QIVIVE using reported *in vitro* CL_{int} from the ToxCast database with a focus on comparing the performance of restrictive vs non-restrictive clearance of the chemicals. Prediction accuracy was evaluated by comparing the predicted total chemical exposure over time (AUC) and maximum plasma concentration (Cmax) with the observed values. Approach 1 with restrictive clearance predicted the AUC of 14 chemicals and the Cmax of 16 chemicals within 5-fold of the observed data. Approach 2 with restrictive clearance predicted the AUC and Cmax of 15 chemicals within 5-fold of the observed data. Approach 3 with restrictive clearance predicted the AUC of 15 chemicals and the C_{max} of 16 chemicals within 5-fold of the observed data and Approach 3 with non-restrictive clearance predicted the AUC of 12 chemicals and the C_{max} of 14 chemicals within 5-fold of the observed data. Overall, modelling restrictive clearance performed better than non-restrictive clearance for this set of chemicals. The higher prediction errors were investigated more closely, identifying contributions of non-hepatic clearances (e.g., active urine excretion, intestinal first pass) and elimination via bile as main reasons for the higher fold differences between predicted and measured AUC and C_{max}. Future work will focus on defining applicability domain for categorizing the compounds to minimize the variability in prediction accuracy to establish animal free risk assessment framework.

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P03-10
Synthetic cannabinoids & neuronal senescence: distinctive responses of *in vitro* models to AMB-FUBINACA

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Among the array of new psychoactive substances, synthetic cannabinoids (SC) stand out as highly popular among consumers. These substances closely resemble, in terms of their pharmacology, Δ^9-tetrahydrocannabinol (THC), cannabis' main active principle, albeit exhibiting full agonism at the cannabinoid receptors 1 and 2. In light of recent scientific findings suggesting that cannabis use can exacerbate ageing-related parameters, the present work was designed to explore whether SC share similar potential effects. For this purpose, we employed two distinct *in vitro* models. The first model involved primary hippocampal cultures (PHC), isolated from Wistar rat embryos at embryonic day 18–19; after seeding, cells were kept in culture and exposed to the popular SC AMB-FUBINACA (AMB-FUB) at 1 pM, 1 nM and 1 μM, starting at day-in-vitro (DIV) 3 or DIV7, and perpetuated until DIV21. DMSO at 0.02% was used as the solvent control. At the end of the exposure, ß-galactosidase activity (a common first-line cell senescence biomarker) was assessed using a commercially-available kit. Our findings under these experimental conditions, revealed that PHC exposed to all AMB-FUB concentrations had less ß-galactosidase activity than the control condition ($p<0.01$, 1 pM; $p<0.001$, 1 nM and 1 μM). The other *in vitro* model used herein was the human neuroblastoma cell line SH-SY5Y. Beginning at passage 24, cells were seeded and exposed to 1 nM and 1 μM AMB-FUB. At passages 24 (48h after drug exposure) and 28, samples were collected for the analysis of several senescence-related endpoints, namely ß-galactosidase activity, cell cycle analysis (via flow cytometry, following DNA staining with propidium iodide) and relative telomere length measurement (using qPCR). Surprisingly, no discernible effect of AMB-FUBINACA was observed for any of the endpoints examined. The apparent observed "anti-ageing" effect of AMB-FUB on PHC warrants further investigation. Moreover, the differential reponse observed between the two *in vitro* models also requires scrutiny, particularly in light of recent published findings suggesting that THC has the potential to increase ß-galactosidase activity. Further experiments will ensue to hopefully shed some light on these interesting results.

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P03-11
Assessing kinetic properties in food and feed: tebuconazole case study

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As part of the ADME4NGRA project, a tiered testing strategy is under investigation to better characterize interspecies differences for ADME properties. The *in vitro* ADME parameters will be used as input for physiologically based kinetic modelling approaches to estimate the bioavailability of substances found in feed and food.

Nine case studies evaluate *in vitro* approaches to measure transport and metabolism of the liver, intestine and kidney. They also take into account metabolism by the gut microbiome using data rich model compounds.

One case study is presented here, which investigates the degradation of the pesticide tebuconazole in different life stages. Metabolism in the adult women is compared to pregnancy, also including the situation of the foetus in the first, second and third trimester.

Tebuconazole is degraded to hydroxy-tebuconazole by CYP3A4 and CYP2C9. Both enzymes are increasingly expressed during pregnancy, while CYP2C9 in particular is significantly less present in the foetus. In the case study, the rate of degradation of tebuconazole by different CYPs is determined experimentally. PBK modelling is then used to model and compare the bioavailable concentrations of tebuconazole in the different phases of life.

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P03-12
Physiologically based kinetic modelling (PBK) using QIVIVE to predict the toxicokinetic profiles of triazoles via oral route of administration

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