



## **INFLAME NEWSLETTER**

**AUTUMN 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 December 15<sup>th</sup> 2013. Please send it to Kate Nauta [K.nauta@bham.ac.uk](mailto:K.nauta@bham.ac.uk)

### **Co-ordinator's view**

Greetings from Birmingham! The highlight of the last few months for me has been the INFLAME Special Session at the Dioxin 2013 conference in Daegu last month. Augmented as it was by several other poster and platform presentations by INFLAME fellows, I think we as a project made an excellent impression. The standard of presentation by the fellows was very high and they were a credit to the individual partners and INFLAME as a whole. You will see mention of the INFLAME presence in Korea at several points in this newsletter. I am glad to see that it wasn't just work ☺

I look forward now to seeing you all again in Stockholm this November. In particular, I look forward to welcoming Boris Krivoshev to the INFLAME team in person. Boris has recently joined the team at Antwerp. You can read a little bit about him on the following pages.

### **INFLAME meetings**

November 14<sup>th</sup> Discussion Group meetings (fellows only), November 15<sup>th</sup> 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 Birmingham

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

*Cassandra* reports:

Since the last update I have presented results generated with the test chamber experiments at three different conferences including a poster presentation at the BFR conference in San Francisco, USA in early April; an oral presentation at the POPs conference in Birmingham, UK in late April; and both an oral and poster presentation at the Dioxins 2013 conference in Daegu, South Korea in August.

In June I undertook a two week secondment to IVL, Stockholm to work with ESR4 to develop a model utilizing emission and partitioning data obtained from chamber experiments. A simple model was developed which can be expanded as more data is produced.

Between conferences and secondments I have been conducting chamber experiments to prepare dust samples to take to NIES, Japan, for a two month secondment where I am currently working. During this secondment to NIES I will be utilizing the emission microscopes and expert analysts to analyse the produced dust samples for Bromine content. Micro-EDX and SEM will locate bromine rich fragments in dust samples, which will be then analysed further with FTIR to search for components such as plasticisers and resins. Samples generated in chamber studies are to be analysed, as well as dust samples from the University of Birmingham sample library, known to contain higher concentrations of BFRs.

*Jinkang Zhang* tells us that after finishing the first subproject of transcriptomic and metabolomic analyses of molecular responses in HepG2/C3A cells exposed to HBCD, a comprehensive supplementary experiment about measuring of the levels of HBCD in cell pellets and cell media after exposure is underway in collaboration with Dr. Mohamed Abdallah in Birmingham. I have started the second subproject which is focusing on metabolic and transcriptomic responses to TDCPP in both HepG2/C3A cells and A549 cells. I have finished instrumental analysis and preliminary data analysis of positive ion mode MS dataset. The remaining part of the data analysis is currently ongoing. The third subproject has also been started while I collaborated with Cassie (ESR2) who helped to prepare dust extracts for further toxicity studies and omics studies.

In terms of meetings, I have attended the Biosciences Graduate Research Symposium and 7<sup>th</sup> POPs network conference in Birmingham after the last INFLAME network meeting in Oslo in April. I also gave a poster presentation titled “Transcriptomic and Metabolomic Analyses of Molecular Responses of Human Cell Lines to A Commonly Used Brominated Flame Retardant: Hexabromocyclododecane” on the 9th Annual Conference of the Metabolomics Society 2013 at the beginning of July in Glasgow. At the end of August, I attended the Dioxin 2013 conference in Daegu, Korea and gave an oral presentation on “Metabolomic analysis of molecular responses of HepG2/C3A and A549 cell lines exposed to Tris (1,3-dichloro-2-propyl) phosphate (TDCPP)”.

