BPA may cause adverse health effects because of its estrogenics properties. The widespread occurrence of BPA in environment and consumer urines has raised concerns among regulatory agencies all over the world. In humans, BPA is metabolized in the liver to its glucuronide forms and eliminate mainly through urine. A quantification of free and conjugated forms of BPA in urine is thus a valuable tool for the assessment of human exposure. The objective of this work was to develop an accurate method for the determination of free and total BPA in urine samples, using liquid chromatography tandem mass spectrometry (LC-MS/MS) and thus to assess occupational exposure via thermal paper.

Conjugated BPA forms were hydrolysed enzymatically from urine to release free forms. BPA was extracted with toluene and derivatized with dansyl chloride before analysis (Fox *et al*. 2011). The liquid chromatography separation was performed in a reversed-phase column and BPA-d_6 was chosen as internal standard. BPA and BPA-d_6 dansyl derivatives were thus analyzed by LC-MS/MS operating in positive mode. The validation criteria of methods were assessed in pools of spiked urine samples.

The LC-MS/MS method was shown to be selective, reproducible, accurate and complied with good laboratory practices. Exogenous contamination due to releasing of BPA from the materials and solvent contamination was minimized. Background levels of BPA from the overall procedure were determined before each run. On account to its sensitivity, our method was thus suitable for biomonitoring of occupational and environmental exposures to BPA.

90 cashiers from various sale areas handling daily thermal paper receipts were recruited for urinary BPA monitoring. We also investigated the urinary BPA levels of 44 non occupationally exposed workers from the same sale areas. In a second time, we investigated the urinary BPA levels of workers from a printing company handling BPA containing thermal paper on rotary press. A significant increase in urinary total BPA concentration for cashiers was observed, compared to non occupationally exposed workers. We also observed an occupational exposure to BPA via thermal paper in printing company.

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Reducing inhalation and dermal exposures to polycyclic aromatic compounds and their metabolites in the urine of hot-mix asphalt paving workers

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Our presentation goal is to summarize a comprehensive study of hot-mix asphalt paving workers that included eight publications and discuss source, nature and biological relevance of dermal exposure in relation to inhalation exposures within the asphalt paving industry.

Four workers from three-asphalt paving crews (12 workers) were monitored for three consecutive days over 4 weeks: baseline, dermal- and respiratory-protection, and biodiesel substitution. Standard working conditions were maintained during three weeks with regard to airborne, dermal and urinary metabolite exposures. During week four, biodiesel was substituted for diesel oil as a cleaning agent. Collection and analytical procedures used included two newly developed methods, 5-layer passive-organic-dermal sampler and enzyme-linked immunosorbent assay kit adapted for measuring polycyclic aromatic compounds (PAC) and their metabolites in urine. Furthermore, 24 individual urinary hydroxy-PACs were tested using isotope-dilution gas chromatography high-resolution mass spectrometry. Key variables examined included application temperature; biodiesel substitution for diesel oil; use of dermal protection, i.e., new clean pants, long-sleeve shirts, gloves, and head and neck coverings; use of respiratory protection, i.e., powered air-purifying respirators.

Of 33 individual PACs measured, fluorene, naphthalene, phenanthrene, and pyrene were most often detected with the remainder generally below detection limits. Hot-mix asphalt application temperatures significantly affected individual and summed four- to six-ring PAC exposures. Multivariate linear mixed-effects models showed substituting biodiesel for diesel oil was associated with significant reductions in total organic matter, summed four- to six-ring PACs, naphthalene, and pyrene concentrations. Under standard conditions, reducing the application temperature from 149°C to 127°C resulted in a reduction of all modeled test results of airborne exposures.

Dermal exposures measured under all conditions via passive-organic-dermal sampler and hand washing were low, with most samples for each analyte being non-detectable with the exception of phenanthrene and pyrene. Increased frequency of glove use was associated with significant reductions for hand-wash and passive-organic-dermal phenanthrene and pyrene concentrations; percent reductions ranged from 40 to 90%. Similar reductions in hand wash concentrations were observed when biodiesel was substituted for diesel oil. Application temperature, asphalt grade, and asphalt application rate (tons per hour) were found to significantly affect exposures. Combined effect of substituting biodiesel for diesel oil, frequent glove use, and reducing the application temperature from 149°C to 127°C reduced dermal exposures by 76–86%, varying by analyte and assessment method.

Post-shift and bedtime concentrations of hydroxy-PACs in urine were significantly higher than pre-shift concentrations. Similarly, over a workweek, there was no significant increase in urinary hydroxy-PACs during any experimental week. Application temperature, total organic matter and smoking status (urinary cotinine) were strongly correlated with urinary hydroxy-PACs. Compared with baseline, urinary metabolites were reduced during the dermal- and respiratory-protection weeks; only 1-naphthol was reduced during the biodiesel substitution week.

Overall, sensitive analytical endpoints and detailed field observations are critical to interpret low exposures. Common sources of worker PAC exposures during hot-mix asphalt paving included- asphalt emissions, diesel oil, lubricating oils, working on broken down equipment, diesel-powered screed systems, and elevated application temperatures. The nature and biological relevance of dermal exposure in relation to inhalation exposures was varied due to these source complications. The relative contribution of inhalation and dermal exposures to hot-mix asphalt paving workers is estimated to be 60:40, although field-parameters are influential.

Modeled data were unable to predict a relative contribution due to relatively low levels of urinary hydroxy-PACs combined with lack of statistical power. We showed reduction in personal exposure among asphalt paving workers by substituting biodiesel for diesel oil as a cleaning agent, decreasing the hot-mix asphalt application temperature, and requiring increased dermal coverage, such as the use of gloves and/or long sleeves.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health. Mention of company names and/or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

Detection of tetrahydroxylated-benzo[a]pyrene isomers in hair as biomarkers of exposure to benzo[a]pyrene and signature of DNA-adduct levels

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Although the analysis of PAH metabolites in urine has long been considered a reference biomarker of exposure to polycyclic aromatic hydrocarbon (PAH) in occupationally-exposed workers, hair analysis was recently demonstrated to enable the biomonitoring of PAH exposure just as accurately with the advantage of wider windows of detection. In addition to the "classically-analyzed" monohydroxylated metabolites, recent studies have highlighted the interest of the analysis of tetrahydrotetrols for the assessment of human exposure to PAHs.
This study addresses the hypotheses that the concentration of tetrahydroxylated-B[a]Ps in hair might be a useful biomarker of human exposure to B[a]Ps, providing quantitative assessment of the internal dose, as well as information on the possible associated toxicity related to an individual's own metabolism. Therefore, by means of an animal model, this work aims at assessing the analysis of tetra-OH-B[a]Ps in hair as new indicator of B[a]P uptake and metabolism, and evaluating the associations between DNA-adducts levels and tetrahydroxylated-B[a]Ps concentration in hair. Twenty adult Wistar male rats were randomly allocated to experimental groups receiving 0 or 10 mg/kg body weight of B[a]P solubilized in vegetable oil, by intraperitoneal injections, 5 days per week over a 28-day period. Control rats received blank vegetable oil only.

Animals were already shaved prior to the beginning of the experiment in oder to ensure that hairs collected at the end of the 28-days exposure only represented the period of exposure.Venous blood was collected 60 min after the last B[a]P injection and DNA was isolated from white blood cells (WBCs) within 24h of the sampling.
A first gas chromatography-tandem mass spectrometry (GC-MS/MS) method was designed for the analysis of B[a]P and its monohydroxylated metabolites in hair. The extraction and purification procedures as well as the analytical conditions used for chromatography and MS/MS detection were recently described (1). Calibration curves were performed using hair specimens supplemented with increased concentration levels of B[*a*]P and their metabolites from 0 to 1 ng per mg of hair. Limits of quantification (LOQs) ranged from 0.05 to 10 pg/mg for the 12 OH-B[a]Ps and B[*a*]P.

Due to the hydrophilic character of tetrahydroxylated-B[a]Ps, another method had to be developed for their analysis after releasing from DNA hydrolysis. Four tetrahydroxylated-B[a]P isomers were investigated : benzo[a]pyrene-r-7, t-8, t-9, c-10-tetrahydrotetrol; benzo[a]pyrene-r-7, t-8, t-9, t-10-tetrahydrotetrol; benzo[a]pyrene-r-7, t-8, c-9, c-10-tetrahydrotetrol; and benzo[a]pyrene-r-7, t-8, c-9, t-10-tetrahydrotetrol. The calibration curve was linear from the LOQ up to 413 adducts / 10^8 nucleotides in DNA and from LOQ up to 1 ng/mg in hair. The coefficient of determination of the calibration curve was above 0.997 for all the analytes investigated regardless of the matrix analyzed. LOQs ranged from 0.5 to 2 adducts / 10^8 nucleotides in DNA and from 0.1 to 0.2 pg/mg in hair.

The method was subsequently applied to the analysis of DNA isolated from WBCs of rats exposed to B[a]P as described above. The study led to the detection of the four targeted tetrahydroxylated-B[a]Ps. The results obtained confirm that this method was sufficiently sensitive to detect environmental exposure levels since all DNA samples measured were above the LOQ for benzo[a]pyrene-*r*-7,*t*-8,*t*-9,*c*-10-tetrahydrotetrol and two of them tested positive for benzo[a]pyrene-*r*-7,*t*-8,*c*-9,*c*-10-tetrahydrotetrol (2). The benzo[a]pyrene-*r*-7,*t*-8,*c*-9,*c*-10-tetrahydrotetrol proved to be the most abundant isomer in both treated and control animals, followed by benzo[a]pyrene-*r*-7,*t*-8,*t*-9,*c*-10-tetrahydrotetrol.

Regarding hair analysis and upon comparison of chromatograms obtained from the analysis of blank and supplemented hair samples (2 pg/mg of tetrahydroxylated-B[a]Ps) and of hair samples collected from animals exposed to B[a]P, the four tetra-OH-B[a]P isomers investigated were detected (Figure 1). Interestingly, benzo[a]pyrene-*r*-7,*t*-8,*c*-9,*c*-10-tetrahydrotetrol, which appears as the most abundant isomer in hair, proved to also be the principal isomer released from DNA adduct hydrolysis in humans (2,3).

Moreover, the levels of benzo[a]pyrene-*r*-7,*t*-8,*t*-9,*c*-10-tetrahydrotetrol concentration in hair were significantly superior to those of 2-OH, 1-OH, 9-OH monohydroxylated forms and similar to 3-OH-B[a]P levels classically

analyzed in urine. The results obtained demonstrated for the first time that the analysis of tetrahydroxylated-B[a]Ps in hair might be a useful biomarker of human exposure to PAHs as it provides quantitative assessment of the internal dose. These new biomarkers might be of particular interest for the assessement of occupationally-exposed workers in the future.

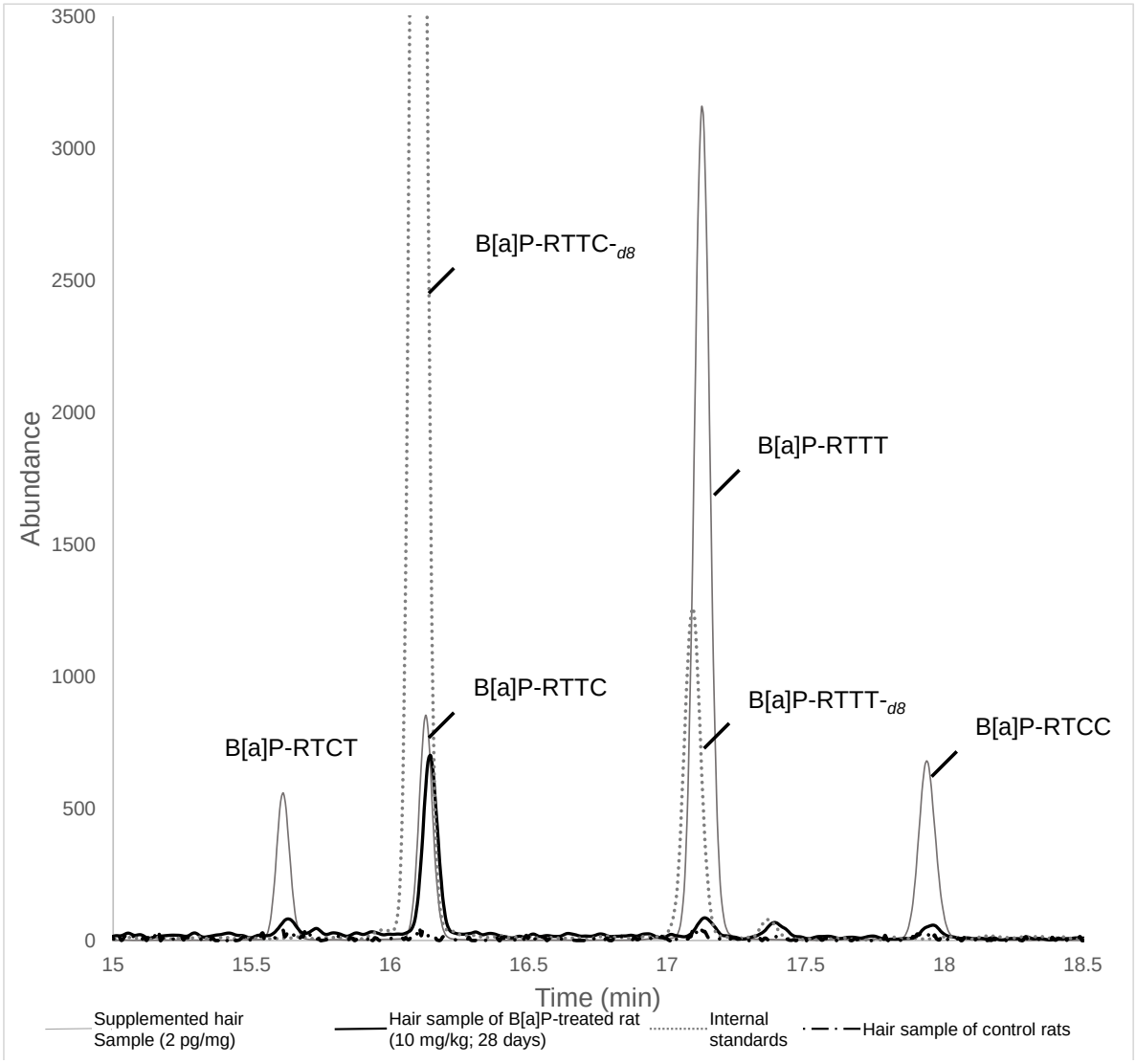

Figure 1: Chromatograms obtained from the analysis of tetra-OH-B[a]Ps in hairs of rat supplemented with 2 pg/mg of each isomers, hair sample collected from control rats and hair sample collected from B[a]P-treated rats (10 mg/kg b.w., i.p. 5 times per week for 28 days).

Acknowledgments: This work was supported by ADEME (agence de l'environnement et de la maîtrise de l'énergie) and ANSES (agence nationale de sécurité sanitaire de l'alimentation, de l'environnement du travail)

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Session 3

NEW APPROACHES IN EXPOSURE ASSESSMENT & RISK REDUCTION

Chairpersons:

Olivier Le Bihan, INERIS, Verneuil-en-Halatte, France
Dominique Thomas, ENSIC, Nancy, France

Monitoring occupational exposure using real-time detection

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Keywords: Solvents, VOC, fumes, vapours, dust, oil mists, IR analyser, PID, OPC, CAPTIV

Within the framework of its occupational health mandate, the interprofessional health service of the Vallée de l'Arve (Rhône-Alpes region) assesses the exposure of its members' employees to different sources of pollution in order to recommend technical and medical prevention measures. For that purpose, it uses traditional indirect methods, but also direct methods such as real-time detection. The activity sectors concern industry with a specialisation in bar turning, woodwork, etc.

With regard to exposure to chlorinated solvent fumes and vapours in the industrial washing activity, the use of IR or PID analysers gives insight into the levels of exposure, and also highlights emissions during the industrial process. These solvents are gradually being replaced by A3 solvents. Monitoring of VOCs emitted by these solvents is done with a PID worn by the washer. These atmospheric measurements may be accompanied by video recordings of the employee. These recordings may be synchronised through data logging using the CAPTIV software. This method is also applied to dust and fumes using an optical particle counter as a sensor. Therefore, real-time detection enables a finer analysis of the activity of employees and their interaction with pollutants. It could also raise members' awareness of their employees' chemical risks, for example, when projecting CAPTIV videos during health and safety committee meetings.

Development and validation of a tool for mapping operator exposure at workstations DACTARI: trajectography acquisition device for individual risk analysis

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In numerous cases, operators are required to move around in a certain area considered their "workstation". When those operators are exposed to pollution, generally the levels of that exposure vary in time and space.
To propose workstation improvements, it is necessary to have a tool that allows for continuous analysis producing this spatial and temporal information.

This type of information is very useful for identifying the areas in which priority action to protect the operator must be conducted because of long presence and/or high exposure levels. The results of this mapping must also enable the positioning of fixed detectors to represent the workstation's overall exposure.

The DACTARI system developed by INRS meets this requirement because it enables simultaneous and synchronised recording of occupational exposure data for operators at their workstations, of their location in the workshop (geolocation) and a video of their activity. The files so obtained can be presented in 3D or 2D false colour graphs (Figure 1). They can then be perused by OSH practitioners in order to propose prevention solutions adapted to the workstation in question.

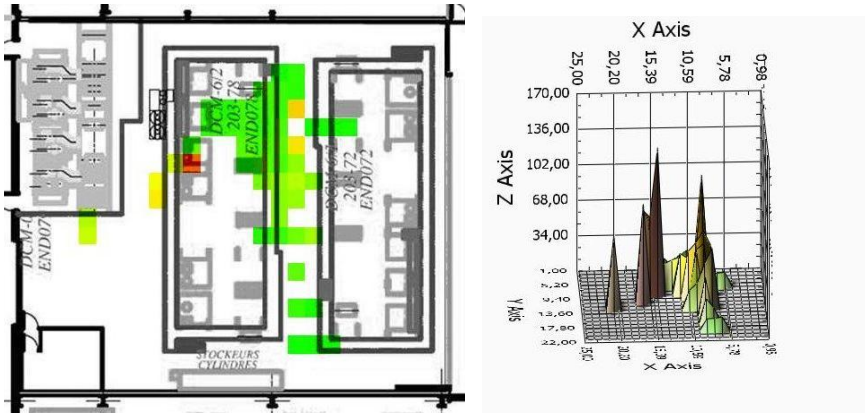

Figure 1. Presentation of the results of measurements taken using DACTARI.

DACTARI is simple to use in the field since it takes only 15 minutes to be set up. It does not require any prior installation in the company and given its small size, it does not cause any obstructions in the workshop in which it is installed. There is also a simplified version of the tool which uses a tablet. Its purpose is to generate quick operator exposure maps (for example during a pre-visit to a company).

This tool was validated in a real industrial situation presenting a risk of exposure to volatile organic solvents. This enabled us to identify the areas and the periods of professional activity during which the operator is particularly exposed.

In addition to the action report presenting the exposure maps, video sequences of the place studied together with real-time exposure measurements can be presented.

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Risk analysis and reorganisation of a workplace dedicated to a nano-ZrO2 process

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The FP7 Project Sanowork supports developments on worker protection and exposure risk management for nanomaterial production. An exposure assessment was implemented at six workplaces in order to evaluate the effectiveness of existing and proposed exposure reduction strategies. Based on this rik assessment, based on qualitative and quantitiative measurement campains, recommendations were provided for reorganisations at some workplaces to improve the safety of workers.

These measurement/risk assessment campaigns aim to identify the potential emission sources, exposure scenarios, environmental factors (e.g. ventilation, safety procedures, personal protection equipments, etc). Some simple measurement devices were used so as to give an indication on the background concentration and particle generation (particle count) during the process. For the relevance of this study, it is necessary to schedule and to perform the measurements when the process is running so as to verify the presence of Engineered NanoMaterials (ENMs) during the main operations like handling, production, cleaning. These measurements essentially focus on near field emissions so as to characterize the source term. A TEM Mini Particle Sampler (MPS®, Ecomesure – R'mili 2013) is coupled to a particle counter (CPC) enables to perform an in-depth off-line analysis (morphology, chemical composition). This technique is inexpensive, portable, and easy to implement.

The measurements were associated with an analysis of the configuration of the workplace (process) and of the worker practices. This information is useful to identify exposure scenarios. Moreover, process information, regarding ventilation, personal protection equipment were used to realize the risk assessment.

The present contribution focuses on a pilot process line that implements a sol-gel technique to recover ZrO2 nanopowders. Two measurement campaigns have been carried out, respectively before and after a reorganisation of the workplace.

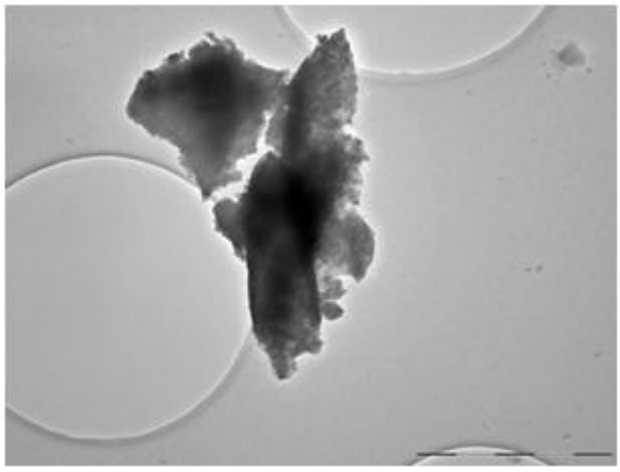

Figure 1. Example of ZrO2 inhalable particle.

The results of the first campaign showed that drying and retrieval of the ZrO2 powders in an oven were the only highly emissive operations with various sizes and types observed (cf. example of ZrO2 particle in Figure 1) in normal conditions of work. Recommendations were given to reduce emissions notably by placing the oven under a fume hood.

The process line was then modified by implementing this recommendation. The second measurement campaign, on the new setup, demonstrated the efficiency of the recommendation with the limitation of the emissions of the nanoparticles (NPs) at the workplace: the measured number concentration in particles over time during the operations of handling and opening the oven door showed no peak or increase in concentration in contrast with the observations made in the first measurement campaign. This was also confirmed by the TEM analysis since the the particles observed on the TEM grid were those commonly found in ambient air (no NPs observed).

So, the remediation recommendation which consists in placing the oven in the fume hood appeared efficient to limit the dispersion of particles in the workplace atmosphere and, as a consequence, the exposure of workers to NPs at the workplace.

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Skin exposure to bitumen: mutual contributions to ergonomics and metrology

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Workers applying asphalt concrete to road surfaces are exposed to bitumen. There is quite a lot of uncertainty surrounding knowledge about the health effects of exposure to bitumen through inhalation and skin contact (CIRS, 2011).

However, given the practical difficulties in particular, there is no standardised method currently available for assessing skin exposure to bitumen. This lack of a common methodology makes data comparison very complicated and even risky.

Without a shared method for assessing this exposure to substances present in bitumen, an ergonomics approach was used to better apprehend situations in which workers are exposed to skin contact. The goal was also to determine the surfaces for possible sampling. To reach these objectives, video data was collected from worksites and analysed using an ergonomics methodology. These analyses identified two phases of activity (working phase and waiting phase), different areas exposed (one hand, two hands, face, forearm) and different objects with which the operators may be in direct contact (shovel, control panels, clothes). A grid for characterising skin contact was made based on these different variables. This characterisation tool is based on the simplified chemical risk assessment method developed by INRS and proposes scores for the surface exposed[1] and frequency of exposure[2] from 1 to 10. The scores established for the situations analysed allow skin exposure to bitumen in work situations to be specified. The skin exposure situations revealed through analysis of activity result from contact between a non-gloved hand and different soiled objects. These exposures vary according to the phases of activity and the task performed.

This data served to specify the areas of the body and the tools from which surface samples could be taken during joint observations by ergonomists and metrologists at the worksite.

Guided by the results of the ergonomics approach, the metrological approach for assessing skin exposure to bitumen covered the hand, identified as a target contact area. Cotton gloves were given to the employees to wear during the different phases of their activity. These gloves were then analysed according to a laboratory-validated protocol. The protocol consisted in depositing known quantities of two types of bitumen on clean gloves, followed by an extraction in THF to determine the recovery rate by liquid chromatography coupled with fluorescence detection. The recovery rates obtained were satisfactory for both types of bitumen considered (i.e. 96% and 105%).

The gloves collected after use by the workers at worksites were heavily soiled, with contamination levels ranging from 1 to 50 mg of bitumen per glove according to the phases of the worksite and the operators' tasks.
Since the analysis method is a global optical method, part of the quantified material may come from the deposit of bitumen fumes on the gloves. However, the very dark colour of the gloves indicates that this is a very small contribution, and that the main part is due to raw bitumen.

Sampling by wiping surfaces or tools in manual contact areas was also performed. Analyses highlighted high quantities of bitumen on these surfaces reaching several mg (i.e. from 1 to 6 mg) with significant contamination of certain tool handles between the start and end of the work day (i.e. Δ = + 5 mg).

[1] Value 1 corresponds to one hand and 10 to the upper limbs and torso, and/or the pelvis and/or legs.

[2] Value 1 corresponds to an occasional frequency < 30 min/day, 10 corresponds to permanent frequency > 6hrs/day

In order to give weight to this metrological data, future toxicological studies aimed at determining the rate of passage of bitumen through the skin are essential.
This will be followed by the establishment of solutions to prevent skin exposure to bitumen.

In order to be efficient, this establishment will be collective and will involve all players (operators, managers, OSH practitioners, etc., and even heads of companies in the sector).
It will be based on the coupling of observation data and measurements resulting from collaboration between ergonomists and metrologists.
This coupling will be considered as a shared framework, a subject for exchange among these different players.

Validation of the test bench for N95 filtering face-piece respirators - Comparison of performance measurements with simulated occupational exposure

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Nanoparticles (NPs), with a diameter of less than approximately 100 nm (ISO/TS 27628, 2007 Standard), have specific properties that are of particular interest to nanotechnology. However, due to their nanometric size, they may be deposited in the respiratory tract and cause certain lung diseases.

According to current studies, exposure to ultrafine particles (UFPs) in the work environment occurs mainly during the use of nanomaterials or during processes that indirectly generate UFPs. Yet, there is almost no specific occupational exposure limit value for nanomaterials adopted through standards or by inspection bodies.

Therefore, respiratory protective devices (RPDs) are generally used in cases where collective protection is not possible or insufficient. Consequently, the efficiency of RPDs is very important for the safety and health of workers likely to be exposed to UFPs.

RPDs are subject to tests aimed at measuring different protection factors (PFs). These PFs are all measured as the ratio of the concentration of a contaminant outside of the RPD, C_0, to the concentration inside the RPD, C_i. Only the situations in which the ratio is measured differentiate those indices. The higher the protection factor, the higher the protection provided by the respiratory protective device.

There is no study that measures the efficiency of N95 filtering face-piece respirators (FFRs) in working situations as regards UFP risks. In our study, we simulated the activity of transforming synthetic material and measured the efficiency of an N95 device in this situation. The performance of the FFR was measured for a "natural" aerosol and a globally neutral aerosol, for three constant flows and one cyclic flow, for particle sizes from 20 nm to 200 nm. This measurement was then compared to the classic measurement of FFRs carried out in laboratories in a test chamber (Mahdavi *et al.*, 2014). In addition, penetration measurements were conducted without considering leakage (the mask was sealed to a dummy) in order to compare both test benches.

According to the results, all the initial penetrations measured are lower than those required by American regulation (42 CFR 84, 1995). Moreover, the results show that there is a correlation between the penetration measurement on the laboratory test bench and in occupational exposure simulation and that the measurements of the most penetrating particle size (MPPS) are not significantly different. The laboratory approach therefore reflects the simulated activity.

Secondly, we measured the effect of the neutralisation of the aerosol on penetration measurement in the case of a simulated activity in the workplace where workers are likely to be exposed to UFPs. The results show that the neutralisation of the aerosol generated represented the most unfavourable case of exposure, as presented in the standard. We measured a slightly higher maximum penetration in the case of a non-neutralised aerosol. It can however be noted that this slight difference did not cause a shift in the MPPS. We measured maximum penetration at approximately 40 nm under all the study conditions.

ISO/TS 27628 (2007) Standard Workplace atmospheres -- Ultrafine, nanoparticle and nano-structured aerosols -- Inhalation exposure characterisation and assessment.

Code of Federal Regulations (1995), 42 CFR, Part 84, Respiratory Protection Devices, Washington, U.S. Government Printing Office.

Mahdavi A, Bahloul A, Haghighat F and Ostiguy C. (2014) Contribution of breathing frequency and inhalation flow rate on performance of N95 filtering facepiece respirators, Annals of Occupational Hygiene, 58:195-205.

