Short Symposium:
Indoor pollutants

8 September 2023, Institute for Medical Research and Occupational Health, Zagreb, Croatia

Organised by

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and
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HrZZ-UIP-2017-05-6713

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PROGRAMME

9:00-9:30	**Introduction and registration**
9:30-9:45	**Darija Klinčić** Presenting the results of the five-year work within the installation research project DeValApp
9:45-10:05	**Karla Jagić** Polybrominated diphenyl ethers in dust from households and human milk as a bioindicator of exposure
10:05-10:25	**Marija Dvoršćak** Getting out of the given framework – the realization of ideas created during the implementation of the DeValApp project
10:25-10:45	**Antonio Zandona** The influence of PBDEs present in house dust and human breast milk on human cells
10:45-11:05	**Ivana Jakovljević** Polycyclic aromatic hydrocarbons – levels, pollution sources, and risk assessment
11:05-11:25	**Coffee break**
11:25-11:45	**Zorana Kljaković-Gašpić** The impact of long-term mining on the pollution of the Raša River with metal(oids): the eel as a biological indicator
11:45-12:05	**Blanka Tariba Lovaković** Exposure to elements from dust – from home to kindergarten (by car)
12:05-12:25	**Jelena Živančev** Human and material resources for environmental contaminant analysis – scientific achievements of the Lab for Chromatographic Analysis of the Novi Sad Faculty of Technology
12:25-12:45	**Nataša Đurišić-Mladenović** Horizon Europe Twinning project as a way of reinforcing capacities towards excellence in analysis of contaminants of emerging concern – TwiNSol-CECs project
12:45-12:55	**Dubravka Rašić** Meet the Toxicity – Live Safely – science in service of the public
12:55-13:05	**Darija Klinčić** Look ahead
13:05	**Lunch break and discussion**

Short Symposium:

Indoor Contaminants

On September 8th, 2023, a Short Symposium entitled "Indoor contaminants" was held at the Institute for Medical Research and Occupational Health in Zagreb. The Symposium's main goal was to share and discuss findings from the project HrZZ-UIP-2017-05-6713 DeValApp. A total of 50 people attended the Symposium, including PhD students and postdoctoral researchers, as well as seasoned scientists who are each experts in their specific fields.

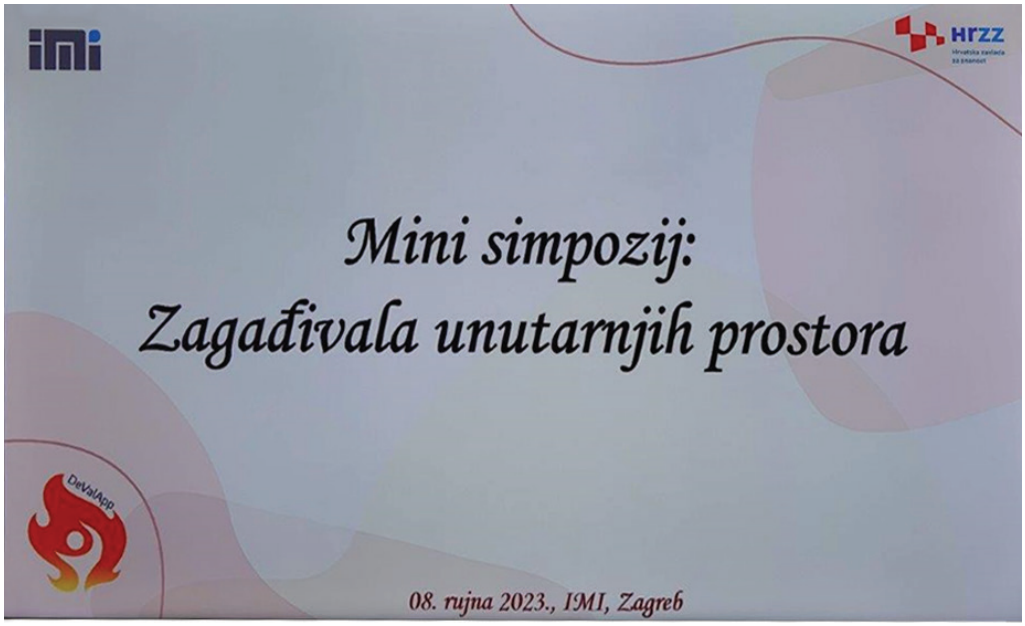
The scientific programme of this Short Symposium was divided into two sessions where the outcomes of the five-year research project were presented. These findings included concepts developed during project implementation that covered novel research areas and opened up opportunities for collaboration with other research groups. Also, participants had the opportunity for a fruitful discussion which opened up new possibilities for further investigations into various environmental and human samples.

