table difference between WT and KO. Overall, we show that the tissue accumulation of uranium is Nrf2- and sex-dependent; that biological disturbances are greater in Nrf2-KO animals indicating a role for Redox control, and that females would be more sensitive to the nephrotoxicity of uranium.

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### P17-010
### Generation and characterisation of induced pluripotent stem cells- derived renal proximal tubular-like cells

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The kidney plays a vital role in whole body homeostasis, via blood filtration, reabsorption of required substances and excretion of excess and waste substances. The proximal tubule region is the major workhorse of the nephron and is also one of the most susceptible regions to injury by xenobiotics. Thus the proximal tubule is an important tissue to assess in integrated testing chemical safety assessment approaches.

The main objective of this study was to explore the possibility of differentiating induced human Pluripotent Stem cells (iPSC) into cells representing a proximal tubule phenotype for application to chemical safety assessment and personalised medicine. iPSC cells were differentiated using a 2-step protocol employing specific small molecules and growth factors. Differentiation was characterised by following the expression of pluripotency markers, renal development markers and proximal tubular markers via immunofluorescence, western blot analysis and RNA sequencing. The data demonstrate a temporal transition from pluripotent tissue, to intermediate mesoderm, renal vesicles and finally to a renal phenotype. The last stage could be maintained for up to 10 days. RNA sequencing was cross referenced with the network biology platform CellNet, which confirmed a renal tissue type and absence of similarities to other organs in the database. The cells were positive for megalin, were sensitive to parathyroid hormone and insensitive to vasopressin which are all characteristic traits of the proximal tubule nephron region. This iPSC derived renal proximal tubular-like model will be further characterised with respect to phase I and phase II metabolism and xenobiotic transport capabilities.

### P17-011
### Investigation on chemical induced mitochondrial toxicity in human proximal tubular epithelial cells

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The proximal tubule performs constitutive reabsorption of water, amino acids, protein, glucose and ions which is driven by energy dependent Na-K-ATPase. The energy required for this process is generated through oxidative phosphorylation and beta oxidation of fatty acids in the mitochondria. Proximal tubule cells have a high content of mitochondria, which make them especially sensitive to compounds which can injure mitochondria or impair their function. Mitochondrial impairment is a frequent mode of toxicity, that is often identified only late in the drug development pipeline. Thus, there is a need to develop a preclinical screen to identify potential renal mitochondrial liabilities.

The human proximal tubular cell line RPTEC/TERT1 was exposed to 22 electron transport chain (ETC) complex inhibitors of complex I, complex II and complex III. Mitochondrial function was investigated by monitoring glycolysis (lactate production, extracellular acidification rates (ECAR)), mitochondrial membrane potential (MMP) and oxygen consumption rates (OCR, Seahorse Bioanalyser). Transcriptomic studies were also performed using TempO-Seq analysis.

Resazurin reduction in combination with lactate production, the JC-1 assay, the seahorse assay and the TempO-seq analysis performed well to detect mitochondrial liabilities and exhibited similar potency rankings. The data will be used to support the development of a renal quantitative Adverse Outcome Pathway for chemical induced mitochondrial renal diseases, such as Fanconi Syndrome.

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## P18 – Reproduction

### P18-001
### GM stack soybean MON87701×MON89788 reproduction toxicity investigation

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The system of genetically modified (GM) organisms safety assessment in the Russian Federation within the framework of new GM lines state registration includes a large-scale toxicological studies. Since 2011, according to established researcher practice, the reproduction toxicity study of GMO (generative function, pre- and postnatal development) is one of the obligatory stages.

This publication presents the results of GM stack soybean MON87701 × MON89788 evaluation in the *in vivo* reproduction toxicity experiment on Wistar rats. The animals were divided into two groups, fed with rodent diet with inclusion of GM soybean ('test' group) and non-GM near-isogenic counterpart ('control' group) soy varieties. The soy was included into the diet at maximum possible level (~44%) not causing nutritional imbalance or metabolic disturbance for the experimental animals. Rats were monitored for body weight, feed consumption, and general health. The assessment of reproductive system was focused on the generative (indices of mating) and endocrine gonads function of parent animals' and on pre-/postnatal offspring's development. Prenatal development was assessed on 14–15 females of each group, that were euthanized on the 20$^{th}$ day of pregnancy (one day prior to the expected day of delivery). Postnatal offspring development was being assessed during the first month of pups' life (29 and 28 litters in test and control group, respectively).

Analyses of reproductive function (mating efficiency level, ranges of serum estradiol, progesterone and testosterone), offspring prenatal development (number of ovarian corpora lutea, resorptions, implantation sites, number of live and dead fetuses, pre- and post-implantation losses), postnatal development (number of live and dead pups, dynamic of body weight and length, physical developmental parameters) revealed no biologically meaningful differences between

test and control groups. All parameters did not exceed physiological range. The results of the reproduction toxicity assessment along with other biomedical research data indicate the safety of the GM MON87701 × MON89788 soybean stack.

