and their new replacement compounds di-isononyl phthalate (DINP) and its primary metabolite mono-isononyl phthalate (MINP) in human hepatic cell models. Specifically, we studied species-specific nuclear receptor activation and mitochondrial bioenergetic functions using the Seahorse XF analyzer. To shed light on wider systemic metabolic changes occurring as a response to phthalate exposure, we also analyzed lipids and metabolic parameters from C57BL/6J mouse tissue samples using histological staining and untargeted metabolomics approach.

Results: In cells, all the tested phthalates activated several nuclear receptors involved in metabolic regulation. Several parameters of mitochondrial bioenergetics were altered dose-dependently or with U-shaped dose response. The effects between parent compounds and their metabolites varied, indicating the importance of considering human metabolism in chemical testing. In the livers of C57BL/6J, the expression and distribution of several classes of lipids was altered by phthalate exposure.

Conclusion: Exposure to phthalates caused alterations in lipid metabolism and mitochondrial function *in vitro* and *in vivo*. These data shed light on cellular targets and functional effects of phthalates. More research is needed to study detailed processes behind adverse metabolic effects induced by common environmental contaminants.

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P11-04

In-depth xenobiotic metabolism characterization of human *in vitro* liver models for toxicology

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The liver is a main metabolic hub in the body, being responsible for central metabolic pathways, as well as xenobiotic metabolism. Having cellular systems able to mimic human hepatic metabolism is a priority in studying bioavailability and potential toxicity of drugs. Despite the existence of several hepatic cell models, many lack essential metabolic activities. We have established two novel human hepatocyte models: the human-induced pluripotent stem cells (hiPSc) derived into hepatocytes (HLCs, Boon et al 2020) and the metabolically matured HepG2 (mHepG2, Boon et al 2020), that we aim to characterize for their xenobiotic metabolism. Of relevance for toxicology, the cytochrome P450 (CYP) superfamily is responsible for the metabolism of most pharmaceutical drugs. In cases of toxicity through bioactivation, CYPs are often involved. This study aims to characterize the xenobiotic metabolism machinery of the hepatic liver models HepG2, mHepG2, HLCs and HepaRG by using transcriptomics, proteomics, and metabolomics. Moreover, multi-omics integration approaches were used to integrate three expression levels and compare in-vitro model's xenobiotic machinery. Our analyses have revealed that mHepG2 and HLCs at late stages of the differentiation significantly increase their expression, protein amount and metabolic activity of both phase I and phase II enzymes compared to HepG2 and earlier stages of HLCs differentiation respectively. Nevertheless, multi-omics integration reveals that transcript, protein and metabolite levels do not always correlate among the studied in-vitro models. A better metabolic characterization of *in vitro* models is an essential milestone for their potential use in drug development programs.

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P11-07

***In vitro* effects of polystyrene microplastics, alone or in combination with environmental pollutants, on viability and lipid content of a human hepatocarcinoma cell line**

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The pervasive water and soil pollution by microplastics (MPs) poses uncertain impacts on human health. Beyond their direct effects, research and discussions have focused on their role as vectors for other environmental pollutants into organisms. The concurrent presence of MPs and environmental pollutants may constitute potential hazards to both human health and the ecosystems. Therefore, to contribute to the understanding of this complex phenomenon, we assessed *in vitro*, on a human hepatocarcinoma cell line (HepG2), the acute toxicity and impact on lipid content of commercial 500 nm diameter polystyrene (PS) MPs. Additionally, we investigated their effects in the presence of commonly found environmental contaminants, such as bisphenol A (BPA) and cadmium (Cd), representing significant organic and heavy metal pollutants, respectively.

Physicochemical characterization of MPs were conducted using dynamic light scattering and electron microscopy, and we evaluated if the presence of Cd or BPA modifies the appearance or aggregation state of the MPs. MPs internalization was assessed by confocal microscopy.

After 24h exposure to MPs w/o each environmental pollutants, cell viability was evaluated by CellTiter-Glo® Luminescent Cell Viability Assay.

The lipid content, both basal and induced (by exposing cells to 24-hour incubation in serum-free MEM containing a 0.5 mM mixture of free fatty acids), was assessed by AdipoRed/NucBlue staining following a 24-hour exposure to MPs with and without various concentration of each of the pollutants. AdipoRed assay quantifies intracellular triglycerides, while the DNA content is estimated by NucBlue staining.

In our experimental model, after 24 hours of contact, HepG2 cells internalize to some extent the 500 nm PS MPs. Despite their internalization, they do not affect viability of HepG2 up to concentrations of 1000 μg/ml.

The toxicity of each tested contaminants was not modified by the presence of MPs and the Bliss independence model confirms that there is no synergistic effect. The MPs do not alter the basal lipid content nor the induced one, either alone or in conjunction with BPA. Conversely, when cells are exposed to MPs and Cd at concentrations of 1–5 microM, the induced lipid content significantly decreases after only 24 hours of incubation. However, this association has no effect on basal lipids. Therefore, our ongoing studies are focusing on lipid transport mechanisms that appear to be altered by the association between non-cytotoxic concentrations of Cd and PS MPs. If this were the case, we could presume that the association of PS MPs with an environmental contaminant alters the impact of MPs on human cells.

