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# An expert-driven literature review of “negative” reference chemicals for developmental neurotoxicity (DNT) assay evaluation

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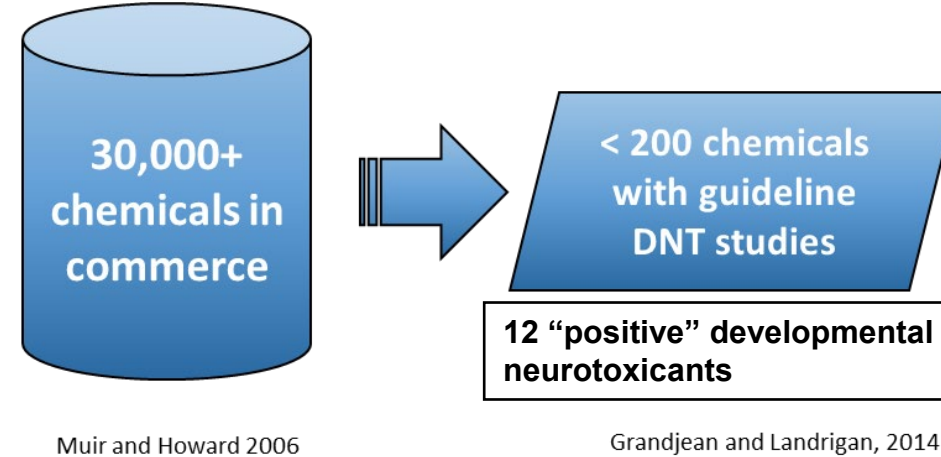
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## Introduction and Background

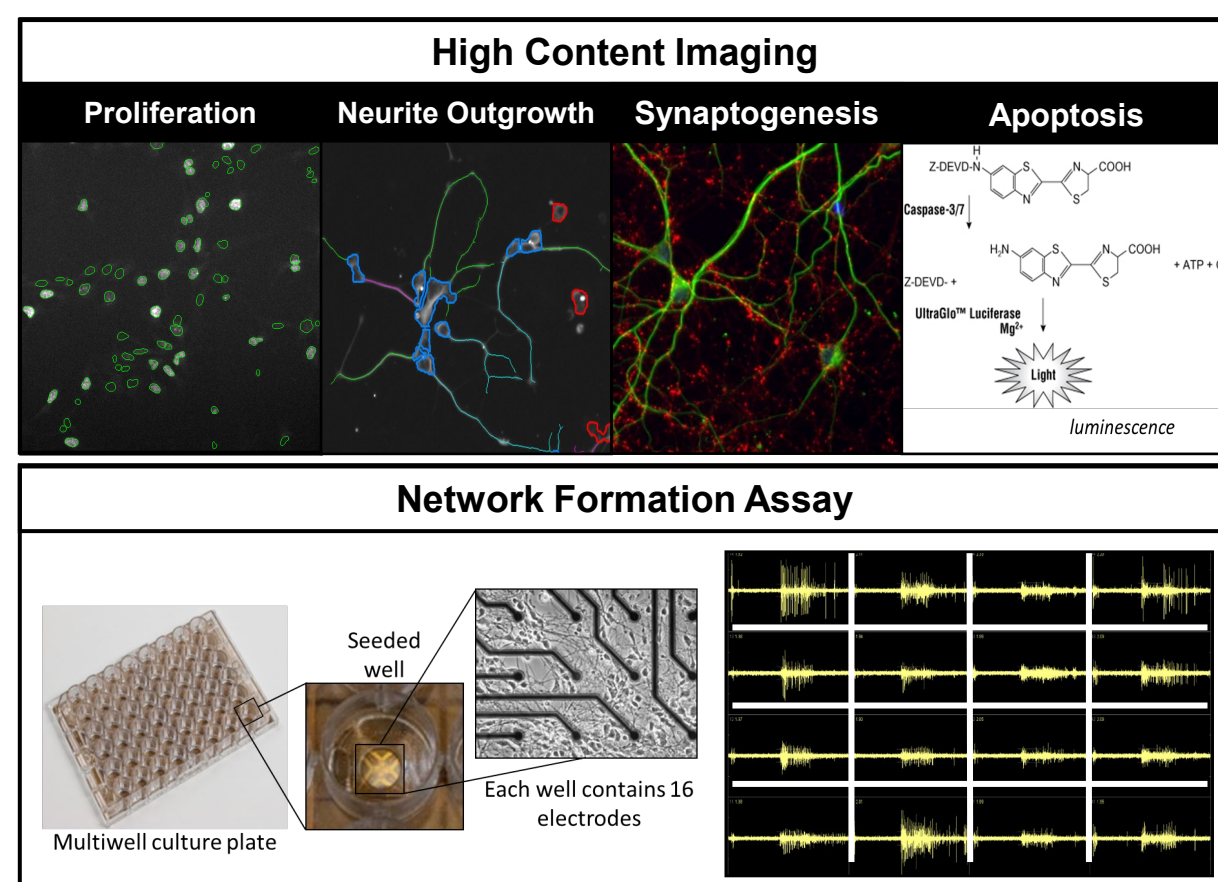
### Developmental Neurotoxicity (DNT) guideline studies

