Iodide Uptake in Thyroid Gland as a Target for Endocrine Disruptors

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Introduction

- Endocrine disruption is a well-known phenomenon, still not much is known regarding the interference of pollutants and environmental exposure mixtures with thyroid hormone regulation (Fig.1).
- ERGO project, which this work is part of, is based on Adverse Outcome Pathway (AOP) concept and it focuses on different Molecular Initiating Events (MIEs; Fig. 2).
- This work focuses on NIS (Natrium Iodide Symporter), which mediates uptake of iodide into follicular cells of the thyroid gland, which is the critical first step in the synthesis of thyroid hormone.

AIMs

- ✓ Development and optimization of assay for the assessment of NIS inhibition by model chemicals as well as environmental pollutant mixtures.
- ✓ Assessment on NIS inhibition by a set of priority chemicals.

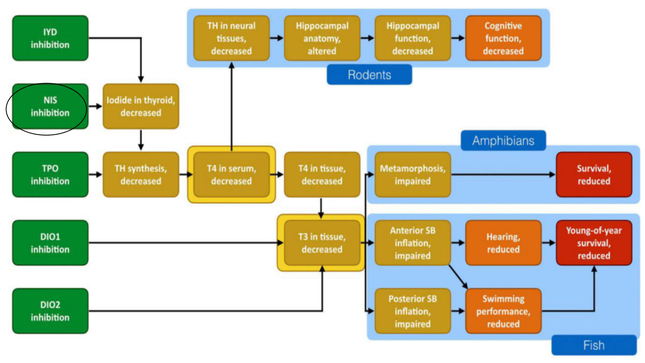

Fig. 2: Adverse Outcome Pathway (AOP) network demonstrating thyroid hormone disruption highlighting NIS (Natrium Iodide Symporter) as molecular initiating event (MIE)

Conclusion

- Developed a novel bioassay focused on an AOP-prioritized endpoint in thyroid hormone disruption - assessment of iodide uptake by thyroid cells mediated by Na^+/I^- symporter (NIS).
- It is based on stably transfected human cell line overexpressing NIS and the detection of iodide levels uptaken by the cells detected by a sensitive non-radioactive Sandell-Kolthoff reaction.
- The results demonstrate the utility of the newly developed bioassay for high-throughput screening of chemicals as well as environmentally relevant complex pollutant mixtures for the characterization of their thyroid hormone-disrupting potential.

Future Plans

1. Assessment of real exposure mixtures from field studies.
2. Assessment of predictability of *in vivo* effects using zebrafish model by the data from the *in vitro* assay.
3. Based on the results acquired, the manuscript will be prepared.

REFERENCES

1. Dong, H. et al. (2019) DOI:10.1016/j.tiv.2019.01.021.

Methodology

- Various thyroid *in vitro* models were characterized regarding gene and protein expression of NIS – none suitable for high-throughput assessment
- Novel transfected HEK293T- NIS *in vitro* model was developed using lentiviral transfection to study NIS inhibition in comparison with rat cell thyroid line (FRTL 5) with intrinsic NIS expression.
- Established and optimized NIS assay based on non-radioactive spectrophotometric method using Sandell-Kolthoff reaction (Fig. 3).

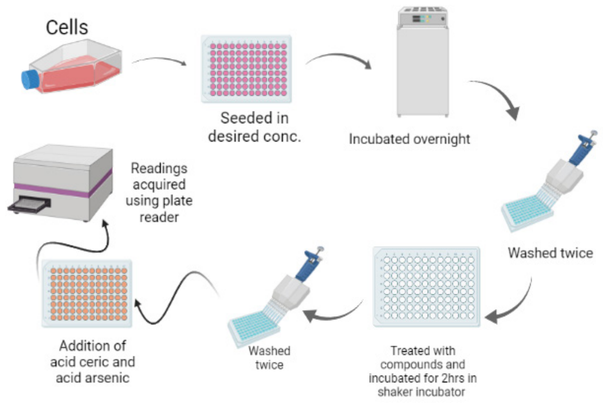

Fig 3: Pictorial representation of assay based on Sandell Kolthoff reaction for the assessment of NIS (Natrium Iodide Symporter), which plays a crucial role in the synthesis of thyroid hormones.

