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Review

Mechanism-based testing strategy using *in vitro* approaches for identification of thyroid hormone disrupting chemicals

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ABSTRACT

The thyroid hormone (TH) system is involved in several important physiological processes, including regulation of energy metabolism, growth and differentiation, development and maintenance of brain function, thermo-regulation, osmo-regulation, and axis of regulation of other endocrine systems, sexual behaviour and fertility and cardiovascular function. Therefore, concern about TH disruption (THD) has resulted in strategies being developed to identify THD chemicals (THDCs). Information on potential of chemicals causing THD is typically derived from animal studies. For the majority of chemicals, however, this information is either limited or unavailable. It is also unlikely that animal experiments will be performed for all THD relevant chemicals in the near future for ethical, financial and practical reasons. In addition, typical animal experiments often do not provide information on the mechanism of action of THDC, making it harder to extrapolate results across species. Relevant effects may not be identified in animal studies when the effects are delayed, life stage specific, not assessed by the experimental paradigm (e.g., behaviour) or only occur when an organism has to adapt to environmental factors by modulating TH levels. Therefore, *in vitro* and *in silico* alternatives to identify THDC and quantify their potency are needed. THDC have many potential mechanisms of action, including altered hormone production, transport, metabolism, receptor activation and disruption of several feed-back mechanisms. *In vitro* assays are available for many of these endpoints, and the application of modern '-omics' technologies, applicable for *in vivo* studies can help to reveal relevant and possibly new endpoints for inclusion in a targeted THDC *in vitro* test battery. Within the framework of the ASAT initiative (Assuring Safety without Animal Testing), an international group consisting of experts in the areas of thyroid endocrinology, toxicology of endocrine disruption, neurotoxicology, high-throughput screening, computational biology, and regulatory affairs has reviewed the state of science for (1) known mechanisms for THD plus examples of THDC; (2) *in vitro* THD tests currently available or under development related to these mechanisms; and (3) *in silico* methods for estimating the blood levels of THDC. Based on this scientific review, the panel has recommended a battery of test methods to be able to classify chemicals as of less or high concern for further hazard and risk assessment for THD. In addition, research gaps and needs are identified to be able to optimize and validate the targeted THD *in vitro* test battery for a mechanism-based strategy for a decision to opt out or to proceed with further testing for THD.

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Contents

1. Introduction

Endocrine disruption (ED) by chemicals is not restricted to the sex hormone system, but also includes thyroid hormone (TH) disruption (THD). THD is defined herein as a change in hormone production, transport, function or metabolism resulting in impaired homeostasis. When the homeostasis is not impaired it is called a hormone modulator. THD can be induced by a variety of causes including diet, disease, and exposure to environmental chemicals.

TH are involved in several important physiological processes such as regulation of energy metabolism (Cheng et al., 2010), growth and differentiation, development and maintenance of brain function and the sympathetic nervous system (Bernal, 2007; Horn and Heuer, 2010; Reinehr, 2010; Warner and Mittag, 2012), thermo-regulation (Ribeiro, 2008), osmo-regulation and renal function (Vargas et al., 2006), regulation of onset and proper function of other endocrine systems including the estrogen system, sexual behaviour and fertility, and cardiovascular functioning (Danzi and Klein, 2012; Krassas et al., 2010; Wagner et al., 2008). Whether during development of the organism, differentiation of cells and tissues, maintenance or alteration of physiological functions of adult individuals, in many cases TH effects can best be characterized as 'permissive hormone action'. This indicates that the TH status of cells, tissues, and organisms provides the background and platform for other biological signals – hormonal, neural, immunological, nutritive and environmental – that are critical for maintenance of both development and homeostasis of the organism as a whole (Lopez-Juarez et al., 2012; Pascual and Aranda, 2012; Sirakov et al., 2012).

The predominant TH in the circulation in the euthyroid situation is 3,3′,5,5′-tetraiodothyronine (thyroxine, T_4), which is the precursor for the most active form of TH (3,3′,5-triiodothyronine; T_3). Most of the known functions of TH are mediated by the interaction of T_3 with the nuclear T_3-receptors (TRs), which act as ligand-modulated transcription factors. While almost none of the genes regulated by T_3 are exclusively responsive to T_3, virtually all molecular, cellular and metabolic events are more or less sensitive to TH (Grimaldi et al., 2012; König and Moura Neto, 2002; Oetting and Yen, 2007).

1.1. Possible physiological consequences of thyroid hormone disruption

Lessons learned from decades of biomedical studies of iodide deficiency, congenital hypothyroidism, genetic diseases related to