Annual Report

LaMer, Ehime University

Date (26/02/2017)

To Director of LaMer

Principle Investigator: Ana Catarina Almeida Sousa

Affiliation: University of Aveiro

Position: Post Doc researcher

Name in print: Ana Catarina Almeida Sousa

1. Project / Meeting title

Environmental risk factors for the development and progression of pulmonary diseases

2. Members of project / meeting

Name	Affiliation	Position	Contribution part
PI:			
Ana Sousa	CICECO and Department of Chemistry University of Aveiro	Post Doc researcher	Recruitment of volunteers', Sampling; chemical analysis; paper preparation
Members: M. Ramiro Pastorinho	Faculty of Health Sciences, University of Beira Interior, Portugal	Invited Assistant Professor	Supervisor for the project in Portugal
LaMer Faculty member in charge	Tatsuya Kunisue	Professor	Supervisor for the project at CMES

3. CONTENTS

3.1. Introduction

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease characterized by progressive airflow obstruction and destruction of lung parenchyma that was the fifth most common cause of death worldwide in 2001, and that it expected to rise to 3rd place by 2030. Whereas tobacco smoking is regarded as the principal risk factor for the development of COPD, 25–45% of patients with COPD have never smoked. Furthermore, recent studies showed that exposure to other environmental contaminants plays an important role in this disease, particularly in intense and frequent exposure scenarios, such as occupational and indoor exposures. Thus, studies on the association between COPD and other risk factors are essential. In this line of thought several studies on the association of COPD and occupational exposures have been performed over the last years. However, household and indoor exposures did not receive the same degree of attention by the scientific community, even though most people tend to spent the vast majority of their time indoors (about 80%) and that 2/3 of that time is spent at home. Hence, studies on the distribution of environmental contaminants in the indoor environment alongside with studies on the levels of those contaminants in human samples are of great importance. Organotins are an important class of semi volatile organic compounds (SVOCs) that were already detected at high concentrations in dust samples. The toxicity of OTs towards humans is still poorly understood but data obtained from experimental models clearly demonstrate that they are potent imunotoxic elements which are capable of interfering with the natural ability of cells to control important immune responses and inflammation. This project aims to quantify the levels of organotins in houses of patients with DPOC and controls in order to identify risks factors for the development and progression of COPD and ultimately to promote health.

3.1.1. Rationale for this study

One of the most important sources of indoor contaminants is house dust. This complex matrix that includes both organic and inorganic materials such as animal fibers, pollen, clay, fungi, amongst other residues, behaves as a concentrator and repository of many persistent and toxic chemicals. The long residence times and the elevated concentrations of contaminants in dust increases the chances of exposure by 1000-fold when compared to outdoor environments. In order to be available for humans to ingest then, dust particles must be small enough to adhere to hands, however the precise size of dust particles that in fact adhere to the surface of hands and might therefore be ingested trough hand to mouth behavior is still not consensual. Several reports have addressed this topic and whilst most of the available literature quantifies the levels of contaminants in the 500um fraction, several authors and regulatory agencies suggest that only the fraction <250um adheres to hands. Recently this size was revised and at the moment recommendations set the 63um fraction as the most appropriate one to evaluate the risk of exposure to harmful chemicals trough dust. In order to compare both fractions we quantified the levels of OTs in the 500um fraction (the fraction generally used for similar studies) and also in the 63um fraction. The obtained results will allow to select the most suitable fraction of dust to be used in future studies.

3.2. Aims

- To compare the levels of organotins in different fractions of house dust samples (500 and 63um fractions).
- To evaluate the levels of organotin compounds in dust samples from houses of patients with respiratory diseases and controls.

