High-Accuracy QSAR *in Silico* Model for Predicting Thyroid Receptor Endocrine Disruption Potential

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Introduction

Interest in the impact of **endocrine-disrupting chemicals** (EDCs) on human health and ecosystems is growing globally. Regulatory agencies have developed frameworks for EDC identification involving *in vitro* and *in vivo* testing of Estrogenic, Androgenic, Thyroid and Steroidogenesis (EATS) modalities. While certain E, A and S receptor assays are fairly well-validated, thyroid receptor-specific assays are underdeveloped and lack comprehensive validation. Current regulatory methods rely on *in vivo* studies of reproductive and developmental toxicity which do not reveal specific interactions with thyroid receptor subtypes (α and β). Such knowledge gaps are especially significant for high-production-volume substances and complex substances *e.g.* petrochemicals.

To address these limitations, new approach methodologies (NAMs), including **high accuracy quantitative structure-activity relationship (HA-QSAR) models**, are essential for rapid, cost-effective **but accurate** screening of **chemical interactions with endocrine system targets**, thus minimizing reliance on animal testing.

Methods

This study introduces a novel HA-QSAR model designed to **predict thyroid receptor-mediated endocrine activity**, underpinned by an innovative data curation methodology. The dataset was primarily sourced from US EPA ToxCast[1] and refined through a **semi-automated curation process guided by expert criteria**, especially **considering cytotoxicity**, ensuring high data quality. The ToxCast dataset was augmented with actives from BindingDB[2] to improve data balance, as most *in vitro* results from ToxCast were inactives. The **data splitting** was then performed using **molecular fingerprints and the Kennard-Stone algorithm** to ensure structural diversity and balanced distribution across the training, validation, and test sets, while also considering the response to achieve better representativity of the target properties. A machine learning algorithm, namely a **support vector machine (SVM)** using circular molecular fingerprints, was trained and validated. A **sophisticated applicability domain methodology** was applied, based on the identification of structural analogues (using k-nearest neighbours' model) and the concordance of results with these analogues.

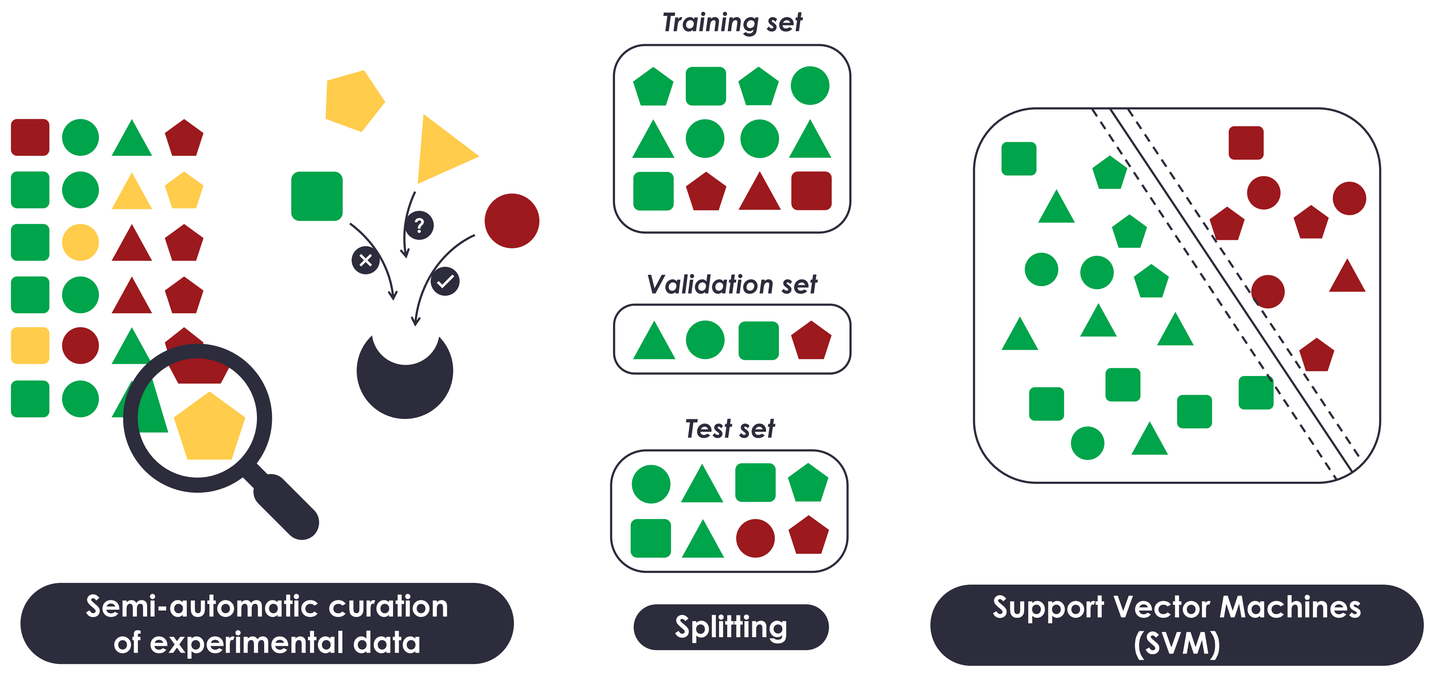

Figure 1. *Development of iVEMPS model (in vitro endocrine modalities prediction by SVM) for agonism/antagonism of thyroid receptors. Substances in red are active, while substances in green are inactive. Yellow substances were reevaluated for endocrine disruption taking into account the cytotoxicity level.*