Respiratory protective devices used during removal of asbestos-containing material: method for assessing their performance in work situations

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In order to protect numerous workers from the risk of exposure to asbestos fibres, the Ministry of Labour set up a regulatory system strengthened over the years to take into account in particular new asbestos toxicity elements and the development of prevention techniques and collective and personal protective techniques. As from July 2015, the occupational exposure limit value for asbestos will drop from 100 f/L to 10 f/L and testing for dust fibres will be done by analytical transmission electron microscopy (ATEM). This regulatory development must be accompanied by the assessment and improvement of worker protection solutions both at collective and personal levels.

This study is focused on the determination of assigned protection factors for the most effective RPDs worn by asbestos-removal operators. The protection factor is a parameter determined experimentally from simultaneous measurements of concentrations outside and inside an RPD, which are, in this study, determined by analytical transmission electron microscopy. This method enables, in addition to chemical characterisation of the fibres counted, the detection of fine and short fibres that are not visible by optic microscopy methods.
A preliminary study served to develop, in collaboration with manufacturers of respiratory masks, a modified prototype enabling the sampling of fibres inside that mask in compliance with the standard XP X 43-269 (2012). Batches of different models of modified masks were then manufactured in collaboration with manufacturers and certified CE according to the PPE directive 89/686/EEC.

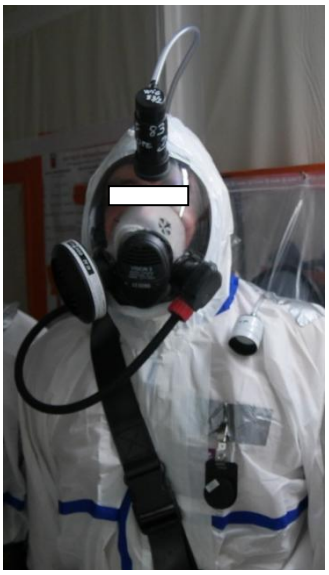

Figure 1. Operator equipped with a modified Vision 3 INRS ® mask enabling assessment of the protection factor.

An initial project was run in May 2014 to validate the protocol for sampling and decontaminating material on supplied-air respirators followed by a dozen projects involving this type of respiratory protection devices to assess their performance. The operations monitored during these projects were varied: removal of asbestos sprayed coatings, asbestos-containing plaster, sound-insulating cladding, coatings, etc. with different removal techniques, which made it possible to assess personal protection for numerous processes. Project configurations were also very varied: inside train cars, renovation of buildings (residential, commercial and public premises), industrial sites, etc.

Session 4

INPUTS FROM NUMERICAL SIMULATION

Chairpersons:

Emmanuel Belut, INRS, Vandœuvre-lès-Nancy, France
Goodarz Ahmadi, Clarkson University, New-York, USA

Computational modeling of particle transport and deposition in indoor and outdoor environments

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Computational modeling of particle transport and deposition in indoor and outdoor environments are presented. The mechanics of particulate pollutant transport and deposition in turbulent flows are discussed. The numerical simulations of airflow through the Reynolds averaged Navier-Stokes (RANS) equation, as well as DNS and LES are described. The stochastic models for simulation of the instantaneous turbulent flow velocity are discussed. The Lagrangian particle trajectory analysis methodology is presented, and the effects of various forces including drag, lift, gravity and Brownian are discussed. It was shown that the particle deposition and removal processes in turbulent flows are strongly affected by the near wall flow structures. Wind tunnel studies of particle transport, deposition and resuspension are also discussed. Examples of computational modeling of gas-solid flows in ducts, as well as, in indoor and outdoor air environment are presented. Simulations for particulate pollutant transport through human upper airways are discussed and sample results are presented. It was shown that computational modeling provided an efficient tool for studying dilute gas solid flows in complex passages.

Estimating emission profiles of a source of particulate matter (transient regime)

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Determining the "source term" responsible for pollution is a core problem in prevention, although it is particularly delicate to achieve in practice. It may be done indirectly with the use of inverse methods. This is how M. Girault *et al.* (Girault, Maillet, Fontaine, Braconnier, & Bonthoux, 2006) adapted techniques initially developed for thermal problems to the case of a gaseous pollutant emitted by a transient source of pollution. The approach used was conclusive and we therefore propose in this presentation to extend its scope of application to pollutants emitted in aerosol form. The process involves two different stages. The first consists in determining the parameters of the inversion relationship using a known aerosol source and the corresponding dust measurements (Figure 1). In the second stage, an unknown aerosol source is reconstructed based on the inversion of the model and the dust measurements (Figure 2).

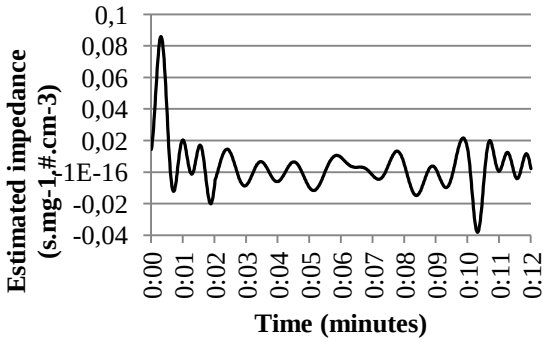

Figure 2: The relationship between the source term and the concentration measurement

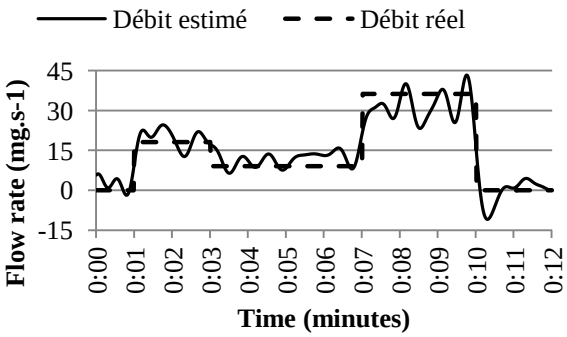

Figure 3: Estimate of a source flow rate by inversion of a measurement

The method used is based on the assumption that the concentration field measured varies linearly in relation to the particle source term. This assumption is valid only if the particles considered are not inertial, not concentrated and if the flow deposited on the walls is proportional to the concentration. Under these conditions, we can consider a convolutive approach to the physical system made up of an entry (pollutant source with flow rate), an exit (Particle concentration) and a transfer function (impulse response), also called impedance. The general temporal relationship between the entry of the system and its exit at a point therefore is as follows:

(1)

The matrix form of the system defined previously is as follows for a total number of time steps of :

, (2)

written as:

(3)

where is the Toeplitz matrix associated with impedance . The relationship (3) is commutative and can conversely be written as:

(4)

The solution to the equation (4) in the first stage of the method allows construction of the impedance using the known source term *Q* and the concentration measured *C*. In a second phase of the method, equation (3) allows the unknown source term *Q* to be estimated based on the impedance *Z* and the concentration obtained by this unknown source.

This method is applied to the experimental case in which a pollutant source is placed in an open ventilated cabin following the configuration in Figure 3. In this configuration, the pollutant source is made up of an aerosol (aluminium oxide powder with an aerodynamic diameter centred on 5µm) emitted by a PALAS RBG 1000 generator. Measurement of the concentration in the far-field region is performed with an APS placed in the cabin's outlet duct.

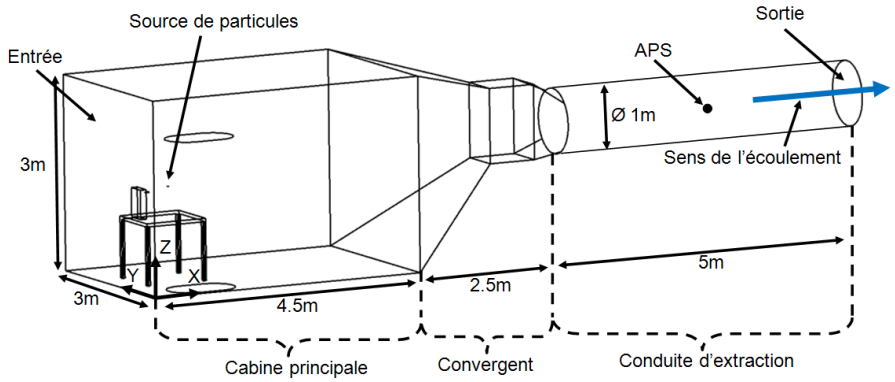

Figure 4: Positioning of the pollutant source in the experimental cabin

The analysis of the results indicates that it is possible to reconstitute the dynamics of time variation of the unknown pollutant source (Figure 2). The method also allows reconstitution to within 1% of the total quantity of pollutant emitted by the source during the length of the experiment.

Girault, M., Maillet, D., Fontaine, J. R., Braconnier, R., & Bonthoux, F. (2006). Estimation of time-Varying Gaseous Contaminant Sources in Ventilated Enclosures Through Inversion of a Reduced Model. *International Journal of Ventilation*, *4*(4), 365–379.

Numerical modelling of transport & deposition of particles in the upper airways

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Aerosol particles present in our environment are increasingly being identified as health risk factors, but aerosols can also be used for diagnostic or therapeutic purposes in treating lung diseases. The small particles that reach the gas exchange surfaces in the alveolar region of the lungs are deemed to be the most dangerous for health. When treating lung cancer, administering medicine by aerosol offers the advantage of reaching the affected regions directly and of requiring lower doses (adverse effects minimised).
The human lung is a sequence of bifurcations into two parts. On average, we have 23 generations of bifurcations, i.e. about 17 million bifurcations. The last five generations are covered with cell structures through which gas exchange takes place with the blood.

On average, an adult breaths in from 10,000 to 20,000 litres of air per day. Air contains a very large quantity of particles in the form of micro-organisms, dust, smoke, and allergens, and other toxic or non-toxic aerosols that can be deposited during the process of inhaling and exhaling. The "total deposit" refers to the particles that collect in all of the airways, and the "regional deposit" is specific to a given region.
The distribution of the deposits of the inhaled particles is determined to a large extent by particle size. The range of particle sizes occurring naturally or of human origin can be considerable. For example:

– Occupational dust: 0.001−1,000 µm
– Pollen particles: 20 – 60 µm
– Aerosol products: 2 – 6 µm
– Most particles in cigarette smoke: 0.2 − 0.6 µm
– Viruses and proteins: 0.001 − 0.05 µm.

The deposit depends on the characteristics of the particles (size, shape, density), on the morphology of the airway and on the respiratory mode (which determines the volume flow rate and the mean residence time of a particle).

Particles are generally deposited under the action of three mechanisms:

- **Impact by inertia:** this mode of deposition is a function of particle diameter and of particle speed. Particles of size greater than 10 µm impact in the throat whereas the smallest particles continue deeper into the bronchial tree.
- **Sedimentation**, resulting from the effect of gravity. It mainly affects small particles (from 1 to 5 µm) and involves only the bronchial tubes and the alveoli. It is maximised at low air flow rates and can be increased merely by marking a pause in breathing at the end of inhalation.
- **Brownian motion or scattering** is due to chaotic agitation of small particles (smaller than 0.5 µm). This mode of deposition is insignificant because 80% of the particles remain in suspension, and are then removed on exhalation.

Understanding the flow structures and the particle transport/deposition in the bronchial system remains a challenge because of the complexity of the geometry of the lungs. The work presented here is based on a large amount of collaboration between medical imaging physicians and fluid mechanics and computational fluid dynamics (CFD) researchers. Four airway configurations (Weibel's generic model, a rat lung obtained by micro-computed tomography (µ-CT), a rabbit geometry obtained by a synchrotron scanner, and a human model proposed by Hiroko Kitaoka) were generated, meshed, and simulated. Then generic flows under realistic breathing conditions were studied, as was the associated particle transport / deposition (Figure 1).

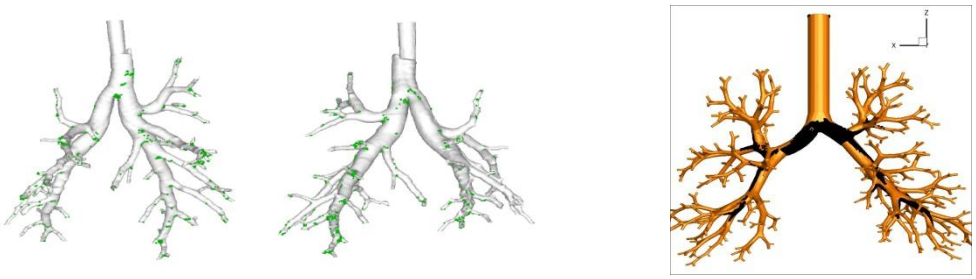

Figure 1. Deposition of particles in an anatomically realistic rabbit geometry and in a human lung model.

Acknowledgements: This study was funded by the *Investissements d'avenir* ("investing for the future") programme of the University of Strasbourg.

Session 5

“OMICS” & ALTERNATIVE MODELS IN TOXICOLOGY

Chairpersons:

Marc Baril, Montreal University, Montreal, Canada
Hakan Wallin, NRCWE, Copenhagen, Danemark

Analysis of global gene expression data for risk assessment and revelation of mechanisms of toxicology of nanomaterials

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Many chemicals around us have not been well examined for toxicological effects and risk assessment is deficient. REACH poses a tremendous challenge of filling the gap. The public increasingly challenge the need to do animal experimentation, which is the corner stone for traditional risk assessment. There is now a rapid development of in vitro tests but these have shortcomings. For nanomaterials it is problem that the interactions and adverse effects of solid materials in organisms are poorly understood. The uptake and bioadministration and long-term deposition are central to understanding how solid materials exert adverse effects. Systematic investigation of the relationship between physicochemical properties in many materials and biological interactions is needed.

Genomics, proteomics, metabolomics and other comprehensive characterization and quantification of biological perturbation may provide tools into identifying critical biological changes and mechanisms. The linking molecular events that are rate limiting or pivotal between exposure and pathologic outcome in animals may provide a shortcut for designing operational principles for risk assessment, design of high throughput testing, cross species extrapolation and design of biomarkers for human monitoring.

Proteome changes in auricular lymph nodes and serum after dermal sensitization to toluene diisocyanate in mice

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Some reactive chemicals, such as diisocyanates, are capable of initiating an allergic response, which can lead to occupational asthma after a latency period. Clinical symptoms such as cough, wheezing and dyspnea occur only late, making it difficult to intervene at an early stage.
So far, most studies using proteomics in lung research have focused on comparisons of healthy vs. diseased subjects. Here, using two-dimensional difference gel electrophoresis (2D-DIGE), we explored proteome changes in the local draining lymph nodes and serum of mice dermally sensitized once or twice with toluene diisocyanate (TDI) before asthma is induced to explore biomarkers of sensitization.

The proteomes of male BALB/c mice (6 weeks old, 20g), sensitized once (n=12) or twice (n=12) with 0.3% TDI, were individually compared with control mice (n=12) in two separate experiments using 2D-DIGE and Decyder 7.0. A commercially available ELISA kit was used to validate the difference in hemopexin levels between TDI-sensitized and control mice. Western blotting was used to validate the results of lymphocyte specific protein 1 and coronin 1a levels. GAPDH was used as an internal standard in Western blot validation experiments. Graphpad Prism 4.01 (Graphpad Software Inc) was used for statistical analysis of ELISA and Western blot data. All data were normally distributed (tested using Kolmogorov-Smirnov). An unpaired t-test was used to compare both groups (AOO vs. TDI). A level of $p<0.05$ was considered significant.

In the lymph nodes, we found 38 and 58 differentially expressed proteins after one and two treatments, respectively, between toluene diisocyanate-treated and vehicle-treated mice. In serum, 7 and 16 differentially expressed proteins were detected after one and two treatments, respectively. We identified 80-85% of the differentially expressed proteins by mass spectrometry. Among them, lymphocyte specific protein-1, coronin 1a and hemopexin were verified by Western blotting or ELISA in an independent group of mice.

This study revealed alterations in the proteomes early during sensitization in a mouse model before the onset of chemical-induced asthma. If validated in humans, these changes could lead to earlier diagnosis of TDI-exposed workers.

Acknowledgement: This work was supported by a grant of the Interuniversity Attraction Pole Program, Belgian State, Belgian Science Policy [P6/35] and from the 'Fund for Scientific Research Flanders' (FWO), [FWO G.0547.08].

Mining brain metabolomic and behavior datasets obtained from adult rats exposed to chemicals like PAHs or a mixture of PCBs, dioxins and furans: a powerful tool to assess the risk for the brain of a chronic exposure to environmental contaminants

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Polycyclic Aromatic Hydrocarbons (PAHs), PolyChloroDibenzo-p-Dioxins (PCDDs), PolyChloroDibenzoFurans (PCDFs) and PolyChloroBiphenyls (PCBs) are ubiquitous environmental contaminants that have been classified as Persistent Organic Pollutants (POPs) by the end of the 1990s. These are detected in numerous foodstuffs including fish, seafood, milk, crops and smoked and/or grilled food products. This raises the question of the risk for the human health of such exposure, especially for the brain which is an organ of high susceptibility to environmental insults. Therefore, the present study aimed to investigate the brain toxicity of the repeated ingestion of milk coming from lactating goats contaminated with mixtures of PAHs or PCDD/PCDF/PCBs in adult rats using brain metabolomic phenotyping and behavioral testing.

Six groups of Wistar male rats were considered for the study: one was administered with methylcellulose 0.3 % (MC, n=10), 3 with different non-contaminated milks (NCGM, n=10 per group), one with milk coming from goats artificially contaminated with PAHs (n=10), and the last one with milk issued from goats poisoned by feeding them with a hay contaminated with a PCDD/F/PCB mixture (PCM, n = 10). Milk or MC was daily administrated during 3 weeks (0.5 ml/100g b.w., p.o.). By the end of the period of exposure, rats were assessed for their locomotor activity in an open-field, their spatial learning and memory performances in a T maze (one session without a delay between the two trials and one session with a 120 s inter-trial delay), and their level of anxiety in the elevated plus maze (EPM) and in the conditioned defensive burying test (CDB). After the behavioral testing, the animals were sacrificed, the whole brain removed and the tissue extracts analyzed using the ^{1}H NMR spectroscopy for achieving metabolomics.

After 3 weeks of administration, PAH-treated rats exhibited a significant reduction of their level of anxiety in the open-field, the EPM and the CDB whereas the PCM animals did not. In the T maze, PAH-contaminated rats showed a significant deficit of spontaneous alternation whatever the conditions of testing used (with or without a delay between the two trials), suggesting the potency of PAHs to impair the learning and memory abilities of these animals compared to NCGM and MC rats. In the same test, learning performances of PCM-exposed animals were also affected but only when a delay of 120 s was interposed between the two trials. Regarding the brain metabolome, the multivariate statistical analysis of the ^{1}H NMR spectra of hydrosoluble brain extracts discriminated PAH, PCM and control groups (MC, NCGM) one from each other according to two orthogonal principal components accounting respectively for 32.3% and 20% of the total modeled variance by a PLS-DA-based regression.

Besides, a regularized canonical correlation analysis (RCCA) was performed using the R software mixOmics package in order to highlight the canonical correlations between the two multidimensional data sets, the first one containing the ^{1}H NMR fingerprints of hydrosoluble brain extracts and the second one referring to the behavioral data. Network plots obtained from the canonical analysis identified highly correlated clusters of differentially behavioral impairments related to anxiety and brain metabolites in both contaminated groups. Thus, metabolic

disruptions related to *myo*-inositol and to 5-oxoproline, a metabolite involved in the regulation of the brain oxidative status, were identified to be linked with the reduction of anxiety with specific variations depending on the anxiety behavioral paradigm used and the group of contaminants.

In conclusion, these results confirm the potent neurotoxicity of PAHs, PCDD/Fs and PCBs through the ingestion of contaminated food and suggest subtle differences in the brain and behavior toxicity induced by these two families of POPs. The identification of the disrupted brain metabolic pathways and the regularized canonical correlation analysis of the two sets of variables should be helpful to precise the mechanisms of action of both xenobiotic families to which organisms are chronically exposed.

Interaction between cells and polymeric nanoparticles: what toxicogenomics can bring

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Genome response following exposure to nanoparticles (NPs) is a topical challenge for toxicologists. One of the focuses for research developed by the research unit EA 3452 is to develop nanoparticles filled with donors of nitric oxide NO• (in particular nitrosothiol derivatives) for cardiovascular applications. Firstly, we determined the toxicity of empty Eudragit® NPs (ENPs), Eudragit® being a biocompatible polymer, and then we used toxicogenomics studies to compare the empty ENPs with ENPs filled with S-nitrosoglutathione (GSNO).

Considering that, regardless of the mode of preparation, a high percentage of the NPs remain empty of active ingredient and that the monocytes/macrophages are the first cells with which the NPs interact after passing through the biological barriers, the first step was to study the effect of empty ENPs on murine (NR8383) and human (THP-1) monocyte lines that had kept the capacity to differentiate into macrophages.

The cells were exposed to the ENPs, and the cell viability after 24 hours was estimated using conventional tests (MTT, WST-1, Trypan Blue). The ENPs led to a decrease in the viability of the NR8383s, and, conversely to an increase in the viability and in the growth of the THP-1s. The modification in the expression of the genes involved in oxidative stress (*NCF1*), inflammation (*NFKB, TNFA, IL1B*), autophagy (*ATG16L*) and apoptotic balance (*PDCD4, BCL2, CASP8*) was studied by RT-qPCR after 4 hours of exposure. The expression of the genes *ATG16L, BCL2* and *TNFA* was increased in the rat cells, thereby facilitating induction of the autophagy and inflammatory processes. The decrease in the expression of *NCF1, NFKB* and *IL1B* in the human cells may explain the increase in the viability and in the growth of the cells. Toxicity and *in-vitro* cell activation were dependent on the cell line used, and this should be taken into account in future applications (Ronzani, 2014a).

For the remainder of the work, we chose to limit ourselves to a single line, and THP-1 cells were chosen as the human cells, in particular because another human line (mammary epithelial cells) has also shown activation in the presence of ENPs (Hussein, 2013). The cell viability, the endocytosis of the ENPs, and the metabolic pathways involved were studied. The WST-1 test showed an increase in the cell viability both with the empty ENPs and with the filled ones. Penetration of the ENPs into the cells was shown by TEM. As of 2 hours of exposure, the cells contained individual or clustered ENPs in the cytoplasm, confirming the observation under confocal microscopy. Lastly, an initial analysis of the transcriptomes of THP-1 cells exposed to the ENPs filled with GSNO or not filled with GSNO, as well as to GSNO on its own, showed a difference in the profiles of genes involved in cellular response depending on the treatment (**Table 1**) (4 hours of exposure). All three conditions resulted in overexpression of genes belonging to the following clusters "Cellular Structure"; "Metabolic Processes"; and, to a varying degree, "Oxidative Stress". Conversely, the genes of the "Immune Response" and "Mitochondrion" clusters were activated only in the presence of GSNO. In the "Immune Response" cluster, GSNO on its own induced overexpression of the genes *CCL4, CCL3, CCL20, INHBA, VEGFA and IL23A* (**Figure 1**) (Ronzani, 2014b), whereas the filled ENPs did not. However, the filled ENPs induced a higher increase in the expression of the genes involved in metabolic processes compared to the empty ENPs or to GSNO on its own. The genes of the "Proliferation" cluster were highly activated by the ENPs on their own, but not activated at all if they ENPs were filled with GSNO (**Table 1**).

Our results show the importance of the choice of the cellular model used for assessing the toxicity of NPs *in vitro*. However, differences in expression of the genes were observed when the filled or non-filled particles were studied, the guest molecule having physiological activity by itself that can, however, differ from the activity of the substance on its own, possibly due to release being slowed down by insertion into the NP. The genomic responses were, however, consistent with the observations and with what is known about the action of NO• donors in cells.

Genomic study is becoming an essential tool for better understanding the interactions between NPs and cells, and it thus has a very full part to play in toxicology.

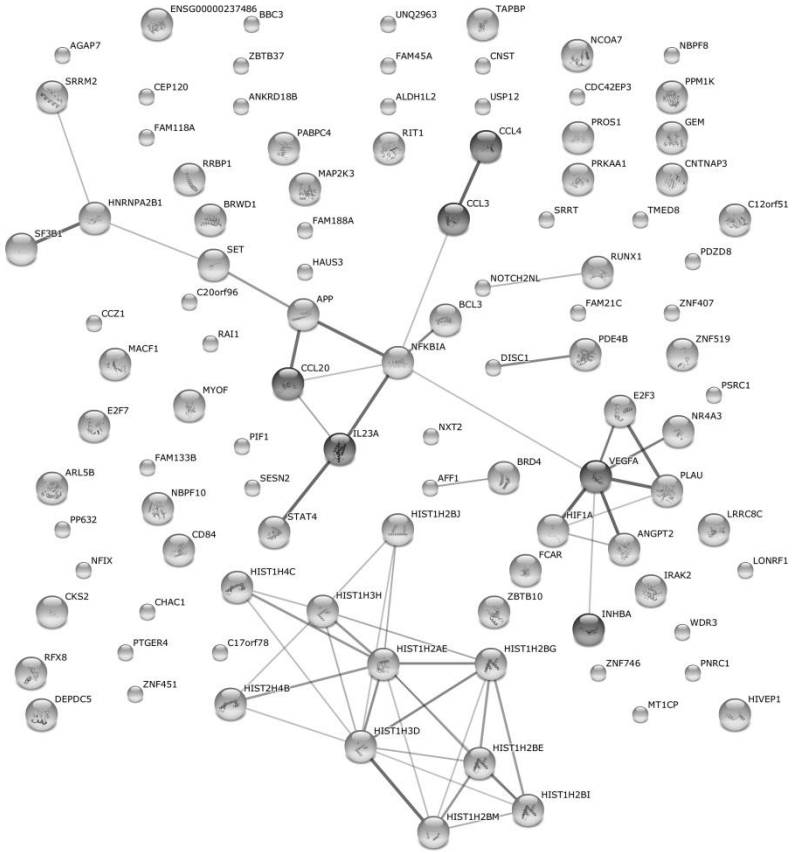

Figure 1: protein-protein interaction of the modified corresponding genes of THP-1 cells exposed to GSNO (String database, http://string-db.org/)

Table 1: Main clusters including the overexpressed genes in the response of THP-1 cells to various treatments

	GSNO alone	**Empty ENPs**	**ENPs filled with GSNO**
Cellular Structure	8	6	9
Metabolic Processes	13	7	26
Proliferation	4	5	0
Immune Response	18	0	1
Mitochondrion	3	0	6
Oxidative Stress	5	1	2

Acknowledgements: This study was funded by the French National Research Agency - *Agence Nationale de la Recherche (ANR NanoSNO)*, and Ramia Safar has a doctoral grant from the Syrian Ministry of Higher Education.

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SiO_2-NPs translocate through human bronchial barrier reconstituted *in vitro*

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Application and safe development of nanotechnologies in many fields require more knowledge about their potential effects on human health. Inhalation is the most frequent mode of non-intentional exposure to nanoparticles (NPs). Currently there is *in vivo* evidence of the ability of nanoparticles (NPs) to cross bronchial epithelial barrier and to reach secondary organs. Our aim was to quantify and characterize the translocation of different SiO_2 NPs across a human bronchial epithelial barrier reconstituted *in vitro*.

In order to form a tight epithelial monolayer, the human bronchial epithelial Calu-3 cell line was grown onto a 3 µm-porous membrane separating the culture well in two compartments, an apical and a basolateral chamber, during 14 days. Its integrity and efficiency as a barrier was assessed by monitoring the paracellular transport of a fluorescent marker (Lucifer yellow) as well as measuring the transepithelial electric resistance (TEER).

We compared the fate of silica NPs of three different sizes (16- 50- 100 nm) and surface charges (SiO_2 or SiO_2^+ whose the surface sites are chemically converted to ammonium groups). These NPs were fluorescently labelled by FITC fluorochrome for facilitating their detection after cellular uptake and quantifying their translocation.

We first investigated the ability of the different NPs to cross the porous membrane in the absence of cells. Whatever their size or surface charge, 24 hr after their application to the apical chamber, the NPs were recovered in the basoalteral chamber with an increasing NP amount while the applied apical concentrations were decreasing.

NPs were non cytotoxic and had no pro-inflammatory effect after 24 h of treatment, at tested concentrations, as shown by studying interleukin-8 release and did not modify the TEER values. Confocal microscopy observations demonstrated that NPs were internalized and exhibited heterogeneous distribution in the epithelial monolayer. By Transmission Electron Microscopy (TEM) experiments, we observed non agglomerated NPs in apical and basolateral parts of the cells included in vesicles. All the studied NPs can translocate through the Calu-3 monolayer but this translocation was increased for the smallest and negatively charged NPs. TEM observations coupled to X-Ray analysis allowed identifying the presence of NPs in the basolateral chamber. The translocation was not associated with an alteration of the epithelial integrity suggesting a transcytosis of the internalized NPs. Modulation of NP corona by addition of foetal calf serum or dipalmitoyl lecithin, modify the NP ability to cross the epithelial barrier depending on their intrinsic properties, making positively charged NPs more prone to translocate. NP translocation can be improved by using chemical agents known to open tight junctions (EGTA and chitosan) and to allow paracellular passage. NP translocation was also modulated creating a pro-inflammatory context, commonly found in lungs, by pre-exposure of cells to Tumor Necrosis Factor alpha or lipopolysaccharide altering the epithelial integrity and inducing transient tight junction opening.