We were very pleased to host eminent scientists Dr Jelena Živančev and Dr Nataša Đurišić-Mladenović from the University of Novi Sad, Faculty of Technology, Novi Sad, Serbia, delivering inspiring lectures presenting Horizon Europe Twinning project (TwiNSol-CECs) as well as their experience in areas of environmental protection and monitoring different classes of contaminants.

The Indoor Contaminants Short Symposium was an enriching scientific experience for us. We express our gratitude to all of the presenters and participants for sharing their research, thereby improving the excellence of the Short Symposium. We also express our gratitude to the Croatian Science Foundation and the Institute for Medical Research and Occupational Health for their support that was crucial to the overall success of our Short Symposium.

Organisers of the Short Symposium
HrZZ project DeValApp

THE SYMPOSIUM IN PICTURES

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ABSTRACTS

Presenting the results of the five-year work within the installation research project DeValApp

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Project "Development, validation and application of analytical methods for PBDE determination" started in 2018 as part of the "Installation Research Projects" programme financed by the Croatian Science Foundation with the main goal of establishing a new research group of young scientists that will engage in innovative research topics. The purpose of the project was to develop and validate analytical methods for determining specific polybrominated diphenyl ether (PBDE) congeners in complex samples of house dust and human milk. Results on PBDE levels and distribution in the obtained samples are the first of their kind in Croatia and were further used to assess the exposure of the general population to PBDEs. The project team successfully completed all of the tasks set in the work plan, and in collaboration with other research groups, further expanded on its research. Until now, project results were published in 11 scientific papers (9 of which were published in journals placed in the first quartile of the category according to Journal Citation Reports), 20 symposium abstracts, 1 doctoral thesis, 1 diploma thesis, and 1 leaflet intended for the general population. During the course of the project, team members participated in several dozen specialized trainings to obtain new knowledge and skills, but they also transferred their knowledge by holding student internships, presenting project results and by bringing science closer to the wider population, including the youngest (children in kindergartens and elementary schools). The Croatian Academy of Sciences and Arts twice supported this project with their grants, and two rewards from the Institute for Medical Research and Occupational Health for publications were received by team members. The presented results show the success of this project, but its greatest value is the research group and the scientific foundation that was created, which enables further research to contribute to general knowledge about pollutants in our environment and their impact on human health.

Acknowledgements: This study was supported by the Croatian Science Foundation project UIP-2017-05-6713

Polybrominated diphenyl ethers in dust from households and human milk as a bioindicator of exposure

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Polybrominated diphenyl ethers (PBDEs) are a class of persistent organic compounds used as flame retardants. They are typical indoor pollutants that accumulate in house dust, the ingestion of which is the most important route of exposure for humans besides diet. The exception are breastfed infants, the most sensitive population group, for whom breast milk is the dominant exposure pathway to PBDEs. In this work, microwave-assisted extraction was used for the analysis of seven PBDEs in house dust and, for the first time, in human milk, with carefully optimized extraction conditions and extract clean-up. Purified extracts were analysed by gas chromatography coupled to an electron capture detector, and in the case of human milk samples, due to low PBDE levels, confirmatory analyses were performed using a gas chromatograph coupled to a tandem mass spectrometer operating in electron impact mode. Thirty matched samples of human milk from women living in Zagreb and its surroundings and house dust from their households were analysed. The median sum of detected PBDE congeners (∑PBDEs) in household dust samples was 4.9 ng/g dust in positive samples, while in human milk it was lower, 0.49 ng/g lipid weight. The determined levels were similar to the rest of Europe and among the lowest in the world. Statistics showed that mothers were the most exposed to the penta formulation in their households, which was mainly used as an additive in polyurethane foam and textiles. Overall, the estimated daily intakes of PBDEs suggested that they do not pose a health risk to infants and mothers in the studied area.

