INFLAME NEWSLETTER

SPRING 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 September 15th 2013. Please send it to Kate Nauta K.nauta@bham.ac.uk

Co-ordinator's view

Hope you are all having a good summer!

As mentioned in the last newsletter I was involved heavily with the preparation of the 2nd year report for INFLAME. This included our 1st report on the financial aspects. For those of not aware of the outcome, it was accepted by the EU, although they "disallowed" €44.10 of our claim. As our total claim exceeded €1.7 million, I think we can claim our efforts to be a success ☺

On a sad note, I have to inform you that for personal reasons, Max Behr has resigned from his ESR position in Antwerp. I for one will miss Max and wish him well in his future career. The Antwerp team are recruiting replacement for Max and news of this will follow in the next newsletter I am sure.

Given the start of numerous new projects at Birmingham, I am pleased to say that I have been able to recruit a full-time project administrator (Kate Nauta) who will allow me to focus more on the science and training aspects of these projects. Amongst (many) other things, Kate will be managing the newsletter from now on, so we will be able to keep up to date in future!

As you know we will be participating as a Consortium at the Dioxin 2013 conference in Daegu in the last week of August. I am sure that our Special INFLAME session will be a success, and I look forward to seeing you all there.

Very best wishes to you all and I look forward to your next updates in mid-September, when thanks to Kate the newsletter will be back on schedule.

INFLAME meetings

November 14th Discussion Group meetings (fellows only), November 15th Network Assembly and Supervisory Board Meeting (fellows AND supervisors). Venue IVL, Stockholm (details to be circulated in due course)

Upcoming Symposia/Conferences

SETAC North America – Nashville, TN, USA 17/11-21/11/13 http://www.setac.org/event/id/244644/SETAC-North-America-34th-Annual-Meeting.htm

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

News from the Participants

University of Antwerp

Alin Ionas (ESR1), Maximillian Behr (ESR10), Adrian Covaci and Ronny Blust

Since the last update *Alin Ionas* reports:

- From January 2013 until March 2013, I have done the first part of my secondment at the IVM (VU Amsterdam)
- I have analysed a few samples of dust (indoors, from the US and from e-waste storage areas – Thailand) and car interiors for exploratory purposes. The techniques employed were LC-TOF and GC-TOF (Agilent 1260 LC System and Agilent 7890A GC System coupled with a Bruker microTOF)
- The statistical analysis of the data for PBDEs, OPFRs and phthalate esters in children's toys is still on-going and the data is being compiled in an article.
- At the moment I am working on analysing new BFRs from soil and sediment samples from e-waste processing areas along with Dr. Masayuki Someya, a guest at out lab within the INTERFLAME project
- An older manuscript was published:

Alin C. Ionas & Adrian Covaci (2013): Simplifying multi-residue analysis of flame retardants in indoor dust, International Journal of Environmental Analytical Chemistry, DOI:10.1080/03067319.2013.763248

The news from *Maximillian Behr* is as follows:

- I established a method for extracting, digesting and labelling proteins from HepG2/C3A cell culture. Protein identification is within the expected range (~500 proteins identified per sample).
- During Jinkang's secondment in February, the first proteomics experiments exposing HepG2/C3A cells to HBCDD and TDCIPP were conducted. The results are not known yet.
- I performed the prokaryotic stress promoter assay experiments for BDE209, TBBPA, HBCDD, TBOEP, TnBP, TCEP and TPHP. Multiple stress promoters show significant induction on exposure to different FRs. The most prominent example is TBBPA which significantly induces promoters known to be activated in case of oxidative stress at concentrations as low as 313 nM.
- I am currently still confirming the results for the OPFRs using the MCF-7 E-Screen for (anti) estrogenic activity. TDCIPP and TnBP show a significant reduction in proliferation of these cells which we suspect to be the result of anti estrogenic activity. This hypothesis is backed by results obtained using the Yeast Estrogen Screen (YES).
- I am planning to present preliminary results of the MCF-7 and YES experiments at the Copenhagen Workshop on Endocrine Disrupters (28-31 May 2013).

