Reproductive Toxicology 80 (2018) 126–130

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Review

The urgency for optimization and harmonization of thyroid hormone analyses and their interpretation in developmental and reproductive toxicology studies

Manon Beekhuijzen[a,*], Steffen Schneider[b], Narinder Barraclough[c], Nina Hallmark[d], Alan Hoberman[e], Sheri Lordi[e], Mary Moxon[f], Deborah Perks[c], Aldert H. Piersma[g], Susan L. Makris[h]

[a] Charles River, 's-Hertogenbosch, The Netherlands
[b] BASF SE, Ludwigshafen am Rhein, Germany
[c] Covance Laboratories Ltd., Harrogate, United Kingdom
[d] Bayer S.A.S. Crop Science Division, Sophia Antipolis Cedex, France
[e] Charles River, Horsham, PA, USA
[f] Regulatory Science Associates, Inverkip, United Kingdom
[g] National Institute for Public Health and the Environment (RIVM), Bilthoven, and Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands
[h] US Environmental Protection Agency, National Center for Environmental Assessment, WA, DC, USA

ARTICLE INFO

Article history:
Received 11 August 2017
Received in revised form 13 April 2018
Accepted 27 April 2018
Available online 2 May 2018

Keywords:
Thyroid hormones
T4
TSH
OECD
Developmental and reproductive toxicology (DART)
Adversity

ABSTRACT

In recent years several OECD test guidelines have been updated and some will be updated shortly with the requirement to measure thyroid hormone levels in the blood of mammalian laboratory species. There is, however, an imperative need for clarification and guidance regarding the collection, assessment, and interpretation of thyroid hormone data for regulatory toxicology and risk assessment. Clarification and guidance is needed for 1) timing and methods of blood collection, 2) standardization and validation of the analytical methods, 3) triggers for additional measurements, 4) the need for T4 measurements in postnatal day (PND) 4 pups, and 5) the interpretation of changes in thyroid hormone levels regarding adversity. Discussions on these topics have already been initiated, and involve expert scientists from a number of international multisector organizations. This paper provides an overview of existing issues, current activities and recommendations for moving forward.

Contents

Abbreviations: ANSES, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (French Agency for Food, Environmental and Occupational Health & Safety); CRO, contract research organization; CV, coefficient of variation; DART, developmental and reproductive toxicology; EATS, estrogen, androgen, thyroid, and steroidogenesis; EC, European Commission; ECHA, European Chemicals Agency; EFSA, European Food Safety Authority; ED, endocrine disruption; EDSP, Endocrine Disruptor Screening Program; ELISA, enzyme linked immunosorbent assay; EOGRTS, Extended One-Generation Reproductive Toxicity Study; EPA, Environmental Protection Agency; ETS, European Teratology Society; GD, gestation day; HPLC–MS, high performance liquid chromatography–mass spectrometry; JRC, Joint Research Centre (European Commission); NGO, non-governmental organization; OECD, Organisation for Economic Co-operation and Development; OPPTS, Office of Prevention, Pesticides and Toxic Substances; PND, postnatal day; RIA, radioimmune assay; T3, triiodothyronine; T4, thyroxine; TG, test guideline; TSH, thyroid stimulation hormone, thyrotropin.

* Corresponding author.
E-mail address: manon.beekhuijzen@crl.com (M. Beekhuijzen).