In conclusion, we provide evidence that SiO_2 NP can translocate across human bronchial epithelial barrier by transcytosis with higher efficiency for the smallest and negatively charged NPs. The use of Calu-3 model could be extended to the evaluation of the translocation of other inhaled NPs in order to predict their biodistribution and provide absorption data to supply PBPK models.

Acknowledgments: This work was supported by grants from *Anses* project, by *Région Ile de France* for I. George PhD funding.

Session 6

LOW DOSES AND STRUCTURE-ACTIVITY RELATIONSHIP

Chairpersons:

Marc Baril, Montreal University, Montreal, Canada
Hakan Wallin, NRCWE, Copenhagen, Danemark

Chemical risk assessment: toxicology and biometrology innovations

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Toxicology is facing many questions such as: the predictive value for humans of animal experiments, animal ethics issues, representativity of in vitro tests etc. It needs to adapt to new regulations: REACH in 2007, the European Directive 2010/063/EU in 2013, etc. Emerging scientific issues should be considered: non-linear dose/effects relationship, low dose toxicology, combined effects of concomitant exposures, study of the toxicity of new substances such as nanoparticles, for which traditional methods cannot provide appropriate responses.

To deal with this situation, new methods have been developed to study the toxicity of chemicals in this new environment. Thus, alternative methods were developed to either replace in vivo methods by other methods: in vitro, in silico (QSAR, PBPK models), ex-vivo, extrapolation by analogy (Read-Across), bio-artificial organs, cultured organs, or reduce the number of animals used: optimization of the use of statistical tests, development of "OMICS" techniques, use of transgenic animals, implementation of integrated testing strategies or refining optimizing the use of animals using imaging or telemetry techniques etc.

The presentation will address these different techniques by introducing the principle and in some cases illustrating with examples.

Toxicology has made a significant effort to adapt, but there is still much to do to be able to control perfectly all these techniques and develop them. They should be validated to be considered as references and possibly in some cases, be subject to guidelines.

Urinary excretion profiles of 42 monohydroxylated metabolites in rats exposed to a mixture of low-dose polycyclic aromatic hydrocarbon for a 90-day period

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On the assumption of relations between exposure to Polycyclic Aromatic Hydrocarbons (PAHs) and certain health disorders, several recent research studies have been dedicated to the development of strategies aimed at assessing human exposure to these ubiquitous pollutants. As part of these research efforts, the usefulness of the analysis of hydroxylated metabolites in urine as reference biomarkers of exposure for the assessment of human exposure to PAHs was clearly demonstrated. Since people are generally exposed to mixtures of PAHs, more extensive methods covering a larger number of PAH metabolites were developed to assess exposure adequately.
In this context, a method was firstly developed for the analysis of mono-hydroxylated-PAHs in urine, based upon gas chromatography tandem mass spectrometry. The method focused on 52 target compounds (2- to 6-aromatic rings) corresponding to the most common metabolized forms of the 16 PAHs listed as priorities for their toxicity by international agencies (e.g. US-EPA) and on which biomonitoring needs to focus (Fig-1A). The limits of quantification (LOQ) ranged from 0.05 to 5 ng/mL. The calibration curve was linear from the LOQ up to 100 ng/mL with a coefficient of determination over 0.99 for most of the analytes tested. The precision and accuracy determined at the LOQ ranged from 1% to 24% and from 75% to 136% respectively.
This method was then applied to the analysis of rat urine submitted to controlled exposure to PAHs in order to allow (i) confirmation of its sufficiently high sensitivity for the detection of low levels of exposure, (ii) assessment of PAH-metabolite excretion in this matrix and (iii) comparison between the OH-PAH excretion profiles at different time points (days 1, 28, 60 and 90). Urine samples were therefore collected from female Lister Hooded rats (n=8 per group) exposed to a mixture of 16 PAHs at seven low doses ranging from 0.03 to 2.4 mg/kg/week, by gavage for 90 days. Eight non-exposed animals receiving vehicle only were used as controls. Monthly urine samples were collected using individual metabolic cages for a 24h period. The results of the present study confirm that these metabolites can be excreted in urine after P.O. administration of the corresponding parents. Forty-one out of the 52 metabolites were actually detected in urine samples corresponding to the 16 native compounds. Regardless of exposure time, the highest concentration levels were observed in ascending order for 1-OH-naphthalene, 9-OH-phenanthrene, 1-OH-phenanthrene, 3-OH-fluoranthene, 4-OH-phenanthrene and 1-OH-pyrene (Fig1-B). Several OH-metabolites corresponding to the same native compounds were also measured in the urine of the control rats, suggesting an environmental contamination of the animals.
On day 90, the mean concentrations of 1-OH-pyrene in rat urine samples exposed to 0.8 mg/kg of PAHs were quantified at 71.2 ± 36 ng/mL corresponding to 1402 ± 399 ng excreted over a 24h period whereas 2.1 ± 0.6 ng/mL corresponding to 52.8 ± 9.9 ng were excreted for 3-OH-B[a]P. A linear dose-response was observed between the PAH concentrations administered and the levels of their metabolites as measured in urine for almost all of the metabolites with fewer than 5 aromatic rings investigated. For instance, a coefficient of determination ranging from 0.88 to 0.91 was calculated for naphthols and from 0.88 to 0.96 for OH-phenanthrenes. Regarding 1-OH-pyrene, it was determined at 0.89 (Fig-1C).
Finally, by following the urinary elimination kinetics of each mono-hydroxylated-PAH detected, the present study aims to evaluate the influence of sampling time on the suitability of the urine matrix for the revelation of rats' exposure. On days 1 and 60, urine fractions produced by rats exposed to PAHs were collected separately within 2h, 4h, 6h, 8h, 12h and 24h of PAH administration (Fig 1-D). The results obtained do not seem to reveal a relation between the peak of excretion, the molecular weight and the log Kow of PAHs. Moreover, a time lag of 2h of the excretion peaks, suggesting metabolism induction, was observed for most of the metabolites of OH-phenanthrenes, OH-chrysenes and OH-benz[a]anthracenes.The toxicokinetic analysis currently in progress should help to confirm these findings.
These results provide interesting data on the suitability of the analysis of OH-metabolites in urine for the assessment of rats' exposure to PAHs, which could be taken into account for the design of occupational epidemiological studies in the future.

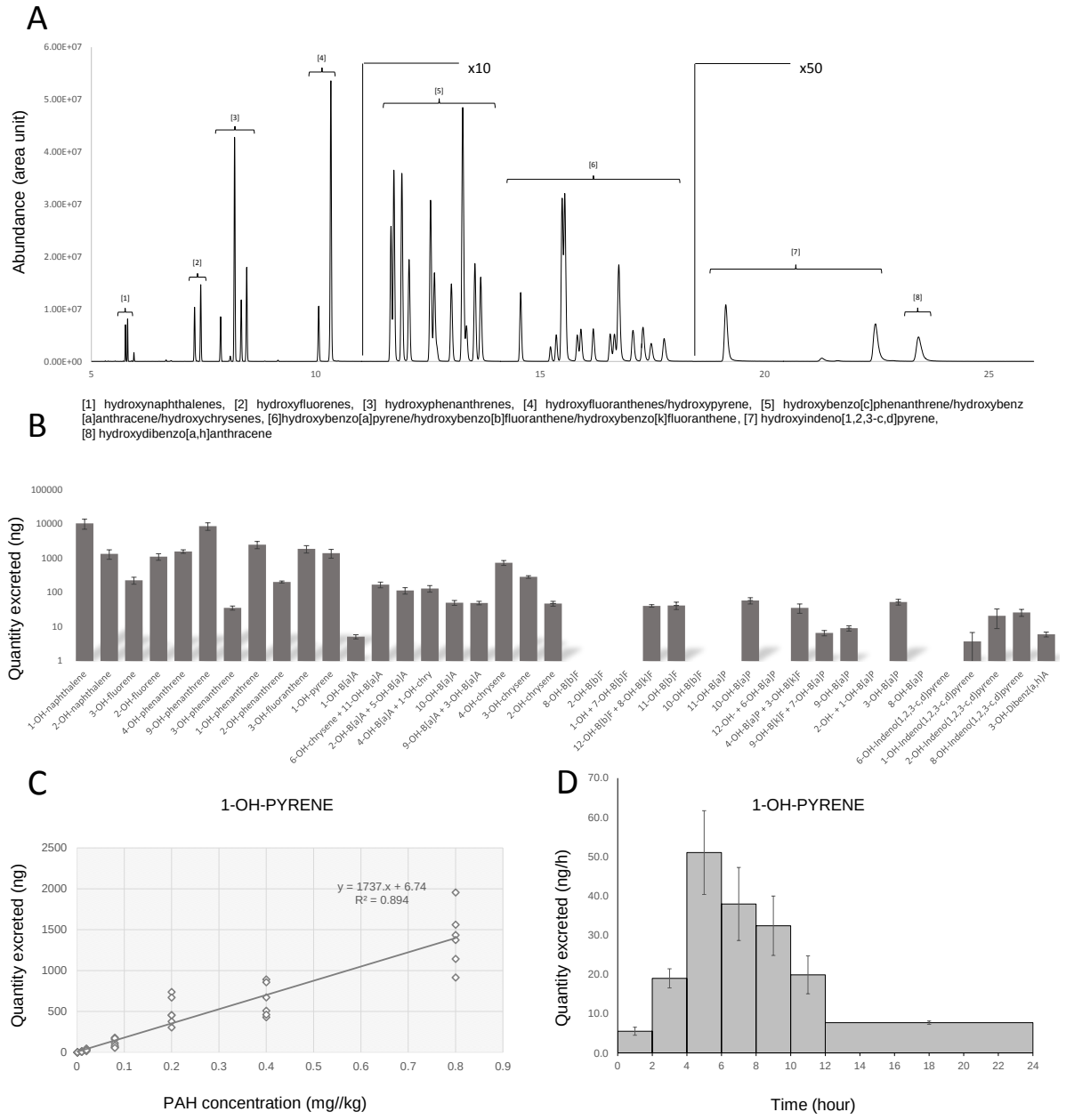

Figure 1. A. Analysis of OH-PAHs in urine sample supplemented at 100 ng/mL. OH-PAH standards were derivatized with N-tert-butyldimethylsilyl-Nmethyltrifluoroacetamide. **B.** Quantity of OH-PAHs excreted in urine of rats exposed to 0.8 mg/kg PAHs on day 90 (results expressed by mean ± SD). **C.** Linear dose-response between the quantity of 1-OH-pyrene excreted and the dose of PAHs administered to rats (n=8 per group). **D.** Urine excretion profile of 1-OH pyrene following a single gavage of rats with a mixture of PAHs at 0.8 mg/kg (results expressed by mean ± SEM).

Acknowledgments: This work was supported by the Luxembourg Ministère de l'Enseignement Supérieur et de la Recherche.

Dose-related assessment of the neurobehavioral toxicity of a 90-day exposure to a mixture of pesticides in adult Wistar male rats

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Occupational exposure to pesticides has been recently established in farmers to be at risk for the brain through association between such exposure and occurrence of neurodegenerative disorders and Parkinson disease. In contrast, little attention is paid to the potential neurotoxicity of pesticides in the general population due to chronic exposure through ingestion of contaminated food, especially for lower levels of exposure. The present study aimed to assess the brain and behavior toxicity in adult rats subchronically exposed to a mixture of pesticides. Adult Lister-Hooded rats were administered p.o. 3 times a week for 90 days with a mixture of 20 pesticides including persistent organochlorines, organophosphate compounds, pyrethroïds, carbamates, azoles, phenylpyrazoles, neonicotinoids and chlorophenols. Seven doses of pesticides covering a wide range of levels of exposure (from 12 µg/kg/week to 1200 µg/kg/week) were tested. By the end of the period of exposure, rats were assessed for their locomotor activity in the open-field, their level of anxiety in the elevated-plus maze, and their learning and memory abilities in the Y maze (short-term memory) and the eight-arm maze (spatial working memory). After the behavioral testing, the animals were sacrificed and the whole brain removed and deep frozen. Thus, frontal sections of 20 µm thick were collected from each brain. Regional cytochrome oxidase (CO) and (AchE) activities were measured within the same animals by using semi-quantitative histochemistry methods with 3-3' diaminobenzidine and S-actetylthiocholine iodide as substrates, respectively. Enzyme activity was quantified in 70 brain regions for cytochrome oxidase and 40 brain areas for acetylcholine esterase using a Biocom image analysis densitometer. Results obtained in the open-field and the elevated-plus maze showed a non-monotonic dose-response with a significant reduction of the level of anxiety in rats exposed to the two lowest doses of pesticides (12 and 30 µg/kg/week) compared to controls and to the other groups of exposure. Locomotor activity and memory performances remained unchanged. Non-monotonic activation of CO activity was measured in motor areas like the substantia nigra, subthalamic and red nuclei, and globus pallidus, and limbic regions including amygdala nuclei and ventral tegmental area. AchE activity was significantly reduced in a dose-dependent manner in fornix whereas a dose-related increase was observed in dopaminergic areas like accumbens and ventral tegmental area. Such results indicate the susceptibility of emotional-related behavior and dopaminergic brain regions to pesticides suggesting the ability of such chemicals to induce neurodegenerative disorders. The use of 7 doses of exposure to assess the neurobehavioral toxicity of pesticides demonstrated a non-monotonic dose-effect relationship and questioned the risk for the brain when exposed to low doses of contaminants.

QSPR models for predicting physico-chemical hazards of substances

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To control the risks to which workers are exposed at their workstations, it is necessary to know the hazardous properties of the substances they handle or use in industrial processes. Most of that information is summarised in the safety data sheets of the substances handled and is generally obtained by experimental tests.

In addition to those tests, INERIS develops models to predict the hazardous physico-chemical properties of chemical substances such as explosibility and inflammability of nitro compounds (Fayet, 2011), organic peroxides (Prana, 2014) and ionic liquids (Diallo, 2012). These quantitative structure-property relationships (QSPRs) are based on correlations between the macroscopic property of a substance and molecular descriptors. In addition, quantum chemistry tools are used both to define molecular descriptors taking into account substance reactivity and to characterise reaction mechanisms involved, for example, in the decomposition of nitro compounds (Fayet, 2009).

Until presently, QSPR methods were mainly devoted to toxicological properties, but are now increasingly being used for physico-chemical properties (Dearden, 2013). The development of such models was encouraged in particular, within the context of the REACH Regulation with principles for validation defined by OECD for the use of these models within a regulatory framework (OECD, 2007).

All of the models developed at INERIS are validated according to those principles so that they may be used within a regulatory framework, proposed to the Joint Research Center (JRC) for inclusion in its QSAR/QSPR model base and made available in existing tools (such as the QSAR Toolbox ECHA/OECD). The QSAR Toolbox (http://www.qsartoolbox.org/), initially focused on (eco)toxicological properties, includes the first model devoted to a hazardous physico-chemical property in its latest version made available in December 2014. This model, developed by INERIS, concerns the prediction of sensitivity to the impact of aliphatic nitro compounds (Prana, 2012).

The predictions resulting from these models are used to anticipate hazardous substances, prior to carrying out tests and/or when tests are not possible (for example for new substances still being synthesised). These tools are particularly valuable in screening procedures to select products with both the desired functional properties and limited hazards for workers. They can therefore be used for the development of safer substances, processes and workstations (safety-by-design).

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Consideration of physical factors during the development of predictive chemical risk models

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Recent regulations, in particular with the entry into effect of REACH in 2007, requires manufacturers to characterise the chemical risk associated with the products they handle. Major work must be conducted, which explains the resurging interest in numerical models for assessing this risk (Lucas, 2014). There are various mechanisms through which a chemical compound may cause fire, explosion or poisoning risks, and it is therefore difficult to connect its structure to its dangerousness through a purely physical approach. Consequently, quantitative structure/toxicity relationships (QSTRs) or more generally quantitative structure/property relationships (QSPRs) developed until presently are empirical. They generalise the quantitative structure/activity relationships (QSARs) used in drug design.

One difficulty associated with the purely empirical character of these models is the risk of resulting in random relationships compatible with the training sets but leading to wrong predictions for new compounds. This risk is greater when the training set is limited. In addition, it increases with the number of descriptors considered and the number of adjustable parameters authorised. The application of these techniques to chemical risk assessment is delicate: the data available is often limited, while the complexity of the properties encourages the use of large descriptor pools and formalisms requiring the adjustment of numerous parameters.

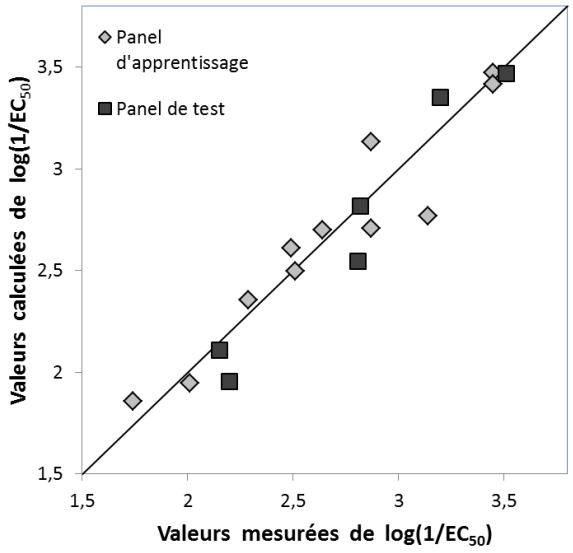

Figure 1. Model obtained by Cartesian genetic programming for nanoparticle cytotoxicity.

By setting constraints for acceptable structure/properties relationships, inclusion of physical considerations should improve the quality of models. This can be done with the choice of the descriptors, through dimensional analysis, or by requiring the relationship to be compatible with theoretical considerations, as illustrated with the example of decomposition enthalpy ΔH measured by DSC. Based on an equation linking ΔH to reactivity indices (Fayet *et al.*, 2009), a simple multiplication of those indices by the number of atoms in the compound - in order to make it consistent with the extensive character of ΔH – significantly improves its performance and robustness. However, a model deduced directly from physical considerations is highly superior while having only one empirical parameter (Mathieu, 2011).

For complex properties, different physical models are often possible based on mechanisms deemed preponderant. In the case of explosives' insensitivity to h_{50} impact, a specific model is distinguished by its remarkable predictive power, thus validating the underlying physical assumptions (Mathieu *et al.* 2014).

With regard to toxicity criteria, their assessment based on a single mechanism is most often impossible. Common modelling techniques postulate an arbitrary mathematical form, which may be linear (multi-linear regression, partial least squares, etc.) or not (neural networks, support vector machines, decisional trees, etc.), which may not be optimal for describing the property to be modelled. This rigidity is compensated by the use of numerous adjustable parameters, which requires a large training set and increases the risk of random relationships. Genetic programming (GP) techniques explore an even greater space, but this space can easily be limited by physical constraints. A specific variant of GP, Cartesian genetic programming (CGP), limits the complexity and the number of parameters of equations, which enables better exploitation of limited data sets. We use CGP associated with the Levenberg-Marquardt algorithm in order to set the values of the equations' numerical constants. Applied to the assessment of the toxicity of metal oxide nanoparticles, this procedure leads to a linear model whose performance - evaluated based on multiple random partitions of the data set as in Figure 1 - is superior to all previous models, thanks to the non-linear transformation of the variables introduced by CGP. This technique appears to be interesting for establishing and validating predictive models based on very small data sets.

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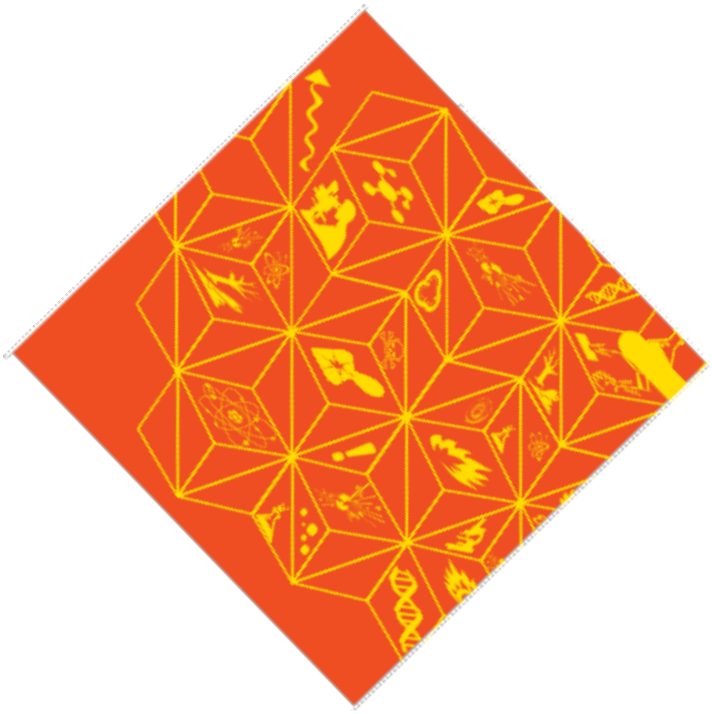

POSTERS

Different methods to determine the oxidative potential of $PM_{2.5}$ as a predictive marker of their toxicity

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Particulate pollution was recently classified as carcinogenic by IARC and is recognized to induce cardiorespiratory diseases. Regulations rely on particulate matter (PM) mass without taking into consideration PM composition. PM is a complex mixture including metallic and organic compounds, with different proportions according to the sources of emissions and climatic conditions (photochemistry). Alternative metrics could be used to predict PM toxicity such as their oxidative potential (OP). Our project aim is to compare different methods to characterize the intrinsic oxidative potential of particles to predict PM toxicity.

Different batches of $PM_{2.5}$ were sampled in Paris in background as well as in traffic sites. They were extracted from the collection filter by sonication and suspended in buffered aqueous solution. They were compared with carbon black nanoparticles (CB) and diesel exhaust particles (SRM1650, DEP) from 10 to 100 µg/mL. Their OP was characterized using different acellular assays: the consumption of dithiothreitol (DTT), a reducing agent, the scission of plasmid DNA and the depletion of antioxidants (AO) (ascorbic acid (AA), uric acid (UA) and glutathione (GSH) in a synthetic lung surfactant. Bronchial epithelial cells (NCI-H292) were exposed for 24 h to particles from 1 to 10 µg/cm². The induction of a pro-inflammatory response was characterized by measuring IL-6 cytokine release by ELISA and expression by RT-qPCR. The expression of xenobiotic metabolism (cytochrome P450 1A1 (CYP1A1), NADPH quinone oxidoreductase (NQO-1) and antioxidant enzymes (Heme oxygenase 1 (HO-1)) were also studied.

The consumption of DTT is induced by all the particles with the following order CB > $PM_{2.5}$ > DEP. We checked that this consumption is related to the production of oxidized DTT. No DNA damage was induced by CB and DEP. It occurs with $PM_{2.5}$ in presence of H_2O_2, suggesting the production of free radicals by Fenton reaction. For both assays, the OP of $PM_{2.5}$ was also observed using a soluble fraction of $PM_{2.5}$ underlining the role of water soluble metals. CB keep their ability to oxidize DTT after washing underlining the role of their surface reactivity. We also investigated the possibility to run these assays using $PM_{2.5}$ on their collection filter and observed the feasibility of this approach that could be very useful for routine studies. For the depletion of anti-oxidants in synthetic lung surfactant, none of the particles have an effect on UA depletion. By contrast we observed the same ranking as for DTT of the different particles: CB > $PM_{2.5}$ > DEP, for their ability to deplete AA and GSH used alone or in mixture. GSH depletion is associated to an increase of GSSG.

$PM_{2.5}$ induced a clear dose dependent release of the IL-6 proinflammatory cytokine by NCI-H292. DEP have a lower effect whereas CB has no effect. HO-1, CYP1A1 and NQO-1 expression allowed discriminating background and traffic PM, the latter being the most inductive. CB has only a low effect on HO-1 expression.

DTT and AO assays provide similar ranking of particles but that is different from the one with plasmid assay. Moreover these acellular assays are not completely matching the observed proinflammatory response of epithelial cells. The surface reactivity of CB highlighted by DTT and AO assays does not translate in a biological response. $PM_{2.5}$ provide positive responses in all the acellular assays and these responses are related to their water soluble metals. However the strong induction of CYP1A1 by $PM_{2.5}$ underlines the role of the organic fraction suggesting that the main drawback of acellular assays is their inability to reveal ROS related to organic compounds metabolism that is cell dependent.

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Assessment of the oxidative potential of nanoparticles: comparison and improvement of methods

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Background: Oxidative stress induced by reactive oxygen species (ROS) is increasingly being demonstrated as playing a key role in the biological response induced by nanoparticles (NPs). Yet, there is a need to adapt or improve current methods to characterize the intrinsic and cellular oxidative potential of NPs and to develop high-throughput assays to allow the rapid screening to classify NPs in terms of toxicity. The acellular oxidant generating capacity of NPs may be a predictive tool for the toxicity of nanomaterials.

Methods: The physico-chemical properties of suspensions of highly oxidative control (Mn_2O_3 NPs) low oxidative but widely used control (CeO_2 and TiO_2 NPs) and negative control NPs ($BaSO_4$ NPs) were characterized. The intrinsic oxidative ability of NPs was measured by the cytochrome c assay. This test was adapted for nanotoxicology purpose and compared to the DTT assay performed in dose-course studies. A high-throughput assay of the cytochrome *c* test was developed in view of its application for nanotoxicology screening strategies. To assess whether the oxidative potential of the tested NPs correlates with cytotoxicity and cellular oxidative stress responses we studied the effect of negative control, high and low oxidative NPs on the human bronchial epithelial cells NCI-H292. The cytotoxicity of the NPs was evaluated by the WST-1 assay. The ability of NPs to induce an oxidative stress *in vitro* was assessed by measuring the induction of antioxidant enzyme expression in NCI-H292 cells treated for different times with NPs and the pro-inflammatory response triggered by NPs was assessed by measuring the Il-8 release by ELISA and mRNA expression by RT-qPCR.

Results: The adapted cytochrome *c* assay revealed efficient to rank the oxidative potential of NPs. Indeed positive and negative control NPs induced expected oxidation levels of cytochrome *c*. Mn_2O_3 NPs induced a dose-dependent oxidation whereas $BaSO_4$ had no effect. CeO_2 and TiO_2 NPs did induce no oxidation of cytochrome c. We successfully established a high-throughput assay of this test and these oxidative potentials of NPs were confirmed by the DTT assay. Besides, Mn_2O_3 NPs induced a dose dependent cytotoxicity and increase of antioxidant enzymes expression in bronchial epithelial cells. CeO_2 and TiO_2 NPs modulated slightly the antioxidant enzyme expression and induced moderate cytotoxicity whereas $BaSO_4$ had no or low effects. Furthermore non-cytotoxic concentrations of NPs led to a dose-dependent increase of the IL-8 release in bronchial epithelial cells except for $BaSO_4$ NPs.

Conclusion: Cytochrome c oxidation could be used for high-throughput screening of the oxidative potential of NPs. Mn_2O_3 NPs revealed good positive control and $BaSO_4$ negative control NPs. The oxidative potential of these two NPs correlated well with their cytotoxicity, pro-inflammatory response and antioxidant defense induced in bronchial epithelial cells. However, the acellular tests revealed not predictive for only moderately toxic NPs with low oxidative capacity. Indeed, the highly used TiO_2 and CeO_2 NPs have shown no intrinsic oxidative potential but induced intermediate cellular responses underlining the need to further study the pulmonary response to these important NPs.

Assessment of chemical risk in a petrochemical analysis laboratory (core library), Algeria

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The central core library in the south of the Algerian region of Hassi Messaoud is considered the largest laboratory for physico-chemical analysis of cores obtained from oil drills. The extensive use of chemicals during these operations makes them dangerous, involving a major risk of exposure to chemicals.
The goal of this work was to assess chemical risk in the petrochemical analysis laboratory using INRS's simplified chemical risk assessment method (Vincent *et al.*, 2007) and measuring mercury and chrome in urine samples.

Inventorying and analysing the chemicals used in the core library revealed different levels of risks according to the products used and the different workshops. Only one workshop presented a high risk requiring priority prevention actions. Different products classified as carcinogenic according to the International Agency for Research on Cancer were found (chloroform, toluene, xylene, chromium and mercury).
Chloroform, xylene, toluene and methanol were found in the plug preparation and cleaning workshop. Mercury was found in the core analysis and petrophysical assessment workshop. In the rock mechanics workshop, polyester resin, styrene monomer, methyl ethyl ketone, silicone, epoxy resin, ethyl cyanoacrylate and isophorone diamine were identified.