3.3 Procedure

Monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), diphenyltin (DPT), triphenyltin (TPT), monooctyltin (MOT) and dioctyltin (DOT) levels were quantified following the protocol described by us with slight modifications. In short, deuterated labeled standards (d_9-MBT, d_{18}-DBT, d_{27}-TBT, d_{10}-DPT, d_{15}-TPT, d_{17}-MOT, d_{34}-DOT) were spiked into the samples (about 0.3 g of dust), as surrogates, before extraction. OTs in the samples were extracted by 1N HBr/methanol-ethyl acetate (1:1) and then transferred into ethylacetate/hexane (3:2) and concentrated by rotary evaporation. They were them ethylated by adding 1mL of 5% tetraethyl sodium borate and afterwards the extracts were cleaned up by SEP-PAK plus florisil cartridge (Waters). OTs were eluted by 5% diethyl ether/hexane and then solutions were concentrated into 1 mL and spiked with 50 ng of deuterated tetrabutyltin used as a recovery standard. The final solutions were injected into a gas chromatograph-mass spectrometric detector (GC-MSD) (Hewlett-Packard 6870 GC system with 5973 mass selective detector and 7683 series auto sampler). OTs were measured by GC-MSD in selected ion monitoring mode (EI-SIM) and quantified by isotope dilution method.

3.4. Results

3.4.1. Organotin levels in 500um dust fraction

Organotins, quantified by GC-MS, were detected in all analyzed samples (n=27) with highly variable values ranging from 570 to 6100 ng Sn.g^{-1} dry weight (dw) [Fig. 1]. Butyltin compounds (BTs) accounted for 57±18% of the total organotin (∑OTs) levels detected in dust. Both MBT and DBT were detected in all the analyzed samples with values ranging from 170 to 3000 and from 26 to 850 ng Sn.g^{-1} dw, respectively. TBT was detected in 81.5% of the samples with values ranging from 1.4 to 870 ng Sn.g^{-1} dw. Octyltin compounds (OcTs) were also detected in all the samples analyzed, with values ranging between 120 and 2300 ng Sn.g^{-1} dw for MOT and between 38 and 1100 ng Sn.g^{-1} dw for DOT. Contrary to butyltins and octyltins; phenyltins (diphenyltin-DPT and triphenyltin-TPT) where not detected in any of the samples.

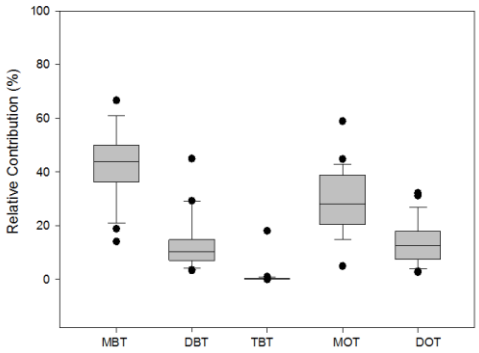

Figure 1. Relative contributions (%) of each individual compound to total organotin levels (Sum OTs) detected in 500um fraction dust samples. .

3.4.1. Comparison of organotin levels between the 500um and 63um dust fraction

Contrary to what was expected the fine fraction didn't always exhibit highest levels when comparing with the 500um fraction. Table 1 depicts the differences observed between the two fractions of the same sample.

Sample	MBT		DBT		TBT		MOT		DOT		DPT		TPT	
code	500um	63 um	500um	63 um	500um	63 um	500um	63 um	500um	63 um	500um	63 um	500um	63 um
1	680	1500	101	262	4.5	5.1	617	1672	486	1009	<10	0.0	<1.0	0
4	1526	970	300	182	6.5	1.5	947	772	545	344	<10	0.0	<1.0	0
5	168	480	91	188	<1.0	1.5	345	774	290	655	<10	0.0	<1.0	0
7	1582	4300	608	705	10	7.0	123	319	72	114	<10	0.0	<1.0	0
9	1770	1200	221	137	10	3.6	666	500	419	184	<10	0.0	<1.0	0
10	692	730	271	162	<1.0	1.1	1169	820	1012	297	<10	0.0	<1.0	0
11	1042	1300	142	173	5.7	9.2	862	1054	191	152	<10	0.0	<1.0	0
12	2181	2000	548	244	14	24.0	677	1300	284	569	<10	0.0	<1.0	0
13	524	1200	126	156	12	0.8	296	809	191	532	<10	0.0	<1.0	0
16	399	4000	60	819	<1.0	14.0	344	1014	188	321	<10	0.0	<1.0	0
18	1108	3900	601	1290	2.4	2.4	669	2208	341	1263	<10	3.0	<1.0	11
19	210	670	26	52	<1.0	0.0	263	631	81	186	<10	0.0	<1.0	0
20	415	2000	140	229	2.0	0.0	353	1127	99	224	<10	1.7	<1.0	0
21	848	3200	548	1703	5.0	3.4	394	1121	152	355	<10	0.0	<1.0	0