## University of Antwerp

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

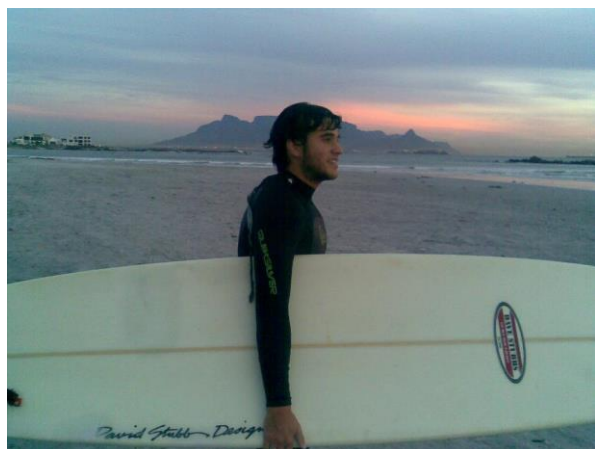
Since the last update *Alin Ionas* reports:

- In April and May 2013, I was busy with analysing the data generated during my secondment at the IVM (VU Amsterdam) and doing some further complementary experiments – I presented this data at the Dioxin 2013 Symposium:
  - Oral presentation: “Identification of novel halogenated flame retardants using spectra-less databases”
  - Poster: “Identification strategies for flame retardants employing spectral and spectra-less databases”
- During the analysis of soil and sediment samples from e-waste processing areas (which we did alongside Dr. Masayuki Someya, a guest at our lab within the INTERFLAME project – some data presented at Dioxin 2013 “Emissions of PBDE and non-PBDE flame retardants from primitive e-waste recycling activities in the northern part of Vietnam”), we realised that the GC-ECNI/MS is not the most adequate technique for analysing V6 (if the liner starts to get dirty, there is a substantial drop in sensitivity). Together with Fuchao Xu (A-TEAM PhD student), we have developed a method on LC-MS/MS for V6 and other emerging PFRs (RDP, BDP, TXP, iDPP). At the moment, we are working on optimising the SPE step of the analysis in order to streamline the process.
- During the secondment of Enrique (ESR6) at the UA, we analysed dust and air samples collected from Norwegian households and schools for a wide array of FRs (PBDEs, NBFRs and PFRs).

## General news from the Toxicological Centre, UA

In the frame of INTERFLAME, we have had 2 secondments to ENTOX (Australia) when we further developed a method for the determination of PFR metabolites in (human) urine. Also there are two secondments ongoing at the Tsinghua University, China on photodegradation of triphenylphosphate.

In June we had to say goodbye to Max, but we are proud to introduce Boris!



Hello, my name is Boris Krivoshiev and I have taken over the ESR10 project at the University of Antwerp in the group of Ronny Blust and Steven Husson. Originally born in Bulgaria, but raised in Cape Town, South Africa, my two passions in life are scientific exploration and surfing. The PhD project, titled "mechanistic profiling of FRs in general systemic stress and endocrine disruption" briefly entails investigating *in vitro* toxicity of FRs whilst also developing mechanistic profiles to further investigate the modes-of-action of these compounds. Looking forward to working within the INFLAME consortium and meeting you all.

## 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 0.1m<sup>3</sup> emission test chamber (ETC) has been ongoing for 140 days. Simultaneously to the emission test in the environmental test chamber, the same material is tested in  $\mu$ -ETC, where the air change rate is higher. This experiment was performed in order to explore the possibilities of using the  $\mu$ -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 PU foams.

In the meantime, the article about “Optimisation steps of an innovative air sampling method for semi volatile organic compounds” in collaboration with Flemish Environmental Agency (VMM) has been published at Atmospheric Environment.

Lazarov, B.; Swinnen, R.; Spruyt, M.; Goelen, E.; Stranger, M.; Desmet, G.; and Wauters, E.; 2013. Optimisation steps of an innovative air sampling method for semi volatile organic compounds. *Atmos. Environ.* 79 (2013) 780-786.

The preliminary results from emission testing of a flame retarded sealing material (PU foam) for 120 days were present at The 33rd International Symposium on Halogenated Persistent Organic Pollutants - Dioxin 2013, Daegu, Korea.

### *II. Perspectives*

- Exploring the applicability of the established draft emission test protocol for assessment of emission rates of other FRs from different treated products.
- Full validation of the newly developed method, specifically for FR detection, focused on a scientific publication.
- My secondment at the University of Stockholm is planned to take place on first half of November, where together with Seth Newton we plan to compare the optimised PDMS/Tenax method and the method used by University of Stockholm for sampling and detecting FRs in air.

