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QSAR models for thyroperoxidase inhibition and screening of U.S. and EU chemical inventories☆

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ABSTRACT

Thyroperoxidase (TPO) is the enzyme that synthesizes thyroid hormones (THs). TPO inhibition by chemicals can result in decreased TH levels and developmental neurotoxicity, and therefore identification of TPO inhibition is of high relevance in safety evaluation of chemicals. In the present study, we developed two global quantitative structure–activity relationship (QSAR) models for TPO inhibition *in vitro*. Rigorous cross- and blinded external validations demonstrated that the first model, QSAR1, built from a training set of 877 chemicals, was robust and highly predictive with balanced accuracies of 80.6% (SD = 4.6%) and 85.3%, respectively. The external validation test set was subsequently merged with the training set to constitute a larger training set totaling 1519 chemicals for a second model, QSAR2, which underwent robust cross-validation with a balanced accuracy of 82.7% (SD = 2.2%). An analysis of QSAR2 identified the ten most discriminating structural features for TPO inhibition and non-inhibition, respectively. Both models were used to screen 72,524 REACH substances and 32,197 U.S. EPA substances, and QSAR2 with the expanded training set had an approximately 10% larger coverages compared to QSAR1. Of the substances predicted within QSAR2's applicability domain, 8,790 (19.3%) REACH substances and 7,166 (19.0%) U.S. EPA substances, respectively, were predicted to be TPO inhibitors. A case study on butyl hydroxyanisole (BHA), which is extensively used as an antioxidant, was included to exemplify how predictions from the developed QSAR2 model may aid in elucidating the modes of action in adverse outcomes of chemicals. Overall, predictions from QSAR2 can for example be used in priority setting of chemicals and in read-across cases or weight-of-evidence assessments.

Introduction

Thyroid hormones (THs) participate in multiple biological processes from early development and throughout adulthood [1–3]. In the fetus and neonate, THs play an essential role in neurodevelopment [4], where they are involved in neuron differentiation, proliferation and migration, dendritic branching and synaptogenesis, and myelination [1,5]. In early gestation, the fetus depends entirely on maternally-derived THs until the fetal thyroid gland becomes functional at approximately gestational week 12 in humans and gestational day 17–18 in rats [1,6,7]. Maternal THs continue to contribute to fetal TH levels throughout gestation in both humans and rats [1,6]. Studies have shown that even a moderate and transient decrease in maternal TH levels during pregnancy is associated with permanent adverse neurological changes in the offspring [8]. In animal models and humans altered cognition, socialization and motor function as well as hearing loss have been observed following moderate to severe hypothyroidism [6,9–17]. Even low levels of TH insufficiency during fetal development

Abbreviations: AD, applicability domain; AOP, adverse outcome pathway; AUR, Amplex®UltraRed; BHA, butylated hydroxyanisole; DNT, developmental neurotoxicity; DTU Food, Technical University of Denmark National Food Institute; EPA, Environmental Protection Agency; HTS, high-throughput screening; IATA, integrated approaches to testing and assessment; KE, key event; LPDM, Leadscope® Predictive Data Miner; MIE, molecular initiating event; NCCT, National Center for Computational Toxicology; OECD, Organisation for Economic Co-operation and Development; PLR, partial logistic regression; PRS, pre-registered substances; QSAR, quantitative structureactivity relationship; SD, standard deviation; TH, thyroid hormone; TPO, thyroperoxidase; WoE, weight-of-evidence

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may result in measurable IQ deficits in children [9,13–18]. In adulthood, dysregulated TH levels can give reversible clinical symptoms of hypo- or hyperthyroidism [8] and are correlated with pathological processes involved in adverse outcomes such as cancer, obesity and type II diabetes mellitus [19,20].

Humans are exposed to tens of thousands of man-made chemicals through food, drugs, air, water and consumer products [21–24]. Large data gaps exist for most of these xenobiotics on their potential thyroid disrupting properties [25]. Xenobiotics can disturb TH homeostasis through many different mechanisms, including altered TH synthesis, transport, metabolism, and thyroid hormone receptor activation as well as disruption of the hypothalamus-pituitary-thyroid axis [10,25–28]. The same xenobiotic may act through more than one mechanism [25]. Because of the severity of the adverse effects that can be expected from chemical disruption of thyroid homeostasis, especially during early development, there is a need to develop a strategy for the identification and testing of thyroid-active compounds. As a step towards replacing expensive and time-consuming whole animal studies with alternative methods in chemical risk assessments, the Organisation for Economic Co-operation and Development (OECD) launched a new program on the development of Adverse Outcome Pathways (AOPs) in 2012 [29]. An AOP describes the sequential chain of causally linked events at different levels of biological organization starting from a so-called molecular initiating event (MIE) going through a number of downstream linked key events (KEs), and ends at an adverse health or ecotoxicological effect [29,30]. According to the OECD, AOPs are the central element of a toxicological knowledge framework to support chemical risk assessment based on mechanistic reasoning. AOPs can help industry and regulators use results from alternative methods, such as *in vitro* and *in silico* methods, in chemical risk assessments [31], e.g. by applying the AOP in OECDs Integrated Approaches to Testing Assessment (IATA) context [29,32,33]. Multiple thyroid-related AOPs have been suggested [34,35]. One AOP under development determined to have a strong overall weight-of-evidence (WoE) describes a series of linked events from the MIE, thyroperoxidase (TPO) inhibition, leading to hypothyroxinemia, and resulting in altered neurodevelopment and neurological dysfunction in the offspring [36, see also 4 and 25]. TPO is a heme-containing multifunction enzyme essential in TH synthesis [37,38]. Recently, a high-throughput screening (HTS) *in vitro* assay for TPO inhibition was developed by the U.S. Environmental Protection Agency (EPA) National Center for Computational Toxicology (NCCT) [39] and used to screen 1,126 ToxCast Phase I and II chemicals including structurally diverse environmental chemicals and failed drugs [34,40,41]. The assay is based on microsomes from rat thyroid tissue and requires the amount from approximately one rat to assess quantitative TPO inhibition of 1.5 chemicals [39]. An additional set of 771 ToxCast chemicals (known as the 'Endocrine 1000' or 'E1K' set) [41,42] was subsequently screened in the same HTS TPO inhibition assay (Simmons et al., in prep).

