INFLAME NEWSLETTER

WINTER 2013

Welcome to the quarterly update newsletter from around the INFLAME participants. This continues to be a (relatively) informal vehicle for updating your fellow INFLAME(rs) about what's going on with your specific project, or related events. The deadline for 'copy' for the next issue will be March 15th 2014. Please send it to Kate Nauta K.nauta@bham.ac.uk

Co-ordinator's view

As the 3rd year of INFLAME draws to a close, I feel it permissible for us look back with considerable satisfaction at our achievements to date as a consortium. This of course has not happened by magic but as a result of hard work by you all and I am grateful to every one of you for your contributions to making INFLAME a success. I'd like to think we've had plenty of fun on the way too and on that note would like to say a big thankyou to Anna and colleagues at IVL for hosting the combined INFLAME and A-TEAM meetings last month, and for arranging the really enjoyable tour of the Vasa Museet. Thankyou all also for responding to my request for information to include in the year 3 Progress Report to the EU; it makes my job much easier.

It only remains then for me to wish you and your families all the very best for the Festive season and a very Happy and Prosperous 2014.

INFLAME meetings

Supervisory Board 28th April 2014

"Fellows-only" Meeting 29th April 2014. Both hosted by VU. Venue to be advised.

Upcoming Symposia/Conferences

- 8th POPs Network Conference – University of Birmingham UK - 8-9/5/14 www.birmingham.ac.uk/nercpops-8th-conference
- SETAC Europe – Basel, Switzerland – 11/5-15/5/14 http://basel.setac.eu/?contentid=636
- BFR2014, Indianapolis, IN, USA – June 22-24 2014 http://www.bfr2014.indiana.edu
- Dioxin2014, Madrid, Spain – 31/8-5/9/14 www.dioxin2014.org

News from the Participants

University of Antwerp

Alin Ionas (ESR1), Boris Krivoshiev (ESR10), Adrian Covaci and Ronny Blust

Since the last update *Alin Ionas* reports:

- In September and October 2013, I finished writing and then submitted the article about the analysis of FRs and phthalates in toys to *Environmental International*. The manuscript came back with major revisions and a revised version has already been resubmitted.
- In November 2013, along with Ana, during her secondment at the UA, I have worked on the identification of some interesting halogenated clusters from electronic consumer products and e-waste samples. The data from previous experiments and the results from these experiments were compiled in an abstract submitted for the SETAC Europe 2014:
  Poster: Ionas AC, Ballesteros Gómez AM, Leonards PEG and Covaci A. "Identification strategies for halogenated flame retardants and related chemicals"
- Another abstract was submitted (also a poster) as a summary of the conclusions from the article on toy analysis: Ionas AC, Dirtu AC, Anthonissen T, Neels H, Covaci A. "Harmful organic chemicals in children's toys due to use of recycled materials"
- I have started compiling the findings from the (Q)ToF screening for identification experiments into an article.
- We are in the planning stages for experiments on FR leaching from hard plastic toys into saliva and on the analysis of FRs from textile materials. These experiments will be conducted together with Dr. Pim Leonards and Dr. Ana Ballesteros during February-March 2014 as the second part of my secondment to VU.

Boris has the following news from his first few months:

Since my arrival in Antwerp in September as the successor of Max Behr, I have been primarily using the bacterial stress profiling assay and I familiarized myself with the so-called E-screen to test the flame retardants (FRs) for their potential estrogenic effects. Max initiated this part and we mainly studied adverse effects of TPP, TBEP, TCEP, TnBP, HBCD and TBBPA. From the bacterial gene profiling study, we report that a majority of compounds show to generally result in oxidative stress, loss in membrane integrity, and protein perturbation. More interestingly, we could show that a majority of compounds tested so far exhibit estrogenic activity. Particularly for TBBPA, this is in disagreement with the literature where all studies show no estrogenic activity associated with the compound. The remaining compounds that are needed for screening (TCPP, TDCPP, TBB, TBPH, BTBPE) have been ordered and are expected to arrive shortly. These would also undergo screening using the bacterial stress gene profiling assay, and hopefully the first paper concerning these data will be submitted in 2014.