The measurement of chromium and mercury in urine samples was carried out for 35 workers. The average concentration of chromium in the urine samples was 0.128 microgrammes/litre. The average mercury concentration was 5.066 microgramme/litre. Those values are lower than the maximum permissible values.
Conclusion: The application of the simplified method for chemical risk assessment in this petrochemical analysis laboratory combined with biological monitoring did not highlight a major risk.

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CHEOPS, a methodological approach to assessing and prioritising chemical risks in the work environment based on toxicity reference value

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The waste treatment activity can expose workers simultaneously to different chemicals likely to have dangerous health effects.

The definition of occupational exposure limit values (OELVs) has been a major breakthrough in employee protection. Nevertheless, it can be considered that OELVs do not sufficiently protect workers' health because they take into account not only scientific and technical criteria, but also social, economic and even psychological factors (INRS, 2012). In addition, certain carcinogenic, mutagenic and reprotoxic agents have no threshold effects, which means that they may produce effects even at very small doses. The limit values which are set for those agents therefore do not provide absolute protection against those risks (INRS, 2012).

Since 2006, scientific expertise for establishing OELVs has been entrusted to Anses (the French Agency for Food, Environmental and Occupational Health and Safety) with the main goal of defining French OELVs for regulatory purposes and of reviewing existing OELVs (approximately 500), issued through circulars (INRS, 2010).

In this context, the Veolia group's objective was to complete the regulatory approach and anticipate the development of OELVs, by defining an integrative risk assessment methodology for its employees. This methodology is based on the toxicity reference values (TRVs) used for the general population and fitted to the work environment considering employees' working time. By crossing the data on the average concentration of chemicals measured in the air in the different work zones and the distribution of employees' working time in those zones, risk levels are estimated based on 1) the chemicals and 2) homogenous exposure groups (HEGs) predefined by the occupational health services.

The estimated risk levels are based on either the hazard quotient (HQ) calculated for substances with threshold effects (see example in Figure 1), or the individual excess risk calculated for non-threshold carcinogenic substances. This risk assessment methodology aims to prioritise the risks depending on the chemicals and the HEGs and then guide in defining prevention actions such as the introduction of collective protective measures or the organisation of working time.

This methodology, currently being developed in the form of a web application called CHEOPS (Chemical risk prioritisation based on exposure at workplaces: an occupational prevention system), is expected to be made accessible to all OSH practitioners. In addition to tracing exposure, this application could be used as a risk management support tool since OSH practitioners may test different exposure scenarios by changing the following parameters: distribution of the HEGs' working time, wearing of a respiratory protective device (RPD), period of exposure. Their influence on the risk levels can then be viewed.

The method currently assesses risks for each separate substance as well as the risks associated with mixtures of non-threshold substances through the sum of excess individual risk. However, the "mixture" effect associated with threshold chemicals is not included in the tool presently. Therefore, one way of improving the tool would be to incorporate this function as is the case for example with the MiXie tool developed by the University of Montreal and IRSST and then adapted by INRS.

In the MiXie application, it is assumed that the substances present in the same work environment and classed in the same effects category have an additive effect: the mixture's exposure index for that effects category is then calculated. CHEOPS and MiXie would thus be complementary.

The methodology developed in CHEOPS will first be presented, followed by a demonstration of the tool based on fictitious data.

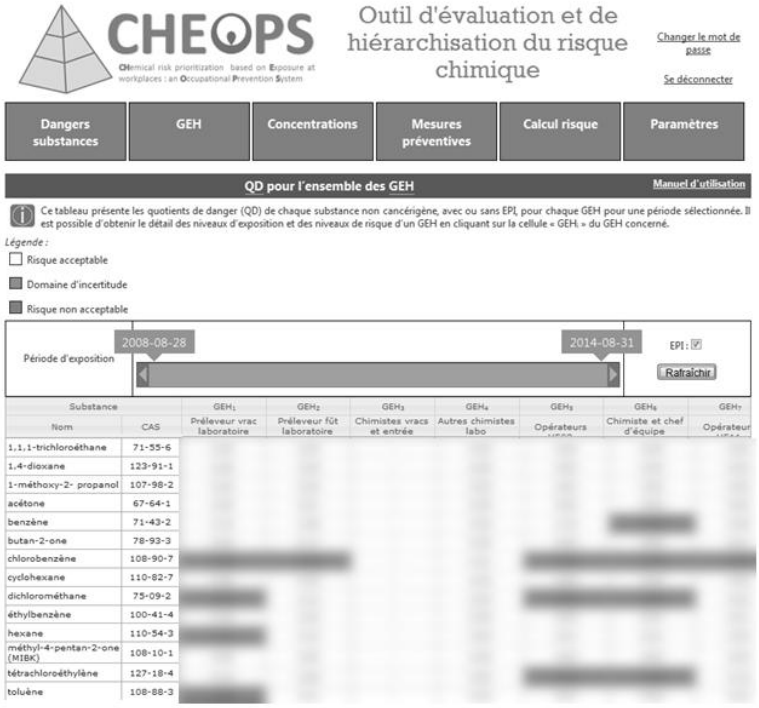

Figure 1. Screen capture of one of the web pages of the CHEOPS tool: hazard quotient for all HEGs, substance by substance.

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Cytotoxic Drugs: Handling practices and clinical manifestations among hospital staff

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Keywords: Cytotoxic Drugs, handling, clinical manifestations, hospital staff

Objectives
To determine the handling practices of cytotoxic drugs and to describe clinical manifestations expressed by hospital personnel of Sidi Bel Abbes during the year 2014.

Methods
Sectional descriptive study conducted in 3 center university hospital units (Hematology, Oncology and Urology) and Gynecology of EHS Sidi Bel Abbes. A questionnaire was administered to hospital workers regulary exposed to cytotoxic drugs. A work-place visit was performed to have an overview about working conditions. The Cytotoxic Contact Index (CCI) was calculated for each nurse on a period of 15 working days. Treatment of the results was done using SPSS software.

Results
The survey reveals that 22 men and 58 women are exposed to cytotoxic drugs for an average of 7 years. Many symptoms such as ocular irritation (38,75%), throat irritation (56,25%), headache (68,75%), dizziness (43,75%), nausea (37,5%), metallic taste (30%), were reported with high frequency. Are noted in the offspring, 3 congenital anomalies,2 diaphragmatic hernia and a cleft palate. The Cytotoxic Contact Index (CCI) was higher than 3 among Oncology nurses and higher than 1 for most of the nurses of Hematology and Gynecology service. The wearing of personal protective clothing was not respected by all workers: (22/23) wear gloves and (20/23) wear a mask,(5/23) wear a cap, (2/23) wear glasses. Only 3 nurses have benefited from continuous training on handling cytotoxic drugs.

Conclusion
This study shows a high occupational exposure risk to cytotoxic drugs among persons handling these drugs and the necessity to apply rigorously all measures related to personal protection awareness and training of personnel to minimize these exposure.

Automated generation of reference samples from particles in suspension using the SAGE generation system

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Assessment of worker exposure to chemical agents present in workplace air requires validated sampling and analysis methods to be developed. Ideally, such methods are validated on the basis of aerosols generated in the laboratory by means of systems making it possible to produce controlled and homogeneous atmospheres and thus to evaluate the performance of a method, from sampling to analysis. However, most laboratories that test occupational exposure to chemical agents do not have such facilities, which are complex and costly. By default, sampling and analysis methods are validated by doping sampling media with solutions of known concentrations. That method is simple and easy to implement but it does not, however, make it possible to evaluate the performance of the method for the phases of sampling and of sample preparation prior to analysis (putting welding fumes into solution is difficult for example). An intermediate technique, enabling sample preparation to be taken into account, is to deposit a suspension of particles rather than a solution. This is a technique that has been used for a long time to produce reference samples for method validation (Rains *et al.*, 1974), but producing a large number of samples with the best possible homogeneity using this technique remains difficult.

The SAGE automated sample generation system, originally developed to fabricate replicas on filters automatically by liquid deposition from solutions (Boulet *et al.*, 2010), was thus adapted to enable particle suspensions to be deposited. A known volume of a doping suspension maintained under continuous agitation is sampled using an automatic syringe and is then deposited on a sampling medium disposed in a cassette that is 37 mm in diameter. Once the suspension has been deposited on the filter, the cassette is transferred automatically to a vacuum suction device that sucks up the suspension liquid. The particles then find themselves at the surface of the filter. The entire system is controlled by computer and is fully automated.

Validation of the SAGE system for depositing suspension was performed in four stages: determining the parameters of the balance; developing deposition suspensions; testing water deposition; and testing suspension deposition. Dust suspensions were produced using a viscosity agent, a surfactant, and a dispersant (polyacrylic acid) at different concentrations. The dust chosen for the testing was a manganese ore. Mn analysis was performed by inductively coupled plasma optical emission spectroscopy (ICP-OES). Two viscosity agents (Xanthane, Carboxymethyl cellulose) and two surfactants (triton X-100, iso-propanol) were tested in order to obtain the best homogeneity over the deposited quantities of particles. All of these tests enabled samples of homogenous concentration to be generated reproducibly (Figure 1).

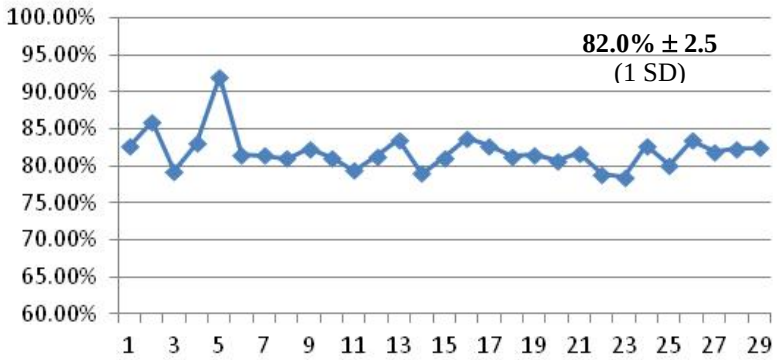

Figure 1. Recovery rate of manganese (in %) during successive depositions (n=29) of manganese ore dust

The tests conducted on the SAGE automated system showed its capacity to produce samples on which dust can be deposited quantitatively. The regularity of the deposition of dust during the doping phases can be a function of the desired precision by modulating the volume to be deposited. This study shows the importance of developing a suspension that is suitable for the dust that is to be deposited, it being possible for numerous substances such as viscosity agents or dispersants to be used. The suspension matrix should be adapted to suit the type of dust deposited. The type of dust should also be of composition adapted to being put in suspension (usually aqueous solution), and be stable over time in the suspension medium. Since the dust suspension cannot be fully homogeneous, the assigned values can be calculated only by analyses and not by computations, as in the case of depositions of solutions. This involves analysing aliquots of the suspension and/or analysing a certain number of samples from among the prepared samples. Particular attention should be paid to how well the suspensions behave over time. In this study, the importance of agitating the suspension was thus demonstrated (magnetic or ultrasonic agitation).

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Prevention techniques of chemical risks in bakery industry

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Occupational health and safety is one of the most important aspects of human concern. It aims an adaptation of working environment to workers for the promotion and maintenance of the highest degree of physical, mental and social well being of workers in all occupations (Tadesse & Admassu, 2006). There are more than 250 million work-related accidents every year. Workplace hazards and exposures cause over 160 million workers to fall ill annually, while it has been estimated that more than 1.2 million workers die as a result of occupational accidents and diseases (Alli, 2001).

Workers in bakeries face many hazards in their work environment; hazards have the potential for causing injury or illness. The baking industry, like, most occupations is prone to occupational health challenges. Most of these hazards are preventable and arise from the neglect of occupational safety measures (Emmanuel & Sussan, 2014).

There are many chemical risks for workers in bakeries. Exposure to flour may cause respiratory system disorders and skin diseases. Many bakers working with some spices suffer from chronic conjunctivitis and chronic rhinitis; allergic skin diseases are sometimes found; after prolonged exposure, respiratory infections, particularly chronic bronchitis and sometimes even bronchial asthma, may develop. Exposure to sugar dust may cause dental caries. In mechanized bakeries, dough which is in an active state of fermentation may give off dangerous amounts of carbon dioxide. Firing equipment which is badly adjusted or has insufficient draw, or defective chimneys, may lead to the accumulation of unburned fuel vapors or gases or of combustion products, including carbon monoxide, which may cause intoxication or asphyxia (ILO, 2000). Some chemicals such as sodium hydroxide and bleach used in cleaning bakeries could cause contact dermatitis and could also be hazardous to the eyes and the respiratory system. Reports have also indicated a higher prevalence of occupational skin diseases among bakery workers than in the general population (Emmanuel & Sussan, 2014).

An effective control and prevention techniques like clean process and purification techniques should be required at the workplace.

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Assaying beryllium using molecular fluorescence

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Beryllium is a light, strong metal which has a high melting point, is resistant to acids, and has high thermal conductivity. These properties make it an element that is attractive for many industrial sectors. However, exposure to beryllium can lead to sensitisation to beryllium that can even result in lung disease characterised by formation of granulomas (berylliosis or chronic beryllium disease). A latency period that can be as long as several tens of years exists between exposure and development of the disease. Beryllium and its compounds are classified as category 1B carcinogens by the European Union and as category I carcinogens by the International Agency for Research on Cancer (IARC). As regards occupational risk prevention, a time-weighted average occupational exposure limit (occupational exposure limit value over 8 hours or "VLEP-8h" in France) was established for beryllium and its compounds at 2 $\mu g.m^{-3}$, but various exposure studies showed that this limit value was not sufficiently effective in preventing new cases of pulmonary berylliosis. That is why in January 2009, the American Conference of Governmental Industrial Hygienists adopted a reduction in the TWA-TLV® (Time-Weighted Average Threshold Limit Value) to 0.05 $\mu g.m^{-3}$ in the United States. In France, in 2010, the French Agency for Food, Environmental, and Occupational Health & Safety (ANSES) recommended lowering the "VLEP-8h" to 0.01 $\mu g.m^{-3}$.

An industrial hygiene analysis method should be capable of achieving quantification limits less than one tenth of the occupational exposure limit, which, in the case of beryllium means a few nanograms or even less deposited on a filter. Such a value is less than the quantification limits achieved by the usual assay methods using atomic emission spectrometry (AES) that are recommended in industrial hygiene (atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES)). The objective of this work is to assess a beryllium assay method that is made more sensitive by using molecular fluorescence. A molecular fluorescence method for quantifying beryllium samples taken from cellulose-based media has recently been developed, validated, and standardised (American Society for Testing and Materials (ASTM)) and published in collections of methods (US National Institute for Occupational Safety and Health (NIOSH)). The fluorescence method studied here is based on instantaneous complexation of beryllium using HBQS (hydroxybenzoquinoline **sulfonate**). The mode of operation starts with an extraction procedure using a 1% NH_4HF_2 extraction solution followed by measurement of the fluorescence of the complex formed by beryllium in the detection solution. However, the high cost of the detection solution is currently limiting the use of this analysis method.

A synthesis protocol for synthesising this detection solution was thus developed, making it possible to reduce its cost considerably. This synthetic solution was compared with the commercially available solution and the analysis method was entirely re-evaluated with this new solution. For this purpose, various collection media were tested (quartz fibres, cellulose membranes, and wipes). The tests were then conducted by depositing solution (BeF_2) or suspension (BeO) directly on the collection media. The full performance of the method was thus evaluated (influence of the matrix, linearity of the response, quantification limits, recovery rate, repeatability, preservation). This method made it possible to achieve quantitative measurement from 0.005 $\mu g/m^3$ to 4 $\mu g/m^3$ but did not make it possible to achieve 0.1 of the "VLEP-8h" occupational exposure limit recommended by ANSES (i.e. 0.001 $\mu g/m^3$) for the two compounds studied. In spite of a value of 16.6% for the lowest concentration, the repeatability of the method is deemed to be correct (mean CV (coefficient of variation) of 4%). The method is thus fully validated relative to the criteria of EN 482 (enlarged uncertainty of 30%) and of EN 13890, and is going to be the subject of a datasheet in the MétroPol database of sampling and analysis methods.

Comparison of environmental and biological monitoring of exposure to xylenes

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The objectives of this study were to study the exposure to xylenes of nine people working in two Tunisian paint companies on different days of the work week and to compare active workplace air sampling with passive sampling and the correlation between the concentrations found for each of the two sampling techniques with the different xylene exposure biological indicators: urinary methylhippuric acids, xylenes in blood, xylenes in urine.

Passive sampling was done with 3M-3500 dosimeter badges while active sampling was performed with NIOSH (100/50 mg) activated charcoal tubes (ACTs). Analysis of xylene in air, blood and urine was carried out by gas chromatography coupled with mass spectrometry (GC-MS) and a headspace injector (HS-GC-MS) for the analysis of urinary and blood xylenes. The determination of the urine concentration of methylhippuric acids was performed by high-pressure liquid chromatography with a diode array detector (HPLC-DAD).

The average exposure levels were relatively low both for the dosimeter samples and the ACT samples (6,8±6,2 ppm -n=28- and 5,1±4,3 ppm -n=14- respectively). The maximum value recorded was for colourists (25.9 ppm). There was no significant difference between the concentrations obtained by the two sampling techniques.

The best correlations between xylene concentrations in the air and biological exposure indicators were obtained for methylhippuric acids (R = 0.55) and urinary xylene at the end of the work shift (R = 0.54). There was a small correlation for blood xylenes (R = 0.1).
Being specific and well correlated to air concentrations, it would appear that urinary xylenes are the most relevant biological exposure indicators. Nevertheless, although they have individual variations, urinary methylhippuric acids could be an interesting alternative, since their concentrations are as well correlated with xylene air concentrations even at low concentrations and are analysed by a technique that is easier to use.

Optimisation of the synthesis of molecularly imprinted polymers for the extraction of urinary glycol ether metabolites

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Amphiphilic in nature, glycol ethers are widely used to make products for industrial use (paints, inks, adhesives, varnishes) and domestic use (cosmetics, household products, pharmaceuticals). Several epidemiological studies have confirmed the toxic effect of certain glycol ethers particularly on reproduction and development (Cicolella., 2006; Welch., 2005). Biological monitoring of occupational exposure to these substances requires a purification stage. Currently, the use of molecularly imprinted polymers (MIPs) for extraction is booming because of their selectivity and low-cost synthesis (Hugon-Chapuis et al., 2007). In this context, this study was conducted to synthesise MIPs to enable the specific extraction of two urinary glycol ether metabolites: methoxyacetic acid (MAA) and ethoxyacetic acid (EAA), in order to analyse them by GC/MS after esterification.

The goal of this work was therefore to determine the optimal synthesis conditions for these MIPs. For that purpose, we used the experimental design methodology, with Doehlert matrices. The factors studied were the quantity of MAA (monomer), the quantity of ethylene glycol dimethylacrylate (EDMA, a cross-linking agent), the volume of solvent (acetonitrile) and the polymerisation temperature. The solutions to the two mathematical models correspond to the peak areas for the MAA and EAA esters in relation to the unit of mass of the synthesised MIPs. In addition, Fourier transform infrared spectrometry (FTIR) analysis of the MAA monomer, the non-imprinted polymer (no addition of target molecules) and the MIPs of the two metabolites was performed in order to ensure that polymerisation was complete.

Statistical analysis of the results was conducted using the NEMROD software. Graphical analysis of the iso-response curves show that retention of MAA and EAA is highest when the quantity of monomer is 0.66 mmol, the quantity of cross-linking agent is 4 mmol, with a volume of solvent of 1 ml and a polymerisation temperature of 40°C.

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Titanium dioxide-induced gene expression profile in rat lung, a sub-acute inhalation study

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Due to the growing use of nanoparticles in industrial processes, the number of workers potentially exposed is increasing while the toxicological properties of these compounds are not fully known. Inhalation represents the main route of occupational exposure, and the first tissues to be exposed are those from the respiratory system. In this respect, the experimental toxicology studies conducted by inhalation in animals appear to be the most relevant for the early evaluation of the hazard associated with exposure to nanoaerosols (Schröder et al., 2014).

Gene expression profiling is a promising approach to study the toxicological effects of nanomaterials since it allows to identify variations in gene expression that may occur while no physiopathological modification is observed or to identify new mechanisms of action of toxicants (Sturla et al., 2014).

In this work we will study the pulmonary toxicological properties at the transcriptomic level of titanium dioxide, one of the most widely used nanomaterial. For this, we exposed Fischer 344 male rats 6 hours/day, 5 days/week for 4 weeks to an aerosol of ~21 nm TiO_2 nanoparticles (10 mg/m^3) using our nose-only inhalation facility.

Lung samples have been collected 0, 3, 30, 90 and 180 days after the end of animal exposure. Biochemical and cytological analyses of the broncho-alveolar lavage fluid (BALF) have been performed and showed a strong neutrophilia up to 3 days which decreased overtime and was already no detectable 90 days after the end of exposure. In addition titanium lung deposition and clearance were assessed by ICP-MS following animal exposure showing a 21 % lung deposition with a slow decrease of titanium burden. These physiopatholological changes are being completed by a gene expression profiling experiment. Total RNAs are extracted, amplified and labelled with Cyanine 3-CTP. The subsequent purified labelled cRNA are hybridized on Agilent 8*60K Rat Oligo Microarray slides. The slides are read using an Agilent Microarray Scanner and the data are extracted using Agilent's Feature Extraction software. Differentially expressed genes are analyzed using dedicated software for Gene Ontology and biological pathways and grouping of genes.

This experimental approach will allow us to better investigate early (acute) and delayed (chronic) pulmonary effects of titanium dioxide nanoparticle in our experimental conditions and then better characterize the mode of action of this nanomaterial. This high content screening method may also help to identify toxicity markers that can be used in *in vitro* models to increase their predictivity.

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In a multivariate world is densisty functional the theory (DFT) a way to theoretical toxicology?

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With diffusion of technology of nanostructured compounds there is some evidence that is difficult to establish some threshold limit value, like number or mass concentration, to perform risk assessment of these chemicals. Nanostructured compounds follow different pathways and show different Mechanism of Action compared to cluster or bulk chemicals. As established in European Rule (EC) N° 1907/2006 (REACH) risk assessment need both hazard and exposure scenario evaluation. This work is focused on use of Quantitative Structure Activity Relationships (QSAR) to evaluate hazard of chemicals. Density Functional Theory has been used in calculation of electronic properties of small organic molecule to use as molecular descriptors related to biological endpoint. QSAR were developed by multivariate analysis PCA and PLSR for dioxin like chemicals. ANOVA shows a relation between considered endpoint and calculated MDs is plausible, but it must be considered non linear. With dioxin like molecules presence of toxicity «activity cliffs» and "outliers" has to be faced. Quantum mechanical approach seems useful in comparing molecules and identify similar MOA rather than in developing predictive toxicity QSAR. Results of multivariate analysis strongly depends from training set choice and Pearson collinearity test shows some correlation between independent variables when calculated by DFT. Small organic molecules were studied, nevertheless multivariate QSAR approach may be useful to perform occupational risk assessment for nanostructured inorganic compounds like TiO2 and SiO2. It is unlikely a single Threshold Limit Value (as number per volume, mass per volume, etc.) will be able to describe toxicity of these chemicals when nanostructured, as dimensions have strong influence on surface chemistry and toxicity Mechanism of Action.

Co-exposure to toluene (or styrene) and methyl ethyl ketone: impact on biological exposure indicators

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Introduction

Mixed-exposures are ubiquitous in occupational environment. They can lead to unexpected interactions at a target organ or organism. Toluene (TOL), or styrene (STYR), and methyl ethyl ketone (MEK) are mixed-exposures which can be easily found in industry. The metabolic interactions of these combinations have been studied in order to evaluate the relevancy of the occupational exposure limits and the biological exposure indicators (BEI).

Methods

Inhalation experiments have been carried out on Brown Norway rats with TOL, STYR and MEK separately and in binary combinations. Blood and brain concentrations, as well as urinary metabolites of the different solvents were monitored. Among all potential biomarkers, special attention has been given to the currently used BEI.

Results

It is well known that TOL and STYR have similar metabolic pathways in humans and rats. In this study, we demonstrated that the metabolism of MEK is similar in human and rat as well, and produces a common main urinary metabolite: 2,3-butanediol. Metabolism of TOL (or STYR) was inhibited by the presence of MEK (and vice versa) resulting in a significant increase in blood/brain concentrations, depending on the proportions of each compound of the mixture. To a lesser extent, this interaction affected the excretion of urinary metabolites.

Discussion

Interaction between TOL/STYR and MEK can lead to an increase in the blood/brain concentrations of the aromatic solvents, even at authorized atmospheric concentrations. This may potentiate their effects and impact employees' health. In case of mixed-exposures, the appropriateness of recommended occupational exposure limits (OELs) can be questioned. Moreover, urinary excretion does not faithfully reflect what happens in the blood. That can also pose a problem for the biomonitoring of employees. The appreciation of the level of exposure to mixtures such as TOL/MEK or STYR/MEK could be undervalued.

Modelling the trend of biomonitoring for occupational exposure in Belgium

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Biomonitoring the exposure to chemicals in the workplaces is an important component of exposure assessment and prevention of adverse health effects. Biological Exposure Indices (BEIs) were established by ACGIH as recommended reference values in industrial settings, but these values are changing over time in part due to the improvement of knowledge and the continuous efforts to reduce exposure. In this context, our objective was to assess the potential changes in exposure in Belgium over the last 2 decades, to determine whether consistent trends have occurred, and examine if this trends could be explained by changes of the BEIs values.

To reach this objective we reviewed the measurements of exposure performed in our laboratory from 1997 to 2014. For this purpose, we have chosen 3 chemical agents (i.e. benzene, toluene and styrene) based on their importance in industrial settings and the available analytical data. The trends of positive samples (considered as the percentage of urinary samples with levels ≥BEI of the total number of samples) were modeled using an integrated random walk model. If the trend slope was positive the percentage of positive samples was increasing and if negative the percentage was decreasing.

From 1981-2000, the **exposure to benzene** was assessed by measuring the urinary content of phenol (BEI, 50 mg/g creatinine). The percentage of positive samples range from 0% (2002) to 2.5% (2001). The number of tests for phenol in urine declined from 1142 in 1998 to less than 200 in 2001, and the trend line had a slope of -0.001 for 1998-2014. Since 2000, the measurement of muconic acid in urine (BEI, 500 mg/g creatinine) was suggested as more suitable biomarker of benzene exposure. Even so, the actual measuring of muconic acid in urine started only in 2005. The percentage of positive samples varied from 2% (2003) to 6% (2009), and the trend line had a slope of 0.002 for 2005-2013.

To evaluate **toluene exposure,** the measurement of hippuric acid in urine was used as biomarker. In 1985, the BEI value for hippuric acid in urine was set at 2.5 g/g creatinine, and in 1997 was further reduced to 1.6 g/g creatinine. The percentage of positive samples varied from 1% (2005) to 3% (2010). Nevertheless, no statistically sustained changes were observed from 1998-2013, since the slope of the calculated trend line was close to zero.

To evaluate **styrene exposure,** the measurement of mandelic acid in urine was used. From 1985-2002, the BEI of urinary content of mandelic acid was set at 800 mg/g creatinine and in 2003, was reduced to 400 mg/g creatinine. For the period 1998-2002 the percentage of positive samples varied from 7% (1998) to 3% (2002), with the slope of the calculated trend line of -0.009. Staring with 2003, the number of positive samples decreased reaching the value of 0% in 2006, and the slope for 2003-2013 changed to -0.003.

On one hand, these results demonstrated that change of BEI values impact the percentage of positive samples (≥BEI). For instance, the BEI for hippuric acid was not changed from 1997-2004 and the calculated trend had a slope of zero, whereas changes of BEI for mandelic acid was followed by a decline in the % of positive samples. On the other hand, it can also be concluded that the replacement of one biomonitoring test with another (e.g. replacing phenol with muconic acid) would not be followed by the industries immediately.

The trend modeling approach of biological monitoring of exposure to benzene, toluene and styrene may shed light on understanding the impact of legislations such as BEIs on the temporal dynamics of biological exposure in industries.

Experimental model of skin/lung responses to chemical exposure

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Diisocyanates are the leading cause of chemical-induced occupational asthma. However, despite reductions in workplace respiratory exposures, isocyanate asthma continues to occur. This has prompted a focus on the skin as a route of sensitisation. We have produced immunologically mediated respiratory responses in mice after initial dermal sensitization using a respiratory sensitizer - toluene diisocyanate (TDI).

Mice were dermally sensitized on days 1 and 8. On day 15, the mice receive a single respiratory challenge. Immediately after the challenge, the 'early' ventilatory response (40 min) was monitored and 22h later airway hyperreactivity (AHR) upon methacholine provocation was measured. Pulmonary inflammation was assessed by bronchoalveolar lavage (BAL) analysis. Analysed immune related parameters comprised total serum IgE and local draining lymph nodes.