3.5. Future perspectives

The obtained results will be discussed under the scope of risk assessment and a research paper will be prepared and submitted to an international Journal. We also intend to present this work at an international conference. Under the framework this project dust samples were already analyzed, however in order to provide the global contamination scenario matched blood samples should also be analyzed. Hence we intend to analyze all matched blood samples and also to increase the number of dust samples in the future.

3.6. Achievements[1]

3.6.1. List of papers[2] published:

Sousa ACA*, Coelho SD, Pastorinho MR, Taborda-Barata L, Nogueira AJA, Isobe T, Kunisue T, Takahashi S, Tanabe S (2017) Levels of TBT and other selected organotin compounds in duplicate diet samples. Science of the Total Environment 574: 19-23; **IF 2015: 4.317, Q1;** http://dx.doi.org/10.1016/j.scitotenv.2016.09.037;

Coelho SD, Maricoto T, Pastorinho MR, Itai T, Isobe T, Kunisue T, Tanabe S, Sousa ACA*, Nogueira AJA (2017) Cadmium intake in women from Aveiro University, Portugal – a duplicate diet study. Journal of Geochemical Exploration; (accepted) http://dx.doi.org/10.1016/j.gexplo.2017.02.003; **IF 2015: 2.749, Q1**

Coelho SD, Sousa ACA*, Isobe T, Kim J-W, Kunisue T, Nogueira AJA, Tanabe S (2016) Brominated, chlorinated and phosphate organic contaminants in house dust from Portugal. Science of the Total Environment 569–570: 442-449; http://dx.doi.org/10.1016/j.scitotenv.2016.06.137; **IF 2015: 4.317, Q1**

Coelho SD, M. Ramiro Pastorinho, Takaaki Itai, Isobe T, Kunisue T, Nogueira AJA, Tanabe S, Sousa ACA* (2016) Lead in duplicate diet samples from an academic community. Science of the Total Environment 573: 603-607; http://dx.doi.org/10.1016/j.scitotenv.2016.08.133; **IF 2015: 4.317, Q1**

*corresponding author.

3.6.2. List of oral presentations in seminars/ symposiums

Sousa ACA, Coelho SD, Nogueira AJA, Taborda-Barata L, Tanabe S, Pastorinho MR (2016) Human exposure to obesogens: Tributyltin levels in duplicate diet and house dust samples. I Simpósio Diabesidade e Fertilidade, 22-23 setembro, Porto, Portugal, p. 46

3.6.2. List of posters in international conferences

Sousa ACA, Isobe T, Coelho SD, Pastorinho MR, Taborda-Barata L, Nogueira AJA, Kunisue T, Takahashi S, Tanabe S (2016) Levels of the obesogen tributyltin and other selected organotin compounds in house dust and duplicate diet samples from Portugal. 5th Conference on Prenatal Programming and Toxicity (PPTOXV), 13-16 November, Kitakyushu, Japan.

[1] With acknowledgment to LaMer funding. All the files can be accessed and downloaded through this folder: https://drive.google.com/open?id=0B7sGfjDz5sziVjVzRXRtdDBBRHM

[2] The impact factor™ (IF) corresponds to 2015 Thomson Reuters metric "5-year impact factor". Quartile values obtained from http://www.scimagojr.com/.