Title

Draft protocol for a systematic evidence map of the effect of endocrine disrupting chemicals on thyroid hormone measurements in mammals

Authors:

Dr Asma Baig, College of Health, Medicine and Life Sciences, Brunel University London
asma.baig@brunel.ac.uk

Dr Isabel Forner-Piquer, College of Health, Medicine and Life Sciences, Brunel University London
Isabel.forner-piquer@brunel.ac.uk

Professor Andreas Kortenkamp College of Health, Medicine and Life Sciences, Brunel University London andreas.kortenkamp@brunel.ac.uk

Funding and sources of support

The time spent on this project by Asma Baig, Isabel Forner-Piquer, Andreas Kortenkamp was funded by the EU Horizon 2020 ATHENA project grant 825161

Competing interests

The authors have no competing interests to declare.

Introduction

Rationale

The Hypothalamic Pituitary Thyroid (HPT) axis is responsible for maintaining correct circulating levels of thyroid hormone (TH) in the body. The HPT axis is a classic endocrine negative feedback loop: when there are high levels of thyroxine (T4) in the blood, there is downregulation of thyroid stimulating hormone (TSH) production in the hypothalamus and conversely, low T4 levels often, but not always, activate increased production of TSH. The thyroid hormone system is sensitive to perturbation by endocrine disrupting chemicals (EDCs) which can cause increased or decreased levels of TH leading to hypothyroidism or hyperthyroidism due to these extraneous factors. Persistent changes in TH levels can lead to adverse effects in an individual or its progeny. We are particularly interested in the mechanisms of hypothyroidism due to chemical exposure, which can lead to impairment in brain development in the foetus and which is underpinned by a need for understanding the mechanistic behaviour of chemicals in the body.

The mechanism of action (MoA) of chemicals affecting the thyroid hormone system varies, resulting in differing outputs for TH measurement; for example, exposure to certain thyroid peroxidase (TPO) inhibitors manifests as lowered serum T4 with raised TSH, while other chemicals including polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) also produce lowered serum T4 but without a concomitant increase in TSH (Bansal et al 2014). The reasons for these diverging effect patterns are unclear, but these observations suggest that there is disruption of the HPT axis and that our understanding of the mechanisms operating within the HPT axis is incomplete. It is therefore important to elucidate the mechanisms by which different chemicals drive these diverging hormonal patterns to be able to develop effective testing strategies for the identification of thyroid hormone-disrupting chemicals (THSDCs).

Aims and Objectives

The aim of producing this systematic evidence map is to obtain a comprehensive picture of effect patterns in the TH-axis produced by chemicals that induce decreases in thyroid hormones T4 and/or T3. For this, we will generate lists of chemicals known to induce drops in T4 or T3 levels, together with their corresponding TSH changes. We will distinguish three possible effect patterns: T4 down, TSH up; T4 down, TSH no change; T4 down, TSH down etc. This will generate a typology of chemicals according to their patterns of TH changes which we will interrogate in terms of differences in chemical features and toxicological patterns in order to develop new hypotheses about their mechanisms and address these with further study. This work will also contribute to risk-management, surveillance and decision-making regarding the use of EDCs.

To meet this aim, the objective of this evidence map is to collate literature in which there are experimental measurements of T4/T3 and TSH from mammalian studies.

Population, Exposure, Comparator, Outcome (PECO) statement

The objectives of this review are defined by the PECO and facilitate the study design and focus the literature screening by applying the inclusion and exclusion criteria to address the specific aims of the study.

The question formulation for systematic evidence mapping follows a similar procedure to that of systematic reviews in general, i.e. using the PI/ECO (population, intervention/exposure, comparator, outcome) statement.

Research Question:

In what way do chemicals which cause a decrease in the levels of T4 or T3 affect TSH levels? The chemicals assessed for this study may increase, decrease or show no change in the TSH levels measured.

PECO statements are given in Tables 1 and 2, separately for human and experimental animal studies.