Mice sensitized and challenged with TDI showed immediate respiratory responses after challenge. Twenty two hours later, these mice showed pronounced AHR, compared to the controls, associated with a severe neutrophilic inflammation. TDI-sensitized and challenged mice showed, in the auricular lymph nodes, an increase in total number of T helper ($CD4^+$), activated/regulatory T ($CD4^+CD25^+$), T cytotoxic ($CD8^+$) and B ($CD19^+$) lymphocytes together with an increased in vitro secretion of IFN-γ, IL-4, IL-10 and IL-13. An increase in total serum IgE was found. These data were confirmed by applying two other respiratory sensitizers in the model – trimelletic anhydride and ammonium persulfate – which both tested positive. If the model (using TDI) was performed in SCID mice (mice lacking T and B lymphocytes), none of the above described responses were found.

We have been successful in our attempt to develop a model of chemical-induced asthma, with initial dermal sensitization, followed by a single airway challenge. Thereby confirming the skin/lung hypothesis, that dermal sensitization can lead to asthma-like responses. Furthermore, we showed that these skin/lung responses depend on the presence of lymphocytes.

Assessing occupational exposure to multiple volatile organic compounds by biometrology: results of an intervention in companies

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Multiple exposure to chemicals is frequently encountered in an occupational setting and is a topical subject. According to the results of the Sumer 2003 study, 20% of workers are exposed to at least 5 different chemical compounds. The chemical compounds with health effects include numerous volatile organic compounds (VOC) which are commonly used in industry and classed as CMR (carcinogenic, mutagenic, reprotoxic) compounds or as ototoxic products. To assess occupational exposure to VOC, an alternative to tracking the excretion of relevant metabolites in urine consists in measuring residual concentrations of non-metabolised VOC in the urine. Assaying these compounds avoids the need for pre-treatment of the urine sample, which is necessary for traditional metabolite assays but often tricky to perform. The literature contains several examples of successful application of the headspace analysis technique in biomonitoring (Ducos *et al.*, 2008), but few methods are available to track multiple exposures (Fustinoni *et al.*, 1996; Kramer Alkade *et al.*, 2004), and these are often limited to a single chemical family.

We therefore developed an analytical method to simultaneously analyse aromatic and chlorinated compounds: benzene, toluene, ethylbenzene, xylenes, styrene, dichloromethane, chloroform, trichloroethylene and tetrachloroethylene. As it is very unlikely that all of these compounds would be found in a single workplace, the field study focused on companies using at least two of them. Interventions have already been carried out in 7 companies.

This poster presents the results obtained in a French paint and varnish manufacturing company, where chloroform, toluene, ethylbenzene, styrene and xylenes are regularly used in several workshops. Six workshops participated in the study. Thirty potentially exposed workers were monitored, along with five smoker controls and five non-smoker controls. All subjects gave a urine sample before and after their occupational activity for three days. A questionnaire was filled out daily to record the activities potentially linked to exposure during the day, the products used, the personal protective equipment used, and the smoking status of participants.

In the case of low-level exposure to aromatic compounds, especially benzene and toluene, smoking is the most important contributor to exposure. For chlorinated compounds, which are not found in tobacco smoke, the results were always independent of smoking status.

The urinary concentrations of ethylbenzene and xylenes were significantly higher at the end of a shift than at the start, clearly indicating occupational exposure. As end-of-shift excretion is considered to be representative of occupational exposure, these values were used to compare the results for different workshops. For aromatic compounds, the levels measured in the test group were only compared to those of the smoker controls to avoid biases due to the smoking status of the potentially exposed workers. Significantly higher end-of-shift excretions were noted for several workshops compared to control samples, in particular for chloroform, ethylbenzene, xylenes and styrene. However, for chloroform and styrene, the levels measured were still quite low in absolute terms.

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Derivation of cumulative toxicity indicators for indoor semi-volatile organic compounds: the case of reprotoxic and neurotoxic mixtures

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Semi-volatile organic compounds (SVOCs) are widely used indoors in consumer products, furniture or building materials and are also produced by combustion processes. They include a large panel of different compounds such as phthalates (plasticizers), polybrominated diphenyl ethers (flame retardants), or pyrethroids (insecticides). These uses can lead to human exposure to numerous molecules via oral, respiratory or dermal routes because of their physico-chemical properties. Indeed, their low to medium volatility yields adsorption in dust or particles, and presence in air. Most of SVOCs are suspected to have reprotoxic or neurotoxic properties but little is known on the health impact on SVOC mixtures present indoor. In order to investigate this impact, a cumulative health risk assessment is needed. This project aims at deriving cumulative toxicity indicators for reprotoxic and neurotoxic SVOCs present in French dwellings. Methods employed are based on the dose-additivity assumption. Compounds were selected since they were detected in more than 10% of French dwellings. They were grouped according to a common effect or a common mode of action linked with reproductive and neurologic effect. Then, dose-response relationships were collected from a literature review and selected if meeting comparability criteria in terms of species, exposures (doses, route, duration), and windows of vulnerability. Benchmark doses (BMD) were derived using the PROAST software (RIVM, 2014) according to the Hill equations largely used in the modelling of continuous data.

Fifty-one SVOCs were selected among 66 measured. Twenty-seven have reprotoxic properties and for 17 SVOCs, the mode of action is based on an impairment of the steroidogenesis, leading to a decrease in testosterone concentrations. Twenty-seven have neurotoxic properties leading to neurobehavioral disorders and for 19, a neuronal impairment was identified. After restricting to comparable dose-response data, it was possible to derive BMDs and associated relative potency factors based on a decrease in testosterone concentrations in laboratory mammals for 6 reprotoxic SVOCs (DEHP, BBP, DEP, benzo(a)pyrene, cypermethrin and bisphenol A). In addition, 95% lower bound of BMDs were derived for 13 neurotoxic SVOCs (DEHP, benzo(a)pyrene, BDE-47, 99, 209, PCB52, 77, 153, deltamethrin, diazinon, chlorpyrifos-ethyl, lindane, dieldrine) having a common effect (neuronal mortality based on an in vitro decrease in cell viability). These BMDs could serve as a point of departure for risk assessment.

The originality of this work was the inclusion of SVOCs from different chemical families detected in French dwellings. The main limitation was the lack of comparable toxicological data in terms of dose level ranges, duration of exposure, species or cell line tested, and window of vulnerability.

These cumulative toxicity indicators enable a cumulative risk assessment. Assessing risks due to SVOCs will help targeting prevention measures through the identification of compounds and exposure media that lead to a greater risk.

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The authors have no conflict of interest.

Assessment of microenvironments contribution on $PM_{2.5}$ and PAHs exposures of population using integrated models

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PAHs are known to induce health effects on population due to the ability of airborne particulates to absorb and transport these species in the lungs. Since some PAHs are potent carcinogens by a genotoxic mode of action that adds to that of fine particles (e.g. $PM_{2.5}$), their levels in air should be kept as low as possible (WHO, 2013). Consequently, the assessment of $PM_{2.5}$ and PAHs exposures of the most sensitive populations living in urban areas represent a relevant issue, which was addressed by the EXPAH project (www.ispesl.it/expah).

An integrated model is presented to estimate $PM_{2.5}$ and PAHs exposures of children and elderly people living in Rome, Italy. It is based on a microenvironment approach accounting for concentrations experienced by the target population in the most visited living environments. The model uses data provided by the EU LIFE+ EXPAH project: indoor/outdoor $PM_{2.5}$/PAHs concentrations collected in homes, schools, offices and traffic (cars, buses) to derive infiltration factors; time activity tables to get information on the prevailing living environments. Ambient concentration fields. are obtained by using a three-dimensional Eulerian chemical-transport model FARM (Flexible Air quality Regional Model), which includes the PAHs reactions with hydroxyl radical and their partitioning between gas and aerosol phases. A Monte Carlo statistical approach has been used to estimate uncertainties in the exposures evaluated. Daily and annual exposures were calculated over one year (June 2011-May 2012). The downtown area was found to be the most contaminated one with concentrations up to 2±1 and 0.6±0.2 ng/m^3, on an annual basis, respectively for PAHs and B[a]P. As for $PM_{2.5}$, up to 16 µg/m^3 are foreseen in the downtown area as yearly average. No differences were found between children and elderly exposures to PAHs and B[a]P exposures, while small variations for $PM_{2.5}$. Seasonality was found to be the strongest contributor to the overall exposure. As for children, mean exposures up to 4.0 and 1.1 ng/m^3 are estimated for PAHs and B[a]P respectively during the heating season (Nov-Feb). Conversely during the non-heating season, substantially lower exposures are predicted (up to 0.6 and 0.15 ng/m^3 for PAHs and B[a]P respectively). Similar but less striking contrasts were found for $PM_{2.5}$. The most contributing microenvironment for children was found to be home followed by school (see figure 1), due to the predominant time spent in these two indoor rooms. The sum of the exposures experienced in them accounts for about 85% of the total daily exposure.

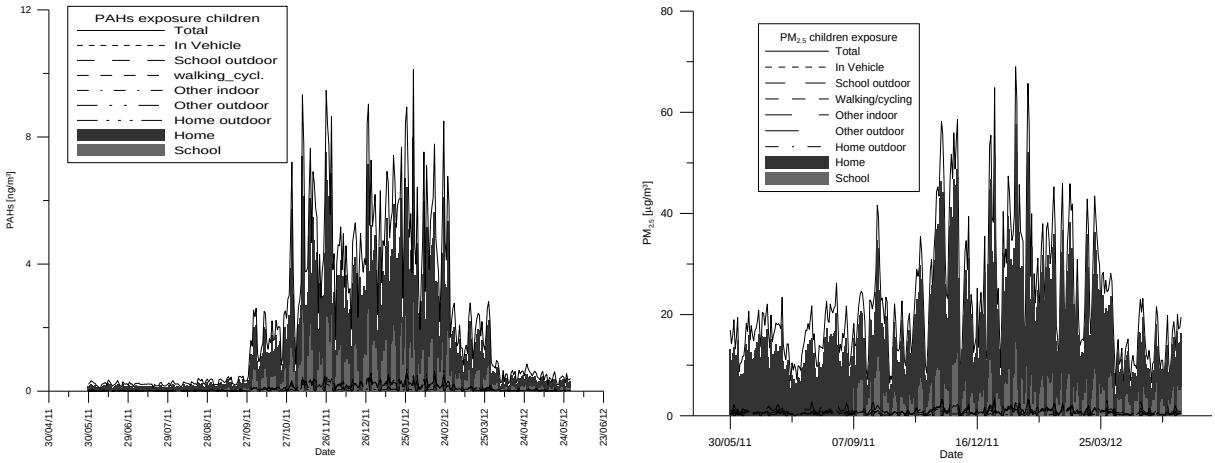

Figure 1. Time series of microenvironments contribution to PAHs (left) and $PM_{2.5}$ (right) exposure for children living in a downtown area of Rome

The model results were compared with personal exposure measurements. The exposure model showed ability to reproduce observed values with satisfactory results of indexes of performance.

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Biological assessment of exposure to di-2-ethylhexyl phthalate (DEHP) in the soft PVC industry in France

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In order to assess exposure to DEHP in the soft PVC industry, the employees of six companies provided their assistance over five consecutive days at the start and end of the work shift. Three urinary DEHP metabolites: mono-(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-carboxypentyl)phthalate (5Cx-MEPP) and 2-ethylhexanoic acid (2-EHA) were quantified in 62 exposed workers and 9 controls. Analyses were carried out by high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (HPLC-MS/MS).

The median urinary concentrations found are as follows: for MEHP, 12.6 and 28.7 µg/L; for 5cx-MEPP, 38.6 and 84.4 µg/L; and for 2-EHA, 20.4 and 70.6 µg/L, at the start and end of the work shift respectively. We observed a significant increase in the excretion of DEHP metabolites in workers at the end of the work shift compared to the controls. A significant increase was also recorded between the start of the work shift and the end of the work shift only for exposed workers. On the basis of this work, urinary concentrations of 250 µg/L for MEHP and 500 µg/L for 5Cx-MEPP (100 and 280 µg/g of creatinine) are proposed as guideline values.

The statistical analysis of the results clearly indicates exposure to DEHP of employees concerned. The guideline values proposed should prevent high levels of exposure in the soft PVC industry, particularly in companies in which compounds and plastisols containing DEHP are made or used.

Individual production of reference materials by using a piezoelectric microdosing system - First tests

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In the field of analytics, quality control has come to play an increasingly important role. Reference materials are just as essential in calibration and quality control as they are for verifying the accuracy and reliability of results obtained.

Due to the complexity of their manufacture, reference materials for substances occurring at workplaces are expensive and available only on a limited scale and for a small number of substances. Finding a possibility of their fast and cheap production is of great interest. Demand for reference materials which are flexible in use and under practical conditions in particular is growing from both laboratories and accrediting bodies. In order to accomplish these requirements, the applicability of a piezoelectric microdosing system was examined.

The high uniformity and small size of piezoelectric droplet generation enables accurate non-contact metering of very small substance quantities with high reproducibility. Droplet formation is highly influenced by the dispensing parameters voltage and pulse width. They have to be adjusted together for each dispensing fluid. Frequency shows hardly an impact on droplet formation.

First gravimetric test series were performed with ultrapure water and aqueous glycerol solution. This method is suitable only to a limited extent for the determination of the reproducibility due to the dependency on the environmental conditions and the influence of evaporation.

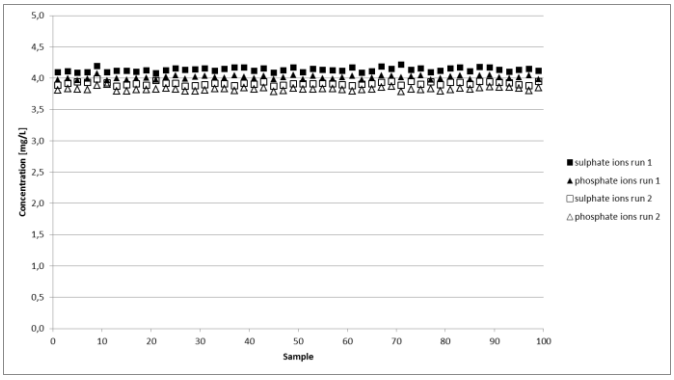

Figure 5: Results of dosing 9000 droplets of a mixture of aqueous sulfuric acid and aqueous phosphoric acid on two different days.

Therefore another analytical method was necessary: dispensing non-volatile inorganic acids with following quantification by ion chromatography led to more accurate results. Daily series of 100 samples were performed. The target value of relative standard deviations below one percent was achieved. Comparison of the results gained on different days shows higher variability because of the influence of the environmental conditions on the droplet generation.

To make use of the piezoelectric microdosing system for the production of reference materials, the temperature influence on droplet formation needs to be eliminated. That means either working under constant conditions or accurate measuring of the actual droplet size and adapting the number of droplets required for a certain volume. Series with an adequate number of samples (> 500) need to be realised with high reproducibility.

Performance of the µ-Cathia aerosol sampler versus conventional thoracic health-related aerosol fraction

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The personal aerosol sampler µ-Cathia was initially developed to measure inhalable aerosol concentrations. It uses a multidirectional sampling inlet studied previously in our laboratory (Görner et al 2008). At international level some aerosol substances have been designated to be sampled in thoracic aerosol fractions. This applies to cotton dust (OSHA 1978, ACGIH 1985), asbestos (Dement 1990, NF X43-050) and sulphuric acid (EU Directive 2009).

The aim of this study is to check that the µ-Cathia is capable of sampling thoracic aerosol fraction as defined by the CEN-ISO standards. A modified µ-Cathia prototype with a patented aspiration slot (Görner et al 2012) was used at 3 L.min[1]. It was challenged by a polydisperse aerosol composed of glass micro-spheres generated by a fluidized-bed aerosol generator in a vertical dust chamber (Figure 1). The reference and sampled size-dependent aerosol concentrations were measured using the TSI Aerodynamic Particle Sizer (APS 3320). The entire experimental method is described elsewhere (Görner at al 2001, 2010).

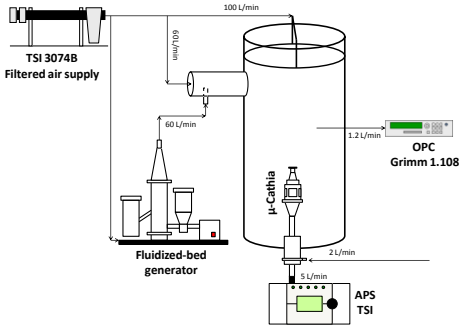

Figure 1. Experimental set up

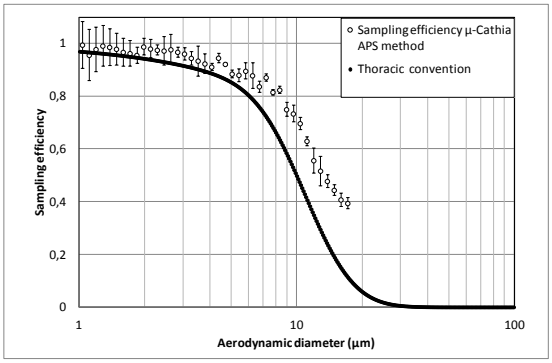

Figure 2. µ-Cathia sampling efficiency

Figure 2 shows that the µ-Cathia sampler oversamples the conventional thoracic aerosol fraction.

Experimental efficiency data are available only up to 20 µm in aerodynamic diameter, which is due to the limited scale of the APS.

With this performance, µ-Cathia has shown itself to be suitable for sampling asbestos fibres since the sampling standard NF X43-050 specifies sampling "at least" the thoracic aerosol fraction. For the H_2SO_4 mist, the thoracic fraction must be measured. Hery et al (1992) measured the particle size distribution of acid mists in a plant manufacturing titanium dioxide using the Marple cascade impactor (Rubow 1987). They found the average thoracic/inhalable ratio to be 0.52. In that case, the thoracic aerosol would be slightly oversampled by the µ-Cathia sampler. To check this statement workplace measurements will be necessary in various occupational situations.

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Setting up of the Bhas 42 *in vitro* cell transformation assay

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Four to 8.5% of cancers in France are assumed to be linked to occupational exposure, i.e. 11,000 to 23,000 new cases per year (Brasseur *et al.*, 2006). From a regulatory point of view, the assessment of the carcinogenic risk of a chemical or physical agent is essentially based on *in vivo* animal carcinogenesis assays. The most commonly used *in vitro* assays that are an alternative to animal experiments aim to identify the genotoxic effects of the agents tested: DNA damage, chromosomal alterations and gene mutations. "Non-genotoxic" carcinogenic agents (i.e. not directly altering the gene) are therefore likely to be overlooked by genotoxicity tests. For this reason, *in vitro* cell transformation assays on different cell models have been developed with a view to detecting the carcinogenic activity of hazardous agents regardless of their original mode of action.

Our project consisted in setting up the Bhas 42 cell transformation assay in the laboratory. This assay was recently evaluated by the European Centre for The Validation of Alternative Methods (ECVAM). The assay uses a line of transgenic cells, Bhas 42, developed from the Balb/c 3T3 murine cell line. The genome of Bhas 42 cells contains the *ras* viral oncogene sequence, which characterises them as "initiated cells" (Sasaki *et al.*, 1990) Compared to other cell transformation models, Bhas 42 are therefore particularly intended for carcinogenic agents capable of activating tumour promotion without necessarily initiating genotoxic events. Bhas 42 cells develop transformed foci when they are exposed to a genotoxic tumour-initiating agent such as 3-methylcholanthrene (MCA) but also to a tumour-promoting agent such as 12-O-Tetradecanoylphorbol-13-acetate (TPA). In comparison, Balb/c 3T3 cells (not initiated) will only be sensitive to MCA (Figure 1).

The transformation assay is currently being validated in our laboratory with the use of a series of reference carcinogenic agents whose mode of action is known (Sakai *et al.*, 2011). Afterwards, we will determine whether this assay can also be applied to particulate agents (including nanomaterials), for which there is little data on cell transformation effects.

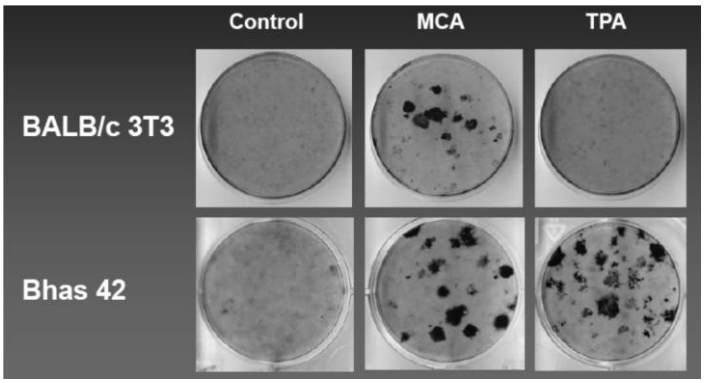

Figure 1: Bhas 42 as model initiated cells. MCA (3-methylcholanthrene), initiating agent; TPA (12-O-tetradecanoylphorbol-13-acetate), promoting agent (Sasaki *et al*. http://www.axlr8.eu/workshops/2012-sasaki.pdf)

Brasseur G, Héry M and Pillière F. (2006) Cancers professionnels, le point des connaissances sur... *Edition INRS*: 1-6.

Sakai A, Sasaki K, Hayashi K, et al. (2011) An international validation study of a Bhas 42 cell transformation assay for the prediction of chemical carcinogenicity. *Mutat Res* 725(1-2): 57-77.

Sasaki K, Mizusawa H, Ishidate M, et al. (1990) Establishment of a highly reproducible transformation assay of a ras-transfected BALB 3T3 clone by treatment with promoters. *Basic Life Sci* 52: 411-416.

Innovative adsorbents for workplace nitrous oxide diffusive sampling

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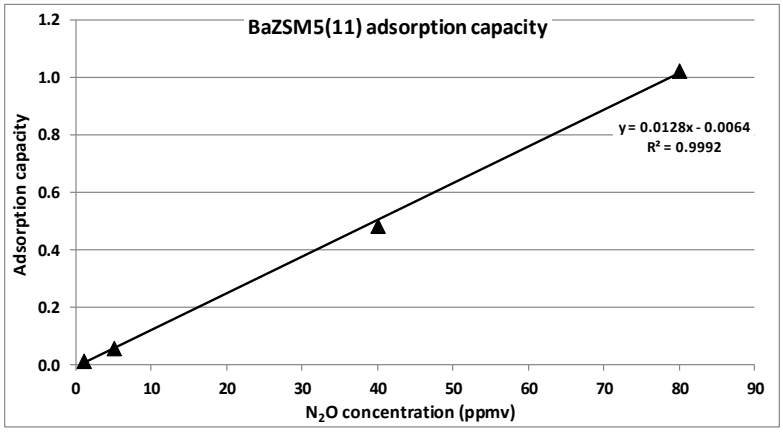
Nitrous oxide (N2O) is used as an anesthetic in medical, dental, and veterinary procedures. Several toxicological studies have shown that occupational exposure to N2O can cause adverse effects such as reduced fertility, spontaneous abortions, neurological disorders, and renal and liver disease (Rowland et al., 1992). The American Conference of Governmental Industrial Hygienists (ACGIH) have established a threshold limit value (TLV) for N2O of 50 ppmv for an 8-hour Time Weighted Average (ACGIH, 1993). In France, decree DGS/3A/667 set the TLV for N2O to 25 ppmv during the maintenance phase of anesthesia.

The current method used to sample N2O is based on diffusive sampling on a 5Å molecular sieve, but this is not ideal as a back-diffusion phenomenon is observed (INRS, 2010).

Previous work (Guillemot et al., 2014) on cationic ZSM5 zeolites have demonstrated that the highest adsorption capacities, in the presence or absence of water vapour, were obtained with Ba-exchanged ZSM5 zeolites with an Si/Al ratio of 11; no back diffusion was observed on this adsorbent. BaZSM5(11) thus appears to be a suitable solid to replace 5Å molecular sieve for diffusive N2O sampling.

Further investigations have been performed to validate the diffusive sampling method on this new adsorbent. N2O dynamic adsorption experiments have shown that the adsorption capacity dicreases as the concentration of nitrous oxide in the atmosphere decreases.



The influence of temperature, level of moisture and the presence of copollutants on the uptake rate of nitrous oxide was investigated in a experimental design.

Rowland, A.S.; Baird, D.D.; Weinberg, C.R.; Shore, D.L.; Shy, C.M.; Wilcox, A.J. Reduced fertility among women employed as dental assistants exposed to high levels of nitrous oxide. *New Eng. J. Med.* **1992**, 327 (14), 993.

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Internalisation of international regulations on chemical risk in Morocco "Case of transportation of hazardous materials"

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Hazardous materials are governed at international level by several agreements, regulations and directives, the goal of which is to control risks inherent to the transportation, handling, use, storage and disposal of these products. Among these texts are the ADR, the REACH regulation, GHS and the Rotterdam, Basel and Stockholm conventions.

In Morocco, production and consumption of these different materials are booming given the main sectors that make up the economic environment of the country (mines, phosphates, fertiliser, agriculture, etc.). This trend is amplified by sector policies targeted at the economic pillars of the country, in particular, structural programmes such as "Emergence", "Maroc Vert", "Halieutis", etc.

On that basis, and following the different free trade agreements signed with international economic partners (EU, USA, Turkey, etc.), it was essential for public authorities to harmonise the legal framework surrounding the management of hazardous materials and waste, especially since most of the fundamental texts are outdated and ill-adapted to the current context (for example the decree of 1922 on poisonous substances).

To that effect, several texts recently emerged, in particular:

Law 30-05[1] on the transportation of hazardous materials, the aim of which is to define specific rules applicable to the road transportation of dangerous goods;

Law 28-00[2] on the management of waste and its disposal, the aim of which is to organise the management of all types of waste to prevent pollution and protect people's health by placing emphasis on legal means which are essential for improving hazardous waste management;

the Labour Code [3] which contains, among other things, certain provisions for avoiding chemical risks by enforcing the use of PPE, protection of employees' health, availability of facilities for reducing this type of risk, information and training, storage and handling of hazardous materials.

The goal of this legal arsenal is to introduce new rules for the management and control of the use of hazardous materials by including current international standards (MSDS, labelling, classification, storage, transportation, disposal, etc.).

1. Decree No. 1-11-37 promulgating law no. 30-05 on road transportation of dangerous goods, published in the Official Bulletin No. 5956bis, 30 June 2011, p.1765 to 1772.
2. Law No. 28-00 on the management of waste and their elimination published in the Official Bulletin No. 5480 of 7 December 2006.
3. Decree No. 1-03-194 of 11 September establishing law No 65-99 on the Labour Code.

Supercritical CO_2 desorption for air sampling analysis

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For organic compounds, atmospheric pollution assessment is based on the adsorption of chemical substances on porous sorbents and subsequent desorption and analysis. Desorption often involves solvents, a step which presents many drawbacks. The use of porous polymers as sorbents prevents the use of solvent extraction, thus other means of desorption are used such as thermal desorption or microwave assisted desorption (Esteve & Langlois, 2012). But there is no alternative and totally universal method of desorption for all pollutants and sorbents, due to the diversity and complexity of the interactions between them. Furthermore, the thermal treatment involved in these methods prevents online coupling with liquid chromatography and the analysis of thermolabile compounds.

In this study, we propose to develop an analytical device able to desorb any organic compound from any sorbent without using solvents and perform online analysis with gas, liquid and supercritical chromatography. Extractions are performed with supercritical CO_2 ($scCO_2$) at high pressure and high temperature, bringing matter to a supercritical state, between gas and liquid states. The supercritical state endows fluids with penetration and solubility properties that are far superior to those of traditional liquid solvents. Extraction using supercritical fluids is thus an extremely high-performance technique (Chaudot *et al.* 1997). CO_2 was chosen because it has a relatively low critical pressure and temperature, 73.8 bar and 31°C respectively, which are common conditions used in analytical laboratories for high performance liquid chromatography (HPLC), for example. Another advantage of this type of extraction is the ease of separation between the solvent and the solutes: simple expansion is enough to bring a supercritical fluid back to its gas state and thus retain only the solutes in the liquid state.

In the first step of this study, we designed a re-usable sampler that can be used either in active or passive mode. We proved the efficiency of $scCO_2$ for BTX extraction on activated charcoal using offline analysis. In this second step, a mass spectrometer was coupled directly to the extractor in order to monitor the desorption kinetics of different sorbent-substance pairs. These desorption kinetic curves give very interesting information for extraction parameter optimisation as a function of the nature of interaction between the substance and the sorbent, as illustrated in Figure 1 for BTX on charcoal. Each sorbent-substance pair used in all the Metropol[3] methods was studied by spiking the substance in vapour phase on the re-usable sampler filled with the relevant sorbent. The optimal extraction conditions were therefore determined according to the extraction profile.