- Epidemiological studies have indicated that developmental exposure to environmental chemicals is associated with developmental disorders
- With > 30,000 chemicals in commerce, < 200 chemicals have been tested in EPA and/or OECD guideline DNT studies.
- Current guideline developmental neurotoxicity (DNT) studies are costly, time-consuming, use large numbers of animals and are subject to methodological and scientific uncertainties.
- Only 12 recognized human developmental neurotoxicants. (Grandjean and Landrigan, 2014)



### New approach methodologies (NAMs)

- To address this gap, a suite of DNT NAMs has been proposed for screening and prioritization.



### Evaluating the performance of the DNT-NAMs using sensitivity and specificity

- True Negative Rate (specificity)** = True negatives/Known Negatives
- True Positive Rate (sensitivity)** = True positives/ Known Positives

“Truth” or “What is known”			
Results from an <i>in vitro</i> NAM	Negatives	True negatives	False negatives
	Positives	False positives	True positives

### Problem: a curated list of negative DNT reference compounds does not currently exist

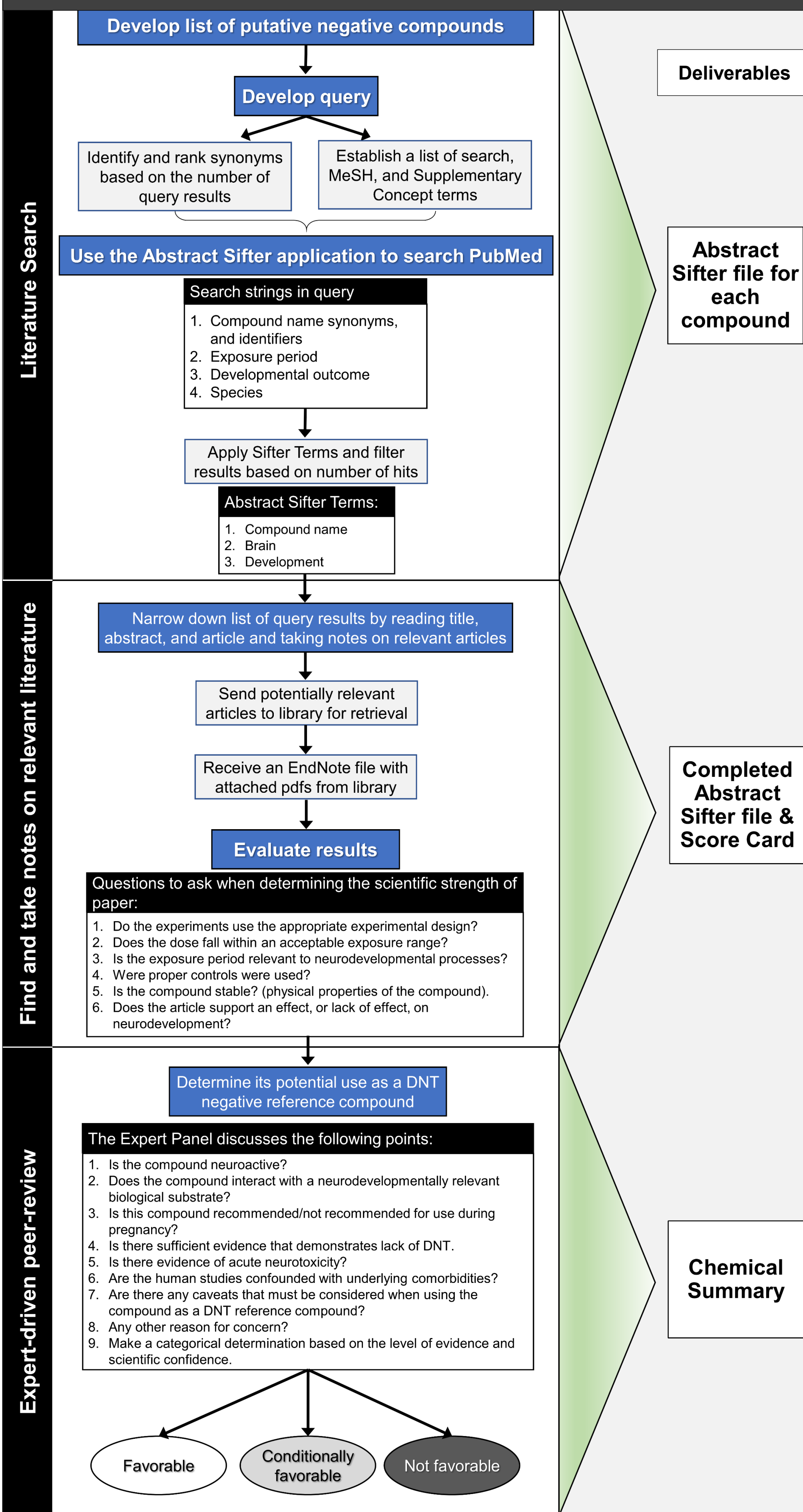
- To evaluate the performance of this battery of *in vitro* assays, we must first establish a set of DNT reference chemicals.
  - DNT positive compounds were vetted in Mundy et. al. (2015)
  - DNT negative compounds have not yet been vetted.

Therefore, the aim of this study is to develop a curated list of negative DNT reference chemicals.

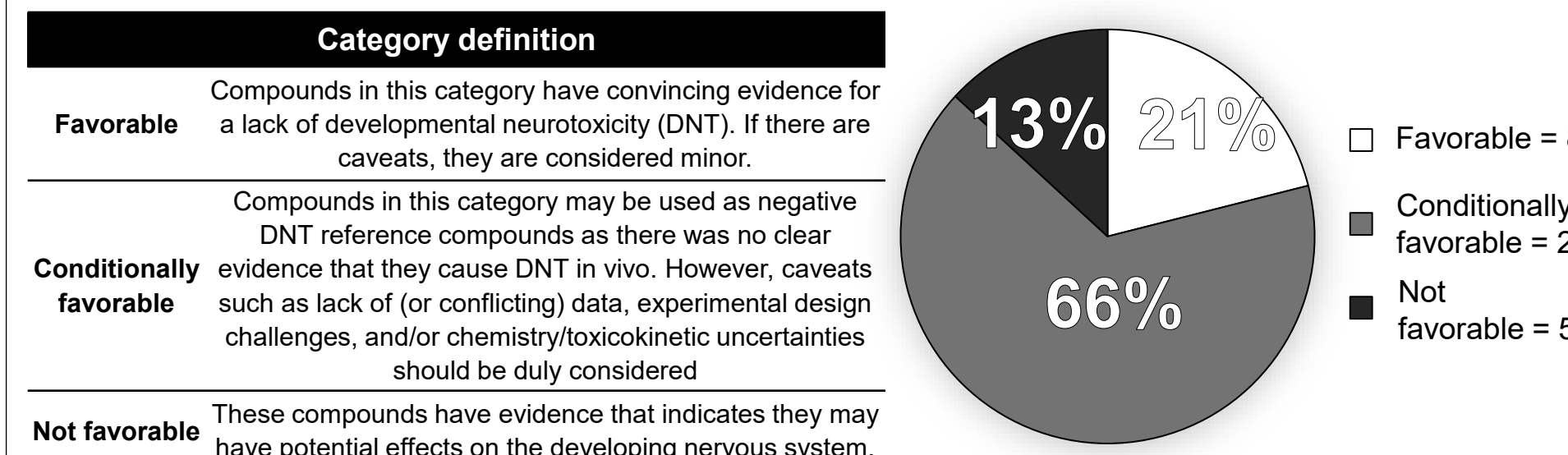
U.S. Environmental Protection Agency  
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## Methods