General news from the Toxicological Centre, UA

- The Toxicological Centre is participating to the "INTERFLAB" inter-laboratory study, within the INTERFLAME project. A wide variety of novel flame retardants were analysed employing GC/EI-MS, GC/ECNI-MS and LC-MS/MS and the results will be reported shortly.
- There is on-going work for the development and application of a GC/EI-MS method for the analysis of PFRs in fish (eel samples).

University of Birmingham

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

Cassandra Rauert's scheduled secondment to VITO was undertaken from September-December 2012 and provided a great opportunity for a comparative study of two different chamber methods. The issues that are common to all chamber experiments, such as sink effects, were highlighted, as were the difficulties with modifying chambers to investigate the migration pathways to dust. The comparative chamber experiments also provided a validation for the in-house designed and built chamber used at the University of Birmingham.

Cassandra also reports:
Curtains treated with the HBCD formulation were obtained and also analysed in both chambers looking at the deposition to dust migration pathway. These curtains were investigated further in the University of Birmingham chamber testing different chamber conditions such as length of experiment and temperatures. Further to this, preliminary experiments have begun looking at the abrasion migration pathway. Another chamber modification has been designed for these experiments and successfully encourages the release of fibres from the treated curtains with visible fibres in the dust- post experiment. Emission Scanning Electron Microscopy (ESEM) has also been utilized to analyse the dust sample generated from an abrasion chamber experiment with the positive bromine identification (and possible indication of BFR presence) of fibres in the sample. More products treated with other BFRs have been obtained for further chamber investigations.
Preliminary investigations of dust particle size fractions have also begun, looking at a difference for a size relationship with BFR concentration.

The migration pathway and chamber comparative studies research will be presented at the BFR2013 conference, San Francisco, in April (via a poster) and also at the POPs conference, Birmingham, in April via an oral presentation. A review paper of published data on chamber experiments of BFRs, including measurement of emission factors and investigations of migration pathways to dust, is also currently underway as a collaboration with ESR3.

Jinkang Zhang tells us that in the last three months, the study of transcriptomic and metabolomic analyses of molecular responses in HepG2/C3A cells exposed to HBCD for 24 hours has been finished. The results showed no significant changes at gene expression and metabolic levels in C3A cells after exposure to HBCD (4μM) to 24 hours, which is similar to previous results in A549 cells.
He sends thanks to everyone for their help in Belgium, where he finished his secondment at University of Antwerp in February. At Antwerp, he and Max (ESR10) focused on proteomics analysis of HepG2/C3A cells exposed to HBCD. Similar designs for cells exposure experiments were used to those employed in Birmingham, so that comparable results could be expected. They finished most of work including protein sample preparation and waited for the final step of instrumental analysis at the proteomics centre. During his secondment, he also visited Adrian's lab where the measurement of amount of HBCD in C3A cell samples has been done. The results showed that there ca. 17% and 20% amount of HBCD (comparing to nominal amount added into exposure media) were detected in cell pellets and cell media separately.
Meanwhile, a 21-month report which is required for accessing progress of PhD project in Biosciences School in Birmingham has been submitted. He will attend and give poster presentations at the Biosciences Graduate Research Symposium in Birmingham and the 7th POPs conference all of which will be held in the coming weeks.

VITO

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

Borislav Lazarov has the following news:

The optimization of the procedure for sampling and detecting the flame retardant chemicals from emission chambers is still ongoing. The preliminary results show a good recovery in sampling of PBDEs on PDMS/Tenax followed by thermal desorption and GC-MS detection. Currently we are exploring the possibilities to apply the method to a wider range of compounds, mainly focused on flame retardants.

In the meantime, the publication for the Environmental Science: Processes & Impacts (previously Journal of Environmental Monitoring), on novel air sampling techniques for SVOC measurements in general, including selected flame retardants, is now submitted.