https://doi.org/10.1016/j.reprotox.2018.04.017
0890-6238/

1. Introduction

Almost 20 years ago, an important endeavor was started by the OECD to update existing test guidelines and to create new test guidelines (TG) for the screening and testing of potential endocrine disruptors. In this context, parameters relevant to the detection of endocrine disrupting activity of test chemicals were added to the OECD TG 416 (*Two-Generation Reproduction Toxicity Study*) in 2001 [1] and the OECD TG 407 (*Repeated Dose 28-Day Oral Toxicity Study in Rodents*) in 2008 [2]. More recently (2011), the OECD TG 443 (*Extended One-Generation Reproductive Toxicity Study [EOGRTS]*) [3] was implemented as a new test guideline to replace OECD TG 416 for industrial chemicals or as an alternative approach for reproductive toxicity testing for agrochemicals. In 2015 and 2016, the OECD 421 (*Reproduction/Developmental Toxicity Screening Test*) and OECD 422 (*Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test*) test guidelines were revised to include estrogen, androgen, thyroid, and steroidogenesis (EATS) relevant endpoints [4,5]. In April 2015, OECD launched a feasibility study for the enhancement of OECD TG 414 (*Prenatal Developmental Toxicity Study*) with selected parameters intended to increase the detection of EATS disrupting potential, and is now in progress [6]. Likewise, a similar endeavor is ongoing for OECD TG 408 (*Repeated Dose 90-day Oral Toxicity Study in Rodents*) [7]. Thyroid hormone analyses are among the parameters added to OECD TG 421, 422 and 443. These test guidelines specify that blood samples be collected from parental animals and pups (generally rats) at various ages, some of which need to be analyzed for thyroid hormone levels (Table 1).

In the US, under the Endocrine Disruptor Screening Program (EDSP), thyroid hormone analyses are included in two test guidelines: OPPTS TG 890.1450 (*Pubertal Developmental and Thyroid Function in Intact Juvenile/Peripubertal Female Rats*) and OPPTS TG 890.1500 (*Pubertal Developmental and Thyroid Function in Intact Juvenile/Peripubertal Male Rats*) [8,9]. In these studies, serum T4 and TSH are assessed at study termination in peripubertal aged study animals that had been administered test substance since weaning (Table 1).

2. Issues

Since many OECD TG 421 and 422 studies are currently ongoing, and in the near future the number of OECD TG 443 studies is expected to increase, there is a clear urgency to address and resolve several issues that have been raised in discussions initiated by OECD and other scientific organizations regarding these thyroid hormone assessments, as noted below.

2.1. Timing and methods of blood collection need consideration

Blood sampling practices should control for experimental, temporal and environmental factors that might influence the absolute concentration measured and the variability of the hormone determination [10]. When best practices are established, consideration should be given to e.g., balancing the time/age of collection of blood and euthanasia across dose groups, collecting samples in as narrow a time period as possible for any given age (because of diurnal variability), minimizing stress during blood collection, pooling of blood from the litter by sex, and the potential impact of anesthesia.

2.2. Standardization and validation of analyses is crucial

Different analytical methodologies are currently being used across labs in the US and Europe. They include ELISA, RIA, Immunoluminescence or HPLC–MS, using different types of human or rat antibody kits, run on various equipment. The different analytical methods vary in the reference calibrator used in quantifying the relative hormone levels and may also have different sensitivities and variabilities. Additionally, transparent reporting of methodological details can sometimes be lacking in the published literature. For these reasons, there is a need for historical controls and comparison of data across studies may be complicated when different analytical testing methods are employed. Further, several labs are experiencing problems with obtaining sufficient sample volume from PND 4 pups. As the reliability of the hormone data is the basis for any subsequent conclusion, standardization of method validation, building up historical control and data interpretation is crucial. While various methodologies may be acceptable, the chosen method should be able to measure normal physiological ranges and detect a defined change of hormone concentrations. Moreover, as the statistical power is relatively low in OECD TG 421 and 422 studies due to the relatively small group sizes (N = 10 per group), it is very important to use an analytical method that is sufficiently sensitive. During the feasibility study for the update of OECD TG 421 and 422, a power analysis was performed (OECD report no. 217; [11]). The statistical analysis showed that detection of a 20% change in T4 levels in a dosed group, compared to control is not likely with 10 litters per group, as this required at least 17 litters per group. The same analysis indicated a high likelihood for detection of a 30% change in T4 with 10 litters per group, assuming average data variability. Unfortunately, the report does not mention which analytical methodologies and equipment for thyroid hormone measurements were used.

2.3. Findings that trigger additional measurements need to be clarified

It is unclear what nature and magnitude of findings should trigger additional measurements. In the OECD TG 421 and 422, it is stated "*Blood samples from the day 13 pups and the adult males are assessed for serum levels for thyroid hormones (T4). Further assessment of T4 in blood samples from the dams and day 4 pups is done if relevant. As an option, other hormones may be measured if relevant*".

