

A Proof-of-Concept Study: Evaluating the applicability of read-across tools and high throughput screening data for food relevant chemicals

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Background

Alternative toxicity methods to characterize the hazards of chemical substances have been proposed to reduce animal testing and efficiently screen thousands of chemicals. Relevant resources include large *in vitro* datasets from efforts such as the high-throughput screening (HTS) Tox21/ToxCast programs and read-across tools such as the Organization for Economic and Cooperation Development (OECD) QSAR toolbox. The goal of this work is to compare the result from traditional toxicity studies with predictions from these alternative testing methods for food relevant chemicals in ToxCast. We used computational models developed using Tox21/ToxCast high-throughput screening (HTS) data to predict the activity of food relevant chemicals against the androgen receptor (AR) pathway. We also used read across approaches to compare the pattern and potency for each target chemical and its respective analogue.

Experimental Flowchart

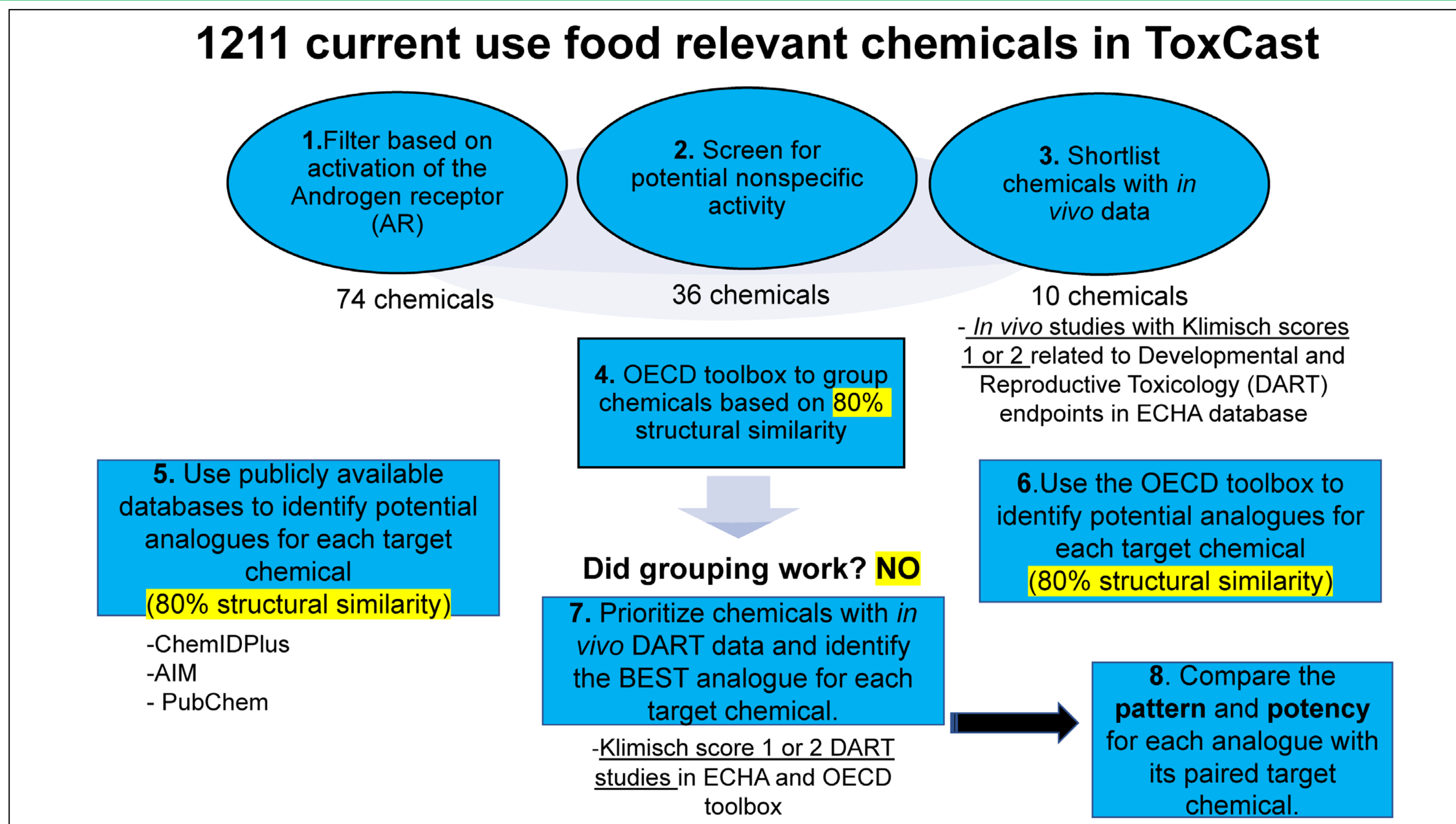


Figure 1. Experimental outline

1. From 1211 food relevant chemicals in ToxCast (Karmaus *et al.* 2017), 74 putatively active AR-active food relevant chemicals were identified.

2. To reduce possible confounding from cytotoxicity and cell stress, the AR model results were filtered based on observed *in vitro* cytotoxicity, which resulted in 36 putatively active, non-cytotoxic food relevant chemicals.

3. Chemicals were prioritized based on the availability of *in vivo* data related to DART, which resulted in 10 putatively active, non-cytotoxic, and DART related chemicals.

4. The OECD toolbox was used to group chemicals based on 80% structural similarity to help identify potential analogues for target chemical.

Results

Target chemical name	Target chemical image	Food relevancy (Karmaus et al. 2017)	Classification from HTS computational models	Best analogue	% Similarity	Analogue images
1. 4-tert-octylphenol		Indirect food additive	AR antagonist	4-tert-pentylphenol	not indicated	
2. Tributyltin chloride		Indirect food additive	AR antagonist	Tetrabutyltin	86.7	
3. Ziram		Pesticide residue	AR antagonist	Thiram	80	

Table 1. Putative endocrine activity and structural similarity of target chemicals and each analogue