The construction of the database with information of treated goods, suitable for emission testing, is ongoing but contains already several interesting products to be studied. Furthermore, the preliminary emission testing of a flame retarded sealing material (PU foam) that contains Tris(monochloropropyl)phosphate (TCIPP) has been initiated. During this preliminary experiment, characteristics like emission test duration, emission test chamber settings (temperature, air change rate, etc.), sink effects, room cleaning, will be explored.

During the last three months *Aga Kucharska* has been working on exposure test with extended group of compounds: PBDEs and OPFRs. Her update follows:

I performed 24h, 48h and 72h exposure tests and as previously I used ice cream boxes and watch glasses spiked with the FR standards (as shown on the pictures below).

In case of OPFRs I obtained results in accordance with my expectations – the longer I exposed hair samples, the higher levels I measured in them. The transfer efficiency is different for each compound that might be caused by different properties (e.g. volatility, polarity). Finally, after the exposure test I compared several washing media: H_2O, hexane:DCM, Methanol, Acetone, Shampoo. Again, my observation leads to the conclusion that water and shampoo are not appropriate media to use for external contamination removal - the exposed hair samples were also analyzed after washing and still FR levels were relatively high when water or Shampoo are applied. The most appropriate medium for washing is Methanol – levels of compounds in the exposed hair after washing in this medium were very low. Only in case of TDCPP and TCPP levels were still relatively high in hair analyzed after the washing – it might also indicate endogenous accumulation in human hair.

It's worth mentioning that in my lab work I have no meaningful problems with blank procedures in the analysis of OPFRs.

The exposure test for PBDEs has to be repeated due to observations that are not consistent with my expectations. In contrast what I expected the longer I exposed the hair the lower levels I measured in them. So far I have no reasonable explanation for this.
I have already validated the method for hair analysis (including digestion, extraction fractionation and clean up).
Currently I'm working on two abstracts which are supposed to be submitted for the conference – Dioxin 2013. The main goal is to present OPFR data of human hair analysis.

IVL
Ioannis Liagjouridis (ESR4) and Anna Palm-Cousins
Ioannis Liagjouridis tells us that:
A draft review paper has been produced summarizing and discussing research findings, aiming for publication in the coming months. Review findings will provide good guidelines for next research stages. Elements of my space plotting work (**Figures 1 & 2**) were presented during the INFLAME mid-term review meeting in October, at Reading. Recent modelling progress involves the development of a coupled emission-indoor fate model based on previous modelling approaches. The aim of this work is to explore and link emission of BFRs from consumer products and goods with indoor concentrations.
Recently, I've completed 2 PhD courses at SU as a part of my research training;

1. "How to use Matlab to solve problems in environmental, climatological, and earth science research''
2. "Modelling of Environmental Pollutants II: Applications''

The first course aimed to improve my modelling skills by introducing a quite powerful and useful tool I could use for my research. A small scale project followed the completion of the course. The second course gave me the opportunity to apply my modelling skills gained so far. For this purpose the coupled emission-indoor fate model mentioned before was developed and applied to explore the role of electronic equipment in indoor emissions and fate of BFRs
Finally a 2-week visit to VITO is scheduled for the end of April-beginning of May. There in close collaboration with ESR3 we will explore the mechanisms and quantify the emission rates of FR release from products with the aid of a specially designed emission chamber.

