

Appendix A

Proposed Positive and Negative Control Compounds for Use in Performance Evaluations of DNT IVB Assays

Proposed Positive and Negative Control Compounds for Use in Performance Evaluations of DNT IVB Assays

The purpose of this Appendix is to provide a list of proposed DNT Reference chemicals. The importance of a list of Reference chemicals cannot be understated as it provides the data necessary to calculate estimates of sensitivity and specificity for both individual assays and the entire DNT IVB. This proposed list was generated to foster international discussion that would lead to a consensus list of chemicals to be used in the development and performance evaluations of assays in the Developmental Neurotoxicity In Vitro Battery (DNT IVB). This includes chemicals deemed to be developmental neurotoxicants in humans, and/or chemicals with evidence of developmental neurotoxicity in mammalian animal models. The majority of chemicals in this list were deemed to have evidence DNT from animal models and were collated from publications that contained expert opinions as well as publication that conducted extensive literature reviews. The minority of chemicals listed as positive in humans were based on mostly on expert opinions

Appendix A.1A provides a list of proposed positive and negative control chemicals used in studies of performance of the DNT IVB. Table 1 lists chemicals used as positive reference chemicals, and Table 2 lists chemicals used as negative reference chemicals.

Appendix A.1B provides the references used to generate the proposed positive and negative chemicals. The list of positive compounds was compiled from the workshop reviews, reviews of published data, and the US EPA's Neurotoxicity Risk Assessment Guideline (see references for details). The table also provides the rationale for the negative reference chemicals, which in some cases differed from lab to lab and may not have been based on known negative in vivo DNT testing. For example, some chemicals may be listed negative in vitro due to lack of metabolism to an active metabolite. Some compounds were deemed to be negative based on a review of literature data (Martin et al., 2021).

References

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Appendix A.1A

Lists of Positive and Proposed Negative Control Compounds for Use in Performance Evaluations of DNT IVB Assays

Appendix A.1A, Table 1. Proposed positive DNT reference chemicals used in studies of performance of the DNT IVB. Details and references describing selection of in vivo positive DNT compounds can be found in Appendix A.1B. An 'X' indicates whether or not the chemical was tested in any of the four published reports. The lack of an X for a chemical indicates that it has not been tested in any of the four publications.

Compound	Harrill et al. (2018)	Frank et al. (2017) & Shafer et al. (2019)	Masjusthusmann et al. (2020)
Acrylamide	X	X	X
Allethrin		X	
Aluminum			
Aminonicotinamide. 6-	X	X	
Amphetamine	X		
Arsenic	X	X	
Aspartame			
Azacytidine			
Benomyl			
Benzene			
Bisphenol A	X	X	
Bis(tri-n-butyltin)oxide	X	X	
Bromodeoxyuridine			
Butylated hydroxyanisole			
Cadmium	X	X	X
Caffeine	X	X	
Carbamazepine	X	X	
Carbon monoxide			
Chlordecone			
Chlordiazepoxide	X	X	
Chlorine dioxide			
Chlorpromazine	X	X	X
Chlorpyrifos	X	X	X
Cocaine	X	X	
Colcemid			
Colchicine	X	X	
Cyclophosphamide	X	X	
Cypermethrin		X	
Cytosine arabinoside	X	X	
DDT, p,p'-			
Deltamethrin	X	X	X
Dexamethasone	X	X	X
Diazepam	X	X	

Compound	Harrill et al. (2018)	Frank et al. (2017) & Shafer et al. (2019)	Masjusthusmann et al. (2020)
Diazinon			
Dieldrin	x	x	
Di-(2-ethylhexyl) phthalate		x	
Diethylstilbestrol	x	x	
Dioxin			
Diphenylhydantoin	x	x	x
Domoic acid		x	x
Epidermal Growth Factor			
Ethanol			
Ethylene thiourea			
Fluoride	x		
Fluorouracil, 5-	x	x	
Fluoxetine	x	x	
Haloperidol	x	x	x
Halothane			
Heptachlor	x	x	
Heroin			
Hexachlorobenzene			
Hexachlorophene	x	x	x
Hydroxyurea	x	x	
Iminodipropionitrile, 3,3-		x	
Ketamine	x	x	x
Lead	x	x	x
Lidocaine			
Lindane		x	
Lysergic acid diethylamide			
Maneb	x	x	x
Manganese	x	x	x
Methylenedioxyamphetamine			
Methadone			
Methanol			
Methimazole		x	
Methotrexate	x	x	
Methylazoxymethanol			x
Methylmercury	x	x	x
Methyl parathion			
Monosodium glutamate			
Methyl-4-phenyl-1,2,3,6-tetrahydropyridine			