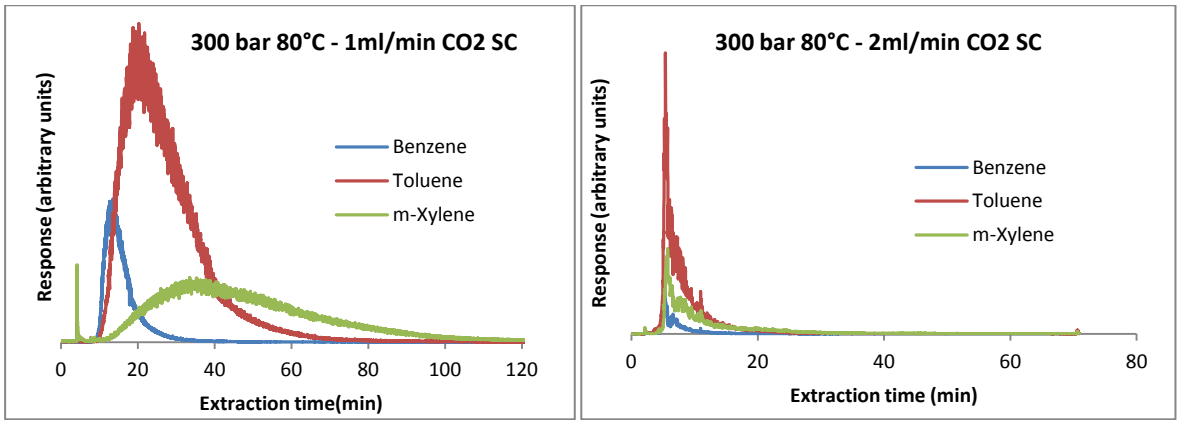

Figure 1: Desorption kinetic curves of BTX on charcoal for different conditions monitored by online mass spectrometry.

In conclusion, the reusable sampler was suitable for all sorbent type fillings, thus extraction conditions were setup for all the organic substances referenced in the MetroPol database without any solvent. Therefore, for all

[1] Metropol: French sampling and analytical method database. Available on http://www.inrs.fr

these methods, solvent extraction and the conventional analytical step could be replaced by online $scCO_2$ extraction and gas chromatography. The next step of the study will be to automate online coupling with chromatography in order to provide a fully automated device.

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Esteve, W. & Langlois E., (2012), *Analytical Methods , 4, p. 2054-2061*.

Adjustment of xorkplace exposure standards for atmospheric contaminants for extended work shifts – Models overview

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The use of unusual or extended work shifts, common on several industries, places workers at increased risk of exceeding recommended levels of airborne contaminants.

Extended work shifts can take many forms, but they generally involve the employee working shifts of greater than 8 hours in length, or a working week of greater than 40 hours. Extended shift causes greater stress on the body's ability to cope efficiently and effectively with toxins.

Longer work shifts and therefore shorter recovery periods between exposures might result in adverse health effects to the worker. Since the standard eight hour day, the epidemiological basis of almost all current exposure standards for atmospheric contaminants, no longer exists in many workplaces, the use of exposure standard adjustments is thus mandatory.

Numerous models, using varying approaches to adjust exposure limits, have been published and discussed in the literature. Some are based on simple mathematical equations that take into account the extended hours of work, while others use more complicated formulas, which take into account variables as rates of uptake and excretion, biological half-lives and health effects.

Among the various methods that have been documented, the most commonly referred are:

- Brief & Scala Model;
- OSHA Model;
- Pharmacokinetic Model of Hickey & Reist;
- Québec Model.

An overview of these four models capable of adjusting exposure standards for use during altered working shifts is presented.

Chemical risk: a global approach for local solutions

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Prevention of occupational risks in the French education system and in the public service on a whole must comply with Part 4 of the French Labour Code. Prevention of chemical risk is included in the Labour Code and concerns all companies.

An education district has tens of thousands of personnel, thousands of educational facilities (primary and secondary schools) and many administrative structures.

Occupational risk prevention is of a general nature regarding the guidelines given and of a very applied nature on a day-to-day basis in workplaces due to the large diversity of work situations.

We will present the way in which the occurrence of individual risk is addressed structurally to provide local solutions.

Proven risks
Concerning chemical risk: what is the reality of this risk?
As soon as they were deployed, occupational safety and health inspectors were immediately called upon for very diverse local issues:

- Presence of wood dust in training workshops.
- Cancers in personnel working in printing.
- Chemical accidents in laboratory and maintenance personnel.
- Investigations into dangerous chemical exposure assessments.

All departments, including teaching departments and administrative services, are concerned by chemical risk. First, emphasis was placed on teaching laboratories, technical teaching platforms (joinery shops, metal workshops and chemistry workshops), repair and maintenance services.

In addition, during their visits to check for compliance with regulations, occupational safety and health inspectors highlighted the personnel's lack of interest in their own occupational risks, being rather more concerned with the safety of the students, which is a characteristic specific to workers in the education system in France.

How to disseminate a culture of occupational safety? How to develop a prevention policy?
The implementation of occupational risk assessment documents (“document unique” that should be established and updated at least once a year) is an important means to raise awareness of the need for prevention action.
However, widening of the use of this approach at local level was relatively slow.
The setting up of a "risks" software specific to the education system in all teaching establishments became a necessity and enabled:

- dissemination across France of the practice of risk prevention
- the possibility of combining the actions to be undertaken.

This software offers the possibility to the head of the establishment or service of distributing risk analysis among the personnel through a "work unit delegation" procedure.
Based on work situations, each unit identifies the occupational hazards and risks and can make proposals to remove or reduce risks. The software can be used to generate documents per work unit or establishment and to edit prevention action proposals.

Essential characteristic of this software: hosted on the academic district's IT department, it offers two advantages:

- data storage enabling instructions to be passed on among department heads.
- the availability of information on risks, enabling local or academic district consolidation and the definition of prevention programmes for the academic district.

And in the future?

For many reasons: structural and societal, awareness of occupational risks is making much progress.
However, there is still the matter of designing prevention solutions and implementing them.
Partnerships between the French education system, which uses the buildings, and local authorities, which own them, add complexity but also proximity to the issues.
With a holistic view, a genuine occupational safety and health department should be set up through the designation and activation of a network of prevention advisors and assistants in liaison with the departments and establishments.

Acknowledgements: The software adapted to different academic districts was initially designed by the IT department of the academic district of Montpellier.

Ref: http://www.ac-grenoble.fr/admin/spip/spip.php?article3226

Draft canister method for sampling and analysis of select volatile organic compounds

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[3]Environmental Health and Safety program, Clarkson University, 13699, Potsdam, NY, USA

The Environmental Protection Agency (EPA) has been monitoring ambient air toxics for years using a canister-based sampling methodology (EPA Method TO-15, 1997). Their focus was on low-level analysis of multiple volatile organic compounds (VOCs) with toxic effects. The Occupational Safety and Health Administration (OSHA) has predominantly focused on high-level exposures to a single analyte using sorbent-based sampling methods, but considering single analyte exposure rarely occurs, the use of a multi-analyte sampling and analysis strategy is warranted. In 2003, OSHA partially validated a canister method (PV2120) for the analysis of only four compounds at ppb-levels. No further work was performed to investigate the use of this method for higher concentrations or alternate analytes.

Researchers at the National Institute for Occupational Safety and Health have recently investigated the use of evacuated canister sampling for a wide range of VOCs. In 2011, Coffey et al. validated the use of fused-silica lined evacuated canisters for chemical warfare agent simulants at ppb-levels. In 2012, LeBouf et al. statistically validated the use of the same canisters for a suite of 14 analytes found in healthcare settings at both ppb- and ppm-levels. The researchers are also investigating an additional three analytes found in workplaces where flavorings are used. Further analytes may be added to the NMAM draft method, as needed, but will require a comprehensive statistical validation.

NIOSH conducted an interlaboratory study to verify performance of the draft canister method for 17 analytes in air by evaluating bias and precision estimates from seven laboratories. In a blind study, three replicates of six different concentrations of 17 analytes (nominal 5, 10, 15, 800, 1300, and 1700 ppb) were sent to seven laboratories. Spike concentrations were generated by diluting a certified gas standard with ultra-high purity (UHP) nitrogen. Canisters filled with only UHP nitrogen were shipped to the laboratories as blanks. Reference canisters were produced during spike generation as a quality control measure for the vapor generation procedure. Canister volumes included both 450 cc and 6 L fused-silica lined canisters depending on the analytical needs of the participating laboratories. Analytes included ethanol, acetone, 2-propanol, methylene chloride, hexane, chloroform, benzene, ethylbenzene, m,p-xylene, o-xylene, toluene, 2,3-butanedione, 2,3-pentanedione, 2,3-hexanedione, methyl methacrylate, limonene, and α-pinene. Relative bias was calculated as percentage difference between the reported and theoretical value. Relative standard deviation was calculated as within laboratory and total variability at each concentration.

Bias and precision were dependent on the following conditions: laboratory, spike concentration, and analyte. Five of seven laboratories had acceptable overall biases of ±10% for most concentrations and analytes and the total relative standard deviation was less than 20%, which was consistent with results from the reference canisters. The highest and lowest average absolute bias for all laboratories was observed for m,p-xylene and n-hexane, respectively. The lower spike concentrations (5, 10 and 15 ppb) had the highest bias and were generally higher than theoretical concentrations. As expected, the worst precision was observed at the lowest spike concentration due to increased variability at the lower limits of the analytical method. Most blanks were below the reported laboratory detection limits for a majority of the analytes.

Bias and precision were dependent on laboratory, spike concentration, and analyte with five of seven laboratories able to achieve acceptable performance. As with any analytical method, laboratory performance should be investigated frequently using proficiency testing programs to verify adequate method performance and to identify areas for improvement. Incorporation of the evacuated canister method into the NMAM and development of an ASTM method will enable a broader use of this methodology for conducting exposure assessments.

Acknowledgement: This work was supported by the National Institute for Occupational Safety and Health.

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Ventilation assessment and improvement using real time techniques in a slate workshop with high rcs (respirable crystalline silica) concentrations

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Slate has a spread use in construction, mainly as a roof tiles. The manufacturing process of this slate tiles, produces a high amount of dust with high respirable crystalline silica content (RCS). This is due to the high amount of RCS in the raw material, and cutting and final product packaging processes. These processes are carried out with a low level of automation, which implies that the operators have to work very close to the dust generation focal point. This is not only a problem associated to marble workshops, but to other sectors like ornamental stone and sectors in which quartz agglomerates are handled. It is an important economic sector in Spain, mainly for the exportation, so it is especially important the adequate control of the exposure risk to RCS. Indeed, it is well known that exposure to this toxic agent can induce silicosis and also lung cancer, among other diseases.

In order to deal with the risk described above, the Technical Department of the National Silicosis Institute of Spain, in collaboration with the Slate Cluster from Galicia (Spain), and based on a grant from the regional Government of Galicia, has started a new project to study the improvements in the preventive measures currently installed in a slate workshop. Within this project, a course of action is the properly assessment of the accuracy of the ventilation system, and its influence in reducing the workplace exposure to respirable dust fraction (RDF) and therefore to RCS. For that purpose, it has turned to the use of real measurement equipment, which is complementary to the conventional DRF and RCS personal sampling. The final object of the study, is to show how to use some tools (different dust meters) to evaluate the installed ventilation and to give recommendations to improve it, when necessary. This is very important, because it is common to find ventilation with low air velocity, whose final results are not the expected.

To study the distribution and evolution of dust concentration, three on line aerosol monitor (optical particle counter. TSI SidePak AM510), one particulate monitor (Optical particle counter. SKC HazDust-IV HD-1004) and one portable aerosol spectrometer (optical particle counter. Grimm model 1.109) were used. To measure the variations in the air flow in the workplace and in different situations, two anemometers (KIMO CV100 y KIMO CV110) were used to quantify the different ventilation scenarios.

Also, to check the influence on the dust concentration of the installed ventilation, trying to verify if it was over-dimensioned, high flow pumps (SKC Leland Legacy 100-3002) were used.

Personal type P sampling pumps (APEX Casella 182000B) were used for 8h RDF (respirable dust fraction) and RCS personal sampling. This personal sampling was carried out to check the global situation, and to compare the exposure to the limit values.

The results show how the ventilation had not been calculated previously, being insufficient to control the dust. In fact, the installation, more than a pure ventilation system, is a dilution system, being more influential in the lowering of the dust the natural ventilation than the installed one.

The main conclusion is the importance on the use of real time equipment to assess the good operation of the preventive measures in the workplace, which is a very useful tool to any hygienist who has to properly assess the exposure risk to RDF and RCS in a workplace.

Assessing chemical risks: an overall management tool

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Decree No. 2003-1254 of 23 December 2003 relating to prevention of chemical risks and which amends the French Labour code (Articles R4412-1 et seq.) requires employers to assess the "risks to the health and safety of workers involved in any activity likely to present a risk of exposure to dangerous chemicals" [1] [2].

The regulations relating to the assessment of occupational exposure to chemicals were also amended, in December 2009, by the adoption of texts reinforcing the overall approach: Decree No. 2009-1570 [3] and the Decree of 15 December 2009 [4] relating to the techniques used to verify occupational exposure limit values (OEL) in workplaces. The circular of application (published 13/04/2010) details these provisions, in particular by requiring the results of any assessment of chemical risks to be recorded to establish a sampling strategy [5].

Finally, exposure to dangerous chemicals was defined as a factor contributing to strain [6] [7]. French companies must therefore determine whether their workers are exposed to levels exceeding the thresholds established for this parameter to allow calculation of their "strain points" from 1 January 2016.

With the aim of helping companies to meet these various requirements, Bureau Veritas has developed a method to analyse chemical risks which can be used for the overall management and follow-up of these risks.

The method developed by Bureau Veritas was inspired mainly by the simplified assessment method for chemical risks described by INRS (French Research and Safety Institute for the Prevention of Occupational Accidents and Diseases) in document ND2233-200-05 [8] and the methodological guides and tools made available by CARSATs (French regional occupational health and pension insurance funds) [9] [10] [11] [12] and the CTN (French national technical committee) for chemistry, rubber and plastics [13]. It can be split into 5 stages (Figure 1):

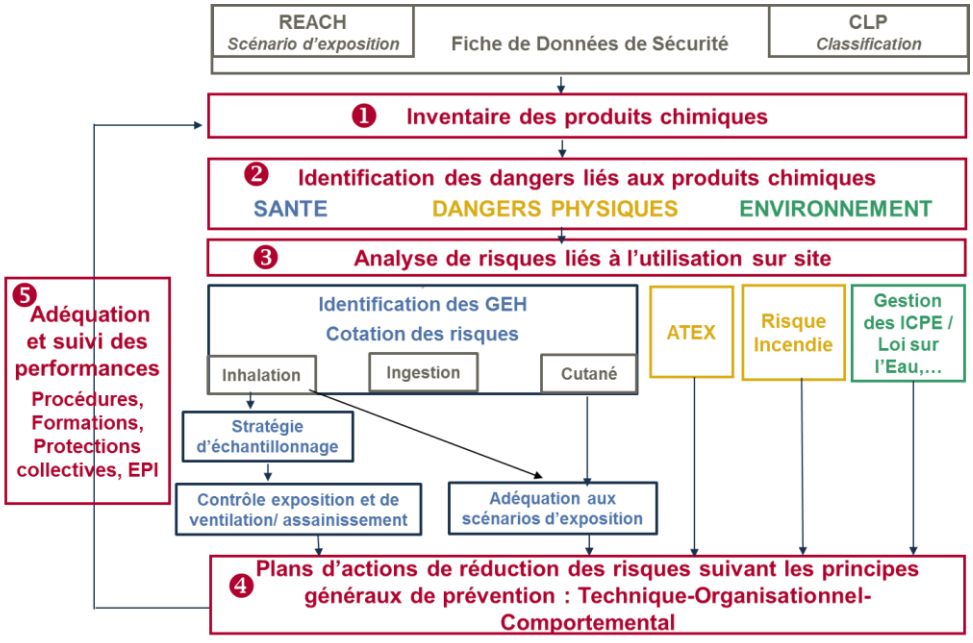

Figure 1: Overall management of chemical risks.

Stages 1 and 2 make an inventory of chemicals and identify the hazards, this information can be used to answer the following questions:

- What chemicals are present in the company?
- What hazards are associated with these chemicals? Are there any CMR (carcinogenic, mutagenic, reprotoxic) products? Are there any nanoparticles?
- Are there regulatory or accepted occupational exposure limits (OELs) for these products?

Stage 3 of the analysis of chemical risks makes it possible to:

- Qualitatively assess the risks to the health and safety of workers using these chemicals at a workstation and/or assess residual risks for operators, based on the exposure conditions and the preventive and collective

and/or personal protective measures implemented. The two aspects "risk by inhalation" and "dermal risk" are studied separately (Figure 2).

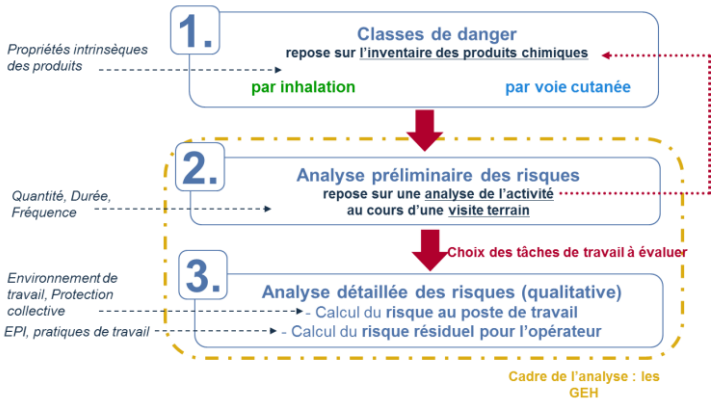

Figure 2: Detailed analysis of the risk for health posed by the chemical

- Identify Homogeneous Exposure Groups (HEGs).
- Establish a sampling strategy based on measurement campaigns for the inhalation risk to take into account variability in worker exposure.
- Measure worker exposure and determine whether or not the occupational exposure limits are exceeded.
- Verify the ventilation and systems to clean the work area, including in terms of retention efficacy.
- Prepare analyses of the impact the chemical risk will have on the installations (ATEX, risk of fire, etc.) and on the environment (ICPE, Seveso 3, etc.)

Stage 4 of the analysis of chemical risks makes it possible to:

- Propose a plan of actions to control the risks identified at stage 3 integrating the various levels of priority: Substitution, Modification of processes, CPE, PPE;
- Help the client to reduce the volume of documentation required: Strain, Assistance with choosing appropriate PPE, Procedure for management of PPE, workstation notices, etc.

Stage 5 is the follow-up stage of the risk-management plan of action, it makes it possible to:

- Verify that the plan is appropriate and ensure that the preventive and protective measures implemented are used;
- Take into account any changes to the activity: Update the inventory of chemicals; take into account new production processes, changes to workstations, results of campaigns measuring worker exposure.

Based on an example, we will present this overall approach for the management of human health risks posed by chemicals, along with the advantages and difficulties encountered in responding to the various regulatory requirements.

[1] Décret n°2003-1254 du 23 décembre 2003 relatif à la prévention du risque chimique et modifiant le code du travail. Version consolidée au 28 décembre 2003
[2] Code du travail. Quatrième partie. Santé et sécurité au travail.
[3] Décret n°2009-1570 du 15 décembre 2009 relatif au contrôle du risque chimique sur les lieux de travail.
[4] Arrêté du 15 décembre 2009 relatif aux contrôles techniques des valeurs limites d'exposition professionnelle sur les lieux de travail et aux conditions d'accréditation des organismes chargés des contrôles.
[5] Circulaire DGT 2010/03 du 13 avril 2010 relative au contrôle du risque chimique sur les lieux de travail
[6] Décret no 2014-1159 du 9 octobre 2014 relatif à l'exposition des travailleurs à certains facteurs de risque professionnel au-delà de certains seuils de pénibilité et à sa traçabilité
[7] Décret no 2014-1156 du 9 octobre 2014 relatif à l'acquisition et à l'utilisation des points acquis au titre du compte personnel de prévention de la pénibilité
[8] INRS. Méthodologie d'évaluation simplifiée du risque chimique. ND 2233. CND, 2005, n° 200, pp.39-62.
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[10] CRAM Alsace Moselle. Outil d'évaluation des risques chimiques Clarice.
[11] CRAM Pays de la Loire. Outil d'évaluation du risque chimique.
[12] CRAM Ile-de-France. Guide de prévention du risque chimique. 2003, 33 p.
[13] CNAMTS. Recommandations adoptées par le Comité technique national (CTN) de la chimie, du caoutchouc et de la plasturgie le 23 juin 2004. R 409. 2004, 48 p.

Assessment of the toxic effect of the endocrine disruptor lead on workers

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Introduction

Over the last few decades, environmental effects on our health have become a major concern for our societies and an active field of research. The consequences of lead exposure on fertility have been the subject of numerous animal and human studies. To date, human studies have produced contrasting results, with some authors highlighting a drop in fecundablity (attested by a lengthening of the time taken to conceive), an alteration of certain semen characteristics and damage to the integrity of sperm DNA.

Objective

This study aimed to assess the effect of lead on sex hormones in male workers exposed to this heavy metal in a lead battery factory.

Findings

The findings show a significant drop in testosterone levels in workers exposed compared to controls. However, the level of LH increased considerably in individuals exposed to lead. A significant difference concerning the level of FSH, prolactin and cortisol was observed. Physiological parameters were also analysed. An increase in blood lead level was recorded (286–796). Urinary delta amino levulinic acid (ALA) exceeded the upper limit of the standard reaching 17.2 mg/100 mL. Zinc protoporphyrin reached substantially high levels, up to 554 mg/100mL in some workers.

Conclusion

Lead pollution in this workplace caused hormonal disruptions in the reproductive axis and also raised cortisol levels, which indicates that workers are subject to stress due to pollution by this metal.

The contribution of durum wheat (Triticum durum) to reducing lead toxicity: a study of some physiological indicators in the Wistar rat

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Keywords: Bilirubin, Ca+2, histological sections, wheat durum, ALP, total protein, lead

Objective

This study aims to assess the therapeutic efficiency of durum wheat (Triticum durum) on the different physiological markers related to lead poisoning in male Wistar rats.

Method

The rats were divided into three groups: the control group, the group administered 600mg/kg of only lead-contaminated food and the group administered a combination of 600mg/kg of lead-contaminated food and 9g/rat/day of durum wheat. Administration lasted six weeks.

Findings

Findings show a significant increase in the level of alkaline phosphatase (ALP) enzyme in the urea and creatinine in the group administered only lead compared to the control group and the lead/durum wheat group. A very significant reduction in the level of Ca+2, Mg+2, total protein and bilirubin was recorded in the group administered only lead. However, a significant difference was not observed in the glucose level.

The histology study shows that the kidney parenchyma presents dilatation of the distal and proximal tubules causing tubular nephropathy in the group administered only lead.

Conclusion

Lead disturbs numerous metabolic pathways and different physiological processes. Supplementation with the *Triticum durum* plant led to considerable improvement which brought the parameters studied back to normal.

Improvement of analytical performance during the use of impactors for characterising nanostructured aerosols

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There is a growing use of nanoparticles for their physical and chemical properties related to their small size. This raises many questions concerning the prevention of the risks they may pose although ultrafine particles (UFPs), by-products of industrial activity, have always been present at workplaces. This prevention can only result from the in-depth understanding of the sources, behaviour and effects of this type of particles. Although there is currently no metrics system for linking exposure to ultrafine particles to toxicity for humans, numerous specific instruments have recently been developed for real-time detection (in number, surface and mass) or for the separation of particles based on their size (impactors, particle size selectors).

Cascade impactors are sampling systems used specifically for characterising (particle size and elementary distribution by chemical or microscopic analysis) particulate aerosols, and in particular nanostructured aerosols. Currently, there are several types of cascade impactors, all based on the same principle of selection by inertial deposition of particles on the sampling media, but different in terms of numerous parameters (personal or static sampling, sampling flow, number of stages, range of each particle size class, greasing of media, etc.). Greasing is often necessary to limit the phenomenon of particle bounce on the impaction medium (larger particles can be carried to the lower stages corresponding in theory to smaller particles). However, the type and quality of grease have a non-negligible influence on the collection efficiency of the sampling device and therefore on the quantitative determination of the mass concentration depending on the size of particles.

The goal of this work is to specify the field of application and of use for cascade impactors by more specifically assessing the performance of collection substrate greasing taking into account the type of grease used (Vaseline, silicone) and the rate of deposition. Tests were done for three different impactors (DLPI, Marple, Sioutas) that are representative of the variability of devices available.

Results show that the variations in mass caused by the deposition of grease are much greater than the uncertainty related to the analysis technique (gravimetry in particular) and may be significant with regard to the particle masses collected. Large variations in mass may be observed for certain types of grease depending on their viscosity and stability as regards airflow in the impactors and temperature. Moreover, the method of grease deposition is a fundamental stage that may cause large variations in mass if the operating method is not controlled. Losses of grease increase, in particular, with the quantity of grease deposited. A systematic control of the greasing rate can however serve to guard against this problem. All of these results were used to define a reproducible greasing protocol applicable to the different impactors tested, which was experimentally validated by sampling and analysis of a polydispersed aerosol produced in a laboratory using a generating device.

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Determination of toxicity of an in-housed synthesized PEGylated Nano Graphene using bone marrow mesenchymal stem cells

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Graphene based research is a rapidly evolving field that has both national and international relevance in the material science as well as in biomedical applications. Graphene is a novel class of two-dimensional carbon-based nanomaterial, which has an enormous potential to serve multifarious applications in the fields of nanoelectronics, biotechnology and biomedicine. Graphene and graphene oxide have been explored with many molecular imaging techniques, including magnetic resonance imaging, optical, photoacoustic and radionuclide-based imaging. They have several unique physico-chemical characteristics and these properties are being exploited in the biomedical field especially stem cell regenerative therapy. Due to their superior mechanical strength, ability to induce stem cell differentiation and proliferation and antibacterial properties, it is used as a coating for tissue engineered scaffolds. However there is a scarcity of literature on biocompatibility of graphene.

The aim of this study is to synthesize PEGylated nano graphene (PNG) and assess its biocompatibility with bone marrow mesenchymal stem cells (MSCs) (Kyung et al., 2011, Julio et al., 2004). PNG was synthesized by reduction of graphene oxide and characterized using TEM, SAED, AFM (Fig 1), XPS, Raman spectroscopy and FTIR.

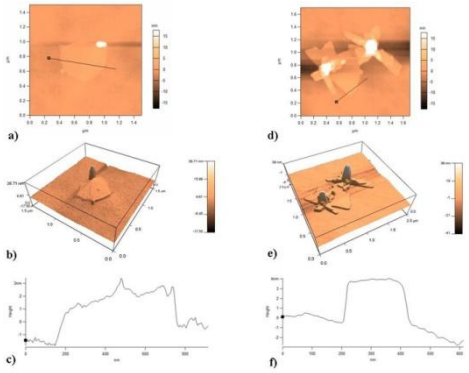

Figure 1: AFM images of PNG

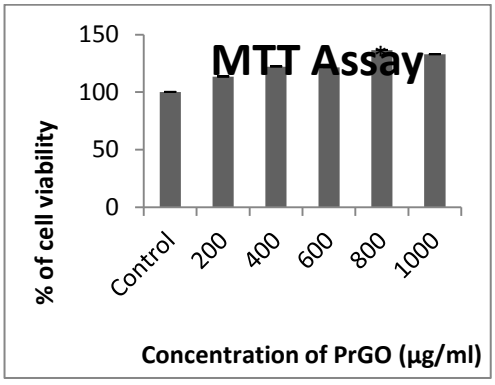

Figure 2: Cytotoxicity assay using MSCs

MSCs were subjected to characterization to evaluate their stemness. The characterized MSCs were exposed to varying concentration of PNG and MTT assay was carried out. It was found that PEG was successfully coated on to nano graphene and MSCs maintained their stemness *in vitro* (Fig 2). Moreover, the PNG was found to be biocompatible and increased proliferation of MSCs. Hence, the present study concluded that, the in-house synthesized PNG was found to be non toxic when exposed to MSCs. This study can help design PNG for safe biomedical applications. Also the MSC can be used as an alternative test system for animal experimentation for the preliminary screening of toxicity.

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Occupational exposure to mycotoxins. Biomarkers and airborne contamination measurements

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Ingestion of mycotoxins from contaminated food products is deemed to constitute the main important source of exposure in the general population. There is also today a growing interest in the role of mycotoxins as health hazards in the workplace. Mycotoxins have been identified in various occupational environments including poultry productions, agricultural and food processing facilities, livestock feed productions, indicating that inhalation and dermal contact may represent an additional route of exposure. To what extent such exposure results in potential risks for health for these workers remains unclear.

