# The role of testicular cell-cell communication in male reprotoxicity of high priority environmental chemicals

MUNI | RECETOX

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## Gap junctional intercellular communication (GJIC) and their critical role in testis

### GJIC – direct cell-cell communication (Fig. 1)

- mediated through intercellular channels build from connexin (**Cx**) proteins (vertebrates) (**Fig. 2**)
- plays a central role in coordinating cell-cell communication
- coordinates collective responses from the cells across a tissue of multicellular organism
- allows an exchange of low molecular weight molecules (<1.2 kDa) between adjacent cells

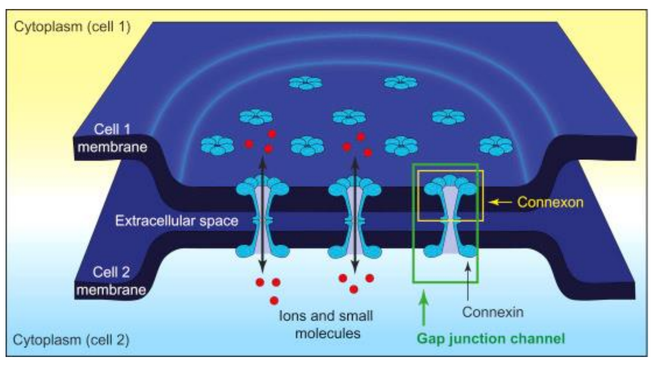



**Fig. 1: GJIC - Exchange of small molecules between neighboring cells through gap junction (GJ) channels [1]**

### GJIC in testes (Fig. 3)

- maintains testicular development & homeostasis
- coordinates proliferation and differentiation of testicular cells
- synchronizes testicular steroidogenesis i.e. regulation of hormone production and release
- controls initiation, regulation and maintenance of spermatogenesis

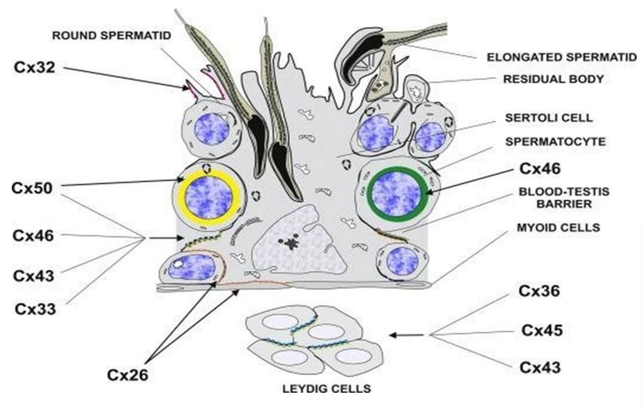



**Fig. 2: GJIC and Cx related dysregulation [3]**

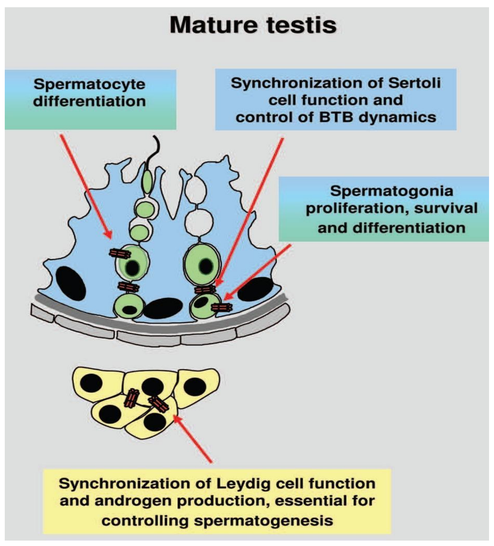



**Fig. 3: GJIC & Critical testicular parameters [2]**

### Untimely dysregulation of GJIC & Cx-related abnormalities may lead to

- Impaired spermatogenesis, increased germ cell apoptosis, spermatogonial arrest, azoospermia, germ cell deficiency
- Loss of blood-testis barrier integrity
- Hyperplasia of androgen-producing Leydig cells
- Leydig cell tumorigenesis
- Impairment of male reproductive capacity and decrease of fertility