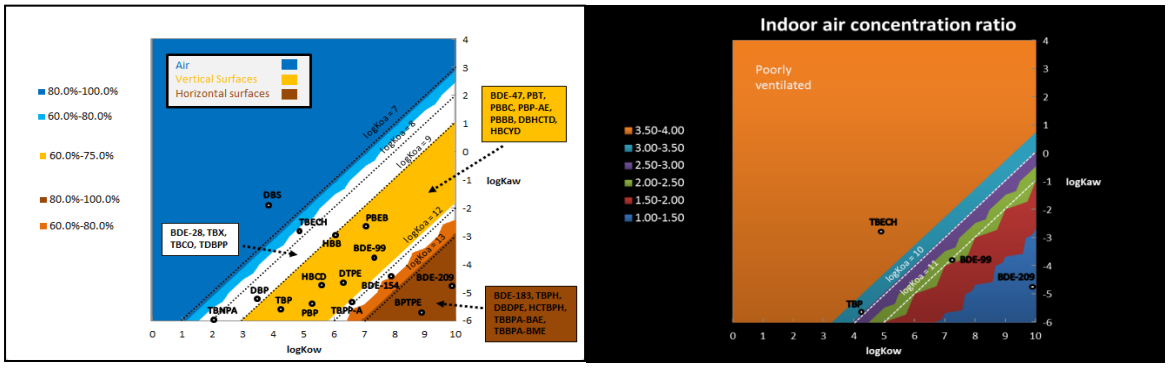

Figure 1: Partitioning behaviour of BFRs in an indoor environment and, **Figure 2**: The effect of reduced ventilation rate on indoor BFR concentrations

ITM, Stockholm University

Seth Newton (ESR5), Fiona Wong (ER1), Cindy de Wit, Matt MacLeod and Ian Cousins

Since the previous newsletter *Seth Newton* has the following to report:

I have completed my six month secondment in Birmingham and began my three month secondment in Beijing, China. Six months of passive air samples at eight sites across a transect of Birmingham were analyzed for HBCDDs and novel flame retardants (NFRs). No NFRs were detected using GC-EI-MS, however, the samples were sent to Stockholm to later be analyzed using GC-ECNI-MS. Detection of NFRs is still a possibility given the lower detection limits of ECNI vs EI. HBCDD data from these samples do not show evidence of an "urban pulse" as has been previously observed with PBDEs and PCBs. However, some seasonal variability was observed. Average concentrations for all sites were lower in the winter months than during the fall.

The secondment in China has had ups and downs already, only a few weeks into it. We had to change many of our plans for our work here when we learned that Chinese customs would not let us send our active samplers or chemical standards to Beijing. Furthermore, a package containing passive sampling equipment was returned to Birmingham. However the package was successful in its second attempt to reach the Chinese capital and we were able to obtain some active samplers to use here, so it all worked out in the end. Our Chinese colleagues in Professor Yu Gang's and Professor Yinping Zhang's groups at Tsinghua University have been very nice and have treated us very well during our stay.

Meanwhile *Fiona Wong* is at her secondment with NIPH from April 15 to May 24. She is going to attend the POPs conference at Birmingham in April 24-25. A paper on "Bounding uncertainties in intrinsic human elimination half-lives and intake of PBDEs in the North American Population" has been submitted to Environmental International and minor revision is required. Fiona is now using the Ritter pharmacokinetic model to understand the exposure, body concentration and elimination of PFASs in the US population.

Fiona has received a two-year post-doctoral fellowship from Stockholm University. She will be studying the fate of chiral and achiral emerging flame retardants in humans the environment after her appointment with INFLAME in October 2013.

Norwegian Institute of Public Health

Enrique Cequier (ESR6), Cathrine Thomsen and Georg Becher

Enrique Cequier tells us that:

Method development for the determination of organophosphate metabolites has being carried out using an UPLC (C18 column) and q-TOF instrument with negative electrospray ionisation. An on-line SPE system has been chosen as clean-up step performed on an Oasis HLB column. Validation of the method and sample analysis will be performed after the secondment in Antwerp, which starts at the end of April and lasts for 6 weeks.

At the University of Antwerp, I will determine tri-aryl and alkyl phosphates, PBDEs and emerging flame retardants in the dust and air samples collected during the sample campaign.

During this winter, we have been preparing two manuscripts for publication: low extraction efficiencies using SPE for highly lipophilic compounds (submitted) and a method paper for determination of PBDEs and emerging flame retardants in serum using GC-MS. In addition, abstracts for two conferences have been prepared and presented: 6th International Symposium on Flame Retardants (April 2013, San Francisco, USA; presented by Cathrine Thomsen) and a poster at the 14th International Conference on Chemistry and the Environment (June 2013, Barcelona, Spain).