However, relevance is not defined. In addition, criteria that prompt the analysis of additional hormones are absent. The OECD TG 443 mentions "*The surplus pups at PND 4 are subject to gross necropsy and consideration given to measuring serum thyroid hormone (T4) concentrations*." It is evident that more guidance is warranted on when to trigger further assessments and what endpoints should be assessed.

*

Table 1
Thyroid hormone parameters indicated in OECD[a] and EPA test guidelines.

	OECD TG 421 and 422[b]	OECD TG 443	OPPTS TG 890.1450 and 890.1500
Parental animals	F_0-males: F_0-females: T4, if relevant	F_0-males and females: T4, TSH F_1-males and females: T4, TSH	n.a.
PND 4 F_1-pups	T4, if relevant	T4, optional	n.a.
PND 13 F_1-pups	T4	n.a.	n.a.
PND 22 F_1-pups	n.a.	T4, TSH	n.a.
Peripubertal animals[c]	n.a.	n.a.	T4, TSH

n.a. = not applicable.
[a] Update of OECD TG 408 and 414 with thyroid hormone parameters is ongoing.
[b] As an option, other hormones may be measured if relevant.
[c] PND 42 females, PND 53 males.

2.4. The need for T4 measurements in PND 4 pups needs clarification

The OECD TG 421, 422 and 443 include blood sampling from PND 4 pups for possible T4 measurements, but T4 is already determined by default from PND 13 pups in OECD TG 421 and 422 studies (and TSH when triggered), and T4 and TSH levels are measured for PND 22 pups in OECD TG 443 studies. As larger blood samples can be obtained from the older pups, TSH levels can also be determined at this age, which collectively provides more useful study data. It is therefore questioned what the added value is for interpretation of PND 4 data when results from the older pups are more robust than the results from the younger pups. Moreover, it might also be considered inconsistent with principles of refinement in animal research [12] to perform a regulated procedure on an animal without analysis of the blood sample as from a regulatory perspective it would not be of added value using the current approach (i.e. to measure it in case an effect is seen for PND 13 pups). The reason for this approach is explained in the OECD TG 421 and 422 feasibility report [11]. A large variation in T4 concentrations for the younger versus older pups is reported. For PND 3–4 pups, the CVs (coefficient of variation) were in the range of 8–77% with median CVs of 8–31% in the data sets. At PND 14–16, the CVs were generally lower, i.e. they ranged from 4 to 33% with median CVs of 6–13%. From this, it was decided that T4 measurements should be included for PND 13 pups by default and for PND 4 pups only if required.

It has been postulated that thyroid hormone data from GD 20/21 fetuses and PND 4 pups are relevant because these fall in a particularly sensitive window and later measurements may not show effects at this developmental stage [13], one might wonder why measurement of T4 levels in PND 4 pups is not added by default. On the other hand, data from neonates appear to be less robust than from older animals, raising more questions about the relevance of thyroid hormone changes than providing answers. The lack of 'robustness' to date, however, may simply reflect data generated from the implementation of methods without the sensitivity to accurately assess low levels of thyroid hormone characteristic of younger animals. It is known that serum T4 values are very low for PND 4 pups (based on the authors own experience). Therefore, if the concern is decreased serum T4, the assay may not have the sensitivity and reproducibility to accurately detect these changes. This is method dependent, and remains a concern until method performance criteria are defined and adopted. This is an ongoing discussion, which has produced different requirements in different test guidelines (OECD) or guidance (EPA) [13], and harmonization of testing and method validation approaches would be needed.