Table 1. PECO statement human studies

Populations	Humans of all ages, particularly pregnant women; epidemiology data
Exposures	All classes of chemicals: chemicals with hormonal activity, natural and synthetically produced, i.e chemicals that decrease serum levels of thyroxine (T4) and/or T3. Exposure through occupation, food, water, location
Comparators	No chemical exposure or placebo or lower exposure. Functioning of the HPT axis in the absence of chemicals; clinically normal thyroid hormone levels or baseline measurements.
Outcomes	• Decrease in T4 and/or T3 levels in blood serum/ cord blood • Measurement of TSH levels (direction of change left open)

Table 2. PECO statement animal studies

Populations	Laboratory mammalian species including rats, mice, rabbits, guinea pigs, dogs and monkeys
Exposures	All classes of chemicals: chemicals with hormonal activity, natural and synthetically produced. Exposure via drinking water or the diet during gestation and postnatal life and when germ cell populations are established. Focus on drop in T4
Comparators	Animals with no chemical exposure or placebo
Outcomes	• Decrease in T4 and/or T3 levels in blood serum/ cord blood • Measurement of TSH levels (direction of change left open)

Methods

This draft protocol has been created with guidance from PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2020 checklist (Page et al 2021) and with reference to the Code of Practice for the Conduct of Systematic Reviews in Toxicology and Environmental Health Research (COSTER) (Whaley et al 2020). The protocol will be registered at Zenodo.

Eligibility criteria

Criteria for the eligibility of epidemiological studies are listed below.

Table 3. Eligibility criteria for human studies

		Inclusion criteria	Exclusion criteria
Populations	Humans of all ages including	Men, women, children, neonates. All demographics	Clinical thyroid disorders Other endocrine disorders
Exposures	All chemicals classes: chemicals with hormonal activity – natural and synthetically produced, that induce drops in serum T4 or T3	Chemical exposures measured in the blood/serum	Exposure information derived from questionnaires or job exposure matrices with laboratory measurement
Comparators	Groups with no or lower chemical exposure, if available.	Sufficient information reported to allow comparison/categorisation of exposures.	Insufficient information reported to allow comparison/categorisation of exposures.
Outcomes	Serum thyroid hormone concentrations	• Total and free T4 • Total and free T3 • TSH • Total and free T4 measured and TSH determined; Total and free T3 and TSH measured	• No T4 measurements • No TSH measurements • No T4 measurements together with TSH measurements
Design		• Case-control studies • Cohort studies • Cross-sectional studies	• Case reports • Reviews

Studies in which patients have an existing clinical thyroid disorder or other comorbidities or disorders which could alter the TH levels will not be considered. The outcomes for each chemical study that gives measurements of both T4 and TSH will be included so that the chemicals can be compared for these two parameters.

Criteria for the eligibility of experimental studies with laboratory rats or mice are listed below:

Table 4. Eligibility criteria for animal studies

		Inclusion criteria	Exclusion criteria
Populations	Laboratory mammalian species including rats, mice, rabbits, guinea pigs, dogs and monkeys	Mammalian species. A few studies are with sheep/cows and should be included as this is a useful model	Non-mammalian test species such as fish or amphibians. Mammals such as whale and seal will be excluded as TH measured in fat is more complex due to fat distribution.
Exposures	All chemicals classes: chemicals with hormonal activity – natural	Administered by gavage, via drinking water or through the	Administered subcutaneously or intraperitoneally; only 1

	and synthetically produced, that induce drops in serum T4 or T3	diet; at least 2 exposure doses.	exposure dose group; in juveniles or adults.
Comparators	Groups with no chemical exposure.	Control group (same species as exposure group(s)	No control group
Outcomes	Thyroid hormone measurements	• Total and free T4 • Total and free T3 • TSH • Total and free T4 measured and TSH determined; Total and free T3 and TSH measured	• No T4 measurements • No TSH measurements • No T4 measurements together with TSH measurements •

Information sources

Searches for peer-reviewed articles will be conducted in the following bibliographic databases:

- PubMed
- Web of Science Core Collection (WoS)
- Scopus

Manual searches of the bibliography and citations of eligible studies will also be carried out and included as references from 'other sources' as described in the PRISMA 2020 flow diagram.

- https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2016.EN-999 Toxicological data collection and analysis to support grouping of pesticide active substances for cumulative risk assessment of effects on the nervous system, liver, adrenal, eye, reproduction and development and thyroid system
- EFSA Journal 2013;11(7):3293
- Collaborate with EFSA who will provide any relevant data on thyroid active pesticides.

Only articles written in English will be included due to limited resource. There will be no restriction on publication date. Articles must describe the results of primary data; reviews will be excluded.

Search strategy

The research question has two main parts: EDCs and THs. Pre-searches to identify relevant search terms, search strategies and information sources were conducted. Relevant search terms were combined using the Boolean operators 'AND' and 'OR' to create a search string in three literature databases: Pubmed, Scopus and Web of Science. An initial search was run as a pilot study in order to test the search string, the screening and data extraction method (Appendix A).