Compound	Harrill et al. (2018)	Frank et al. (2017) & Shafer et al. (2019)	Masjusthusmann et al. (2020)
Naloxone	x	x	
Naltrexone			
Nicotine	x	x	x
Ozone			
Paraquat	x	x	x
Parathion		x	
Penicillamine			
Perchlorate			
Perfluoroalkyls			x
Permethrin	x	x	
Phenobarbital	x	x	
Phenylacetate			
Polybrominated diphenyl ethers	x	x	x
Polychlorinated biphenyls			
Propranolol			
Propylthiouracil, 6-	x	x	
Retinoic acid	x	x	x
Tebuconazole	x	x	x
Tellurium			
Terbutaline	x	x	x
Tetrachloroethylene			
Tetrahydrocannabinol			
Thalidomide	x	x	
Toluene			
Triamcinolone			
Tri-n-butyltin			x
Trichlorfon			x
Trichloroethylene			
Triethyl lead			
Triethyltin	x	x	x
Trimethyltin	x	x	
Valproic acid	x	x	x

Appendix A.1A, Table 2. Summary of proposed negative DNT reference chemicals used in studies of performance of the DNT IVB. Details and references describing selection of in vivo negative DNT compounds can be found in Excel file xxx.

Compound	Harrill et al. (2018)	Frank et al. (2017) & Shafer et al. (2019)	Masjusthusmann et al. (2020)
Acetaminophen	x	x	x
Acetylsalicylic acid		x	x
Amoxicillin	x	x	x
Ascorbic acid		x	
Buspirone			x
Captopril	x		x
Chloramben	x		
Chlorpheniramine			x
Cotinine	x		
Diethylene glycol	x		x
Doxylamine succinate			x
Erythromycin		x	
Famotidine			x
Fluconazole	x		
Folic acid		x	
Glycerol		x	x
Glyphosate	x	x	
Ibuprofen			x
Isoniazid	x		
Loperamide	x		
Mannitol, D-		x	x
Metformin			x
Metoprolol			x
Penicillin VK			x
Phenol	x		
Propylene glycol		x	
Saccharin	x	x	x
Sodium benzoate	x	x	x
Sorbitol, D	x	x	x
Tetracycline		x	
Warfarin			x

Appendix A.1B

Lists of Positive and Proposed Negative Control Compounds
for Use in Performance Evaluations of DNT IVB Assays with
References and Rationale

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Benomyl	Positive				x									
Benzene	Positive				x									
Bisphenol A	Positive				x			x	x					
Bis(tri-n-butyltin)oxide	Positive				x			x	x					
Bromodeoxyuridine	Positive				x									
Butylated hydroxyanisole	Positive				x									
Cadmium	Positive		x		x h	x		x	x	x				
Caffeine	Positive				x			x	x					

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Carbamazepine	Positive				x h			x	x					
Carbon monoxide	Positive				x									
Chlordane	Positive		x		x									
Chlordiazepoxide	Positive				x h			x	x					
Chlorine dioxide	Positive				x									
Chlorpromazine	Positive				x	x		x	x	x				
Chlorpyrifos	Positive			h	x h	x		x	x	x				
Cocaine	Positive	x h			x	x h		x	x					

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Colcemid	Positive				x									
Colchicine	Positive				x			x	x					
Cyclophosphamide	Positive				x			x	x					
Cypermethrin	Positive				x				x					
Cytosine arabinoside	Positive				x			x	x					
DDT, p,p'	Positive		x	h										
Deltamethrin	Positive				x			x	x	x				
Dexamethasone	Positive				x h	x		x	x	x				