Methods

In order to obtain some data about occupational exposure to mycotoxins, the french occupational safety and health institute INRS sets up a project to assess external and internal exposure to some mycotoxins in various workplaces with a focus on feed processing facilities, livestock and poultry farming. For external exposure, airborne contamination will be determined by personal and ambient air sampling. To investigate the respiratory and dermal intake, human biomonitoring of mycotoxins from a cohort of workers will be implemented. Ochratoxin A, aflatoxin B1, fumonisin, zearalenone, deoxynivalenol and their metabolites will be determined in urine using a multimycotoxin method by Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS). For an interpretation of human biomonitoring data, the results will be compared with those obtained from non occupationally exposed persons and the relation between airborne contamination and measured biomarkers will be examined.

Results – Conclusions

This survey, which will take place between 2015 and 2017, would allow the mapping of mycotoxins occurrence in occupational settings and further progress in assessing mycotoxins health impact in some typical occupational environments.

Updating of OELs for complex hydrocarbon solvents

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The RCP (Reciprocal Calculation Procedure) method is used by several countries or organisations to calculate lump occupational exposure limits (OELs) for mixtures of certain refined hydrocarbon solvents derived from petroleum (AGS, 2006; 2012; McKee et al., 2005). These solvents consist of fractions of aliphatic, cycloaliphatic, and aromatic hydrocarbons. Under REACH such solvents are usually registered as substances of unknown or variable composition (UVCB). Because of the varying composition of these complex solvent mixtures and the lack of toxicological data for each individual hydrocarbon substance, the conventional RCP scheme subdivides the broad range of hydrocarbons into four groups, which are regarded as structurally similar. For each of these group a "reference value" based on toxicological considerations has been assigned, which should be representative for other substances of the respective group according to a "read across" approach.

However, recent experimental studies or epidemiological observations on single substance hydrocarbons or mixtures raised concern about (a) the level of some the group reference values, (b) the demarcation of the groups (hydrocarbon fractions with assumed similar toxicological properties), (c) crucial toxicological endpoints, and (d) specific substances to be exempted because of their individual toxicological profile (not suitable for "read across" inclusion compared to hydrocarbons with identical or similar carbon chain length).

Based on a comparison of current OELs for particular hydrocarbons and an evaluation of most recent toxicological findings on single substances and solvent mixtures we propose a revised grouping of C_5-C_{15} hydrocarbons resulting in different health-based lump OELs. As before, certain substances with specific toxicological features, e. g. n-hexane, decalin or naphthalene have to be excluded from the general model and should be assessed on an individual basis. For reasons of technical feasibility, it is recommended to measure C_5 aliphates (pentanes) as well as C_7 and C_8 aromates (toluene, xylenes, ethyl benzene) separately. Long-chained hydrocarbons (>C_{15}) acting predominantly as liquid aerosols rather than in the gaseous phase are beyond the scope of this concept. Hence, lump OELs have to be established for C_6 to C_{15} aliphates and for C_9 to C_{15} aromates.

Key discussions will include the relevance of hepatic changes as being an adaptive or rather an adverse effect, on recent findings on acute neurotoxicity of standard or dearomatised "white spirit" or other hydrocarbon solvents and the extrapolation for those findings from acute to chronic exposure duration.

The suggested concept which is subject to further scientific discussions and tripartite negotiations in Germany is to be presented in more detail at the INRS 2015 Occupational Health Research Conference.

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Use of an effective concentration approach to classify alloys in 2015

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The majority of metals placed on the market are used as alloys, a special mixture of metals with properties that are different from those of the metal components.

As with pure substances, appropriate chemical management practices for alloys are essential to ensure their safe use. Several regulatory frameworks require assessment & communication of the mixtures' hazards: e.g., UN's Global Harmonised System of Classification and Labelling (GHS, 2013) and the EU Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP). As of June 2015, mixtures must be compliant with CLP classification and this information needs to be communicated to the supply chain.

Alloys being mixtures, the default approach for their classification is to directly compare the alloy content of classified elements to the classification criteria (i.e., summation rules or additivity formula for acute toxicity classification and cut-off concentration/limit approaches for the other toxicological endpoints). In the European Union, REACH designates alloys as a form of "special preparation" referring to the potential difference in properties from their constituents and recognised that specific assessment methods are required.

In general, the toxicity of metals and alloys is considered to be related to the bioavailability of the metal ion. The extent of the metal ion release from alloys (and thus the bioavailability of the metal ion) can however be significantly different from the releases from their pure metal constituents, due to the alloys properties. Bioaccessibility testing measuring metal ion releases in synthetic body fluids (bioelution) is a tool that can be used in the refinement of the hazard identification and classification of alloys in various ways. Bioaccessibility provides a conservative estimate of bioavailability. Bioaccessibility has already been incorporated into decision-making in some regulatory areas and guidance on bioelution testing of alloys is available.

An overall framework for the human health classification of alloys is proposed, starting from the collection and evaluation of available data and following tiers of evaluation. Tiers: I) an alloy-specific approach can be applied when toxicity data on the alloy itself are available, II) where toxicity data on alloy itself is not available but toxicokinetic or bioaccessibility data are available to group and classify alloys, the use of bridging principles should be considered for the classification of similar alloys, III) bioaccessibility data in relevant biological fluid allows the calculation of the effective concentration of the metals in the alloys. To determine the classification of the alloy, the effective concentrations of classified constituents can be compared to the GHS (and CLP) criteria (i.e., cut-off concentration) in an analogous way as simple mixture concentrations, IV) When few or no alloy-specific data are available and bioaccessibility testing is not an option, the default approach is applied considering the alloy components as a simple mixture. In every tier, a weight of evidence approach encompassing all available data has to be applied.

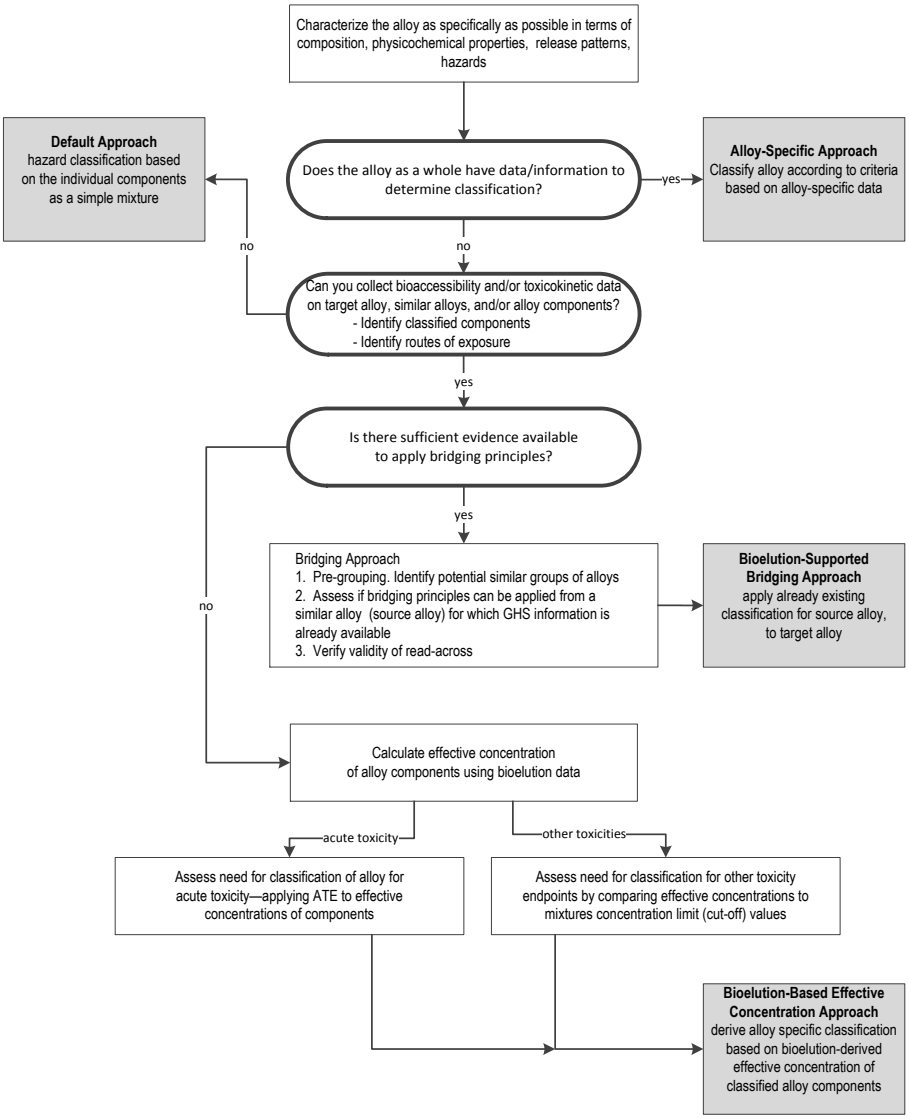

Figure 1. Conceptual tiered approach for human hazard identification and classification of alloys.

EU (2009). CLP Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006,

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Correlation between mass and number concentration of dust at different workplace scenarios with regard to ultrafine particles

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Dust regains more attention recently. Especially the fractions dealing with small particles are in focus of regulation. E. g. in Germany the limit value for the respirable fraction has been reduced and the treatment of ultrafine particles is under discussion. The measurement techniques have to be adapted to the decreasing limit values. The number concentration seems to provide some advantages for very low concentrations and for the identification of sources, nevertheless the mass concentration is the measurand of choice for toxicology at the moment and the state of the art.

To investigate the correlation between number concentration and mass concentration of dust at workplaces the IFA has been collecting results of number concentration measurements in addition to common mass concentration measurements over the last six years. The observed tasks in this study were not restricted to industrial branches or to specific workplace scenarios. Due to different projects the majority of measurements describe workplace conditions during thermic processes, mainly metal working. However other scenarios are the handling of very fine-grained powder, surface finishing and coatings. One aim of this study was to screen whether a risk assessment based on number concentration possibly comes to the same results as a risk assessment based on mass concentration and if non correlation can be verified to describe the differences.

This study was executed within the framework of the MGU (Messsystem Gefährdungsermittlung der Unfallversicherungsträger / Measurement system for exposure assessment). For the mass concentration a filter based sampler (FSP) was used. The number concentration was determined with a CPC (Condensation Particle Counter) or DiSCmini (Diffusion Size Classifier). For selected workplaces also the particle size distribution was measured with a SMPS (Scanning Mobility Particle Sizer). All measurements were carried out simultaneously and at the same spot. During the project period the development of measurement equipment was taken into account and e. g. in the late phase of the project the CPC was frequently replaced by the DiSCmini due to the larger measurement range.

For some kinds of welding (MAG, MIG, TIG) no direct correlation between mass and number concentration could be proven. But a correlation between particle size and mass concentration is quite strong. Therefore in further investigations other exposure scenarios will be tested for the existence of correlations of number and mass concentration and the dependence on other parameter.

For compliance testing the measurement of number concentration might not be first choice but for the understanding of sources and for minimizing of exposure it is a useful und effective tool.

CFD simulations of air distribution and thermal comfort when using textile air ducts

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Textile air ducts are round, semi-round, or quarter-round ducts made of a light-weight textile material. Their advantages compared to ventilation systems with metal ducts and diffusors are e. g. the easy installation and low purchase and maintenance costs. Textile ventilation systems are often used in areas of thermal comfort like offices, laboratories, and conference rooms as they should distribute fresh air homogenously and draught-free on the principle of displacement ventilation. But there are hardly any sound studies known investigating the thermal comfort in rooms with textile ventilation systems.

Therefore, the thermal and air flow conditions using textile air ducts were examined and calculated by Computational Fluid Dynamics (CFD) simulations. Different types of applications like room settings and utilisation and thermal loads were taken into consideration including a two person office, an open plan office, and a laboratory. The basic parameters were: simple textile air ducts at the ceiling, cooling case, preset room temperature of 26 °C, clothing insulation of 0.7 clo, and metabolic rate of 1.2 met (light activities, seated) and 1.6 met (light activities, standing).

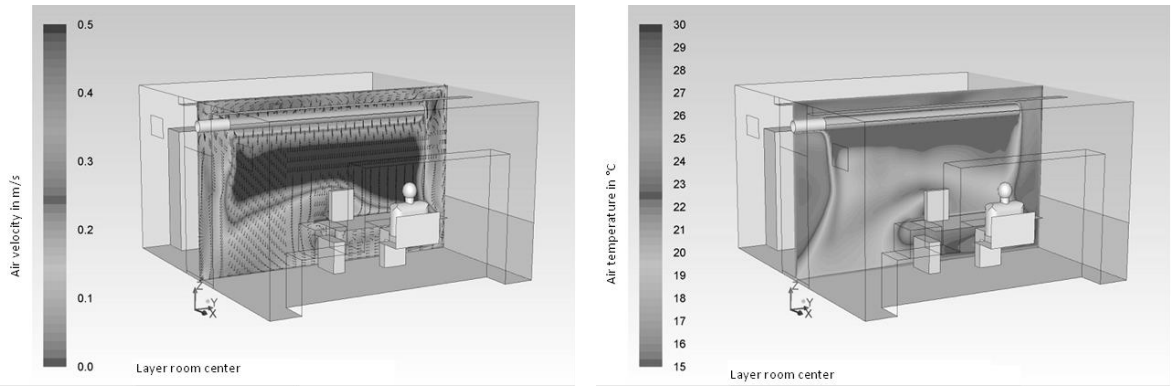

Figure 1. CFD simulations of air velocity (left) and temperature (right) at the workplace in an office.

The results showed mainly two drawbacks of using textile air ducts in areas of thermal comfort. First, if the textile air ducts are installed at the ceiling it is not possible to establish proper displacement ventilation with a layer of fresh air in the occupied area as the thermal updraft from persons and equipment counteracts the supply air flow. Second, great differences between supply air temperature and room temperature lead to cold air flows falling down and causing thermal discomfort at the workplace.

Therefore, textile air ducts are of limited suitability to remove high thermal loads as, otherwise, great temperature differences and high air velocities will occur in the area of the supply air flow and will lead to thermal discomfort if there are workplaces in this area.

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Development of a monitoring strategy based on UPLC-MS/MS for the assessment of occupational exposure to airborne pharmaceutical compounds

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One of the major industrial hygiene challenges in drug manufacturing industry is dust control during solid handling, which generally contains potent therapeutic material. The development of an adequate monitoring strategy is therefore required to evaluate this specific exposure (Tartre, 1992; Van Nimmen et al., 2006). Occupational exposure to active pharmaceutical ingredients (APIs) can cause unintended health effects in workers handling these compounds. Occupational health professionals in the pharmaceutical industry have responded to this hazard recognition by employing strategies for the risk evaluation and management of potent compounds (Van Nimmen & Veulemans, 2004; Calhoun et al., 2011). Few APIs have an OEL (Occupational Exposure Limit), set by regulatory bodies. Therefore in-house OELs are established (Van Nimmen et al., 2006) that generally dictate the level of containment required to assure worker safety that is achieved through the use of engineering controls and safe handling practices (Naumann et al. 1996).

In this context, the present study's main objective was to provide a measurement strategy that reflects actual exposure to a workplace contaminant, more precisely to APIs. Prior to the actual sampling of pharmaceutical compounds in industrial settings, analytical methods have to be developed and validated for the assessment of the respiratory exposure.

Thus, an air monitoring strategy based on ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS) has been developed for the independent or simultaneous analysis of 20 APIs comprising different chemical classes as follows: 7 azoles (imazalil sulphate, isoconazole and isoconazole nitrate, miconazole, parconazole hydrochloride, mitratapide, and tetrahydrozoline hydrochloride), 3 ketones (bromperidol decanoate, haloperidol decanoate and pipamperone hydrochloride), 2 anilides (acetaminophen and closantel), 2 piperidines (fluspirilene and ketanserin tartrate) and 6 miscellaneous (abiraterone acetate, clazuril, decitabine, mebendazole, rilpivirine hydrochloride, triamcinolone acetonide). Different sampling filters were evaluated and the most suitable were found to be polytetrafluoroethylene (PTFE) filters for 13 of the target compounds and glass fiber (GF) filters for the rest of 7 API's. The optimized UPLC-MS/MS method has been further validated by assessing the linearity of the calibration curves and the limits of quantification (LoQ), as well as the extraction recovery and analytical precision using spiked filters at 4 different levels, corresponding to 0.1 OEL, 0.5 OEL, 1 OEL and 2 OEL. Briefly the validation parameters are as follows: R^2 for the calibration curves was higher than 0.9956, LoQ's values were ranging from 0.11 ng/mL for fluspirilene to 55 ng/mL for isoconazole nitrate, the percentages of extraction recovery were ranging from 94.8 to 106.5% and the RSD values for the analytical precision were ranging from 0.6 to 7.8%. Moreover, the stability of the compounds on filters during simulated air sampling (240L) and storage under different conditions was also determined. The most suitable storage temperature for a period longer than 7 days was found to be -20°C.

In conclusion, in the present study a comprehensive air monitoring strategy based on UPLC-MS/MS for the assessment of occupational exposure to airborne pharmaceutical compounds from different chemical classes was developed and validated. For optimal recovery, the use of two different types of filter should be considered.

Nevertheless, since PTFE filters had overall good extraction recoveries for all compounds targeted in the present study, they can be used as a good compromise sampling filter whenever multi-residue exposure assessment is required. Furthermore the developed method has been used in real industrial settings to assess worker exposure to pharmaceuticals (e.g. abiraterone acetate, haloperidol decanoate) as well as for containment testing using acetaminophen.

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Quantitative cancer risk assessment for occupational exposures to asphalt fumes during built-up roofing asphalt (BURA) operations

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In a 2013 monograph, the International Agency for Research on Cancer (IARC) characterized occupational exposure to oxidized bitumen and bitumen emissions during roofing as probably carcinogenic to humans (Group 2A) based on limited evidence in humans, limited evidence in animals for oxidized bitumens, and sufficient evidence in animals for fume condensates from oxidized bitumens. Roofing workers are exposed to these materials, and it is of interest to develop a basis for estimating the quantitative elevation in lifetime cancer risk that might result from such exposure. IARC conducted a qualitative evaluation of potential carcinogenic risks based on human and animal data with inhalation and dermal exposure to oxidized bitumen emissions, but did not conduct a quantitative risk assessment for built-up roofing asphalt (BURA) applications (as the use of oxidixed bitumen in flat-roof construction is termed in North America). In the present analysis, we focus on finding the most reliable and rigorous method to define a basis for quantifying cancer risk in roofing workers exposed to BURA and BURA emissions, using as a basis data from the studies that IARC considered qualitatively. Some particular challenges arise. The exposures are to a complex mixture that varies somewhat in composition depending on bitumen source and temperature of use. Many of the key skin-painting studies examine but a single dose, yet parallel positive control exposures to benzo[a]pyrene (B[a]P) show markedly nonlinear dose-response. The rapidity of appearance of skin tumors is an important aspect of potency in addition to the final counts of tumors produced. There are also some opportunities for novel analytical approaches. Time-of-tumor-appearance information is available, as is parallel testing of different fractions of oxidized bitumen fume condensate alone or in various combinations. Appropriately measured fluorescence of mixures appears to correlate strongly with dermal carcinogenic potency and with mutagenicity. We examined existing chemistry, exposure, epidemiology, and animal toxicity data to provide a comprehensive risk assessment for occupational exposure to BURA. We conducted a number of dose-response analysis techniques to characterize the carcinogenic potency of oxidized bitumen fume via inhalation exposure or dermal exposure to fume condensate, including analysis of the potency-additivity of fractions, the time-to-tumor patterns and their impact on relative potency, the potency effects of compositional variation of the mixture, and the use of borrowed information on dose-response curve shape from the extensively characterized case of B[a]P to inform tumor dose-response for bitumen fume mixtures of various composition. We develop a method to adjust potency estimates for compositional variation using measured fluorescence. The results are combined with estimates of roofer exposure to characterize the cancer risk potential from occupational exposure to oxidized bitumens and their fumes, resulting in risk estimates that are within a range typically acceptable within regulatory frameworks. Collectively, the analyses provide a novel approach to assessing cancer risks from bitumens that could be applied more widely to PAH mixtures in a way that acknowledges and adjusts for variations in composition of a complex mixture.

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The contribution of molecular modelling in assessing product and process safety

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The assessment and control of risks in the industrial environment require fast and precise identification of the physico-chemical hazards of chemical products to which workers are exposed. Among the most dreaded accident scenarios for laboratories and industrial facilities are chemical reactions involving unstable substances or incompatible products.

Until presently, the identification of hazards relied heavily on laboratory tests or on large-scale testing, to study the physico-chemical properties of products and reactions. In addition to experimental characterisation methods (calorimetry methods in particular), INERIS uses quantum chemistry methods to characterise chemical reactivity mechanisms such as the process of ageing, chemical incompatibility and explosive decomposition.
The presentation will illustrate the contribution of molecular modelling (in addition to the experiment approach) in improving identification and control of industrial risks based on the research carried out at INERIS in collaboration with ParisTech:

- the first study dealt with the chemical reaction mechanisms involved in the peroxidation of ethers (Di Tommaso, 2011). Ethers are one of the organic chemical species that may become unstable when stored inappropriately or when they remain in contact with air for long periods of time. They then form hydroperoxides and peroxides which are responsible for many accidents, especially in laboratories. After studying diethyl ether oxidation mechanisms, oxidation inhibition mechanisms (through addition of antioxidant chemical species) were characterised both theoretically and experimentally (Di Tommaso, 2014, a).

- the second study aimed to understand the mechanisms of chemical incompatibility between two chemical substances at molecular level in order to predict reactivity between two substances. In particular, the case of ammonium nitrate, a product widely used in industry (as fertiliser for example), known for its long list of incompatibilities and involved in numerous major accidents, was examined. After characterising the radical mechanism of decomposition of ammonium nitrate alone in gas phase (Cagnina, 2013), the reactivity of the ammonium nitrate/sodium dichloroisocyanurate mixture, a possibility explored in the analysis of the AZF factory accident in Toulouse in 2001, was studied (Cagnina, 2014).

In both cases, a detailed theoretical study based on density functional theory (DFT) calculations aimed at identifying reaction pathways, the (hazardous) products formed as well as the heat released by reactions, was conducted, and clarified or identified the reaction mechanisms involved. The molecular modelling results were compared satisfactorily to results of experiments (Di Tommaso, 2014, b). These two illustrations encourage the use and development of these theoretical methods to improve the understanding of reaction mechanisms in the field of industrial risk prevention.

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Interaction between cells and polymeric nanoparticles: Contribution of toxicogenomics

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Nanoparticles (NPs) are increasingly used in medicine (diagnosis, imaging, drug targeting). Due to their dimensions, they present new properties, in particular an increased surface reactivity, which may modify their biological activities and enhance the therapeutic index of a drug. However, few studies regarding potential adverse effects of these particles were conducted. In EA 3452 CITHEFOR, polymeric nanoparticles loaded with nitric oxide (NO•) donors (nitrosothiols) were developed. Among them, the S-nitrosoglutathione (GSNO) seems a good candidate for NO• delivery in several diseases (cardiovascular diseases, neurodegenerative diseases, diabetes, cancers, etc). Therefore, a nanoformulation of GSNO was developed to increase stability and bioavaibility of NO•. The aim of the present work was to study toxicity mechanisms of Eudragit RL polymeric nanoparticles loaded (NP-GSNO) or not (NP-ERL) with S-nitrosoglutathione on Caco-2 cells as intestinal cell model (HTB-37™).

After 24h exposure, cell viability was determined by different cytotoxicity tests (MTT, Neutral Red, WST-1). In order to find metabolic pathways altered in Caco-2 cells to these NPs, we studied gene expression by transcriptomics (Agilent microarray) after 4h exposure.

NPs induce toxicity beyond 500 µg/mL of polymer. Interestingly, an increase of mitochondrial activity was observed at low dose (50 µg/mL) after 24h of exposure. Transcriptomes were analyzed to identify any similarities or differences. We are especially interested in early expression altered gene clusters (4h exposure).

This study allowed us to explain the adaptive response mechanism observed in our cytotoxicity studies. To specify cellular and molecular toxicity mechanisms induced by nanoparticular formulations, it appears important to use « omics » technologies which transcriptomes is part of.

Particle-induced cell migration assay (PICMA): rutile TiO_2 and SiO_2 but not anatase TiO_2 and $BaSO_4$ can induce migration of NR8383 alveolar macrophages

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Chronic exposure towards particles can lead to occupational lung diseases such as pneumoconiosis or even lung tumors. These diseases are caused by complex inflammatory reactions which can occur at high continuous particle exposure and are only partially understood. Particle-induced inflammation of the lung is initiated by the recruitment of macrophages and neutrophilic granulocytes. We designed a new predictive cell migration assay to study the effect of respirable particles on the migration of macrophages *in vitro*.

NR8383 alveolar macrophages were challenged with following particles overnight: SiO_2 (5 µm and 10 - 20 nm), anatase TiO_2 (5 µm and < 25 nm), rutile TiO_2 (5 µm and 100 nm) and $BaSO_4$ (4 µm), concentrations ranging from 32 to 96 µg cm^{-2}. All particles were characterized with respect to IC_{50} values, crystallinity, size in the dry state and size distribution in culture medium (Westphal et al., 2014). The supernatants were used to induce cell migration of NR8383 macrophages. Chemokine and cytokine levels inside the supernatants were detected by real-time PCR arrays.

Challenge of NR8383 macrophages with particles in concentrations of 32 - 96 µg cm^{-2} resulted in cell supernatants which were able to induce dose-dependent cell migration of macrophages (fine SiO_2 > fine rutile > coarse rutile > coarse SiO_2 > coarse anatase ≈ fine anatase ≈ $BaSO_4$). Chemokines and cytokines which are known to be involved in inflammatory cell signaling increased considerably and dose-dependently in response to particle exposure.

Migration of macrophages *in vitro* varies depending on both the type of particle and its concentration. Since $BaSO_4$ showed no and anatase only weak effects, PICMA may be able to differentiate between inert and inflammatory particles. Cytokines that play an important role in the inflammatory process inside the lung were upregulated. This new *in vitro* model is easy to handle, shows differentiated effects in a highly reproducible way and can be useful for further investigation of inflammatory reactions towards particles.

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Development of a co-culture model to study the genotoxicity of particulate matter

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In many industrial activities, workers are exposed to particles that are either manufactured and used for their physicochemical properties or generated unintentionally during industrial processes. Since particles may get aerosolized, the main exposure route for employees is inhalation. In order to predict the human health hazard of these materials, toxicological studies performed by inhalation or intratracheal instillation on laboratory animals represent the most relevant approach. However, the commitment of the international scientific community to reduce the use of laboratory animals has led us to develop an alternative predictive model for the study of the pulmonary toxicity of particles.

We therefore propose to develop a co-culture model (macrophages, granulocytes and alveolar epithelial cells) designed to be more representative of the inflammatory pulmonary response occurring *in vivo*. Phorbol 12-myristate 13-acetate (PMA)-differentiated THP-1 cells were used as a surrogate for macrophages, All-trans retinoic acid (ATRA)-differentiated HL60 were used as a surrogate for granulocytes and A549 were used as epithelial alveolar type II cells. A crystalline silica sample DQ12 was used a prototypical particle for its capabilities to induce DNA damage, inflammatory response and oxidative stress in epithelial cells; its polyvinylpyridine-N-oxide (PVNO)-surface modified counterpart was also used as a negative particulate control (Schins et al., 2002).

Cells in mono, bi or triculture were exposed to DQ12 or DQ12-PVNO for 24 h. Following exposure, the culture supernatants were collected for the measurement of pro-inflammatory cytokines and A549 cells were harvested for the analysis of DNA damage (Comet assay and γH2AX foci formation), gene expression and eventually oxidative stress.

The exposure of A549 to DQ12 induced a significant change in interleukin-8 (IL-8) protein level which was exacerbated when differentiated THP-1 and HL-60 were added. In addition while no production of TNFa was detected in monoculture of A549, elevated levels of this cytokine were observed in the co-culture system.

Even though further investigations are underway, it appears that the addition of the phagocytic cells modulate the extent of DNA damage induced by DQ12.

This work, like others, shows that a cell culture model which takes into consideration the complexity of the pulmonary inflammatory response might be more reliable to study the toxicological properties of particles than "simple" monoculture models.

