Antenatal depression, a prevalent complication of pregnancy, poses significant risks for both mother and the developing fetus when left untreated. Consequently, expecting mothers globally are often prescribed antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs). Reported by us and others, SSRIs have the potential to alter placental metabolic functions by affecting placental transporters and enzymes. However, thorough placental metabolic profiles of pregnant individuals using SSRIs are lacking. Therefore, we performed a comprehensive determination of the placental metabolome by non-targeted liquid chromatography-mass spectrometry (LC-MS) metabolomics approach, which allows elucidating metabolic pathway regulation and diverse exposures.

A total of 48 placental samples from individuals using SSRI medication throughout the pregnancy (n=24) and non-depressive controls without antidepressant medication (n=24) were included in the study. The LC-MS analysis employed hydrophilic interaction chromatography for more hydrophilic compounds and reversed-phase chromatography for more lipophilic compounds. Both methods were coupled to a high-accuracy, high-resolution mass spectrometer. Additionally, data were acquired using both positive and negative electrospray ionization modes. The method allows global characterization of the metabolites present in the sample without prior bias towards any specific molecules. Open-source software MS-DIAL was used for peak picking and feature alignment, as well as for identification of molecules, and notame R-package was used for data preprocessing and statistical analysis.

The initial results show a decreased level of oxidized glutathione (FDR-corrected q-value <0.001), phosphocreatine ($q=0.03$) and an increased level of reduced glutathione ($q=0.003$), cysteinglycine ($q<0.001$), monoacylglycerols (1-monostearin and 1-monopalmitin, both $q<0.001$), and serylleucine ($q=0.01$) in SSRI users. To conclude, the profiling of the placental metabolome of SSRI users revealed alterations in pathways related to oxidative stress, ATP homeostasis, and lipid metabolism.

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P10-18
Bayesian benchmark dose modelling of hypothalamic–pituitary–ovarian axis endpoints to assess DEHP exposure effects in female mice

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Purpose: Endocrine-disrupting chemicals (EDCs) affect female reproduction, yet efficient regulation is hindered by a lack of regulatory-relevant information. For example, it is unclear which female reproductive endpoints are most sensitive to EDCs. The current work assessed the relative sensitivity of various hypothalamic–pituitary–ovarian (HPO) endpoints in female mice. In this study, di(2-ethylhexyl) phthalate (DEHP) was studied as a prototypical EDC model compound. We identified dose-response studies in the literature involving DEHP in adult mice and modelled the HPO endpoints using the Bayesian benchmark dose (BBMD) approach.

Methods: Studies included sexually mature female mice exposed to DEHP, or a mixture containing 20.8% DEHP, for 30 days, at doses ranging from 0.02 to 200 mg/kg bw/day. The modelled HPO endpoints encompassed ovarian follicle counts and percentages (total, primordial, primary, antral, and preantral follicles), serum hormones (estradiol and progesterone) and estrous cyclicity (measured as% days in estrus and metestrous/diestrous phases). BBMD was performed on www.benchmarkdose.org to determine the credible interval of the benchmark dose (BMD), specifically the benchmark dose lower limit (BMDL) and benchmark dose upper limit (BMDU). Model averaging was performed using equal prior weights. The benchmark response (BMR), reflecting the change from the unexposed control group, was set at 10% for all endpoint**s.**

Results: Dose-dependent changes were observed in all modelled endpoints, although with different levels of uncertainty for the credible interval. For the ovarian follicle counts and percentages, the BMDLs were 0–176.2 and 0–198.0 mg/kg bw/day, respectively. For the reproductive hormones, the BMDLs were 0–55.1 mg/kg bw/day. Finally, for the estrous cyclicity endpoints, the BMDLs were 0–236.9 mg/kg bw/day. The results show that there was dose-dependency, for DEHP and the mixture, in all modelled HPO endpoints, including serum hormones, ovarian follicles staging, and estrous cyclicity.

Discussion & Conclusions: Using a BMR of 10%, BBMD modelling suggested differences in the sensitivity of classical HPO endpoints to DEHP. The results suggest that DEHP and the phthalate mixture exposure have a significant impact on the estrous cycle, hormones, and follicles in female rats. The findings may have implications for human health, as DEHP is a common environmental contaminant that is found in many consumer products, and can potentially be a reproductive toxicant. In conclusion, BBMD modelling is valuable in comparing endpoints to identify critical effects in reproductive toxicity testing. It may also be suited for comparing the effects of different EDCs and their mixtures, as well as for comparing the sensitivity of reproductive endpoints for male and female mice.