Acknowledgements: this study was supported by the Croatian Science Foundation project UIP-2017-05-6713.

Getting out of the given framework – the realization of ideas created during the implementation of the DeValApp project

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People spend a significant portion of their day in different indoor microenvironments besides their homes where they may also be exposed to polybrominated diphenyl ethers (PBDEs) accumulated in indoor dust. In order to obtain most accurate data on human exposure to PBDEs from dust, samples from different types of microenvironments were collected (cars, working places, kindergartens, and schools). The widest range of the sum of mass fractions of detected PBDE congeners (∑PBDE) was observed for dust samples collected from different working places (from <LOD to 313.75 ng/g dust), which is explained by the fact that the dust was collected in facilities where various activities are performed, from metal machining companies, through various service activities, to computer repairing sites. The narrowest range of ∑PBDE was obtained for kindergartens (3.11 – 14.42 ng/g) which indicated a uniform intake of PBDEs in that type of microenvironment. The most interesting results were obtained for car dust samples. When divided into three groups according to the year of manufacture of the car, the median of the detected ∑PBDEs was 316.53, 10.16, and 6.73 ng/g for cars manufactured between 1989-2000, 2001-2010, and 2011-2020, respectively. This was in agreement with the ban of penta and octa commercial PBDE mixtures use by the European Union in 2004. Human exposure via dust ingestion was assessed for three population groups, namely adults, school children and young children (1 to 6 years old). The amount of time spent in a specific indoor space, including the household, was taken into consideration for each population group. Our results confirmed that young children were the most affected population group. Nevertheless, all of the calculated EDI values when compared to the corresponding threshold reference value suggested by the USEPA were orders of magnitude lower, indicating no health risk from overall exposure to PBDEs contained in dust. In cooperation with the colleagues from several units of the Institute for Medical Research and Occupational Health, the presence of PBDEs in one aquatic and one terrestrial animal species was investigated. The European eels (*Anguilla anguilla*) were selected as a bioindicator for the aquatic environment due to their specific life cycle, while the obtained data on PBDEs in brown bear (*Ursus arctos*) fat tissue samples are one of the few of its kind in the world. Very low PBDE levels were detected in both sample sets, which was in accordance with our expectations, considering that eels were sampled in locations characterised by low population density and no industry, and brown bears generally live in the wild, far from human influence.

Acknowledgements: This study was supported by the Croatian Science Foundation project UIP-2017-05-6713.

The influence of PBDEs present in house dust and human breast milk on human cells

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In this study, we assessed the detrimental impact of eight polybrominated diphenyl ethers (PBDEs) congeners (BDE-28, -47, -99, -100, -153, -154, -183, and -209) on human cells. These persistent organic pollutants found in environmental and biological matrices present a risk for human health, so all aspects of their effects should be evaluated. As our results indicate, exposure to these PBDEs in lung epithelial cells (A549) led to membrane disruption, oxidative stress via reactive oxygen species (ROS) formation, and diminished mitochondrial membrane potential. In particular, select PBDEs activated apoptotic markers. Time-dependent toxicity was unveiled for certain congeners, indicating enduring negative effects even at lower concentrations. This toxicity was substantiated using actual house dust sample extracts, reflecting sustained exposure to PBDE congeners through varied pathways. Furthermore, we extended our study to the synergistic effects of PBDEs and polychlorinated biphenyls (PCBs), also persistent organic pollutants. Here, human neuroblastoma (SH-SY5Y) and kidney (HEK293) cells were exposed to ΣPBDE and ΣPCB mixtures, simulating the concentrations detected in human milk. A combination of these pollutants resulted in a statistically significant viability reduction in both cell types, implying potential synergistic influence on the cellular homeostasis. The findings underscore the urgency of comprehensive research into the intricate mechanisms of PBDE/PCB-induced toxicity and synergistic effects, given the bioaccumulative nature of these pollutants, to understand the impacts on human health.

