

was moderately decreased at the highest concentrations (2.5 and 5 µg/mL) of experimental alkylphenols (24 h) with significant ($P < 0.001$) disruption at 5 µg/mL of 4-n-octylphenol after 48 h. Analyses of intracellular communication revealed that a prolonged exposure period of 48 h caused significantly ($P < 0.01$; $P < 0.001$) inhibition at 2.5 or 5 µg/mL of 4-t-nonylphenol and 4-n-octylphenol. Taken together, the results of our in vitro study reported herein are consistent with the conclusion that selected alkylphenols are able to affect cellular parameters in time- and dose-dependency. However, more detailed and systematic research in reproductive toxicology is definitely required for a better understanding of risks associated with endocrine disruption in humans and wildlife.

Keywords: nonylphenol, octylphenol, Leydig cells, viability, membrane integrity, intracellular communication.

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P07-13

Microplastics in mussels (*Mytilus galloprovincialis*) farmed in Emilia-Romagna (North-Adriatic Sea)

S. Rubini¹, E. Baldini², M. Munari¹, F. Lo Chiano¹, L. Fiorentini³, B. Romano¹, M. Baruffaldi¹, P. Bonilauri⁴, M. Mistrì⁵, S. Manfredini², S. Vertuani²

¹Experimental Zooprophyllactic Institute of Lombardy and Emilia Romagna, Sede territoriale di Ferrara, Ferrara, Italy;

²University of Ferrara, Department of Life Sciences and Biotechnology, Master Course in Cosmetic Sciences, Ferrara, Italy;

³Experimental Zooprophyllactic Institute of Lombardy and Emilia Romagna, Sede territoriale di Forlì, Forlì, Italy;

⁴Experimental Zooprophyllactic Institute of Lombardy and Emilia Romagna, Sede territoriale di Reggio Emilia, Reggio Emilia, Italy;

⁵University of Ferrara, Department of Chemical, Pharmaceutical and Agricultural Sciences – DOCPAS, Ferrara, Germany

Purpose: The marine environment has become sinks for large quantities of anthropogenic marine debris. Plastic represents the most abundant source, accounting for 60–95% [1] of marine litter. Plastic marine pollution includes microplastics (0.1 – <5,000 mm) [2]. Microplastics (MPs) include primary and secondary ones. The first ones were intentionally created of small dimensions and next introduced in the environment. On the contrary, secondary MPs derive from degraded macroplastics, fragmented because of physical and chemical stress factors. MPs can occupy all the water columns accumulated at different depths in order of their density. They can be a vehicle for other contaminants or bacteria, creating a dangerous surface layer. The aim of the project was to analyze the presence of MPs in locally farmed mussels. North Adriatic represents a site with a high risk of plastic contamination due to increasing touristic pressure during summer and intense fishing activity. Mussels are perfect bioindicators to detect MPs, as filter feeders MPs can be ingested very easily. In fact, several MPs were founded in those mollusks, that can have a dramatic impact both for human and sea life as it is largely demonstrated that MPs are bioaccumulated and biomagnified inside the marine food chain.

Methods: A total of 101 samples were collected from different breeding sites. Each sample was processed, extracting 100 g of flesh from those mussels and homogenizing them. MPs were determined in only 1 g of homogenate. A 10% potassium hydroxide (KOH) solution was added to digest tissue in the shortest time without damaging the main plastic polymers [3]. After it, samples were filtrated on glass

fibers filters (1 µm pores). Finally, those membranes with retained materials were microscopically observed. As a control to confirm and identify the chemical composition of polymers, particles were analyzed by Raman spectroscopy.

Results: The analyses have detected Mps in 11 samples (10.89%). The number of identified Mps was 1 items/g of homogenate. The most frequent types of MPs were fragments (63.6%) followed by fibers (36.3%). Furthermore, the identified colors were: blue (45.4%), green (45.4%) and transparent (9.0%), and their sizes were between 32–581 µm. The final Raman analyses associated different specters with the most probable chemical composition of those MPs, and it found polymers as Polystyrene (PS), Polypropylene (PP), and Polyvinylchloride (PVC).

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P07-14

Associations of contaminant metal(loid)s with reproductive hormones in the hair of European brown bear from Croatia and Poland

M. Lazarus¹, A. Sergiel², M. Ferenčaković³, S. Reljić⁴, T. Orct¹, L. Pađen⁴, T. Zwiżacz-Kozica⁵, F. Zieba⁵, N. Selva², Đ. Huber²

¹Institute for Medical Research and Occupational Health, Zagreb, Croatia;

²Institute of Nature Conservation of Polish Academy of Sciences, Krakow, Poland;

³Faculty of Agriculture, University of Zagreb, Zagreb, Croatia;

⁴Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia;