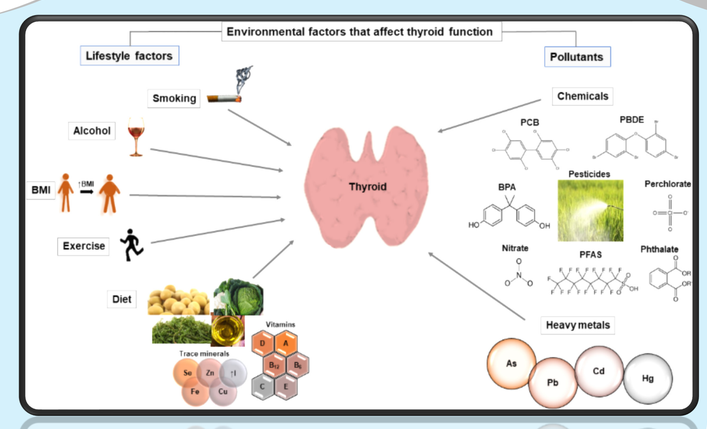

Fig. 1 Environmental factors that affect thyroid function.

Assay Development

- SeqAPASS analysis (US EPA) was conducted to assess the percent similarity of NIS protein across species (fig. 4) showing conserved character of the protein across vertebrates.
- Novel human cell-based NIS-transfected cell model was established and characterized in detail.
- To confirm the successful transfection, qPCR was performed to detect the expression of SLC5A5 gene encoding NIS (Fig. 5) and protein expression analysed.

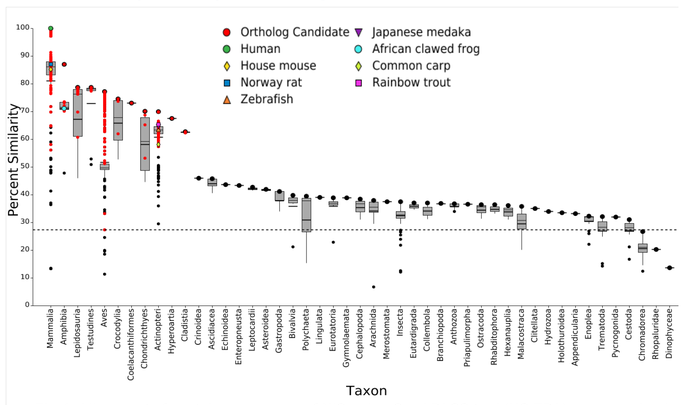

Fig 4: Alignment of protein sequences of NIS using SeqAPASS tool (US EPA) across animal classes (% similarity across species).

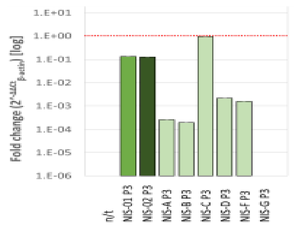

Fig 5:gene expression in different transfected cultures was done to confirm the presence of NIS as compared to NIS expression in the human thyroid gland (red line)

Results

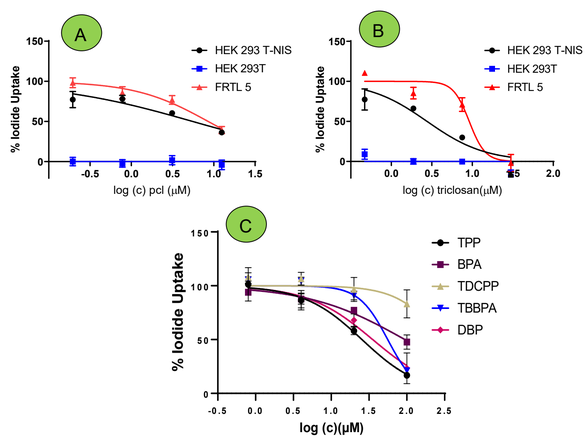

Fig.6: Inhibition of iodide uptake by perchlorate (PCL; A), triclosan (TCS; B), in novel cell model transfected with human Natrium Iodide Symporter (NIS; HEK293T-NIS), non-transfected cells (HEK293T) and rat FRTL-5 cell line with intrinsic NIS activity.
(C): inhibition of iodide uptake in HEK293T-NIS cell in presence of model chemicals; triphenyl phosphate (TPP), bisphenol A (BPA), tris 1,3 dichloro-2-propyl phosphate (TDCPP), tetrabromobisphenol A (TBBPA), dibutylphthalate (DBP).

- The transfected human HEK cells (HEK 293T-NIS) worked similar to rat FRTL 5 cells (with endogenous NIS expression), while there was no NIS activity in non-transfected HEK cells (HEK 293T) (Fig. 6A and 6B)
- Prioritized chemicals were tested on this transfected model (HEK 293T-NIS), selected results are shown in Fig. 6C.
- The results document that some wide-spread pollutants can inhibit NIS and thus thyroid hormone synthesis