**Our research hypothesis: Testicular GJIC plays a critical role in endocrine-disrupting activity of chemicals**

## Summary & Conclusion & Future perspective

- Our study supports that **environmental factors are likely one of the major causes of male reproductive dysfunctions**
- ¨We found supporting evidences for our research hypothesis, i.e. **GJIC and Cxs are important, but overlooked functional biomarkers of male reproductive toxicants in somatic testicular cells**
- **Well-recognized or potential endocrine-disrupting chemicals (EDCs, Fig. 4)** such as vinclozolin, methoxychlor, triclocarban or triclosan **induce a rapid dysregulation of GJIC in testicular somatic cells**, Leydig TM3 and Sertoli TM4 cells
- EDCs cause their male reprotoxicity through **disturbance of MAPK Erk1/2** (mitogen-activated protein kinase extracellular signal-regulated kinases 1/2) **and PKC** (protein kinase C) **signaling pathways and junctional and/or non-junctional functions of Cx43**
- **GJIC** should become **an integral part of male reprotoxicity assessment and relevant *in vitro* test batteries**
- We are currently **linking GJIC with functionality of somatic testicular cells responses such as steroidogenesis, proliferation and apoptosis in 2D and 3D cultures of testicular cells** to improve male reprotoxicity assessment

## Methodology

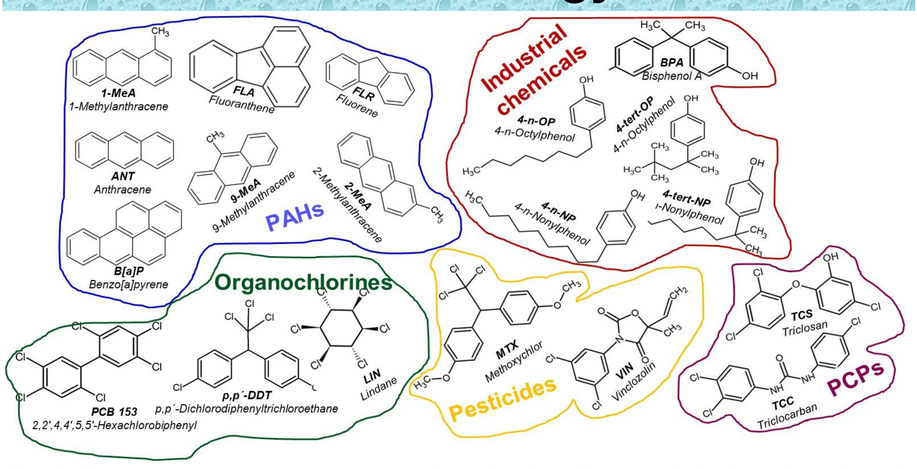
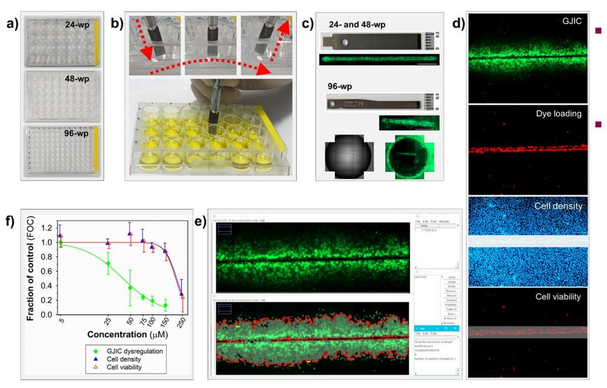



**Fig.4: Studied chemicals** – representatives of polycyclic aromatic hydrocarbons (PAHs), industrial chemicals, organochlorines, pesticides and personal care products (PCPs) ingredients

*Dydowiczova 2020, Sci Report 10*

### SL/DT MULTIPARAMETRIC ASSAY




- an integral component in modern toxicity testing
- Improved multiparametric scrape loading-dye transfer assay for a simultaneous **high-throughput analysis of gap junctional intercellular communication, cell density and viability**