General news from the Toxicological Centre, UA

- During October - November 2013, we received Dr. Ana Maria Ballesteros-Gómez for her secondments at the Toxicological Center. In collaboration with INFLAME ER1 Alin Ionas and the other researchers of the group, namely A-TEAM ER3 Claudio Erratico and Nele Van den Eede, she has investigated the *in vitro* metabolism studies of 2-ethylhexyldiphenyl phosphate (EHDPP) with human liver microsomes and S9 fractions.
- On the 13th November 2013, the prize of the Research Council of the University of Antwerp will be awarded to Dr. Alexander van Nuijs to acknowledge him for his original contribution to the scientific discipline of sewage analysis to estimate illicit drug consumption in the general population.

University of Birmingham

Cassandra Rauert (ESR2), Jinkang Zhang (ESR11), Stuart Harrad, Mark Viant and Kevin Chipman

Jinkang Zhang reports:

After returning from the Dioxin 2013 conference, I focused on transcriptomic analyses of HepG2/C3A cells and A549 cells exposed to TDCPP. High dose (100uM) exposure could alter the gene expression profiles in both two cell lines; while no significant changes of gene expression in medium dose group (10uM). More bioinformatics analysis and following supplemental experiments will be employed to confirm these results in TDCPP study. Meanwhile, the third sub project focusing on toxicity of extracts of dust sample will start soon after several very detailed discussions with supervisors and colleagues from Stuart's group. We are going to use the synthetic mixture of several FRs (HBCDs + three PFRs) to represent predominant FRs in dust extraction for exposure experiments. Comparing with exposure to original extract of reference dust sample, metabolomic analysis of cells exposed to FRs mixture will be conducted while metabolism of FRs during exposure will also be investigated by collaborating with Stuart's group.

In terms of meeting/conferences, I attended the INFLAME network meeting in Stockholm in November.

Cassie Rauert tells us:

Since September I have completed my secondment to NIES, Japan, conducted further chamber experiments, and started a collaboration with ESR7 (University of Reading).

Secondment to NIES, Japan

A two month secondment to the National Institute for Environmental Studies (NIES), Japan, was undertaken to use forensic microscopy techniques to analyse dust samples. Dust samples generated via the abrasion chamber method (fibres of a HBCD treated curtain removed into a dust mass) were analysed using three microscopic techniques and an FT-IR. Three dusts from the Birmingham sample archive (known to contain very high concentrations of BDE-209) were also analysed with these techniques. The high level 'real' dust samples were identified to be contaminated with tiny pieces of plastic (an acrylic copolymer) treated with BDE-209 and Antimony Trioxide. The high levels in these dust samples were caused by abrasion of a plastic product (possibly a computer or TV housing) that abraded fine particles into the dust, lending further confidence to the hypothesis that high levels dusts are likely contaminated via the abrasion pathway rather than solely volatilisation with subsequent partitioning to dust.

Bioaccessibility study with University of Reading

A collaborative study with the ESR7 has started, assessing the bioaccessibility of dusts contaminated with either the volatilisation/partitioning or the abrasion migration pathway. Dusts have been produced in the chamber using the HBCD treated curtains as the BFR source for both migration pathways. The dusts are to be assessed for differences in bioaccessibility, in dust contaminated by

different pathways, at the University of Reading then returned to the University of Birmingham for quantification of sample extracts

Publications

Rauert C., Lazarov B., Harrad S., Covaci A., Stranger M., "A review of chamber experiments for determining specific emission rates and investigating migration pathways of flame retardants" *Atmospheric Environment,* 2014, **82**, pp 44-55.