2.5. Expert guidance should be provided on the relevance and adversity of findings

More guidance is needed for evaluation of the obtained thyroid hormone data. At the time, that OECD TG 407 (*Repeated Dose 28-Day Oral Toxicity Study in Rodents*; [2]) was updated in 2008, the OECD TG 407 validation data were judged insufficient to support inclusion of the thyroid hormone analyses as mandatory due to uncertainty about their sensitivity. The OECD TG 407 validation report [14] states that in the adult rats following 28 days of exposure *"thyroid histopathology was consistently the most reliable and most sensitive endpoint for the detection of thyroid modulation. Thyroid weight was reliable, but was somewhat less sensitive when compared to thyroid histopathology. Circulating thyroid hormone levels (T3, T4, and TSH) were not always reliable and sensitive, but the standard operating procedures for blood sampling and for thyroid hormone analyses were not standardized to reduce stress induced variability and to reduce analytical variability, respectively. Circulating T4 levels were the most promising of the three thyroid hormonal values"*. Based on this information, one might conclude that the outcome of histopathological evaluation of the thyroid should always be interpreted as more rigorous and relevant than the interpretation of any findings in thyroid hormone levels, and that in the presence of thyroid histopathology the measurement of these hormone levels might be considered redundant. However, the situation was considered different when the new guideline for the extended one-generation study was developed in 2010–2011 and assessment of thyroid hormones was included as mandatory and later also included in OECD TG 421 and 422 studies. The change in view over the years is not clearly described, as the methods are still not standardized. On the other hand, there are examples where changes in circulating levels of T4 are observed to be more sensitive outcomes than histopathological findings in the thyroid gland, likely related to the specific mode of action of thyroid toxicity [15,16]. Moreover, changes in thyroid histopathology are typically driven by increases in TSH (although direct acting toxicants may affect thyroid histopathology), not T4, and many examples exist where chemicals induce a T4 drop in the absence of a change in TSH and therefore no histopathological changes would be expected [17–19]. Additionally, T4, not TSH is the hormone of importance for development. Moreover, thyroid histopathology might not be assessed in the OECD 421 study as the guideline mentions "may be examined when necessary". This issue should be clarified.

Another important point to mention is that thyroid disruption occurs when tissue levels of T3, the active form of thyroid hormone, are decreased sufficiently (magnitude, duration and timing of thyroid hormone are all relevant factors) [20,21]. Serum T4 levels may not reflect tissue levels of T3 due to differences in transport proteins, membrane transporters, deiodinase levels in tissues, etc. Therefore, serum T4 levels are a surrogate measure that may not adequately capture the relevant biology [20,21]. A better biomarker of thyroid hormone status might be needed.

An additional issue for interpretation is the measurement of thyroid hormone on PND 13 in the OECD TG 421 and 422 studies versus PND 22 in the OECD TG 443 study. If a change in T4 is seen during the OECD TG 421/422 study, but a change is not seen on PND 22 in the follow-up OECD 443 study, will this negative result cancel

the earlier positive result seen on PND 13? This is a crucial point as often times and exposure methods may vary (e.g., gavage for 421/422 vs. diet for 443). Furthermore, there are marked changes in T4 during the postnatal period with a T4 peak around PND 15 and levels that look more similar to adults on PND 21.

3. Current activities

Currently, an OECD expert group on reproductive and developmental toxicity is looking into the possibility of providing some guidance on best practices for thyroid hormone sampling, measurement, and analysis within the context of OECD TG 421, 422, and 443, including an emphasis on the importance of demonstrating laboratory proficiency through method validation using positive control substances. In addition, the OECD expert group suggested that recommendations could be added to OECD guidance document No. 150 on *Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption* [22] that is at the time of publication under revision. To support this recommendation, it was suggested that empirical data be collected and a historical control database be developed in order to help the laboratories demonstrate their proficiency. This needs to be further discussed and the feasibility of the project assessed. The database would consist of positive control data from various laboratories, including reports on variability and details on the technology used for the measurements, as well as other study characteristics. Based on these discussions, both industry and US EPA will be preparing manuscripts for publication that summarize assay methods for T4 and historical control values of different ages (including PND 13 pups). These papers will be referred to on the OECD webpage in relation to OECD TG 421 and 422.

In 2017, meetings that focused on thyroid hormone assessments included a roundtable session ("Implementing developmental thyroid toxicity guidance into practice: what's working, what's not, and how can we do better?") at the Society of Toxicology annual meeting in the US, the EC/ANSES workshop ("European workshop on thyroid disruption") in Paris [23], the EC workshop ("Setting priorities for further development and validation of test methods and testing approaches for evaluating EDs") in Brussels (reporting ongoing), and initiation of an EFSA/ECHA/JRC effort to gather and compile information on hormone assessment of chemical substances. For all of these, follow-up activities are planned. In addition, on behalf of the EFSA/ECHA/JRC drafting team, the German Federal Institute for Risk Assessment (BfR) performed an anonymous survey (not published) followed by an expert hearing in 2017 with a follow-up plan to prepare a guidance document on best practices for hormone measurements. Moreover, together with the ETS task force on thyroid hormones, HESI-DART started to collate an industry wide historical database on thyroid hormones in order to establish 'biologically relevant ranges' for each assessment.