### Workflow for the identification of negative DNT reference compounds



### Almost 90% of the compounds had “favorable” or “conditionally favorable” profile



Case studies		
<b>Saccharin</b>	<b>Penicillin VK</b>	<b>Acetaminophen</b>
<ul style="list-style-type: none"><li>A non-nutritive artificial sweetener often used to mask the bitter taste of ethanol and nicotine</li><li>Developmental saccharin exposure failed to alter spontaneous locomotor activity, anxiety-like behavior, spatial working memory, object-based attention, recognition memory and impulsive-like behavior in the adult mouse (Zhang, et al. 2018)</li><li>Peri-adolescence saccharin exposure found no effects on reward-seeking behavior (Ivanson et al., 2015) or locomotor activity (Savolainen, et al. 2015).</li><li>Saccharin levels in the fetal brain were found to be relatively low (Pison, et al. 1973).</li><li><b>Categorical determination = Favorable</b></li></ul>	<ul style="list-style-type: none"><li>An antibiotic used against Gram positive bacteria; inhibits the final stage of bacterial cell wall synthesis, leading to cell lysis</li><li>Widely used in cell culture media to prevent bacterial contamination (e.g. Pen/Strep)</li><li>Only one relevant DNT study<ul style="list-style-type: none"><li>Suggests lasting effects on gut microbiota, increases cytokine expression in frontal cortex, modifies blood brain barrier integrity and alters behavior; increased anxiety-like and social behaviors, increased aggression (Lindsey, et al. 2017)</li></ul></li><li>Can possibly be used as a negative reference chemical, however, our results are confounded due lack of replicated or convincing results, potential indirect effects (gut-brain axis), and that it is often used in culture media.</li><li><b>Categorical determination = Conditionally Favorable</b></li></ul>	<ul style="list-style-type: none"><li>Medication used to treat fever and provide pain relief</li><li>Mechanism of action: selective COX-2 inhibitor; also modulates endocannabinoid system via TRPV1 and CB1 receptors</li><li>The endocannabinoid system is present during early brain development and is important for cell proliferation, neuronal migration, and axonal and neurite outgrowth</li><li>Over 20 clinical and preclinical studies reported that acetaminophen produces adverse neurodevelopmental outcomes<ul style="list-style-type: none"><li>Increases the risk of attention-deficit hyperactivity, hyperactivity, or autism spectrum disorders and bipolar emotional and communication skills (Shen, et al. 2016; Newman, et al. 2015)</li><li>Increases the risk of fetal death (Shen, et al. 2015)</li><li>Increases the risk of fetal death (Shen, et al. 2015)</li><li>Increases the risk of fetal death (Shen, et al. 2015)</li></ul></li><li><b>Categorical determination = Not Favorable</b></li></ul>