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Diazepam	Positive				x			x	x					
Diazinon	Positive				x									
Dieldrin	Positive				x			x	x					
Di-(2-ethylhexyl) phthalate	Positive				x				x					
Diethylstilbestrol	Positive				x			x	x					
Dioxin	Positive				x									
Diphenylhydantoin	Positive	x h			x h	x		x	x	x				
Domoic acid	Positive				x	x			x	x				

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Heptachlor	Positive				x			x	x					
Heroin	Positive	x h			x	x h								
Hexachlorobenzene	Positive				x									
Hexachlorophene	Positive				x h	x h		x	x	x				
Hydroxyurea	Positive				x			x	x					
Iminodipropionitrile, 3,3-	Positive				x	(negative)			x		Neurotoxicant requiring metabolic activation. Low toxicity if test system lacks			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
											activating enzymes			
Ketamine	Positive				x	x		x	x	x				
Lead	Positive	x h	x	h	x h	x h		x	x	x				
Lidocaine	Positive				x									
Lindane	Positive					x			x					
Lysergic acid diethylamide	Positive				x									
Maneb	Positive				x	x		x	x	x				
Manganese	Positive			h	x h	x		x	x	x				

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Thalidomide	Positive				x			x	x					
Toluene	Positive			h	x	x								
Triamcinolone	Positive				x									
Tri-n-butyltin	Positive				x					x				
Trichlorfon	Positive				x					x				
Trichloroethylene	Positive				x									
Triethyl lead	Positive				x									
Triethyltin	Positive				x	x		x	x	x				

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Acetaminophen	Negative							x	x	x	Negative in most systems up to mM levels, but has been discussed as <i>in vivo</i> DNT toxicant	Assay negative control	Shafer-not tested	Two criteria: 1) absence of effects in any of the USEPA ToxCast <i>in vitro</i> bioactivity assays (https://comptox.epa.gov/dashboard), or 2) lack evidence of developmental neurotoxicity in a review of the published literature (Pub Chem).

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Acetylsalicylic acid	Negative								x	x	Unknown	not tested	Commonly used substances and/or drugs that are used during pregnancy without established adverse neurodevelopmental outcomes.	
Amicillin	Negative													
Amitryptiline	Negative										Drugs that are acceptable			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
											during pregnancy			
Amoxicillin	Negative							x	x	x	Drugs that are acceptable during pregnancy	GRAS	Shafer - not tested	GRAS and/or FDA Pregnancy Risk Category #2.
Anthracene	Negative										Polycyclic aromatic hydrocarbon; may act via Ah receptor, but has no target in many human DNT/NT test systems			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Ascorbic acid	Negative						x		x		No evidence from previous work of neurite outgrowth inhibition	not tested	commonly used substances and/or drugs that are used during pregnancy without established adverse neurodevelopmental outcomes.	
Atropine	Negative										Drugs with low likelihood to affect DNT test systems due to their			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative					
										well characterized side effects and mode of action (may have direct effects on neural networks)									
Bismuth	Negative																		
Buspirone	Negative								x	Unknown									

Captopril	Negative							x		x	Drugs with extracellular targets			Two criteria: 1) absence of effects in any of the USEPA ToxCast in vitro bioactivity assays (https://comptox.epa.gov/dashboard), or 2) lack evidence of developmental neurotoxicity in a review of the published literature (Pub Chem).
Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Chloramben	Negative							x						Two criteria: 1) absence of effects in any of the USEPA ToxCast in vitro bioactivity assays(https://comptox.epa.gov/dashboard), or 2) lack evidence of developmental neurotoxicity in a review of the published literature (Pub Chem).
Chlorpheniramine	Negative									x	Unknown			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Cotinine	Negative							x						Two criteria: 1) absence of effects in any of the USEPA ToxCast in vitro bioactivity assays (https://comptox.epa.gov/dashboard), or 2) lack evidence of developmental neurotoxicity in a review of the published literature (Pub Chem).
Dabigatran	Negative										Drugs with extracellular targets			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Deferoxamine mesylate	Negative										Iron chelator, tolerated at mM levels			
Deprenyl	Negative										Antidepressant/parkinsonian drug, inhibitor of monoamine oxidase-B (1 mM range)			
Diethylene glycol	Negative							x		x	No pronounced bioactivity, sometimes not entering cells, tolerated to mM level;			Two criteria: 1) absence of effects in any of the USEPA ToxCast in vitro bioactivity assays (https://comptox.epa.gov/dashboard)