Since the last newsletter *Aga Kucharska* updates:

I have optimized and validated a method for simultaneous determination of two groups of compounds (PBDEs and PFRs) from low sample intake of human hair samples. The method was applied to hair samples collected from 20 individuals. In most samples PBDEs were not detected or below LOQ<sub>m</sub>. Only in few samples were BDE-47, BDE-99 detected at relatively high levels. In the same samples, meaningfully higher levels of PFRs were found and they ranged between 4.1 – 5032 ng/g hair. Levels of PFRs in human hair were in the range of the levels found in dust (1). Moreover, results show the current trend – PFRs being used as PBDE replacements are commonly present in the environment and detected at higher levels than PBDEs. Chlorinated PFRs (TDCPP, TCPP, TCEP) considered as cancerogenic were major compounds detected in most of the samples. The method can be useful for further tests and significantly contribute to the human biomonitoring studies.

I also participated in 33rd International Symposium on Halogenated Persistent Organic Pollutants – Dioxin 2013, where I presented my results in the form of an oral presentation (Non-invasive biomonitoring for PFRs and PBDEs: new insights in hair analysis) and poster presentation (Tackling the low sample amounts in biomonitoring studies: broad spectrum flame retardant analysis in hair samples).

Currently, I am also writing my part for a review about human biomonitoring.

Pictures from my holidays in South Korea ☺



### **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 have completed a secondment in Beijing, China, and returned to Stockholm after a long period away. In China, an experiment to compare air and dust sampling methods was completed. Two passive air samplers, one with a glass fiber filter and one without, were deployed alongside an active air sampler which was run for a short period every day to mimic the passive samplers. Samples were taken for 28 days and three sampling periods were completed in three offices. At the end of each sampling period, floor dust and above ground dust was sampled using both a cellulose filter and a nylon sock (4 samples) from each office. During this time, I have also written a large part of my licentiate thesis which I plan to defend within the next couple months. I have attended and given a presentation at Dioxin 2013 along with other INFLAMeRS (good job everyone!) and also attended an INTERFLAME workshop in Beijing. At the moment, I am preparing to teach the laboratory portion of an Environment Chemistry course in our department in which we will analyze PCBs in sediment from the Baltic Sea.

Meanwhile *Fiona Wong* has attended the Dioxin conference, August 25-30<sup>th</sup> in Daegu, South Korea where she presented one platform and one poster. A paper on "Bounding uncertainties in intrinsic human elimination half-lives and intake of PBDEs in the North American Population" has been published in *Environmental International*. Currently, she is investigating the sex difference in the elimination of PFASs in humans using a modelling approach.

## IVL

### Ioannis Liagjouridis (ESR4) and Anna Palm-Cousins

*Ioannis Liagjouridis* tells us that:

- Completed a two week visit to VITO at the end of April-beginning of May where I was introduced to the emission chamber testing. There, together with ESR3, we performed preliminary emission test runs for FRs emitted from a foam sealant product.
- Developed a preliminary emission chamber model along with ESR2 during the latter's 2-week visit to Stockholm in June. This model aims to simulate the chamber experiments carried out by ESR2 in Birmingham and will be optimised and further evaluated during my coming 2-week visit to Birmingham in November. A publication will possibly be considered.
- A poster presentation summarising the key findings of my reviewing work on BFR indoor fate and modelling approaches was made at the DIOXINS 2013 Conference in Daegu, South Korea.
- A final manuscript of the above reviewing work is expected to be submitted for publication within the next couple of weeks.

### Other news from IVL

- Final conference of the Swedish research programme ChEmiTecs, Emissions of Organic Chemicals from Technosphere articles **"About the chemical footprint of consumer goods"** will be held/was held on Sep 9-10, at Teaterskeppet, Stockholm Sweden. This Workshop is organised by IVL and will focus on four main questions:
  - How large is the problem with emissions of organic chemicals from consumer articles, and how large is it expected to become in the future?
  - What combinations of substances, articles and use patterns contribute most to exposure of humans and the environment in short and long perspectives?
  - Which phases in the life cycle are most critical for release of high risk chemicals?
  - What measures are needed to minimise the risks associated with chemicals in articles to fulfill the requirements on a sustainable society?
- Anna defended her doctoral thesis on 10th June, at ITM Stockholm University, title: "Environmental fate of chemicals released from consumer products – Multimedia modelling strategies" (Opponent: prof. Antonio di Guardo), INFLAMERs Ioannis Liagkouridis, Cassandra Rauert, Cynthia de Wit, Fiona Wong, Matthew Macleod and Ian Cousins helped making it an unforgettable day. Thesis available online at <http://su.diva-portal.org/smash/record.jsf?searchId=1&pid=diva2:617448>
- Due to the big interest in A-team nationally (people have been contacting us with requests to donate blood for the project!) – we have set up a Swedish reference group made up of participants from industry and authorities. We plan to hold regular meetings to inform them about the activities and outcome of the programmes (both INFLAME and A-Team) with a special emphasis on the Swedish contribution, but as far as possible promote all the work done. The first meeting will be held next week at IVL where both IVL and ITM researchers will present their work and Anna will introduce the project.