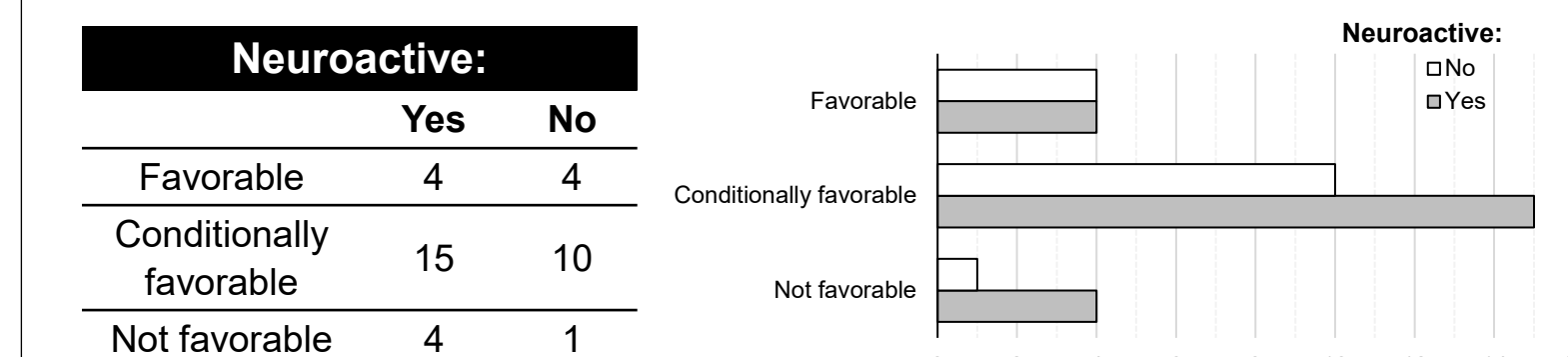
Compound name	CAS RN	DTXSID	Categorical determination
Acetaminophen	103-90-2	DTXSID2020006	Not favorable
Amoxicillin	26787-78-0	DTXSID3037044	Conditionally favorable
Ampicillin	69-53-4	DTXSID4022602	Conditionally favorable
Anthracene	120-12-7	DTXSID0023878	Conditionally favorable
Aspirin	50-78-2	DTXSID5020108	Conditionally favorable
Bismuth	7440-69-9	DTXSID3052484	Conditionally favorable
Buspirone	36505-84-7	DTXSID2022707	Conditionally favorable
Captopril	62571-86-2	DTXSID1037197	Conditionally favorable
Chloramben	133-90-4	DTXSID2020262	Conditionally favorable
Chlorpheniramine maleate	113-92-8	DTXSID4020321	Conditionally favorable
Cotinine	486-56-6	DTXSID1047576	Conditionally favorable
D-Glucitol	50-70-4	DTXSID5023588	Conditionally favorable
Diethylene glycol	111-46-6	DTXSID8020462	Conditionally favorable
Dinotefuran	165252-70-0	DTXSID7034549	Favorable
D-Mannitol	69-65-8	DTXSID1023235	Favorable
Doxylamine succinate	562-10-7	DTXSID7020552	Conditionally favorable
Erythromycin	114-07-8	DTXSID4022991	Conditionally favorable
Famotidine	76824-35-6	DTXSID5023039	Conditionally favorable
Fluconazole	86386-73-4	DTXSID3020627	Conditionally favorable
Galactosamine hydrochloride	1772-03-8	DTXSID4031356	Conditionally favorable
Glycerol	56-81-5	DTXSID9020663	Favorable
Glyphosate	1071-83-6	DTXSID1024122	Conditionally favorable
Ibuprofen	15687-27-1	DTXSID5020732	Favorable
Isoniazid	54-85-3	DTXSID8020755	Not favorable
L-ascorbic acid	50-81-7	DTXSID5020106	Favorable
Loperamide	53179-11-6	DTXSID6045165	Conditionally favorable
Metformin	657-24-9	DTXSID2023270	Conditionally favorable
Metoprolol	51384-51-1	DTXSID2023309	Not favorable
Mifepristone	84371-65-3	DTXSID5023322	Not favorable
Omeprazole	73590-58-6	DTXSID6021080	Favorable
Penicillin VK	132-98-9	DTXSID7021102	Conditionally favorable
Phenol	108-95-2	DTXSID5021124	Conditionally favorable
Saccharin	81-07-2	DTXSID5021251	Favorable
Selegiline hydrochloride	14611-52-0	DTXSID9044584	Favorable
Sodium benzoate	532-32-1	DTXSID1020140	Conditionally favorable
Sulfisoxazole	127-69-5	DTXSID6021292	Conditionally favorable
Tetracycline	60-54-8	DTXSID7023645	Conditionally favorable
Warfarin	81-81-2	DTXSID5023742	Not favorable