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P12 | Liver toxicology

P12-01
Moderate intake of beer improves nonalcoholic fatty liver disease (NAFLD) in a high fat diet (HFD)-induced mouse model

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Purpose: Both beer and some of its components, particularly polyphenols and iso-alpha-acids, have proven to be able to attenuate hepatic lipid accumulation or perturbed blood parameters in different rodent models through different putative mechanisms [1,2,3]. The current study was carried out within the NUTRATGE project (https://nutrage.it/) and aimed to evaluate the anti-steatotic capacity of beer in an HFD-induced NAFLD mouse model.

Methods: The beer was characterized for bioactive molecules content and individual phenolic compounds using UHPLC-ESI-MS/MS. In the *in vivo* study, forty-eight six-weeks-old male mice (C56BL/6) were randomly divided into four groups and supplemented daily during 10 weeks as follows: 1) normal diet (CTR); 2) a CTR diet and 0.14 ml/day beer (CTR+Beer); 3) a HFD (HFD); 4) a HFD and 0.14 ml/day beer (HFD+Beer). Prior to sacrifice, the weight of each animal was recorded, and blood was collected. We quantified liver lipids, performed histopathological evaluation using hematoxylin and eosin staining, and analyzed biomarkers of oxidative stress. Additionally, analysis of gene expression and DNA methylation of hepatic tissue was performed by RNA-Seq and Reduced Representation Bisulfite Sequencing.

Results: The beer displayed a good content in total phenols (25.01±1.27 mg GAE/100 ml), flavonoids (3.17±0.17 mg CE/100 ml) and flavonols (3.07±0.23 mg QE/100 ml). Among the single phenolic compounds, isoquercetin emerged as the predominant polyphenol (14.68±2.68 mg/100 ml). Compared to CTR, HFD group showed significantly higher levels of AST, ALT, TC, LDL-C, glucose, body weight and liver lipids, indicating the presence of steatosis, confirmed also by histological analysis. In HFD+beer group all the parameters returned to levels similar to those of CTR. All groups exhibited comparable levels of both protein carbonylation and lipid peroxidation in the liver, suggesting that our model represents an early stage of NAFLD with no oxidative stress. Analysis of transcriptomic and CpG methylation profile showed a clear separation between CTR and HFD groups. Beer consumption only partially affected gene expression whereas specifically changed the DNA methylation profile. RNA-Seq revealed 162 differentially expressed genes (DEGs) between CTR and HFD, whose biological function was related to cellular inflammatory processes and regulation of lipid metabolism. Beer consumption ameliorated the HDF effect (CTR*vs*HFD+beer, DEGs=43) showing alteration in the inflammatory response but not in the lipid homeostasis. RRBS profile identified 562 (CTR*vs*HFD), 429 (CTR*vs*HFD+beer), 469 (CTR*vs*CTR+beer) and 860 (HFD*vs*HFD+beer) differentially methylated cytosines (DMCs). DMCs target genes related to acyl glycerol and lipid biosynthetic process for CTR*vs*HFD+beer and insulin signaling for CTR*vs*CTR+beer comparisons. In summary, beer was capable to improve NAFLD likely due to the ability of polyphenols to modulate lipid metabolism.

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P12-02
effects of high-fat diet and streptozotocin-induced diabetes on CYP2E1 protein expression in rat liver

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The starting point of our study was the demonstration in various studies that CYP2E1 enzyme expression is affected by diabetes. As a toxicological concern, CYP2E1 is of interest because it metabolizes and activates a wide range of toxicologically significant compounds, including ethanol, carbon tetrachloride, acetaminophen, benzene, and halothane. Additionally, procarcinogens such as nitrosamines and azo compounds are among the substrates of CYP2E1 [1]. The metabolism of these compounds by CYP2E1 generates toxic intermediates and excessive levels of reactive oxygen species. As a consequence of its ability to produce reactive oxygen species at high levels, CYP2E1 has been linked to a wide range of pathological conditions, including diabetes, non-alcoholic steatohepatitis, and cancer [2]. All this information indicates that CYP2E1 is an important microsomal source of oxidative stress and lipid peroxidation [3]. For all of these reasons, our study examined the expression changes of CYP2E1 in liver tissues from Spraque-Dawley rats with type 2 diabetes caused by a high-fat diet combined with streptozotocin. On the other hand, we also highlight, for the first time, the effect of dapagliflozin, which is used to treat type 2 diabetes, on CYP2E1 expression. In our study, 32 maleSprague-Dawley rats were randomly divided into four groups: control, high-fat diet and streptozotocin-induced diabetes, dapagliflozin treated control, and dapagliflozin treated diabetes. In the microsomes obtained from the livers of these rats, the protein expression levels of CYP2E1 were determined by western blot. In our study, hepatic CYP2E1 expression level increased in control rats compared to the other three groups, but this increase is not statistically significant This result contrasts with previous studies reporting that hepatic CYP2E1 expression enhanced in diabetes [4]. Further research with a larger sample size is needed to clarify these conflicting results. From the result, hepatic CYP2E1 protein expression levels in the diabetic group treated with dapagliflozin were increased compared with in diabetic group. Although there was not statistically significant difference between two groups, this finding might indicate that increased CYP2E1 expression with the use of dapagliflozin under diabetic conditions may significantly affect impact