A snapshot from the big day!



The excitement was clearly too much for the opponent!

## **Norwegian Institute of Public Health**

**Enrique Cequier (ESR6), Cathrine Thomsen and Georg Becher**

*Enrique Cequier* tells us that:

The experimental work of the project is basically finished. Serum and urine samples have been analysed in Oslo and air dust during a secondment in Antwerp. Currently, I'm focusing on the data analysis, i.e. processing raw data and using statistical methods to investigate associations. Concurrently, I am preparing manuscripts for publications. Recently we have published a method paper for the determination of flame retardants in serum:

Cequier, E.; Marcé, R. M.; Becher, G.; Thomsen, C. Determination of emerging halogenated flame retardants and polybrominated diphenyl ethers in serum by gas chromatography mass spectrometry. *Journal of Chromatography A*. **2013**, *1310*, 126-132.

We were able to report for the first time the occurrence of Dechloranes 602 and 603 in human serum.

Further, we have under revision a manuscript entitled: "Low extraction efficiencies of highly lipophilic flame retardants from serum" for Environmental Science and Technology Letters.

Together with the other students from the INFLAME team, I attended the 33<sup>rd</sup> International Symposium on Halogenated Persistent Organic Pollutants - Dioxin 2013 in Daegu, South Korea and gave an oral presentation entitled: "Determination of emerging and common flame retardants in Norwegian serum samples".

## **VU-IVM**

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

*Ana Ballesteros* reports as follows:

We have developed new screening tools for flame retardants in e-waste based on gas chromatography (GC), two-dimensional gas chromatography (GCxGC) and direct probe (DP) coupled to atmospheric pressure chemical ionization (APCI)-high resolution (HR)-time-of-flight (TOF)-mass spectrometry (MS). The results of these investigations were accepted on 9<sup>th</sup> September for publication in Analytical Chemistry under the title "Novel Analytical Methods for Flame Retardants and Plasticizers based on Gas Chromatography, Comprehensive Two-Dimensional Gas Chromatography and Direct Probe Coupled to Atmospheric Pressure Chemical Ionization-High Resolution Time-of-Flight-Mass Spectrometry", and were presented at the Conference DIOXIN in August. This publication is online at <http://pubs.acs.org/doi/abs/10.1021/ac9016264>

We will soon send another article for publication based on ambient mass spectrometry for fast screening of flame retardants. One important finding of my research during the last months has been the detection of a new triazine-based flame retardant in a variety of plastics and dust. Results on this finding will also be sent soon to a special issue in Chemosphere related to the Conference BFR2013. Besides this, I have been working on bioassays for assessing the toxicity of e-waste extracts and I will move soon to Belgium (October-November 2013) to do a secondment at the UA with prof. Adrian Covaci and A. Ionas focused on microsomes studies.



## University of Reading

Sonia Garcia (ESR7) and Chris Collins

Sonia Garcia informs us that:

In Reading during May I did several *in vitro* experiments to assess the oral bioaccessibility of PBDES and OPFRs from:

- 8 dusts (previously characterized in my first secondment in Vito) corresponding to 4 houses and 4 shops.
- Dust reference material SRM 2585.
- Dust reference material SRM 2585 spiked with low levels of PBDEs and OPFRs.
- Dust reference material SRM 2585 spiked with high levels of PBDEs and OPFRs.