## Results

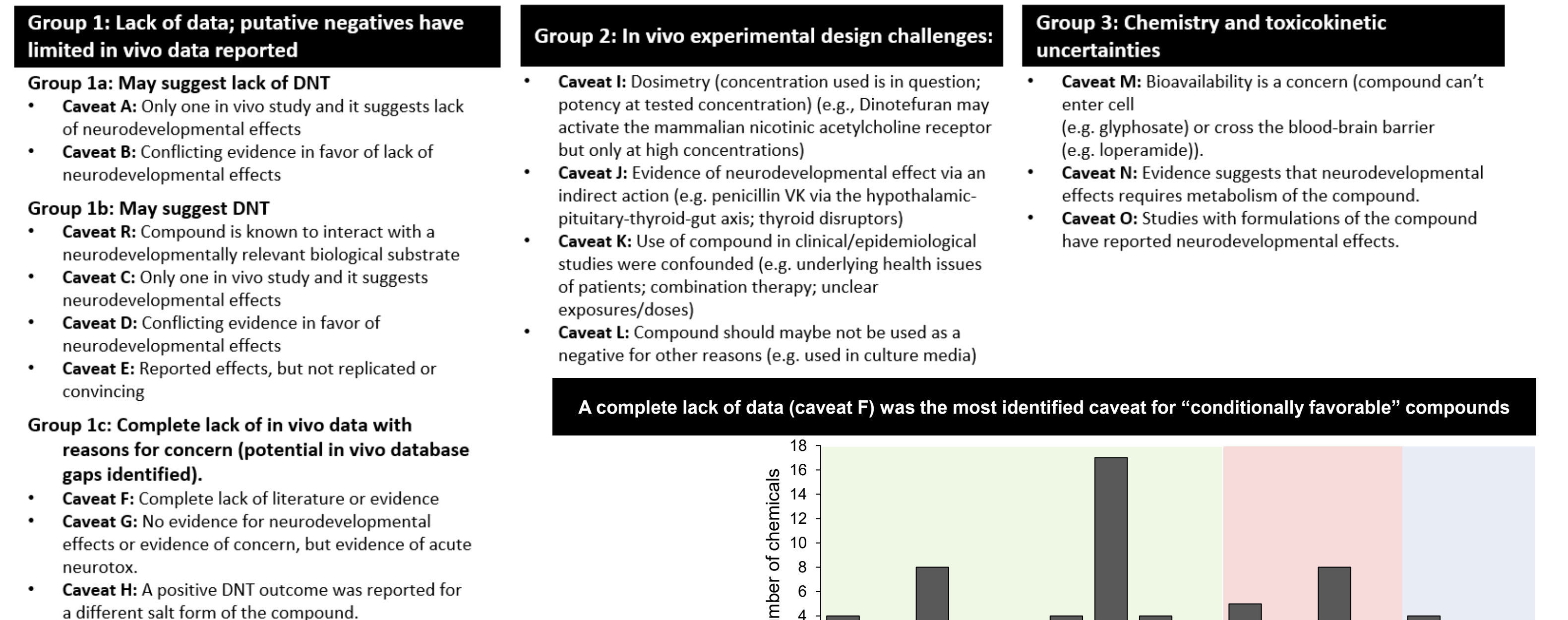
### Favorable compounds are not more likely to be U.S. FDA pregnancy category A or B

U.S. FDA Pregnancy Category					
Determination	A	B	C	D	X
Favorable	1	0	4	0	0
Conditionally favorable	0	9	2	2	0
Not favorable	0	0	2	0	2