Search string for pilot study:

Search string: (("Endocrine Disruptors"[Mesh] OR "endocrine disrupting chemical*" OR EDC OR "endocrine disruptor*" OR "thyroid disrupting chemical*" OR "thyroid disruptor*") AND ("Thyroxine"[Mesh])) AND ("Thyrotropin"[Mesh] OR "thyroid stimulating hormone")

Search term development

From the pilot study, we found that the search string could be enhanced to include further terms for the material sources of chemicals and further biological targets to encompass the TH-axis and therefore capture a greater amount of literature on this subject. The terms included do not name specific chemicals to avoid bias, instead include terms for materials that are known to include EDCs.

Search string:

(("Endocrine disrupt*" OR EDC OR "thyroid disrupt*" OR estrogen* OR fungicid* OR insecticid* OR rodenticid* OR bactericid* OR antimicrobial* OR herbicid* OR pesticid* OR biocid* OR cosmetic* OR "personal care product*" OR "UV filter*" OR pharmaceutical* OR "heavy metal*" OR flam* OR mycotoxin* OR microcystin* OR xeno* OR plastic* OR textil* OR clothing* OR furniture OR pack* OR "canned food*" OR bottle OR container OR "medical device*" OR paint* OR adhesiv* OR solvent* OR propellant* OR coating* OR electronic* OR household OR product* OR toy OR dust OR waste) AND (TSH OR thyrotrop* OR thyroid* OR "HPT axis" OR "nuclear hormone receptor") AND (T4 OR thyroxine* OR tetraiodothyronine OR T3 OR triiodothyronine OR "thyroid hormone*" OR "TH"))

Results:

	Pubmed	WoS	Scopus
No. of 'hits'	15,817	11,891	13,545

Data management

References will be added to Cadima for screening against the eligibility criteria. The identified literature and all systematic review processes will be managed and coordinated using the freely available online tool CADIMA - established from a collaboration between the Julius Kühn- Institut and the Collaboration for Environmental Evidence. CADIMA - EvidenceSynthesisDatabase Area

Relevance screening

The list of eligibility criteria will be applied to the merged reference list by two reviewers working independently. First, only titles and abstracts will be checked for relevance to the study question and irrelevant studies will be excluded. After title/abstract screening, the list of included references will be determined for inclusion by reading of the full text. The reason for exclusion of studies after assessment of the full text will be recorded and any disagreement between the two evaluators will be resolved by a third party. For quality control purposes, the percentage agreement between the two independent evaluators or kappa statistic will be reported.

Data extraction

An initial data extraction template has been designed in Excel to capture salient information from the references which will allow us to categorise the different chemicals and hormonal measurements. The information extracted includes:

- meta-data (authors, date, journal name, article title, funding)
- information about the test system (mammalian species; human demographic)
- Information about the chemical exposure (life stage, duration, exposure regimen, dosing route, dose level).
- Information about the experiment setup (controls, histopathology, Hepatic T4-UDPGT).
- Chemical information (name, CAS, class, source, purity, solvent carrier).
- Method for measuring chemical exposures
- Method for measuring circulating thyroid hormone levels and in tissues, where measured.
- Statistical methods
- Thyroid hormone measurements (T4, FT4, T3, FT3, TSH) and indicate if increased, decreased or no change

Data Analysis and Reporting

Data analysis for this systematic evidence map will involve collating the data and evidence from the data extraction step to obtain a map of the effect patterns of EDCs on TH and TSH from the different studies. The results will be analysed and compared visually in tables. Analysis will be in terms of:

- chemical structures,
- molecular initiating event in inducing TH changes,
- comparisons between patterns in human studies and in animal studies.

The systematic evidence map will be used to inform the work carried out in the H2020 ATHENA project and it will also be written in the form of a peer-reviewed scientific article.

References

Bansal, R.; Tighe, D.; Danai, A.; Rawn, D.F.K.; Gaertner, D.W.; Arnold, D.L.; Gilbert, M.E.; Zoeller, R.T. (2014) Polybrominated diphenyl ether (DE-71) interferes with thyroid hormone action independent of effects on circulating levels of thyroid hormone in male rats. *Endocrinology* 155: 4104.

Page M et al (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews *BMJ* 2021; 372 doi: https://doi.org/10.1136/bmj.n71

Whaley P et al (2020) Recommendations for the conduct of systematic reviews in toxicology and environmental health research (COSTER). *Environment International* 143: 105926