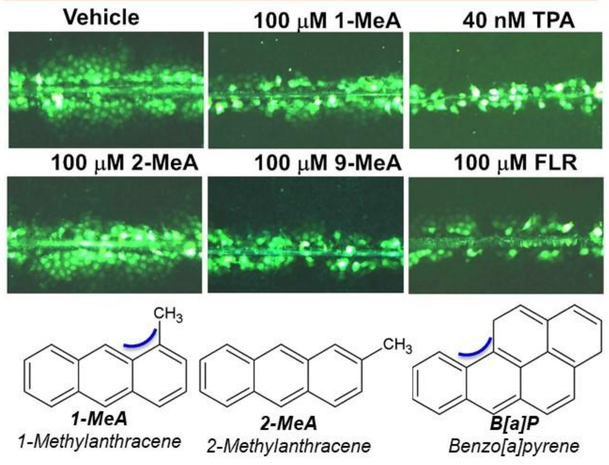
## Results

**GJIC-inhibitory activity of selected EDCs in testicular cells**

| | | $EC_{50}$ (µM) | Leydig TM3 cells | | | Sertoli TM4 cells | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0.5-h | 6-h | 24-h | 0.5-h | 6-h | 24-h |
| PAHs | | Anthracene | >200 | >200 | >200 | >200 | >200 | >200 |
| | | 1-Methylanthracene | 49 | 40 | 41 | >200 | >200 | >200 |
| | | 2-Methylanthracene | >200 | >200 | >200 | >200 | >200 | >200 |
| | | 9-Methylanthracene | 45 | 52 | 43 | >200 | >200 | >200 |
| | | Fluorene | 27 | 31 | >100 | >200 | >200 | >200 |
| | | Fluoranthene | 44 | 42 | 38 | >200 | >200 | >200 |
| | | Benzo[a]pyrene | >200 | >200 | >200 | >200 | >200 | >200 |
| Industrial chemicals | | Bisphenol A | >200 | 200 | 100 | >100 | 100 | 72 |
| | | 4-*n*-Nonylphenol | 46 | 46 | 51 | n.t. | n.t. | n.t. |
| | | 4-*tert*-Nonylphenol | 34 | 28 | 34 | n.t. | n.t. | n.t. |
| | | 4-*n*-Octylphenol | 42 | 48 | 31 | n.t. | n.t. | n.t. |
| | | 4-*tert*-Octylphenol | 25 | 35 | 33 | n.t. | n.t. | n.t. |
| Organichlorines | | Lindane | 132 | 102 | 171 | 85 | >100 | >100 |
| | | PCB 153 | 100 | >100 | >100 | >100 | >100 | >100 |
| | | p,p´-DDT | 64 | 44 | 41 | 45 | 40 | 46 |
| Pesticides | | Vinclozolin | >200 | >200 | >200 | 100 | >100 | >100 |
| | | Methoxychlor | 53 | 56 | 42 | 30 | 41 | 32 |
| PCPs | | Triclocarban | 51 | 48 | 34 | 13 | 14 | 6 |
| | | Triclosan | 30 | 29 | 32 | 20 | 19 | 13 |

*n.t. ... not tested*

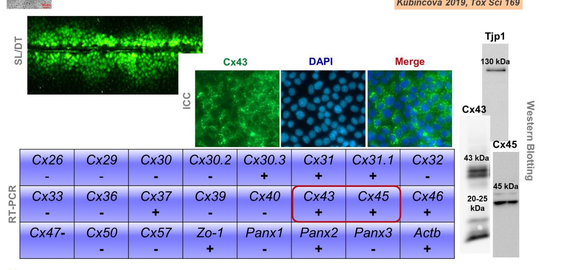
### Structure-dependent GJIC activity of PAHs in Leydig TM3 cells




- **15 of 19 EDCs** had **GJIC-inhibitory activity in testicular cells**
- **Rapid** GJIC-inhibitory activity (within 5-30 min) with $_{0.5h}EC_{50}$ **= 6-200 µM**
- **GJIC dysregulation ⇒ reversible process** (the exposed cells can recovered within time)