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P10-19
A comparative study of the cytotoxic effects of cannabidiol and minor cannabinoids on placental trophoblast cells

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Cannabinoids are the main compounds produced by *Cannabis sativa*, a commonly used illicit drug among pregnant women, particularly for relieving nausea in the first trimester of gestation [1]. Placental extravillous trophoblasts cells (EVTs) invade the uterus, participating in the remodeling of maternal spiral arteries to reduce resistance to blood flow [2]. It is known that cannabinoids can cross the placental barrier, which may result in pregnancy complications, such as intrauterine growth restriction and miscarriage [3]. We already reported the possible impact of the major cannabinoids cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) on placental development [4]. However, the effects of minor cannabinoids remain unknown. Thus, the aim of this work was to compare the effects of CBD with the minor cannabinoids cannabichromene (CBC), cannabidivarin (CBDV), cannabigerol (CBG) and cannabinol (CBN) on HTR-8/SVneo cells, a representative model of EVTs. All cannabinoids induced a dose-dependent decrease on cell viability at 24 and 48 h of incubation, which was confirmed by MTT assay. Lactate dehydrogenase (LDH) release was observed for CBC and CBN at 5 and 10 μM, while for CBD, CBDV and

CBG it occurred only at 10 μM. The dependence of the cannabinoid receptors CB1 and CB2 and of the transient receptor potential vanilloid 1 (TRPV1) for the cell viability loss observed at 48 h of treatment was also evaluated, using the antagonists AM281, AM630 and capsazepine, respectively. CBN effect was CB1-dependent, CBC and CBG were CB2 and TRPV1-dependent, while CBD and CBDV effects were independent of receptors activation. Moreover, CBD and CBG were able to cause mitochondrial depolarization and to generate reactive oxygen/nitrogen species, as well as CBDV, which were assessed using the $DiOC_6$ and the DCDHF-DA fluorescent probes, respectively. Nevertheless, only CBD activated the apoptosis-related effector caspases-3/-7, an effect that was promoted by autophagy, as confirmed through observation of orange-stained acidic vacuoles (fluorescence microscopy) and increase of p62 gene expression (qPCR). On the other hand, the minor cannabinoids CBDV and CBG increased the mRNA levels of the endoplasmic reticulum (ER) stress markers BiPand spliced-XBP1, evaluated through qPCR. Hence, the results suggest that cannabinoid exposure during pregnancy may differently impair the normal trophoblast remodeling through alterations on key biochemical processes for placental development, such as cell death and ER stress, compromising pregnancy success.

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P10-20
Comparative gonadotoxic activity study of two generic pesticides epoxyconazole on female rats

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Aim: This study evaluated gonadotoxic activity after exposure to two generic pesticides, namely epoxiconazole (Epox), in female rats. The test substances, epoxiconazole technical, were obtained from different manufacturers, with the purity of the active ingredient measured at 98.7% (Epox-1) and 97.3% (Epox-2). Animal studies were conducted following the requirements and provisions of the Commission for the Ethics of Medical and Biological Research of L.I. Medved Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine and the European Convention for Protection of Animals used for Experimental and Other Research Purposes.

Materials and methods: The test substances were orally administered as an aqueous emulsion by gavage daily for nine weeks until the mating period to two groups of animals, each composed of 20 females. The doses administered were 0.5 and 2.0 mg/kg body weight. Control animals, consisting of 20 females, received an equivalent volume of solvent: distilled water with an emulsifier. Following the exposure period, functional indicators of gonadal state and animals' reproductive ability were assessed. The estrous cycle, as well as the duration and frequency of each stage, were studied. The reproductive function status was evaluated on the 20th day of pregnancy in experimental females impregnated by intact (untreated) males. At the same time, the number of corpora lutea in the ovaries, the number of live, dead, and resorbed fetuses and embryos, fetal body weight, total litter weight, and the presence of gross developmental anomalies were recorded. Mating, conception, fertility, and pregnancy indexes were determined, considering the duration of the pre-coital interval.

Results: Results revealed that female rats exposed to the test substance Epox-1 exhibited reproductive toxicity at a dose of 2.0 mg/kg. This dose group of females observed a significant increase in the number of preimplantation losses, a decrease in the number of live fetuses per female, and a decrease in the total weight of the offspring. The test substance Epox-2, at both studied doses, did not adversely affect the reproductive function of female Wistar Han rats.

Conclusions: The results suggest that epoxiconazole has the potential to impact female reproductive function adversely. Moreover, discrepancies in the intensity of toxicity observed after exposure to Epox-1 may be ascribed to its higher purity compared to Epox-2 in this context. This highlights the importance of evaluating generic pesticides containing differing levels of impurities.

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P10-21
Spinosad – mode of action and human relevance assessment of dystocia in rats

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Spinosad technical, a natural product insecticide derived from fermentation, associated with treatment-related adverse pregnancy outcomes which manifested as dystocia in the rat. A robust mode of action (MOA) programme was initiated to determine the MOA for dystocia in rats and relevance of this hazard to humans using the (WHO)/International Programme on Chemical Safety (IPCS) framework. A number of dose-related key events have been identified that characterise the rat MOA for Spinosad-induced dystocia. Dystocia was characterised by prolonged parturition which was associated with peri-partum maternal death and other peri-partum effects. Using *in vivo* and ex vivo contractility experiments, it was concluded that parturition became protracted due to inhibition of uterine muscle contraction, arising due to a pharmacological/receptor-mediated inhibition of action potential generation in uterine smooth muscle cells (myometrial cells). By using competition binding experiments with receptor ligands, it is hypothesized that the Spinosad receptor mediating uterine effects may be Translocator Protein (TSPO). The initial dynamic molecular initiating event of Spinosad binding to TSPO requires uterine exposure to Spinosad above a certain tissue concentration threshold. With pharmacokinetic studies, uterine exposure to Spinosad has been unequivocally demonstrated in the pregnant rat after Spinosad oral administration.