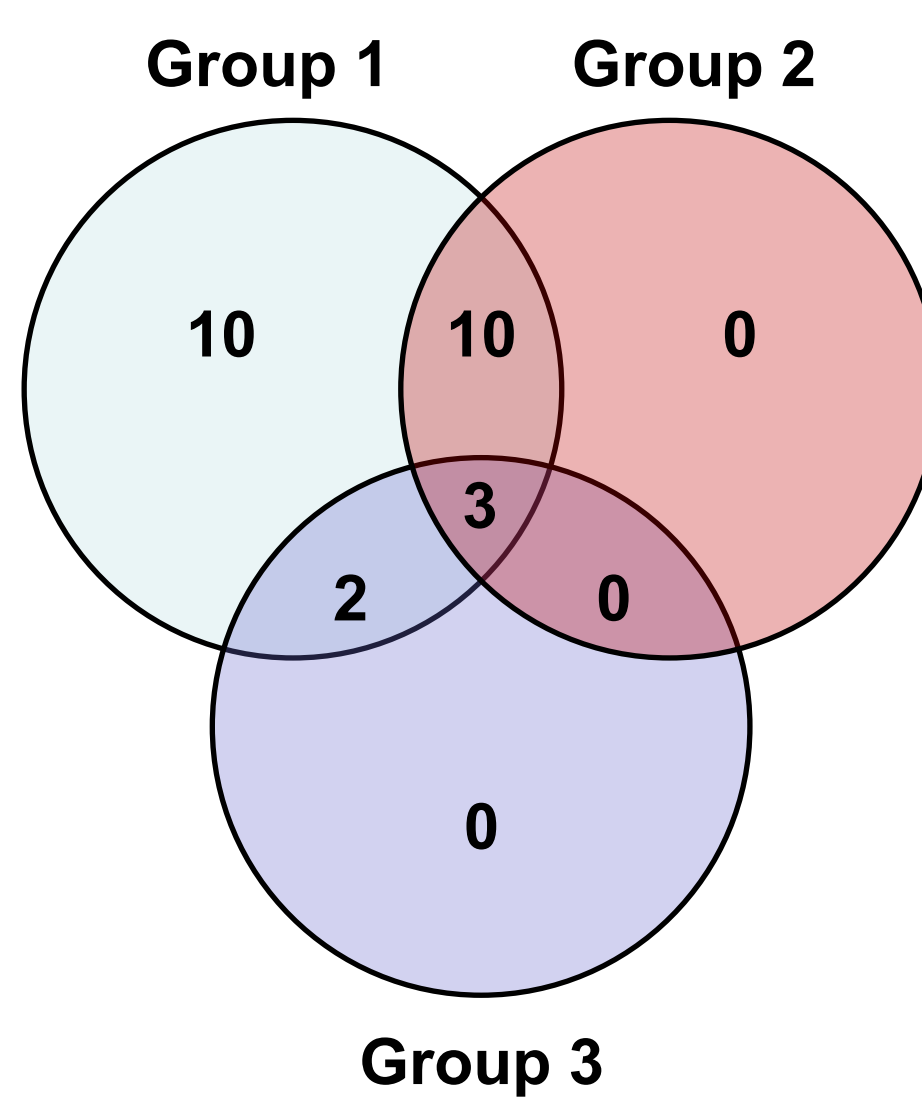
### Neuroactive compounds are less likely to be categorized as favorable



### Identified caveats for "conditionally favorable" compounds



All compounds that had one or more caveat in groups 2 and/or 3 also had caveat(s) in group 1



	Compound Name	Caveats															
		A	B	R	C	D	E	F	G	H	I	J	K	L	M	N	O
Antibiotics	Amoxicillin																
	Ampicillin																
	Erythromycin																
	Penicillin VK																
	Sulfisoxazole																
Drugs	Tetracycline																
	Aspirin																
	Buspirone																
	Captopril																
	Chlorpheniramine maleate																
	Doxylamine succinate																
	Famotidine																
Food	Fluconazole																
	Loperamide																
	Metformin																
	Sodium benzoate																
	Bismuth																
Metal	Anthracene																
	Cotinine																
	D-Glucitol																
	Diethylene glycol																
	Galactosamine hydrochloride																
Other	Phenol																
	Chloramben																
Pesticide	Glyphosate																

## Conclusion

- A set of 38 candidate negative compounds were evaluated using the Abstract Sifter Excel-based application to identify relevant studies which were then reviewed by a panel of experts.
- The panel determined that 8 out of 38 compounds could be categorized as “favorable” negative DNT reference compounds, whereas 6 compounds were categorized as “not favorable” for use in a DNT reference chemical set. 24 compounds were determined to be “conditionally favorable” given that one or more caveats were identified.
- This reference chemical set may be customized to support performance evaluation of specific DNT NAMs, depending on the assay principle and key processes included. Further, this work suggests that additional approaches to DNT NAM performance evaluation may be required.