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Polycyclic aromatic hydrocarbons – levels, pollution sources, and risk assessment

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Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic compounds with two or more benzene rings present in both outdoor and indoor air. PAHs have mostly been studied in ambient air, while studies on indoor dust are very rare, especially for Croatia. Since people spend 60 % or more of their time indoors, they are inevitably exposed to indoor pollutants coming from building materials, electronics, toys, furniture, carpets, paints, household chemicals, and domestic combustion activities (e.g., cooking, heating, smoking). The aim of this study was to determine the mass fractions of eleven PAHs in dust samples collected from different microenvironments including workplaces, kindergartens, households, and car interiors, and estimate potential risks for human health. Approximately 0.5 g of each dust sample was extracted by accelerated solvent extraction (ASE). Extracts were evaporated to dryness and resolved in acetonitrile. A total of 11 PAHs were determined by high-performance liquid chromatography (HPLC) with a fluorescence detector. Mass fractions of ΣPAH were detected in the range from 92.9 to 1504.1 ng/g, 244.9 to 833.0 ng/g, 230.5 to 5632.7 ng/g, and 395.6 to 12114.8 ng/g in households, kindergartens, workplaces, and car dust, respectively. Results of diagnostic ratios and factor analysis showed traffic and biomass combustion as dominant sources of PAHs in all dust samples except for car dust, where analysis showed only one dominant source, traffic/road dust. This study demonstrates that exposure of people to contaminants present in indoor dust can have adverse effects on human health. The highest PAH concentrations were measured in car dust and in such cases of high concentrations or prolonged exposure, human health may be at risk.

Acknowledgements: This study was supported by the Croatian Science Foundation project UIP-2017-05-6713.

The impact of long-term mining on the pollution of the Raša River with metal(oids): the eel as a biological indicator

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For generations, coal mining and associated activities, as well as the use of coal in thermal power plants and households, have polluted the region of the Labin basin (Istria, Croatia), which is bounded in the west by the Raša River valley. Despite the significant deposition of coal and other terrigenous material containing inorganic elements in the Raša Bay and the Raša River estuary, which serve as a unique natural habitat for several animal species, there has been a lack of research on the elemental composition of the biota in the upper and middle reaches of the Raša River. This study examined the impact of anthropogenic activities on the aquatic environment of the Raša River using the yellow European eel (*Anguilla anguilla* L.) as a biological indicator of pollution at two sites: upstream near the Podpićan settlement (site 1) and 6 km upstream of the Raša River estuary (site 2). The data obtained for 22 major and trace elements in muscle and liver of eels indicated a generally low contamination status in the surrounding area. With the exception of the downstream site, where only a select few elements (Cd, Cu, Fe, Na, Se, U, V, and Zn) accumulated in the liver with fish growth, the majority of metal(loid)s decreased or remained constant as the size of the fish increased in both the muscle and the liver of the fish. The spatial analysis revealed a higher concentration of Ag, Cd, Cr, Mo, Tl, U, and V in the downstream location, indicating that the historical coal mining industry may have had a limited impact on the lower Raša River reaches. Nonetheless, the overall concentrations of metal(oid)s in eels were low, representing the lower end of the concentration ranges reported in literature for other European regions, indicating good status of the Raša River ecosystem regarding inorganic elements. In addition, our results confirmed that European eels in their yellow phase are an effective biomonitoring tool for detecting chemical contamination in the environment.