VITO

Borislav Lazarov (ESR3), Aga Kucharska (ESR9), Marianne Stranger, and Stefan Voorspoels

Borislav Lazarov has the following news:

I. Progress to date

The preliminary emission testing of a flame retarded sealing material (PU foam) that contains Tris(monochloropropyl)phosphate (TCIPP) in a 0.1 m³ emission test chamber (ETC) has been completed after 213 days. Simultaneously to the emission test in the environmental test chamber, the same material was tested in µ-ETC, where the air change rate is higher. This experiment was performed in order to explore the possibilities of using the µ-ETC for preliminary (screening) assessments of FRs emission profile after a multitude of air changes. We are currently processing the data from those experiments to establish the first draft of an emission test protocol for TCIPP from other building materials, consumer products and PU foams.

In the meantime, my first scheduled secondment at Stockholm University was completed. The aim of the secondment was to compare the applicability of the new method for sampling of FRs from gaseous phase on PDMS/Tenax (Lazarov et al. 2013) with the reference sampling method on PUF. In a two week comparison experiment, three sets of samples were taken in parallel in a selected indoor environment in Stockholm. Currently we are busy with analyzing the samples and processing the data. The results of this study will be reported at near future (as part of a paper on the validation of the new sampling method).

The next experiment, which is currently in preparation stage, is to explore the applicability of the established draft emission test protocol for assessment of emission rates of other FRs from different treated products.

II. Perspectives

- Exploring the possibilities to modify the test protocol to assess the emissions from treated products in a first phase, and then also at dynamic use conditions (e.g. the dynamic of emission rates at the moment of pressing treated PUF to simulate sitting on it, thus simulating a use scenario)
- Exploring the influence of the presence of particulate matter to the FRs emission rates from treated products.

Since the last newsletter *Aga Kucharska* updates:

Recently, I have submitted two papers entitled:

1) Tackling the low sample amounts in biomonitoring studies: broad spectrum flame retardant analysis in hair samples (in Analytical Bioanalytical Chemistry)
2) Non-invasive biomonitoring for PFRs and PBDEs: new insights in hair analysis and poster presentation (in Science of the Total Environment).

In the meantime I performed additional tests for human hair sample with the use of SEM (scanning electron microscope). I tested the structure of hair after treatments in different media usually used for hair denaturation and extraction, namely acids (HNO_3, HCl), organic solvents (methanol, hexane:dichloromethane, acetone), their mixtures and combined agents (acids with the mixture of hexane:dichloromethane). The treatment with the different agents was tested also at variable conditions (25 min extraction in ultrasounds at 25 °C and extraction overnight at 60 °C). This test

showed that diluted acids do not change the surface of hair as we previously expected. It might therefore indicate that direct extraction of hair with the use of appropriate solvents might be sufficient at hair sample extraction especially when easily hydrolyzed compounds (such as PFRs) are analyzed.
In September I attended the Course of Interpretation of MS-MS Mass Spectra (at VITO; Hyphen MassSpec; Prof. Dr. Wilfried Niessen) 23 – 25 September 2013
Currently I'm participating in a project and I have already analyzed a part of human samples (hair, urine, earwax) collected from workers from burning sites in the Middle East. First insights into the results showed that levels of analyzed compounds namely PFRs (in hair and earwax) and phthalates (in urine) are not meaningfully higher than in samples of general population (e.g from Belgium).

ITM, Stockholm University
Seth Newton (ESR5), Fiona Wong (ER1), Cindy de Wit, Matt MacLeod and Ian Cousins
Seth Newton has the following to report:
Since the last newsletter I taught a laboratory course with Fiona Wong on the analysis of PCBs in sediment. Boris Lazarov was in Stockholm for two weeks for a secondment during which we sampled my apartment using two types of samplers for comparison - PDMS/tenax used for emission chambers at VITO and PUF/filter system we use at Stockholm University for all of our air sampling. I have also printed and am preparing to defend my licentiate thesis on December 19th. I have had a paper accepted to Environmental Science: Processes and Impacts:
Atmospheric deposition of persistent organic pollutants and chemicals of emerging concern at two sites in northern Sweden. Newton S, Bidleman TF, Bergknut M, Racine J, Laudon H, Giesler R, Wiberg K
I have also been preparing outdoor air and dust samples for analysis which were taken in Stockholm last year. This will complete my Stockholm samples and I plan to prepare a manuscript on the data when the analysis of these samples is finished.