The goal of the present study was to use the ToxCast data to develop *in silico* models, and apply the models to large inventories of man-made chemicals to predict their potential to inhibit TPO. For this purpose, we first used experimental TPO inhibition results for 1,126 ToxCast Phase I and II chemicals to prepare a training set of 877 chemicals, which was then used to train and cross-validate a global binary Quantitative Structure-Activity Relationship (QSAR) model. QSARs are mathematical models that relate chemical structure descriptors with an experimental continuous (e.g. EC_{50}) or categorical (e.g. positive/negative) activity. Once established, these *in silico* models can be used as a non-testing approach to predict the activities of untested chemical structures (an introduction to QSAR can e.g. be found in [43] and [44]). The E1K dataset was used to prepare a test set of 646 chemicals, which was applied to externally validate the QSAR model. Next, the test set was merged with the training set to form a larger training set of 1,519 chemicals, which was subsequently used for training and cross-validating a second QSAR model. An analysis of the structural features in the second QSAR model was performed to identify the top features that discriminated TPO inhibitors from non-inhibitors. Both QSAR models were used to screen two large EU and U.S. chemical inventories containing man-made substances potentially present in e.g. the environment and consumer products for their possible TPO inhibition activity. The screened EU inventory consist of 72,524 REACH pre-registered substances (PRS) extracted from the online Danish (Q)SAR Database structure set [45,46]. Briefly, REACH pre-registration concerns existing substances that companies plan to register under REACH as so-called phase-in substances and the full PRS list contains a total of 145,299 unique substances/entries [47]. The U.S. inventory was originally curated by the U.S. EPA as a part of the CERAPP project [48] and contains 32,464 unique structures to which humans are potentially exposed. The structures were curated from sources such as the ACToR CPCat database [21], the DSSTox database [49], the Canadian Domestic Substances List, the Endocrine Disruption Screening Program set and EPI Suite training and test sets [41,42,48]. Predictions from these screenings will inform a tiered approach to prioritize possible thyroid modulating chemicals for further evaluation and could be used, together with relevant AOP(s), in IATA WoE assessments [29,33,50]. We also conducted a case study to highlight how the developed QSAR models for TPO inhibition can support hypotheses regarding the mode of action for chemical-induced adverse outcomes observed in *in vivo* studies.

Materials and methods

Experimental datasets

We used two datasets provided by U.S. EPA NCCT with chemical structure information and HTS screening results for TPO inhibition *in vitro* to train and validate two QSAR models. The chemicals screened contained diverse chemical structures including environmental and industrial chemicals, as well as some failed drugs [41]. The chemicals in both datasets were not selected specifically for this project or based on suspected TPO inhibition activity, and the original datasets include internal replicated samples. The experimental results consisted of data from the HTS Amplex®UltraRed-thyroperoxidase (AUR-TPO) *in vitro* assay [39], which had further undergone a selectivity filtering procedure to identify potentially false positive results due to non-specific activity decrease in the AUR-TPO assay [34]. Briefly, all chemical structures were initially screened at a single, high concentration (~87.5 μM). The chemicals associated with 20% or greater decreases in maximal TPO activity were subsequently screened for possible concentration-response. The concentration-response data were processed as described previously using the ToxCast data pipeline whereby each chemical was assigned a 'hit-call' of 1 if active in AUR-TPO, or a 'hit-call' of 0 if inactive in AUR-TPO [51]. Actives in the AUR-TPO assay were further processed through a selectivity filtering algorithm, which integrates results from cytotoxicity and luciferase inhibition assays to identify possible non-specific positive results in the AUR-TPO assay [34]. The chemical structures, assays, data analysis and selectivity filtering procedure have been described in more details previously [34,39,40,51]. We classified the chemicals into three categories (Fig. 1): 1) chemicals that had a < 20% activity decrease in the single, high concentration screening or had been assigned a 'hit-call' of 0 in the concentration-response AUR-TPO screening were classified as inactive in this assay; 2) chemicals with a 'hit-call' of 1 in AUR-TPO and a selectivity score greater than 1 were classified as active for TPO inhibition; and 3) chemicals with a 'hit-call' of 1 in AUR-TPO but with a selectivity score of 1 or less were classified as inconclusive for TPO inhibition.

The first dataset provided to the QSAR model developers at the Technical University of Denmark National Food Institute (DTU Food) consisted of structure information and experimental results for 1,126 ToxCast Phase I and II chemicals [34,40,41], including replicates, and was used for preparing a training set referred to as training set 1