⁵Tatra National Park, Kuznice, Poland

The terrestrial habitat is contaminated with non-essential metal(loid)s (e.g. arsenic, cadmium, mercury, lead) known to have potential for endocrine toxicity, among others adverse health effects. Impairment of the hypothalamic-pituitary-gonadal (HPG) axis, including steroid hormone homeostasis, may heavily impact individuals and populations, while depleting reproduction, development and adaptation to environmental stress. Organic and inorganic (mercury) pollutant-related changes in reproductive hormones in ursids have thus far been investigated and reported only for polar bear populations. We investigated associations of hair arsenic, cadmium, mercury and lead with progesterone and testosterone levels, taking into account biological and sampling-related variables in two populations of free-living European brown bear (*Ursus arctos*), Dinara-Pindos (N = 46) and Carpathian (N = 27), captured during the 2010–2019 period. Metal(loid)s were quantified using inductively coupled plasma mass spectrometry (ICP-MS) and hormones were measured via enzyme immunoassay (EIA). The best among generalized linear models (GLM) constructed with the above predictor variables were selected comparing the Akaike Information Criterion (AIC) values. For progesterone in hair, the breeding period and body condition index (BCI), but not metal(loid)s were significant variables explaining 24% of variation. As expected, cubs and yearlings exhibited lower progesterone than subadult and adult bears in both the non-breeding (beta = 0.427, 85% confidence interval - CI: 0.207 to 0.646) and breeding period (beta = -0.295, CI: -0.407 to -0.183). Bears with higher BCI appeared to have lower progesterone in hair (beta = -0.062, CI: -0.100 to -0.025). Hair testosterone variation (30%) was explained by the hair

growth period (beta = 0.065, CI: 0.319 to 0.097; higher testosterone in growth phase compared to quiescent phase), Hg (beta = 0.036, CI: 0.006 to 0.065), interaction of age and Pb (beta = -0.948, CI: -1.54 to -0.360) and interaction of Pb and Cd (beta = 0.903, CI: 0.080 to 1.73). Our findings confirmed the well-known influence of biological variables on reproductive hormones, and pinpointed complex interaction of contaminant metal(loid)s with brown bear testosterone levels.

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Biometrics for the impact of acute air pollution on human peripheral immunity

A. Mohamed¹, S. Ohtonen¹, L. Giudice¹, A.-M. Schroderus², T. Závodná³, Z. Krejčík³, P. Rössner⁴, K. M. Kanninen¹, T. Kinnunen², J. Topinka³, A. Muala⁵, T. Sandström⁵, P. Korhonen¹, T. Malm¹

¹University of Eastern Finland, A.I.Virtanen Institute for Molecular Sciences, Kuopio, Finland;

²University of Eastern Finland, Department of Medicine, Unit of Clinical Medicine, Kuopio, Finland;

³The Institute for Experimental Medicine (IEM), Czech Academy of Sciences, Department of Genetic Toxicology and Epigenetics, Prague, Czech Republic;

⁴The Institute for Experimental Medicine (IEM), Czech Academy of Sciences, Department of Nanotoxicology and Molecular Epidemiology, Prague, Czech Republic;

⁵Umeå University, Department of Public Health and Clinical Medicine, Umeå, Sweden

Air pollution is an ever-increasing global problem. Undeniably, it is harmful to human health and a critical environmental health risk factor^{1,2}. Diesel exhaust (DE) is a common manifestation of air pollution. It belongs to Particulate Matter 2.5 (PM_{2.5})³. With a small diameter of the ultrafine and nanoparticles of the DE (< 2.5 µm), its penetrative power extends to deposit deeply in lungs, pass to the blood stream and the blood brain barrier as well as damaging DNA^{3,4,5,6,7}. Currently, a growing body of evidence from epidemiological and controlled animal studies show that exposure to air pollutant increases the prevalence of multiple diseases with significant impact on health economy such as exacerbations in respiratory and cardiovascular diseases triggering hypertension, asthma, ischemia, coronary artery thrombosis, myocardial infarctions and even aging^{3,5,7,8,9,10}. Yet little is known on the impact of diesel exhaust on blood transcriptomic profile. Biomarkers for air pollution risk prediction do not currently exist, thus hindering the identification and disease predictions of individuals at risk for harmful air pollution effects.

Here, we aim to reveal mechanistic effects of air pollution on blood cell signatures and profiles and aim to discover novel biomarkers for air pollution prediction. We use a unique cohort of blood samples from acute in-vivo exposure of healthy human volunteers to clean air and DE³. Briefly, healthy volunteers were placed on an exposure chamber for one hour with moderate exercise. Blood samples were obtained for prior to and 18 hours after the exposure, then peripheral blood mononuclear cells (PBMCs) were isolated. Bulk RNA and miRNA-seq were performed. The preliminary analysis showed more than 190 RNAs and 450 miRNAs that are differentially expressed between pre- and post-DE exposed PBMCs¹¹. Ingenuity pathway analysis (IPA) and pathway network analysis on integrated RNA and miRNA data highlighted pathways related to phagosome, inflammation, interleukin signaling and nervous system^{12,13,14}.

The results will be further investigated in detail with BD Rhapsody targeted single cell RNA-seq platform to check how DE inhalation

exposure alters PBMC immunophenotypes and profiles. This study provides insights on how air pollution affects peripheral immunity, possibly providing mechanistic explanation on the increased risk of health effects.

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Genomic differences between hedgehog populations from southern Sweden in accordance with their pollutant load

N. Molbert¹, H. Ghanavi², M. Hansson¹

¹Lund University, Centre for Environmental and Climate science, Lund, Sweden;

²Lund University, Biology Department, Functional Zoology Unit, Lund, Sweden

Urban environments are under considerable anthropic pressures, including chemical exposure. While metals and legacy pollutants have been well documented, the fate and impact of emerging contaminants on urban wildlife have received little attention, especially on mammals. Incorporating genomic approach in ecotoxicological studies has the potential for unveiling the genome-wide response to environmental pollution. In this study, we therefore aim to characterize chemical exposure in wild European hedgehogs, *Erinaceus*