VU-IVM

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

Ana Ballesteros reports as follows:

During the last months I have finished with the development and validation of three new analytical techniques for screening flame retardants based on GC, GCxGC and direct probe coupled to APCI-HRTOF-MS. The techniques were applied to e-waste and car interiors samples. GC-APCI-HRTOF was found to be advantageous in terms of sensitivity compared to LC-APCI/APPI/ESI-HRTOF-MS for a wider range of compounds. The coupling of two dimensional GC to a high resolution TOF by using a soft ionization source was developed for the first time and proved to be a promising tool for wide screening purposes by obtaining comprehensive information, while direct probe APCI-HRTOF provided a fast qualitative screening tool for rapid identification (few seconds). TBBPA, PBDEs, BTBPE and organophosphates were found at high concentrations (at mg/g to μg/g levels) in the samples.

I recently gave an oral presentation about these results in the conference BFR2013 (San Francisco, CA, 7-10th April, 2013) and we will submit them soon for an article. I also supervised together with Pim Leonards, the secondment of Alin Ionas (ESR1, January-March 2013) applying these techniques for screening flame retardants including unknowns in dust and car interior samples.

I also started a course about cell culturing and toxicity assays for later assessing the toxicity of e-waste extracts and developing clean-up fractionation methods.

University of Reading

Sonia Garcia (ESR7) and Chris Collins

Sonia Garcia informs us that:

I have been in VITO doing my first month of secondment (15 February-15 March) where I have analyzed the bioaccessible fraction of PBDEs in the samples prepared before the last report (different natural indoor dusts). I have also analyzed the properties of new indoor dusts collected by me from houses and shops (carbon content, organic content and particle size) and extracted the PBDEs and OPFRs in a combined method described by Adrian consisting in a fractionation on Florisil. The PBDEs where analyzed in the HR-GCMS and the OPFRs in the HPLC and the results have been shown in San Francisco at the BFR2013 conference and also will be shown in SETAC. Knowing the levels of PBDEs and OPFRs of the dusts, the next experiments will be focused on the bioaccessible fraction of these dusts. I will also do some experiments spiking dust with PBDES and OPFRs at different concentrations to see the absorption patterns. All the samples will be analyzed during my second visit in VITO (1st June-31st July).

At the university I attended a one day practical course of Environment Consultant.

University of Amsterdam

Derya Canbaz (ESR12) and Leonie van Rijt

Derya Canbaz updates that:

We previously found that HBCD can enhance IL-17A production by the lung draining lymph nodes of the mice sensitized with HBCD/House dust mite (HDM) pulsed dendritic cells (Asthma mouse model). It has been described that IL-17A is associated with severe asthma in human and airway hyperreactivity in mice.

Next, we tried to investigate the direct effect of HBCD on the immune response to HDM by giving HBCD/HDM directly to the airways of the mice. Although we didn't observe any

effect of HBCD in this model, we did see increases in both IL-4 levels in the draining lymph node and IgE levels in serum, which are the biomarkers of allergic response, by exposing mice with HBCD twice after the inflammation by HDM. In the repetition of this experiment, we will also measure HBCD effect on airway hyperreactivity in these allergic mice. As a conclusion, HBCD can enhance the allergic response to HDM in different ways, suggesting that HBCD might increase the symptoms in allergic response to HDM in mice.

In the asthma mouse model, we also investigated BDE-153 effect on the allergic response to HDM. Although we observed that BDE-153 enhanced the maturation and activation of the dendritic cells, we did not observe any effect of BDE-153 on the inflammation by allergic response to HDM in mice sensitized with those dendritic cells. We will try to find out the effects of BDE-153 on the allergic response to HDM in the other mouse models mentioned above.

PHOTO FUN SECTION

Just for some fun (sorry Chris), overleaf is a picture of Chris in the Vigeland Sculpture Park in Oslo in April, modelling my 8 year-old daughter's woolly hat. Well, it was cold!!