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**P18-002**
**Two-generation reproduction toxicity studies of novel food sources: chronobiologic features**

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The procedure of new food sources safety assessment includes a two-generation reproduction toxicity study on laboratory animals. A duration of such studies determines the need of exogenous factors background effects standardization (fluctuations of atmospheric pressure, humidity, geomagnetic activity, etc.) during the experiment. Since the development of adaptation to these factors has been formed throughout the whole period of mammals evolution, the seasonal variability of some physiological and biochemical parameters cannot be mitigated even in the controlled laboratory conditions. Thus, when analyzing the results of the reproduction toxicity experiments it is necessary to take into account the chronobiologic features of laboratory animals.

This publication presents the results of research, that was pointed at investigation of seasonal factors influence on the reproductive system function of Wistar rats. The reproductive function in the autumn/winter seasons and spring/summer seasons was evaluated with the indices of mating, postnatal development of the offspring (number of live and dead pups, dynamic of body weight and length, physical developmental parameters).

All parameters did not exceed physiological range and did not form clearly traceable trends. The indices of mating were ~94% regardless of season of the year. The offspring born in the autumn/winter and spring/summer seasons showed the survival rate as 99.4% and 99.7%, and the males/females ratio in litter as 56/44 and 53/47, respectively. Analyses of body weight and length dynamic also revealed no biologically meaningful differences between groups.

Thus, the analysis of the obtained data did not reveal a correlation with seasonal factors. Values of all studied parameters did not fall outside the limits physiological norm and did not form obviously traced tendencies.

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**P18-003**

*This abstract has been withdrawn.*

**P18-004**

*This abstract has been withdrawn.*

**P18-005**

*This abstract has been withdrawn.*

**P18-006**
**Validation of a novel human stem cell-based gene expression assay for *in vitro* DART assessment**

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Testing for developmental and reproductive toxicology (DART) is a crucial part of the toxicological risk assessment. Today, DART mostly relies on animal testing, although alternative *in vitro* tests, such as embryonic stem cells based assays, are used. However, these *in vitro* assays often do not provide mechanistic insight and the results are difficult to translate to human risk due to inter-species differences.

To improve *in vitro* identification of developmental toxicants, we identified potential biomarkers in human induced pluripotent stem cells (hiPSC), marking different developmental stages from pluripotent stem cells to terminally differentiated cells. To test whether compounds affect development, first we optimised the differentiation protocols for hiPSC towards cardiomyocytes, hepatocytes and neural rosettes and confirmed the expression of selected biomarkers (OCT4, BMP4, MYH6, FOXA2, SOX17, AFP, ALB, PAX6) by qPCR. During differentiation, expression of the pluripotency marker OCT4 decreased, while expression increased for matured tissue markers MYH6 in cardiomyocytes, ALB and AFP in hepatocytes and Pax6 during neuronal rosette formation.

Next, we exposed differentiating hiPSC cells to 15 teratogenic and non-teratogenic compounds. We observed a marked downregulation of the cardiomyocyte-specific biomarker MYH6, hepatocyte-specific markers ALB and AFP and/or neural specific biomarker PAX6 during teratogenic compound treatment 5-FU, thalidomide, retinoic acid, diphenylhydantoin, bitertanol, triadimenol and methoxyacetic acid. The late differentiation markers were not affected after mono-butylphthalate treatment, but the early mesoderm specific marker BMP4 was down-regulated. Two potential teratogenic azole fungicides, fluconazole and carbendazim, did not reduce the expression of any of the biomarkers. Three out of five non-teratogenic compounds, acrylamide, dimethyl phthalate and saccharin, did not reduce the biomarker expression in either of the three differentiation protocols and were correctly identified as non-teratogenic

Following the differentiation program by using selected biomarkers allows the quantitative analyses of potential teratogen exposure and provides mechanistic insight into the potential teratogenic mode of action of compounds.

**P18-007**
**Copper nanoparticles alter cell viability and steroidogenic activity of gonadal cells**

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With the rapid development and widespread use of nanoparticles (NPs) in many industrial and biomedical applications, the environmental and occupational exposure of humans and animals to NPs is dramatically increasing. The results of recent studies have reported that NPs may pose adverse effects on male and female reproductive health by altering normal testis and ovarian structure, spermatogenesis and sperm quality, oogenesis, follicle maturation and sex hormone levels. The present study aimed to investigate dose-dependent and time-course effects of copper (Cu) NPs of different size on viabil-