Expanded Methodology

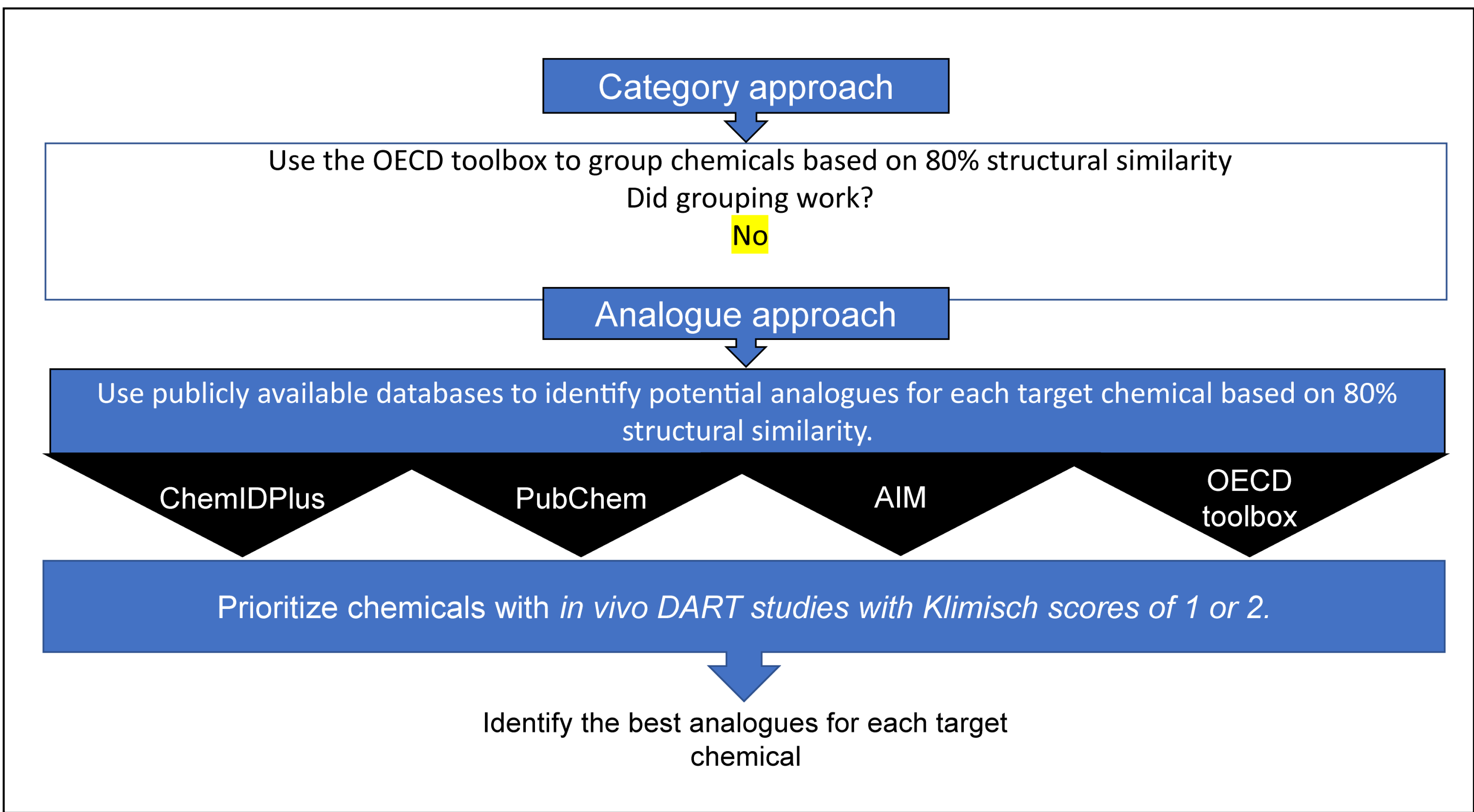


Figure 2. Proof of concept study

Structural similarity and similar mode of action were used to identify potential analogues for the 10 target chemicals.

	CASRN	Chemical name	% similarity	Similar Tox endpoints	Relative potency
1	140-66-9	4-(1,1,3,3-Tetramethylbutyl) phenol	target	acute toxicity	The analogue is more potent
	80-46-6	p-(1,1-dimethylpropyl) phenol	not indicated		
2	1461-22-9	Tributyltin chloride	target	acute toxicity, repeat dose tox, and sensitization	The target is more potent but they share similar potencies in other categories
	1461-25-2	Tetrabutyltin	86.7		
3	137-30-4	Ziram	target	irritation/corrosive, acute toxicity, sensitization	The target is more potent but they share similar potencies in other categories
	137-26-8	Thiram	80		

Table 2. High quality *in vivo* data for each target chemicals and its respective analogue

	CASRN	Chemical name	% similarity	Similar DART endpoints	Relative potency	H code DART classification
1	140-66-9	4-(1,1,3,3-Tetramethylbutyl) phenol	target	DART	similar potency	none
	80-46-6	p-(1,1-dimethylpropyl) phenol	not indicated			
2	1461-22-9	Tributyltin chloride	target	additional study details required	n/a	Repro. 1B H360
	1461-25-2	Tetrabutyltin	86.7			Repro. 1B H360; Repro. 2 H361
3	137-30-4	Ziram	target	DART	The target is more potent	none
	137-26-8	Thiram	80			

H360: may damage fertility or unborn child; H361: suspected of damaging fertility or unborn child

Table 3. Comparison of DART potencies for each target chemical and its respective analogue

Conclusions

- Using structural similarity and high quality *in vivo* data related to DART endpoints as our primary criteria, we identified 8 target chemicals for which the analogue approach could be employed.
- In terms of DART endpoints, the analogue approach helped protectively predict the potential endocrine activity of 3 out of 8 target chemicals.
- This study demonstrates that Tox21/ToxCast HTS assay data could be useful for prioritization along with weight of evidence from read-across tools to evaluate food relevant chemicals.

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This work does not necessarily reflect EPA policy.