Meanwhile *Fiona Wong* has finished her post-doc position with INFLAME. She will work at Stockholm University as a post-doc for the next two years, investigating chiral and achiral flame retardants in indoor and outdoor environment.

IVL
Ioannis Liagjouridis (ESR4) and Anna Palm-Cousins
Ioannis Liagjouridis tells us his highlights of the past few months:

- Completed a 1-week visit to University of Birmingham where ESR2 is carrying out chamber experiments looking at the different release mechanisms of BFRs from treated materials. Of particular interest were abrasion experiments which indicated the potential of a much higher chemical transfer from the source than volatilisation. A chamber model developed together with ESR2 before is currently revised and further developed based on the results of those ongoing experiments.
- Submitted a critical review paper on modeling approaches of emissions and indoor fate of BFRs. Paper is in the review process.
- Presented an overview of my research to date during the PhD seminar day held at ITM, SU.
- Submitted an abstract for a poster presentation in the forthcoming SETAC conference in May 2014, in Basel.
- Currently taking a PhD statistics course at SU.
- Working with modeling the indoor and outdoor fate of alternative BFRs, OPFRs and inorganic FRs.

Norwegian Institute of Public Health
Enrique Cequier (ESR6), Cathrine Thomsen and Georg Becher

Enrique Cequier tells us that:

The experimental part of my project is finalised. Currently, I'm immersed on statistical analyses and writing the manuscripts for scientific publications which contain the experimental data obtained during the last months. As mentioned in the previous newsletter, we published a method article on the determination of halogenated flame retardants in human serum by means of GC-LRMS in *Journal of Chromatography A*. A second article, describing the low extraction efficiencies of highly lipophilic flame retardants from serum, is published online in *Environmental Science and Technology Letters*. Moreover, two more manuscripts are in the final phase, one presenting levels of brominated and organophosphate flame retardants in indoor environments in Norway and another on an analytical method for the determination of organophosphate triester metabolites in urine by means of LC-HRMS. We plan to write two more papers next year. In addition, I will soon start writing the thesis which I intend to defend in September 2014 in Tarragona (Spain).

Merry Christmas and a happy New Year INFLAMERS!

VU-IVM
Jocelyn Ulevicus (ESR8), Ana Ballesteros (ER1), Margot van den Bor, and Pim Leonards

Ana Ballesteros reports as follows:

During the last few months I have completed my secondment (October-November 2013) at the Toxicological Center of the University of Antwerp (Belgium) under supervision of Prof. Adrian Covaci and in collaboration with ER1 Alin Ionas and the other researchers of the group, namely Claudio Erratico and Nele Van den Eede. The secondment was focused on in vitro metabolism studies with human liver microsomes and S9 fractions for 2-ethylhexyldiphenyl phosphate (EHDPP). A number of around 30 metabolites including mono- and dihydroxylated metabolites, ketones and carboxylic metabolites, glucuronide conjugates and hydrolysis products have been identified by UPLC-QTOF-MS. The metabolism of the novel BFR 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine (TTBP-TAZ) was also studied. In this case, only one major ketone metabolite was identified. I have also completed and submitted three articles on the results of the analysis of RDP and BDP in plastics (Chemosphere), the applicability of ambient mass spectrometry for screening flame retardant (Analytical and Bioanalytical Chemistry) and about the detection of the previously unreported TTBP-TAZ triazine flame retardant (Environmental Science and Technology). Finally, I have been working on the submission of two research proposal (VENI from NWO, Netherlands) and a short Pegasus Marie Curie fellowship (FWO, Belgium).