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Exposure to elements from dust – from home to kindergarten (by car)

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Given the large amount of time people spend indoors, exposure to indoor contaminants causes increasing public health concerns. Indoor dust is a heterogeneous and complex mixture of organic and inorganic particles and may present an important source of daily exposure to toxic elements. Children are the most vulnerable population group for adverse health effects that toxic elements may induce due to their extensive interactions to floor and dusty surfaces as well as hand-to-mouth activities typical for that age. This work presents the levels of 18 elements measured in dust samples collected from 68 households, 10 kindergartens and 21 cars from the area of Zagreb, Croatia. Based on the obtained data, the health risks from overall daily exposure to trace elements was assessed for children aged 2–6 years considering three pathways of dust intake – ingestion, dermal absorption and inhalation, and two exposure scenarios – central and worst-case scenario. Based on the obtained data from dust analysis and the questionnaire on the house characteristics and habits of the residents, possible indoor sources of elements were also evaluated. The median concentration of most elements was significantly higher in dust obtained from cars compared to households and kindergartens, especially in the cases of Co, Cr, Cu, and Mo. The house age and house area were identified as the most important contributors for most trace elements. Oral intake was identified as the most important exposure pathway, except for Cr, Ni, and Sb where dermal contact was the main route of exposure. Health risk assessment considering dust ingestion, inhalation, and dermal absorption of analysed dust indicated that no adverse effects are expected for young children from overall daily exposure to trace elements in the Zagreb area.

Acknowledgements: This study was supported by the Croatian Science Foundation UIP-2017-05-6713 and the Ministry of Science and Education of the Republic of Croatia (through Institutional Funding made available to the Institute for Medical Research and Occupational Health).

Horizon Europe Twinning project as a way of reinforcing capacities towards excellence in analysis of contaminants of emerging concern – TwiNSol-CECs project

Nataša Đurišić-Mladenović*, Jelena Živančev, Igor Antić, Biljana Pajin, Nikola Maravić, and Zita Šereš

University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

The European Commission recognized the need for reinforcing the existing research capacities of less developed countries, so-called Widening Countries, in order to connect these research "pockets" to top leading research institutions from different Member States or Associated Countries and harmonize the R&D systems across the continent making it more competitive within the global research landscape. To address this need, there emergerd the horizontal component "Widening Participation and Strengthening the European Research Area" within the Horizon Europe (HE) programme and the so-called twinning calls for projects through which it is expected that coordinating institutions from Widening Countries collaborate with at least two advanced partners in the form of joint research, new research endeavours, transfer of advanced knowledge, and experience in research management and administration. The main objective of the HE twinning project called TwiNSol-CECs is to raise the scientific and innovation excellence of the Faculty of Technology, Novi Sad (FoT NS) in various aspects of research on contaminants of emerging contaminants (CECs) integrated in broader EU networks of excellence, with two prestigious partners from the EU, CSIC-IDAEA, Barcelona and NOVA School of Science and Technology, Lisbon. The expected outcome of TwiNSol-CECs is to contribute to national and regional scientific and economic growth and well-being and the harmonization of advanced research and innovation efforts important for the overall faster and sustainable transition of Europe foreseen by the European Green Deal (EGD) towards zero-pollution and a toxic free environment. The presentation gives insight into the gaps within the field of CEC research, and how TwiNSol-CECs is going to contribute to narrowing the observed gaps by reinforcing the surveillance of CECs in environmental samples in Serbia.

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SYMPOSIUM ABSTRACTS
Arh Hig Rada Toksikol 2023;74:A37-A50

Human and material resources for environmental contaminant analysis – scientific achievements of the Lab for Chromatographic Analysis of the Novi Sad Faculty of Technology

Jelena Živančev*, Igor Antić, Maja Buljovčić, Dušan Rakić, and Nataša Đurišić-Mladenović