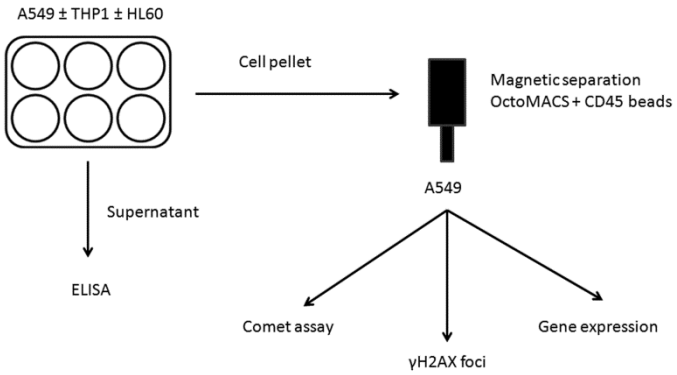

Fig 1: Schematic diagram of the experimental procedure

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Carbon Nanotubes: A PERSPECTIVE FOR THE FUTURE

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Keywords: Toxicology, asbestos, crystalline silica, carbon nanotubes

Materials may be defined as substances that have properties that confers them various uses in products, devices, structures and machines. Only after several properties and characteristics of the materials be discovered and put into practical, tests are made, in order to detect the real risk that these products can exhibit living beings, having checked with different materials such as crystalline silica and asbestos.
Nanotechnology presents itself as a new technology, often described as a promoter of a revolution in human approaches to common problems. The aim of this study is related, therefore, the realization of a forecast of what may turn out to be the results of future epidemiological studies, which look to reveal the actual toxicity of a specific type of nanoparticles, carbon nanotubes. Toxicological and epidemiological data, related to crystalline silica and asbestos, are described in the present study, yet, it was not possible to conduct a relationship with toxicological studies on nanoparticles, due to the disparity of results. However, scientific evidence has been found that the pathogenicity of carbon nanotubes is comparable to asbestos fibers, as well as its structure.
Although there is still no specific legislation for nanomaterials, some EU directives can be applied, being employers who should assess and manage the risks of these materials in the workplace.

Since there are no studies to verify the content of these hazardous materials, no, equally, regulations that ensure both consumer protection, as their handlers, industrial level. Therefore, new approaches are necessary and urgent to the topic in order to protect public health.
This study therefore aims to carry out a forecast of what may turn out to be the results of epidemiological studies, trying to verify the actual toxicity of a specific type of nanoparticles, carbon nanotubes. Ie, verifying the existence of a relationship between the development of studies on asbestos and silica, with the development of studies on carbon nanotubes.

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Development of biotests to ensure quality, safety and improvement of packaging intended for food contact

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Materials intended to come into contact with food (food contact materials - FCM) must comply with European Regulation No 1935/2004 and its Article 3 in particular. Within this framework, "materials and articles [...] shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could [...] endanger human health...".

For several years now, numerous alerts pertaining to these materials have involved non-intentionally added substances (NIAS) present in the packaged food. These substances, which may migrate from the packaging to the foodstuff, may be impurities, newly-formed contaminants emerged during the packaging production chain, residual products formed during synthesis, contaminants from recycled materials, etc. Several research teams (Grob et al., 2006; Skejevrak et al., 2005) showed that NIAS could account for more than half of all migrating substances. Considering FCM as a complex mix of substances, this project proposes a methodology for studying the toxicity of the food packaging as a whole, in particular, paper and board packaging. A battery of in vitro biotests was set up in order to assess the toxicity of the migrating substances, in addition to the chemical analysis. Genotoxicity, cytotoxicity and endocrine disruption are three toxicological targets to be assessed since they correspond to the notion of low-level exposure which is the case with FCM.

Cytotoxicty tests assessing disruption of cell homeostasis, genotoxicity tests highlighting alterations to genetic material and an endocrine disruption test are under way. These biotests are carried out in vitro on different cell lines: hepatic HepG2 and HepaRG cells, the Caco2 cell line derived from a human colorectal adenocarcinoma and the HeLa 9903 line derived from a cervical tumour.

In terms of cytotoxicity, the Alamar blue assay is a colorimetric assay to measure cell viability, and the assay for determining the kinetics of total RNA synthesis (EN 15845 standard) measures the rate of tritiated uridine incorporation during RNA synthesis in cells (Valentin et al., 2001). The potential genotoxic effects of NIAS are assessed using the in vitro comet assay in order to detect damage and breaks to cells' genetic material (Speit and Hartmann, 2006). Endocrine disruption is studied using ERα-mediated transcriptional activation (OECD guideline 455).

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Analysis of the effect of para-phenylenediamine on the osmotic stability of human erythrocyte through electrochemical oxidation

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Keywords: Para-phenylenediamine (PPD), cyclic voltammetry, voltammogram, electrodes, platinum, red cells, humans erythrocytes, glutathion, mercaptoethanole

Para-phenylenediamine (PPD) is a mineral-based aromatic amine derived from aniline, currently used in industry. Its extensive use led to the discovery of its toxic effects. It is responsible for severe poisoning cases that could be fatal despite the therapeutic measures [1].

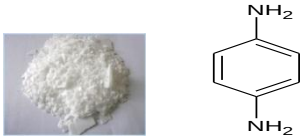
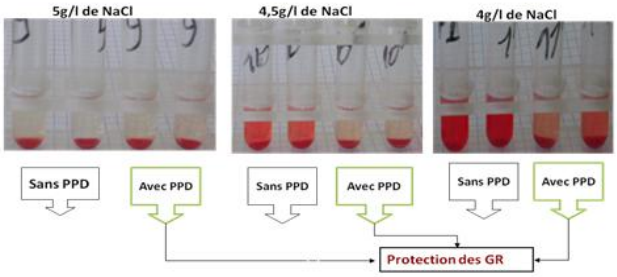


Figure 1: Physical aspect and chemical formula of para-phenylenediamine $C_6H_4(NH_2)_2$

In the course of this work, we explored the effect of PPD on the resistance of human red blood cell membranes in an aqueous environment (NaC1). The low doses of PPD used in this study (5 µg/ml) resulted in protection of human erythrocytes against hypotonic shock for two hours. However, weakening of the membrane was observed after a long period of exposure to low doses of PPD and immediately after exposure to concentrations exceeding 2.5 mg/ ml [2]. This double effect of PPD is linked to its high reactivity and its self-polymerisation property. One of the hypotheses we support is that the stabilising effect of the membrane is related to the formation of PPD polymer films: physical barrier, retention of water molecules, etc.



In order to understand this phenomenon, we studied the electrochemical oxidation of PPD on different types of electrodes. Cyclic voltammetry was used as the electrochemical technique to follow this oxidation. We also used ultraviolet-visible spectrophotometry (UV/Vis) to follow auto-polymerisation (auto-oxidation) of PPD in the presence and in the absence of antioxidants such as glutathion and mercaptoethanol.

Discussion and conclusion

- In this study, we showed that the PPD oxidation on platinum appears at 560 mV/SCE and is rapid and controlled by diffusion.
- Potential cycling, repeated between 0.8 and 1.35 V/SCE, shows a second partially irreversible oxidation of PPD accompanied by the forming of PPD on the electrode (or polymerisation).

- The spectra obtained by UV-Visible showed first of all that in the presence and in the absence of glutathion (GSH), the formation of Bandrowski's base (BB) by oxidation of PPD was stopped in the presence of these antioxidants. Second, the addition of antioxidants to the PPD+RBC mixture resulted in the lack of protection of red blood cells caused by PPD.

We can therefore conclude that the protective effect of PPD at concentrations lower than 2.5 mg/ml for the erythrocyte membrane is related to the poly-PPD formation mechanism. The haemolytic action of concentrations exceeding 2.5 mg/ml may be linked to cytotoxic effects.

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Aromatic solvents disturb the stapedial reflex involved in hearing

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The olivocochlear and stapedial reflexes are both involved in hearing physiology: the first one is mainly dedicated to the high frequency discrimination, whereas the second protects the cochlea by contracting the middle-ear muscles. In the present study, the measured effects of the olivocochlear reflex are negligible regarding those of the stapedial reflex (SR). For this reason, we will speak about acoustic reflex (Rumeau, 2011), a more general concept which can be considered as the stapedial reflex in our experimental context.

For over thirty years, it is known that anesthetics inhibit the SR function (Farkas, 1983). Recently, Campo et al (2013) demonstrated that aromatic solvents can counterbalance the inhibitory effects of anesthetics on the reflex. However, the mechanism of the interaction between solvents and anesthetics are still questionable; it would depend on either the structure, or the physico-chemical characteristics of the solvents (Campo, 2007).

In the present study, the mechanisms of the interaction between anesthetics and solvents were studied to conceive a model capable of characterizing the action of the solvent on the SR and thereby to predict a possible synergy of noise and chemical effects on hearing. Given the large number of occupational chemicals, the model would allow to screen the chemicals capable of potentiating the noise effects.

Hearing from Brown Norway rats was tested by measuring the amplitude of oto-acoustic emissions generated by two pure tones: f1 & f2, with f1/f2=1.2. The oto-emissions were measured at the frequency 2f1-f2 and were called distortion product emissions (DPOAEs). As regards the SR, it was triggered for 3 s every 30 s by a contralateral stimulation at a constant intensity of 95 dB SPL. In this experimental context, the SR amplitude was stabilized at approximately 1.5, 1.8 dB SPL by adjusting the anesthesia depth with a ketamine and xylazine mixture administrated by ip with a driven syringe. Once the amplitude of the SR was stabilized, the animal was intratracheally exposed to a solvent (figure 1).

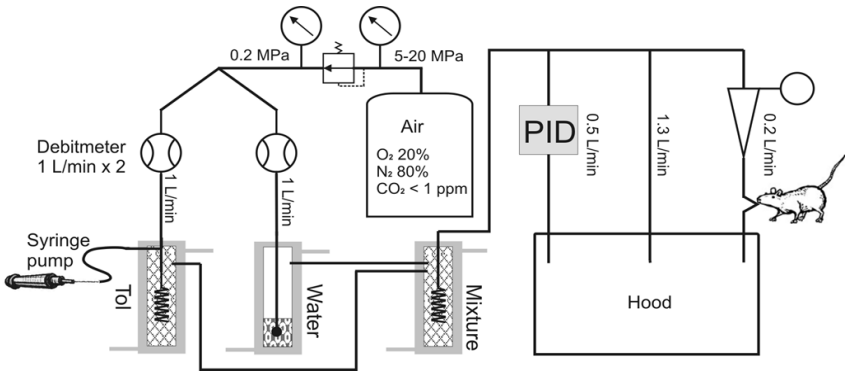

Figure 1: Intratracheal exposures to solvents. PID: Photonionization detector.

Six aromatic solvents were tested at a constant concentration of 3000 ppm on anesthetized animals. The choice of the solvents was based on either the lipophilocity (log Kow), or the structure of the molecule: the position of the methyl groups around the benzene ring.

In spite of a constant atmospheric concentration, the first results showed that the brain concentration of ethylbenzene was higher than those of toluene and styrene. On the other hand, the SR response of ethylbenzene was also different from that of toluene and styrene. Ethylbenzene increases the SR threshold of the anesthetized animal, whereas toluene and styrene decrease it. Since the log Kow of ethylbenzene (3.15) is higher than that of toluene (2.65) or styrene (3.02), it could explain the difference of concentration in the brain and its specific action on the SR (figure 2).

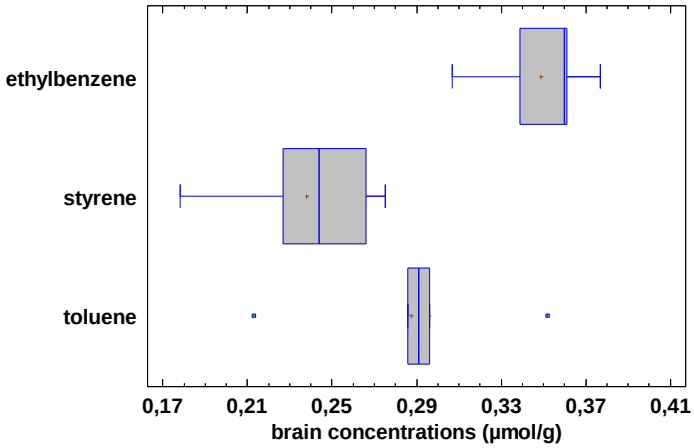

Figure 2: Brain concentrations of toluene (n = 5), styrene (n=5) and ethylbenzene (n=5), *p=0.05*

But we must underline that the lateral chain of ethylbenzene has an additional carbon compared to that of styrene, and two compared to toluene. If the Log Kow values of solvents can explain the differences of cerebral concentrations, the structure of the molecules could also impact the SR in anesthetized animal. The supplementary carbon on the lateral chain could increase the space occupied by the molecule and decrease its action on the protein-binding sites with the anesthetics.

Instead, the results obtained with xylenes allowed the mechanisms of the interaction between aromatic solvents and anesthetics to be better understood (figure 3).

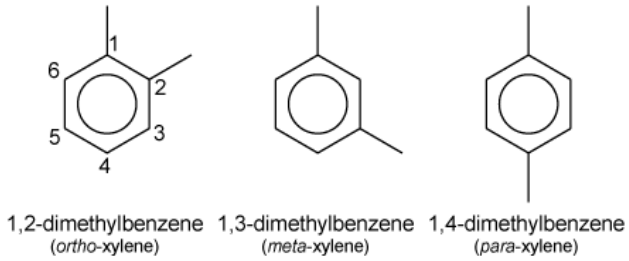

Figure 3: Chemical structures of the three xylene isomers.

Le *p*-xylene decreases the threshold of the SR trigger. As a result, the amplitude of the reflex increased with constant intensity of 95 dB. On the contrary, l'*o*-xylene increases the SR threshold since the amplitude decreased at the term of the inhalation period.

The structure of the solvent appears as the parameter playing a major role on the SR of the anesthetized animals. This pharmacological effect will analyzed to screen the aromatic solvents as a function of their impact on the SR. Then, the model will be used to evaluate the actions of a chloride solvent on the reflex and thereby to foresee the effects of a co-exposure "chlorinated chemical plus noise".

Acknowledgments: The study is financially supported by INRS.
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Optimisation of the filtration of ultrafine metallic particles by granular bed

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Numerous industrial processes generate high concentrations of ultrafine particles, i.e. with a diameter less than 100 nm, for example, arc welding, metal cutting (arc/air processes) and thermal metal spraying. Filter cartridges are the most commonly used method to limit worker exposure and environmental contamination. Initially, these pleated fibre filters are very efficient but generate major pressure drop because of fast and irreversible clogging and additional cost for their replacement (Bémer *et al.*, 2013). Among the alternatives are bubble columns (Charvet *et al.*, 2011, Cadavid-Rodriguez *et al.*, 2014) and granular beds. Granular beds have a high mechanical resistance (compared to fibre filters) and can easily be declogged in order to regain an acceptable pressure drop.

During previous work, it was highlighted that the deposit of ultrafine metallic particles takes place mainly on the first few layers of the granular bed (Bémer *et al.*, 2013). The fact that a shallow depth is effectively exploited reduces the duration of use between each declogging operation and obstructs the performance of the process. To optimise filtration, tests were carried out on a granular bed comprising three levels, each of which were filled with stainless steel balls of descending diameter (1 mm, 0.8 mm, 0.5 mm). Since filtration efficiency is a decreasing function of the size of the collectors, this type of configuration enables the deepest layers of the granular bed to be clogged first.

By way of comparison, the change in efficiency and pressure drop was also monitored in a configuration in which all three layers contained balls with a diameter of 0.5 mm. Although there was a 20% drop in initial efficiency for the optimised granular bed compared to the conventional bed, this difference quickly decreases when we examine the dynamics of such a process (i.e. 13% after 30 minutes and 0% after 40 minutes). Moreover, at the same time, the difference in pressure loss increases continuously. The value reached after 40 minutes drops by 70% with the optimised granular bed.

According to these results, optimisation of filtration by granular bed using a collector diameter gradient appears promising and could increase the duration of use limited by the increase in pressure drop. However, other points still need to be studied such as the optimum combinations of the different collector diameters and coupling with declogging techniques.

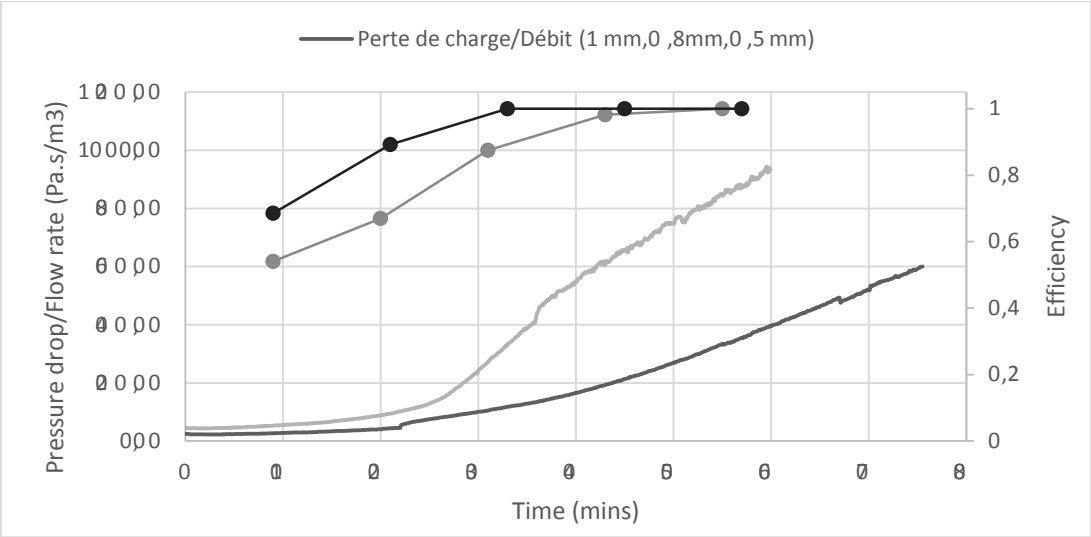

Figure 1. Change in the efficiency and pressure drop of optimised and traditional granular beds.

Bémer, D., Subra, I., Morele, Y., Charvet, A., & Thomas, D. (2013). *Journal of Aerosol Science*, 63, 25-37.
Charvet, A., Bardin-Monnier, N., & Thomas, D. (2011). *Journal of Hazardous Materials*, 195, 432-439
Cadavid-Rodriguez, M.C., Charvet, A., Bemer, D., & Thomas, D. (2014). *Journal of Hazardous Materials*, 271, 24-32

ProtecPo : a software for the selection of skin protective materials

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The selection of chemical protective materials is a major problem due to the number of chemicals used in occupational settings. No protective polymer material exists that protects against all classes of chemicals. There are some Databases containing information on permeation tests for a limited number of both chemical substances and glove materials. Also, glove and protective material manufacturers provide information on their products for a certain number of chemical substances and some mixtures. However, this information is limited with respect to the huge number of chemicals and mixtures used in workplaces. So another innovative approach is required to enhance skin protection.

ProtecPo, a predictive software, was developed in an international collaboration between the INRS in France and the IRSST from Quebec-Canada. ProtecPo is a tool that allows selecting the best protective glove material for exposures to single or mixed chemicals. This modeling software is based on the Hansen Solubility Parameters approach HSP. The more similar the solubility parameters δ of the chemical and polymer is, the more soluble the chemical will be in the polymer ("Like dissolves like"), and consequently the less resistant the material will be.

The total solubility parameter δt of a chemical compound is the result of the contribution of three types of interactions: dispersion forces δ_d, polar interactions δ_p, hydrogen bonds δ_h.

In the ProtecPo modeling approach, each chemical can be represented by a point with three-dimensional coordinates in a figure with δ_d, δ_p, δ_h axes. Polymers can be represented by spheres. The centre of a sphere corresponds to the HSP parameters δ_d, δ_p, and δ_h of a polymer with radius R that corresponds to the limit of solubility of chemicals into the polymer.

Figure 1 is an example of the sphere representation for the calculated HSP values and radius R for the Latex glove material.

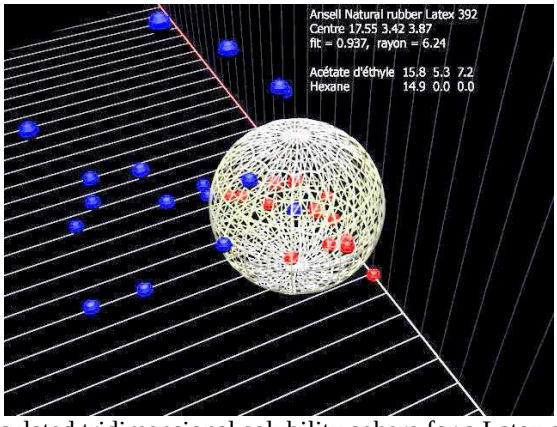

Figure 1: Calculated tridimensional solubility sphere for a Latex glove material

The developed algorithm was validated by comparing ProtecPo's predictions to experimental results found in the scientific literature, in guides published by protective glove manufacturers, as well as by comparing these predictions to experimental permeation measurements.
The algorithm can be refined as new experimental data become available and with user's feedback. Thus ProtecPo will growth to be a better and better representation of reality closer to skin preventive issues.

AUTHOR INDEX

A

Abdennour C. 140, 141
Adamo C. 153
Ahmadi G. 75
Antipov A. 67
Antoine G. 144
Appenzeller B. 60, 95, 97
Arbi R. 108
Armstrong T.W. 31
Arnone M. 38
Arnone M. 33
Ashley K. 54
Aubin S. 52
Audry E. 97

B

Bachir Bouiadjra S. 108
Baeza-Squiban A. 90, 103, 104
Bahloul A. 71
Bahri S. 113
Barbotin J.S. 65
Bardin-Monnier N. 164
Baril M. 23
Baumont E. 121
Beausoleil C. 57
Beghdadli B. 105
Bellasri H. 129
Belut E. 77
Bémer D. 164
Ben Abdelkader N. 113
Ben Khalifa E. 114
Berrubé A. 106
Bertrand N. 34, 57
Binet S. 115, 156
Boivin A. 126
Boland S. 90, 104
Bonfanti E. 117
Bonvallot N. 121
Bouillaud-Kremarik P. 97
Boukarma Z. 140
Boularas E.A. 108
Boulet A. 109
Bressot C. 67
Breuer D. 125
Brisson M.J. 54
Brochard C. 117
Brochot C. 71
Brüning T. 155
Bryk O. 155
Buchs D. 41
Buffa C. 116
Bulut M. 111
Bünger J. 155
Burgart M. 117
Burn D.A. 135
Butler E.L. 152

C

Cagnina S. 153
Caillaud F. 138
Campo P. 117, 162
Canlet C. 86
Capitaine L. 34
Carabin N. 112
Castel B. 128
Cecinato A. 122
Chagnon M.C. 159
Chakroun R. 113, 114
Charvet A. 164
Chata F. 77
Chazelet S. 72
Chézeau L. 115
Chollot A. 166
Chouvet M. 42
Clerc F. 34
Cloutier Y. 52
Clynen E. 85
Collaerts P. 150
Correzzola C. 116
Cosnier F. 115, 117
Cossec B. 112, 117
Crobeddu B. 103

D

Darne C. 127, 156
Delaval M. 104
Delbeke K. 146
Denis F. 124, 144
Di Tommaso S. 153
Diab R. 88
Djebara A. 71
Domange C. 86
Drolet D. 31, 166
Duca R. 97, 118, 150
Duhoux M. 142
Dupasquier F. 138

E

El Yacoubi A. 129
Erb A. 120
Estève W. 69
Evrard P. 106
Excoffon E. 133
Ezanno F. 42

F

Faidi F. 113, 114
Faucher E. 37
Fauchille P. 42
Fayet G. 67, 98, 153
Fedutik Y. 67
Feidt C. 86
Feng H.A. 135
Fernández Pérez A. 137
Ferrari L. 88, 154
Ferreira A. 158
Figueiredo J.P. 158
Fontana C. 127
Forastiere F. 122
Fournier K. 121
Frijns E. 44

G

Gabriel S. 33, 38
Gagné S. 52
Galea K. 39
Galland B. 66
Gariazzo C. 122
Gasic B. 41
Gaté L. 115, 156
Gaudin R. 124
Geerts L. 44
Gemise-Fareau C. 106
George I. 90
Gherardi M. 122
Ghomari O. 105
Giesen Y. 125
Glorennec P. 121
Godderis L. 118, 150
Goodman J.E. 152
Görner P. 126
Grandemange S. 88
Grossmann S. 117
Grova N. 60, 95, 97
Guichard Y. 127
Guillemot M. 128

H

H Abti N. 160
Haenen S. 85
Haghighat F. 71
Hanninen O. 122
Hardy E. 97
Hardy M. 60
Haredasht S.A. 118
Heinälä M. 56
Heine K. 145
Heussen G.A.H. 35
Hoarau Y. 79
Hoet P. 85, 119

I

Ibnlfassi A. 129

J

Janssens H. 118
Jargot D. 53, 57, 144
Jayabalan T. 67
Johnen G. 155
Joubert O. 88, 115, 154
Judon N. 69

K

Kalberlah F. 145
Kandouci A.B. 105
Keller F.X. 77
Kevin Ashley K 51
Koppisch D. 33, 38, 148
Kriech A.J. 58

L

Labelle B. 86
Lafon D. 57
Lamb J. 39
Lambert O. 90
Lamberti M. 122
Landsiedel R. 104
Langlais C. 156
Langlois E. 130
Lara J. 166
Laranjeira P. 132
Larnaud H. 133
Le Bihan O. 67
Le Bouf R.F. 135
Le Faou A. 88
Lekhchine F. 34
Liukkonen T. 56
Lorcin M. 156

M

Mabrouk A. 113
Mac Calman L. 39
Madera Garcia J. 137
Magalhaes-Antoine I. 138

Ma-Hock L. 104
Maisonneuve C. 37
Malard S. 34, 57
Mansouri-Bentayeb O. 140, 141
Marc F. 34
Marrier G. 37
Marsan P. 120, 124
Martin P. 66
Matera V. 142
Mathieu D. 99
Mayfield D.B. 152
Melin S. 53
Menéndez Cabo P. 137
Michaux S. 117
Micillino J.C. 156
Miller B. 39
Mohanan P.V. 143
Monta N. 166
Mornet S. 90
Mosqueron L. 106
Mouton S. 97
Mullot J.U. 37

N

Naimi Y. 160
Nascarella M.A. 152
Naudin G. 90
Ndaw S. 57, 124, 144
Nehdi H. 113, 114
Nemery B. 85, 119
Nies E. 145
Nouagui H. 113, 114
Nunge H. 117

O

Oller A.R. 146
Olry J.C. 97
Olsen L.D. 58
Osborn L.V. 58

P

Pacault S. 164
Parietti-Winkler C. 162
Paris A. 86
Pelletier E. 130
Pelliccioni A. 122
Peltier L. 42
Pelzer J. 148
Peters S. 149
Piccioni A. 116
Pinar E. 111
Poels K. 118, 150
Pol G. 116
Porras S.P. 56
Porta D. 122
Priymenko N. 86
Puisney C. 154
Puscasu S. 52

R

Racordon D. 41
Raschick F. 125
Ravera C. 130
Rémy A. 57
Reshma S.C. 143
Rezk-Kallah B. 108
Rezk-Kallah H. 108
Rhomberg L.R. 152
Rihn B. 88, 115, 154
Robert A. 57, 120, 124, 144
Roger M. 37
Ronzani C. 88
Rosenkranz N. 155
Rossner A. 135
Rotureau P. 98, 153
Rousset D. 109, 112, 142
Rychen G. 86

S

Saad E. 129
Safar R. 88, 154
Saidi M. 141
Salquèbre G. 95, 97
Santonen T. 56
Sarazin P. 52
Saurat D. 37
Savic N. 41
Schmitt N. 34
Schoofs L. 85
Schremmer I. 155
Schroeder H. 86, 97
Sébillaud S. 156
Seddiki S. 105
Servatius C. 148
Séverin I. 159
Shulman S.A. 135
Silibello C. 122
Silva F. 158
Silvente E. 72
Simões H. 158
Simon X. 126
Simonnard A. 93
Smola T. 33
Snawder J.E. 58
Snijkers F. 44
Souton E. 159
Srhayri R. 160
Stefaniak C. 138
Stockmann R. 149
Syama S. 143

T

Takky D. 160
Taniere A. 77
Tebby C. 121
Terwoert J. 35
Terzetti F. 127
Thomas D. 164
Thomassen Y. 49
Tomaz L. 158
Toulemonde N. 34
Tuomi T. 56

V

Van Gelder R. 38
Van Tongeren M. 39
Van Tra H. 52
Vanoirbeek J. 85, 118, 119, 150
Venet T. 162
Verbist K. 33, 35
Vernez D. 25, 41
Verougstraete V. 146
Villamur T. 34
Vincent R. 34
Visser R. 33
Vranckx K. 150

W

Wallin H. 83
Wathier L. 162
Weber D.G. 155
Weltens R. 44
Westphal G.A. 155
Whitney G.E. 54
Williams D.R. 152
Wingert L. 164
Witters H. 44
Wohlleben W. 104

Y

Ylinen K. 56

Z

Zimmermann F. 166
Zmirou-Navier D. 121