From 3<sup>rd</sup> June to 26<sup>th</sup> July I stayed at VITO doing my last secondment and there I proceeded to do the clean-up of all the extracts, in the same way as described in the previous report in April, doing the fractionation of PBDEs and OPFRs in Florisil cartridges. I analyzed the PBDEs in the HR-GCMS and the OPFRs in the LC-MS. These results were shown at Dioxins 2013 conference in Daegu.

As a summary, I correlated the bioaccessibility of PBDEs and OPFRs with the particle size and carbon content of the dusts and also with the Log  $K_{ow}$  and I show that all of these factors affect the bioaccessibility:

- Bioaccessibility of PBDEs have no correlation with the carbon content of the dust but the bioaccessibility of OPFRs have a positive correlation ( $R^2 = 0.64$ ).
- The particle size of the dusts is negatively correlated with the bioaccessibility of PBDEs ( $R^2 = 0.782$ ) and positively correlated with the bioaccessibility of OPFRs ( $R^2 = 0.6$ ).
- Plotting the Log  $K_{ow}$  of all the compounds analyzed versus the bioaccessibility we get a negative correlation ( $R^2 = 0.69$ ).

From the spiking experiments at high and low levels, I saw that PBDEs and some OPFRs have a bioaccessibility limit when the concentration in dust increases, but OPFRs with low  $K_{ow}$  and molecular weight (TCEP, TnBP), as they are more water soluble, don't have this saturation in the gut fluids.

In the next 3 months I will do my last experiments of this PhD, firstly CaCo2 experiments and then other bioaccessibility experiments (possibly PBDEs from dusts with fibers, PBDEs from oily food, DBDPE from dust).

## University of Amsterdam

### Derya Canbaz (ESR12) and Leonie van Rijt

Derya Canbaz updates us with:

#### *I. Progress to date*

We have completed the studies to examine the effects of HBCD on the immune response to house dust mite (HDM) in mouse models for different phases of HDM induced asthma. To investigate the effect of HBCD on allergen presentation of dendritic cells, we exposed HDM pulsed dendritic cells by HBCD invitro and administered to mice and we found increased production of IL-17A by lung draining lymph nodes. It has been described that enhanced levels of IL-17A is associated with severe asthma in humans and airway hyperreactivity in mice. Next, we examined the direct effects of HBCD by exposing naive airway epithelium simultaneously with HDM and we observed that HBCD increased IL-13 production and eosinophils in bronchoalveolar lavage fluid. We also used HBCD with HDM 'lotox' extract which has very low amount of endotoxin to investigate whether HBCD can act as an 'danger' signal for DCs in this mouse model. We observed that HBCD increased IL-17A and IL-4 levels in lung draining lymph nodes. When we exposed mice with HBCD during inflammation to HDM, HBCD enhanced IL-4 levels in lung draining lymph nodes, IgE levels in serum and thus airway hyperreactivity of mice as a functional consequence.

As a conclusion we suggested that HBCD can contribute to an aggravation of the immune response to HDM at different stages of the response.

We have an another project which aims to evaluate in a well-characterized birth cohort whether development of allergic asthma is positively associated with early exposure to flame retardants in dust of mother's mattresses, collaborated with Prof. Dr. Pim Leonards. For this purpose, I worked six weeks (July-August 2013) in the Department of Environmental Sciences (IVM) in VU to extract PBDEs and PFRs from the dust of mattresses (220 case-control samples). To detect levels of FRs in these dust samples, GC-MS measurements of FRs is still going on at IVM.

#### *II. Activities (trainings, workshops, courses, conferences)*

- **D. Canbaz**, A. Logiantara, R. van Ree, L.S. van Rijt. A novel mouse model for house dust mite driven allergic asthma. The EAACI-WAO World Allergy and Asthma Congress 2013 in Milan, Italy, 22-26 June 2013, *Poster presentation*.
- **D.Canbaz**, A. Logiantara, R. van Ree, L.S. van Rijt. Flame retardant HBCD enhances the immune response to house dust mite in mice. The 33rd International Symposium on Halogenated Persistent Organic Pollutants and POPs, Daegu, Korea, 25-30 August 2013, *Oral presentation*.

### PHOTO FUN SECTION

This photo came into Stuart's hands in Daegu, and even though it dates from 2012 we just couldn't resist including it here. A large Swedish beer in Stockholm in November goes to the winner of the best caption!