University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

The Faculty of Technology (FoT NS) is the leading entity within the University of Novi Sad, with the highest number of published papers (0.93) in international journals (IJs) per researcher in 2020. Located in Novi Sad, the administrative center of the Vojvodina Province, known as the main Serbian area for agriculture, food, chemical, and oil-petrochemical industries, FoT NS positioned itself as a confident partner for numerous industry players, innovations, and technical solutions in these domains. Furthermore, FoT NS as a modern academic institution has coordinated numerous domestic and international projects in the fields of basic sciences and technological innovations. Thus, FoT NS has immense experience in project management, training development and lecturing, active interaction with different research stakeholders, being an important part of the wider EU research network. Team members of the Lab for Chromatographic Analysis of the Faculty of Technology, Novi Sad belong to the leading research group at FoT NS in the domain of the environmental protection and monitoring of different classes of contaminants in environmental samples and food, including persistent organic pollutants, heavy elements, and contaminants of emerging concern (CECs). The Lab for Chromatographic Analysis of the Faculty of Technology, Novi Sad is equipped with highly sophisticated instruments such as GC-MS, UHPLC-QqQ-MS, and UHPLC-Orbitrap-HRMS, as well as sample preparation equipment, which are ideal analytical combinations for the determination of regulated and unregulated, new classes of contaminants, as well as for target and suspect surveillance of the known and unknown groups of CECs and their metabolites in very complex samples. Additionally, an advanced software tool – Compound Discoverer Software installed on a high-performance PC for high-resolution mass spectrometric data processing, upgrades the capabilities of the UHPLC-Orbitrap-HRMS for a wide screening of contaminants, including suspected and nontargeted analysis. The team members of the Lab for Chromatographic Analysis comprise two senior researchers with significant experience in the domain of environmental pollution, contaminants monitoring, risk assessment, and instrumental analysis of inorganic and organic micropollutants, also with an important background in multi-partner international and national research projects on development of analytical methods for determination of environmental contaminants, risk assessment, food safety, etc., also having experience in knowledge transfer activities towards different target groups (students, pupils, teachers, academic staff, stakeholders); two early stage researchers, highly trained in GC-MS, UHPLC-QqQ-MS, and UHPLC-Orbitrap-HRMS analysis, also with experience in the multi-partner international and national project, and one young researcher.

Acknowledgements: Funded by the European Union. The views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or EU executive agency. Neither the European Union nor the granting authority can be held responsible for them. This work is conducted under the project TwiNSol-CECs that has received funding from Horizon Europe programme under grant agreement no. 101059867.

Meet the Toxicity – Live Safely – science in service of the public

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Meet the Toxicity – Live Safely (MeeTox) is a project funded by the Erasmus+ Small Partnerships Program for Adult Education, with the Serbian Society of Toxicology and Prof Danijela Đukić Ćosić, PhD leading the project. The other partners on the project are the Croatian Society of Toxicology, the Faculty of Pharmacy at Belgrade University, and the Institute for Medical Research and Occupational Health in Zagreb, i.e. scientists from Croatia and Serbia involved in different fields of toxicology. The project's duration is two years, starting on September 1, 2022. The aim of the project is to examine the understanding of toxicity in the general public, assess the need for education on toxicity and educate the general public about basic toxicology principles in order to improve the safety of everyday interactions with chemicals and further public health. The basis for the creation of educational materials will be toxicity knowledge surveys completed by the general population. The final goal is to prepare education materials on different toxicology topics available to the public for self-learning, including a booklet with information about common toxic substances widely distributed in the environment. A large group of ubiquitous environmental contaminants are persistent organic pollutants, among which polybrominated diphenyl ethers stand out as indoor pollutants. Since they are accumulated in fatty tissue, and eliminated from the body within human milk, it is important to study their toxic effects, and to inform the public about their prevalence and sources of exposure. The results of the project will be disseminated to the scientific community, but also to the general public, and employees in healthcare and education. The purpose of presenting scientific results to the public is to strengthen media literacy and prevent the spread of misinformation.

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Look ahead

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In the near future, at least four scientific papers that are currently under peer review should be published, reporting the project's results. The analytical methods developed during the project's implementation will be applied for further investigation of various environmental and human samples. To secure funding for future research, the project team recently submitted a project proposal entitled "Flame retardants in children's toys: determination of their levels and possible endocrine disrupting properties". In the coming period, the goal is to develop methods for the analysis of currently used flame retardants that have replaced the banned PBDEs and assess their impact on human health. At the end of the Symposium, the project leader Darija Klinčić, PhD expressed her gratitude to the research team, all collaborators and colleagues from the Institute, and other institutions who in any way helped achieve the project's goals. A special thanks goes to everyone who donated dust and/or milk samples or helped in the recruitment of donors.

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