Meanwhile *Jocelyn* updates:

Having just submitted our research protocol to the medical ethics committee here at the VU, we aim to begin recruitment for our environmental exposure study here in Amsterdam in the New Year. Strategies for research recruitment have been established, and we are pleased to note that we have received donated gifts to deliver to families who participate in the study. I am currently working on a review paper that will address exposure to dust as a mechanism for human exposure to flame retardants, with an explicit focus on hand-to-mouth contacts as the primary pathway for this exposure. Our research focus is on toddlers, as the physiological and behavioural characteristics associated with early life may mediate exposure to both dust, and dust laden materials via the hands, which may contribute to an overall greater risk of exposure to both dust and further, flame retardant materials. We are using a mixed methodological approach for this assessment, combining observational research with systematic data collection. With our subsequent findings, we hope to provide an estimate of exposure via hand to mouth activities in our Dutch cohort using a modified exposure calculation used by Stapleton et al (2008) and Hubal et al (2000).

Other activities have included participation in the most recent INFLAME meeting held in Stockholm in November 2013. I have also hired two interns for this coming spring. These two students will both devise and carry out, their own research project based on my protocol (and the available literature). I will supervise them in their methodological development, their data collection, analysis, and final write up of results.

University of Reading
Sonia Garcia (ESR7) and Chris Collins
Sonia Garcia informs us that:
During these last 3 months I have set up the experiments with CaCo2 cells: I have exposed them to PBDEs and OPFRs separately during 32 h to a concentration of 1 µM and I have seen that this concentration is not toxic (the cells continue growing). I have extracted the compounds from the cells and from the cell culture media and at the moment I am waiting for the results. If there is an uptake I will do the exposure from 0.5 to 16 h. When exposing the cells to OPFRs I have observed that the cells don't sediment easily in the pellet after the centrifugation, this doesn't happen when they are exposed to PBDEs, and so I have found difficulties separating the cells from the media when working with OPFRs.
Cassie and I have also been setting up an experiment based on the bioaccessibility of HBCD from dusts with fibers from a curtain. The dusts have been created in Cassie's chamber in different processes of abrasion and volatilization. We will continue working on this experiment during the next 2 months after holidays.
I am also collaborating in a bioaccessibility review within Chris' group and writing the thesis.

University of Amsterdam
Derya Canbaz (ESR12) and Leonie van Rijt
Derya Canbaz updates us with:
I. Progress to date:
I have completed my secondment at Institute for Environmental Studies (IVM) in VU University in Amsterdam. We aimed to evaluate in a well-characterized birth cohort (BAMSE-Barn, Allergy, Milieu Stockholm Epidemiology) whether development of allergic asthma is positively associated with early exposure to flame retardants in dust of mother's matrasses.
For this purpose, I worked six weeks (July-August 2013) to extract PBDEs and OPFRs from the dust of matrasses (220 case-control samples). To detect levels of FRs in these dust samples, GC-MS measurements were applied.
As a result, we assessed the level of 7 OPFRs (TCEP, TCPP1, TDCPP, TBEP, TPP, EHDP, MMP-TCP) in house dust collected at the time of birth of 110 children that would develop asthma between 4 and 8 years old and 110 matched controls. Controls were matched for sex, social economic status and atopy in parents. Triphenylphosphate (TPP) and di-ethylhexyldiphenylphosphate (EHDP) were detected in all dust samples (median value controls: resp. 619 and 172 ng/g and cases: 419 ng/g and 159 ng/g dust). Trisbutoxyethyl phosphate (TBEP) was detected in 91.4% of the samples but in a 10-20 fold higher concentration compared to the other organophosphate flame retardants. No associations between levels of organophosphates in house dust and development of childhood asthma were observed in this case-control study.
GC-MS analyses of PBDEs is still on-going.

II. Publications

Papers in preparation:

1- IL-33 influence on the induction of immunoglobulin production in dendritic cell driven mouse model for allergic asthma – submission is due at the end of 2013.
2- The effect of HBCD on the development of allergic airway inflammation to house dust mite in mice
3- Exposure to organophosphate flame retardants in indoor dust and childhood asthma
4- Exposure to PBDEs in indoor dust and childhood asthma

PHOTO FUN SECTION

A prize to the 1st person to guess which INFLAMEr is "sad" enough to take the photo below on their holidays?