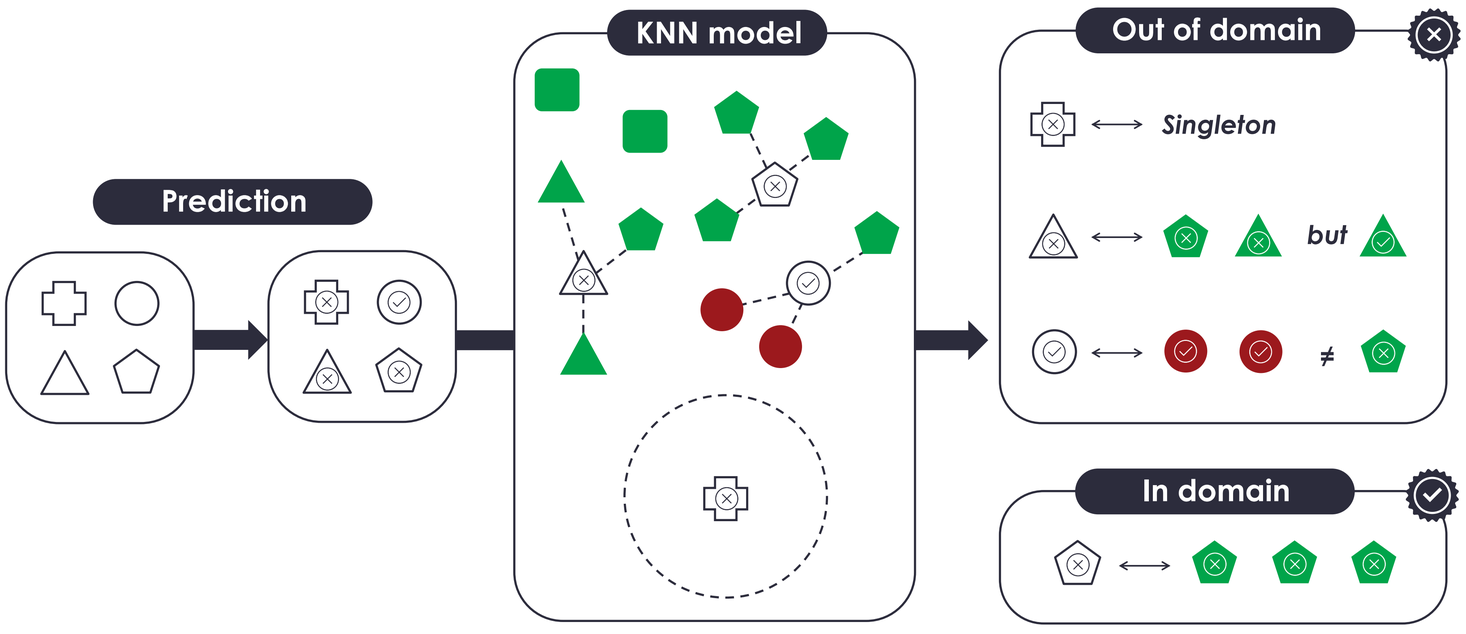

Figure 2. *Applicability domain is defined by a k-nearest neighbours' model (KNN). The predicted substance (white shape) is defined as out of domain whenever no analogues (coloured shapes) are retrieved. To be in domain, at least one analogue must be retrieved. Furthermore, prediction for each analogues (in white, within the coloured shape) must be similar to their respective experimental value (green for inactive, red for active) and match the prediction for the query substance.*

Table 1. *Statistics of the iVEMPS model for thyroid receptor agonism/antagonism.*

	Concordance or accuracy	Error rate	Sensitivity (actives)	Precision (actives)	Specificity (inactives)	Precision (inactives)	Balanced accuracy
Training set (goodness-of-fit)	99.97%	0.03%	99.34%	100%	100%	99.97%	99.67%
Test set (after applying AD)	99.62%	0.38%	92.06%	98.31%	99.93%	99.67%	95.60%

Results

The model demonstrated robust performance, with sensitivity, specificity and f1-score values of 99%, 100% and 99.6% for the training set, and 84%, 99.6%, and 87% for the external test set. A defined applicability domain further enhanced prediction reliability (Figure 3). A QMRF report is available, meeting OECD principles for QSAR validation and ECHA regulatory standards, making this model suitable for regulatory submissions.

Experimental inactive	1724	7
Experimental active	13	68
Before applying AD	Predicted inactive	Predicted active

Experimental inactive	1496	1
Experimental active	5	58
After applying AD	Predicted inactive	Predicted active

Figure 3. *Confusion matrix for the external validation set before (top) and after (bottom) applying applicability domain (AD)*

Conclusion

The HA-QSAR model's unique curation methodology improves data quality, facilitating more reliable predictions and filling critical gaps in thyroid receptor research. This **HA-QSAR efficiently identifies thyroid receptor activity for a wide range of organic chemical structures.** The same methodology will be extended to other EATS endpoints, **supporting regulatory and industrial assessments**, allowing prioritisation of chemicals with lower EATS modality risk, and **promoting faster hazard assessments** while **reducing animal testing**.

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[1] US EPA (2024). CompTox Chemicals Dashboard. CompTox Chemicals Dashboard v2.5.0. https://comptox.epa.gov/dashboard/.

[2] Gilson, M.K., and Liu, T. (2023). BindingDB: Measured Binding Data for Protein-Ligand and Other Molecular Systems. (UC San Diego Library Digital Collections). https://doi.org/10.6075/J0HD7VVF https://doi.org/10.6075/J0HD7VVF.

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