4. Recommendations for moving forward

There is an imperative need for clarification and guidance regarding the collection, assessment, and interpretation of thyroid hormone data for regulatory toxicology and risk assessment, particularly in regard to study designs that include perinatal or pre-weaning thyroid hormone evaluation. There are currently cross-laboratory differences in the methods being used to measure thyroid hormones in young rodents, as well as in the success of obtaining reliable data. Even though publicly available regulatory test guidelines and guidance address study design, they present varied approaches to thyroid hormone measurement in young rodents, and an optimal study design or logical approach to thyroid hormone testing in young rodents has not yet been established. Validity, consistency, and sensitivity of the assays are issues of concern. It is not clear to what extent variability in the data can be attributed to methodological issues or to innate biological variability. The intra- and inter-laboratory historical control data that are needed to address this question are not generally available as these data have only been recently added as requirement to the guidelines. When apparent treatment-related effects in thyroid hormone levels are observed, questions often arise regarding how much of a change, in what endpoints, should be interpreted as an adverse outcome with potential developmental consequences.

In summary, there are a number of important topics that need to be discussed and addressed, including but not limited to:

- Study design, including determining the need for the conduct of specific assays, minimizing animal use, and optimizing the impact of data collected from each animal
- Sampling techniques, including consideration of issues specific to the test subject age, scheduling (dose group and age distribution, timing), minimization of stress, pooling samples, sample storage and processing
- Assay selection and appropriate validation criteria.
- Historical control data collection and its use in the characterization of innate biological variability for thyroid hormone measures at various ages and under various study conditions
- Evaluation and interpretation of the data for consideration and use in risk assessment, including considerations of adversity

This conversation has already been initiated, and involves expert scientists from a number of international multisector organizations (e.g., government agencies, industry, academia, CROs, NGOs), participating in projects with diverse objectives. These current individual efforts, projects, and workgroups are important, even critical, to the resolution of the issues identified in this paper. There needs to be a concerted continuation of these individual efforts, and cross-sector and/or cross-project collaboration to maximize intellectual expertise and contributions. Communication of information to the larger scientific and regulatory communities is of prime importance.

The European Teratology Society (ETS) has initiated a Thyroid Hormone Task Force in 2016 to facilitate communication on this issue among society members and collaboration with other organizations. Plans have been developed to incorporate this topic into the programs of upcoming ETS annual meetings. The ETS Thyroid Hormone Task Force encourages sister scientific organizations and interested parties to raise awareness of issues and ongoing activities in this area of reproductive and endocrine toxicology, and to consider development of action plans and activities commensurate with their programmatic missions.

Note

The authors are members of the European Teratology Society (ETS) Task Force on thyroid hormone analyses in developmental and reproductive toxicology studies.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

References

[1] OECD, OECD Guideline for the Testing of Chemicals. No. 416. Two Generation Reproduction Toxicity Study, 2001, 22-January-2001 http://www.oecd-

ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.
[2] OECD, OECD Guideline for Testing of Chemicals No. 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents, 2008, 16-October-2008 http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.
[3] OECD, OECD Guideline for the Testing of Chemicals. No. 443. Extended One Generation Reproductive Toxicity Study, 2012, 28-July-2011 (former) and 02-Oct-2012 (updated) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.
[4] OECD, OECD Guideline for Testing of Chemicals No. 421. Reproduction/developmental Toxicity Screening Test, 2016, 27-July-1995, 28-July-2015 (former) and 29-July-2016 (updated) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.
[5] OECD, OECD Guideline for Testing of Chemicals No. 422. Combined Repeated Dose Toxicity Study with the Reproduction/developmental Toxicity Screening Test, 2016, 22-March-1996, 28-July-2015 (former) and 29-July-2016 (updated) http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.
[6] OECD, OECD Guideline for Testing of Chemicals No. 414. Prenatal Developmental Toxicity Study, 2001, 22-January-2001 http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.
[7] OECD, OECD Guideline for Testing of Chemicals No. 408. Repeated Dose 90-Day Oral Toxicity Study in Rodents, 1998, 21-September-1998 http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.
[8] US EPA, Endocrine Disruptor Screening Program Test Guidelines. OPPTS TG 890.1450 Pubertal Developmental and Thyroid Function in Intact Juvenile/Peripubertal Female Rats, 2009, October 2009 https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines.
[9] US EPA, Endocrine Disruptor Screening Program Test Guidelines. OPPTS TG 890.1500 Pubertal Developmental and Thyroid Function in Intact Juvenile/Peripubertal Male Rats, 2009, October 2009 https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines.
[10] Döhler, et al., The rat as model for the study of drug effects on thyroid function: consideration of methodological problems, Pharmacol. Ther. 5 (1979) 305–318.
[11] OECD, OECD Series on Testing and Assessment. Feasibility Study for Minor Enhancements of TG421/422, 2015, ENV/JM/MONO(2015)24. 14-Dec-2015 http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.
[12] Buchanan-Smith, et al., Harmonising the definition of refinement, Animal Welfare 14 (2005) 379–384.
[13] US EPA, Guidance for Thyroid Assays in Pregnant Animals, Fetuses and Postnatal Animals, and Adult Animals, Office of Pesticide Programs, Health Effects Division, Washington, DC, 2005 https://www.epa.gov/sites/production/files/2015-06/documents/thyroid_guidance_assay.pdf.
[14] OECD, Report of the Validation of the Updated Test Guideline 407: Repeat Dose 28-day Oral Toxicity Study in Laboratory Rats. Series on Testing and Assessment No. 59, 2006, 08-Sep-2006. ENV/JM/MONO(2006)26 http://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmentpublicationsbynumber.htm.
[15] G. Coelho-Palermo Cunha, B. van Ravenzwaay, Evaluation of mechanisms inducing thyroid toxicity and the ability of the enhanced OECD Test Guideline 407 to detect these changes, Arch. Toxicol. 79 (2005) 390–405.
[16] J.M. Manson, Mechanism of nitrofen teratogenesis, Environ. Health Perspect. 70 (1986) 137–147.
[17] R.T. Zoeller, R. Bansal, C. Parris, Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxin, and alters RC3/neurogranin expression in the developing rat brain, Endocrinology 146 (2005) 607–612.
[18] S.C. Chang, J.R. Thibodeaux, M.L. Eastvold, D.J. Ehresman, J.A. Bjork, J.W. Froehlich, C.S. Lau, R.J. Singh, K.B. Wallace, J.L. Butenhoff, Negative bias from anolog methods used in the analysis of free thyroxin in rat serum containing perfluorooctanesulfonate (PFOS), Toxicology 234 (2007) 21–33.
[19] L.M. Zorrilla, E.K. Gibson, S.C. Jeffay, K.M. Crofton, W.R. Setzer, R.L. Cooper, T.E. Stoker, The effects of triclosan on puberty and thyroid hormones in male Wistar rats, Toxicol. Sci. 107 (2009) 56–64.
[20] N.Y. Choksi, G.D. Jahnke, C. St Hilaire, M. Shelby, Role of thyroid hormones in human and laboratory animal reproductive health, Birth Defects Res. B Dev. Reprod. Toxicol. 68 (December (6)) (2003) 479–491.
[21] R.T. Zoeller, J. Rovet, Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings, J. Neuroendocrinol. 16 (October (10)) (2004) 809–818.
[22] OECD, Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption. Series on Testing and Assessment No. 150, 2012, 24-Aug-2012. ENV/JM/MONO(2012)22 http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)22&doclanguage = en.
[23] A. Kortenkamp, O. Martin, A. Baynes, E. Silva, M. Axelstad, U. Hass, Supporting the Organisation of a Workshop on Thyroid Disruption – Final Report, DG Environment, European Commission, 2017, http://dx.doi.org/10.2779/921523 https://publications.europa.eu/en/publication-detail/-/publication/472d2c88-a8b1-11e7-837e-01aa75ed71a1/language-en.