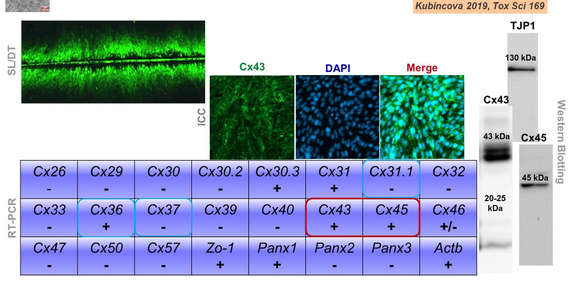
### LEYDIG TM3 CELLS AS MODEL FOR GJIC ASSESSMENT

*Kubincova 2019, Tox Sci 169*




### SERTOLI TM4 CELLS AS MODEL FOR GJIC ASSESSMENT

*Kubincova 2019, Tox Sci 169*

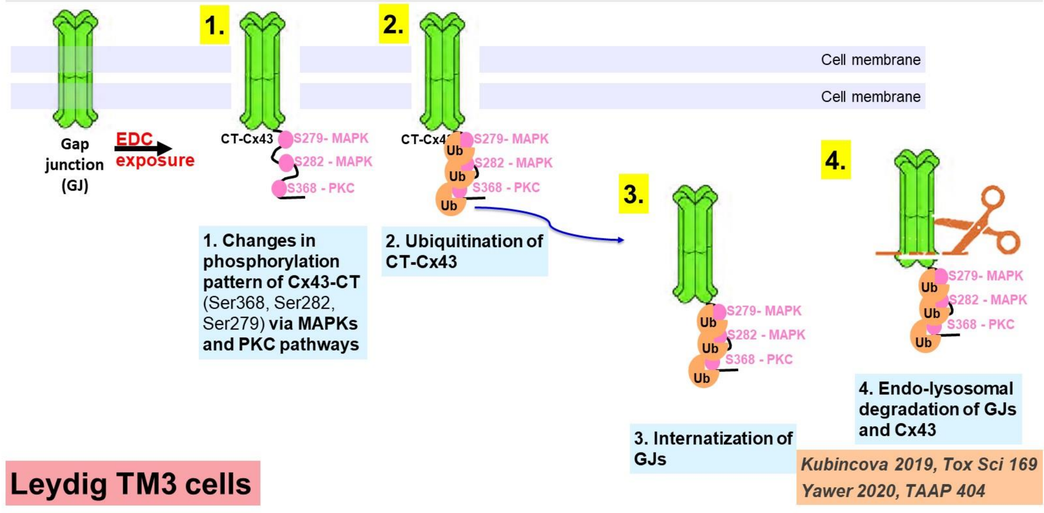



- **Mouse Leydig TM3** (ATCC® CRL-1714™)
- **Mouse Sertoli TM4** (ATCC® CRL-1715™)

⇒ continuous, non-transformed and **non-tumorigenic cell lines** derived from immature BALB/c mouse testis GJIC-proficient cell lines with dominant Cx43 and Cx45

⇒ A good models of immature testicular cells **[3-7]**

### MOLECULAR MECHANISMS - SUMMARY




- Chemical exposure changed phosphorylation pattern of **Cx43 via MAPK-Erk1/2 and PKC signaling pathways** (1) and its reduction in membrane (2-3)
- **Cx43 ubiquitination (2) and subsequent endo-lysosomal degradation (4) likely are involved in this process**

**Acknowledgment:** This project was supported by the Czech Science Foundation Project No. GA16-10775Y

**Reference:** **[1]** Synaptic transmission 2019**; [2]** Kidder et al. 2016, *Semin Cell Dev Biol* **50**: 22**; [3]** Mather 1980, *Biol Reprod* **23**: 243; **[4]** Mather *et al.* 1982, *Ann N Y Acad Sci* **383**: 44; **[5]** Beverdam *et al.* 2003, *Cytogenet Genome Res* **101**: 242; **[6]** Wang *et al.* 2016, Reproduction **152**: R31; **[7]** Nygaard *et al.* 2014, Sci Rep **5**: 10364.