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
											belongs to “trivial” controls (low usefulness for specificity calculations) with solvents			, or 2) lack evidence of developmental neurotoxicity in a review of the published literature (Pub Chem).
Dimethylformamide	Negative										Generally low toxicity up to mM range			
Dimethylsulfoxide (DMSO)	Negative										Generally low toxicity up to mM range			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Dinitrofur n	Negative						x				Neonicotinoi d pesticide without DNT effects in many systems (may however affect neuronal network assays)			
diphenhyd ramine	Negative										Drugs that are acceptable during pregnancy			
Doxylamin e succinate	Negative									x	Unknown			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Erythromycin	Negative								x			not tested	commonly used substances and/or drugs that are used during pregnancy without established adverse neurodevelopmental outcomes.	
Famotidine	Negative									x	Unknown			

Fipronyl	
Negative	Positive or Negative
	Rees et al (1990)
	EPA Neurotox RA Guideline (1998)
	Grandjean papers (2006, 2014)
	Mundy et al List 1 (2015)
	Aschner et al (2017)
	Martin Negatives (2021)**
	Harrill et al (2018)
	Frank et al. (2017) & Shafer et al (2019)
	Masjusthusman et al (2020)***
Pesticide tested clearly negative for DNT; may be cytotoxic at > 10 µM; may have indirect effects through cramp induction (zebrafish)	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)
	Frank et al Rationale for Use as Negative
	Shafer et al Rationale for Use as Negative
	Harrill et al 2018 Rationale for Use as Negative

Fluconazole	Negative		Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	x	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative Two criteria: 1) absence of effects in any of the USEPA ToxCast in vitro bioactivity assays (https://comptox.epa.gov/dashboard), or 2) lack evidence of developmental neurotoxicity in a review of the published literature (Pub Chem).
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Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Folic acid	Negative								x			not tested	commonly used substances and/or drugs that are used during pregnancy without established adverse neurodevelopmental outcomes.	
Furosemide	Negative										Drugs with low likelihood to affect DNT test systems due to their			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
											well characterized side effects and mode of action (may have direct effects on neural networks)			
Galactosamine hydrochloride	Negative													
Glucosamine	Negative										No pronounced bioactivity, sometimes not entering			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
											cells, tolerated to mM level; belongs to “trivial” controls (low usefulness for specificity calculations) with solvents			
Glycerol	Negative						x		x	x	No pronounced bioactivity, sometimes not entering cells, tolerated to mM level; belongs to	not tested	commonly used substances and/or drugs that are used during pregnancy without established	

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
										“trivial” controls (low usefulness for specificity calculations) with solvents		adverse neurodevelopmental outcomes.		
Glyphosate	Negative							x	x		Pesticide tested negative for DNT; low cytotoxicity	listed as UNKNOWN in Frank	Not tested nor listed as negative in Shafer	Two criteria: 1) absence of effects in any of the USEPA ToxCast in vitro bioactivity assays (https://comptox.epa.gov/dashboard), or 2) lack evidence of developmental neurotoxicity in a review of the

Ibuprofen	Negative					x			x	Drugs that are acceptable during pregnancy				
													published literature (Pub Chem).	
Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative

Isoniazid	Negative		Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	x	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative Two criteria: 1) absence of effects in any of the USEPA ToxCast in vitro bioactivity assays (https://comptox.epa.gov/dashboard), or 2) lack evidence of developmental neurotoxicity in a review of the published literature (Pub Chem).
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Lctose	Compound
Negative	Positive or Negative
	Rees et al (1990)
	EPA Neurotox RA Guideline (1998)
	Grandjean papers (2006, 2014)
	Mundy et al List 1 (2015)
	Aschner et al (2017)
	Martin Negatives (2021)**
	Harrill et al (2018)
	Frank et al. (2017) & Shafer et al (2019)
	Masjusthusman et al (2020)***
No pronounced bioactivity, sometimes not entering cells, tolerated to mM level; belongs to “trivial” controls (low usefulness for specificity calculations) with solvents	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)
	Frank et al Rationale for Use as Negative
	Shafer et al Rationale for Use as Negative
	Harrill et al 2018 Rationale for Use as Negative

levetiracetam	Compound
Negative	Positive or Negative
	Rees et al (1990)
	EPA Neurotox RA Guideline (1998)
	Grandjean papers (2006, 2014)
	Mundy et al List 1 (2015)
	Aschner et al (2017)
	Martin Negatives (2021)**
	Harrill et al (2018)
	Frank et al. (2017) & Shafer et al (2019)
	Masjusthusman et al (2020)***
Drugs with low likelihood to affect DNT test systems due to their well characterized side effects and mode of action (may have direct effects on neural networks	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)
	Frank et al Rationale for Use as Negative
	Shafer et al Rationale for Use as Negative
	Harrill et al 2018 Rationale for Use as Negative

Loperamide	Negative		Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	x	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative Two criteria: 1) absence of effects in any of the USEPA ToxCast in vitro bioactivity assays (https://comptox.epa.gov/dashboard), or 2) lack evidence of developmental neurotoxicity in a review of the published literature (Pub Chem).
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Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Mannitol, D-	Negative						x		x	x	No pronounced bioactivity, sometimes not entering cells, tolerated to mM level; belongs to “trivial” controls (low usefulness for specificity calculations) with solvents	not tested	commonly used substances and/or drugs that are used during pregnancy without established adverse neurodevelopmental outcomes.	
Metformin	Negative									x	Unknown			
Metoclopramide	Negative										Drugs that are			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
										acceptable during pregnancy				
Metoprolol	Negative								x	Drugs that are acceptable during pregnancy				
Mifepristone	Negative													
Naloxon	Negative									Drugs with low likelihood to affect DNT test systems due to their well characterized				

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
											side effects and mode of action (may have direct effects on neural networks)			
Omeprazole	Negative						x				Drugs with primary target only in stomach/liver; low likelihood to have DNT effects			
Penicillin VK	Negative									x	Unknown			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Phenol	Negative							x						Two criteria: 1) absence of effects in any of the USEPA ToxCast in vitro bioactivity assays (https://comptox.epa.gov/dashboard), or 2) lack evidence of developmental neurotoxicity in a review of the published literature (Pub Chem).
Pomalidomide	Negative										Thalidomide analog, no			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
											DNT up to 200 µM			
Propylene glycol	Negative								x			GRAS	not tested nor listed as negative in Shafer	
Propylthiourcil	Negative										Hormone modifiers little relevant to <i>in vitro</i> DNT test system targets			
RU38486	Negative										Hormone modifiers little relevant to <i>in vitro</i> DNT test			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
											system targets			
Saccharin	Negative						x	x	x	x	Artificial sweetener, very low toxicity	listed as UNKNOWN in Frank	not tested nor listed as negative in Shafer	GRAS and/or FDA Pregnancy Risk Category #2.
Selegiline hydrochloride	Negative						x							
Seroquel	Negative										Drugs with low likelihood to affect DNT test systems due to their well characterized side effects			

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
											and mode of action (may have direct effects on neural networks)			
Sodium benzoate	Negative							x	x	x	Unknown	GRAS	not tested nor listed as negative in Shafer	GRAS and/or FDA Pregnancy Risk Category #2.
Sorbitol, D (glucitol, D)	Negative							x	x	x	No pronounced bioactivity, sometimes not entering cells, tolerated to mM level;	GRAS	commonly used substances and/or drugs that are used during pregnancy without	GRAS and/or FDA Pregnancy Risk Category #2.

Statins	Negative										Drugs with low likelihood to affect DNT test systems due to their well characterized side effects and mode of action (may have direct			
											belongs to “trivial” controls (low usefulness for specificity calculations) with solvents		established adverse neurodevelopmental outcomes	
Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
											effects on neural networks			
Sulfisoxazole	Negative													
Sumatriptan	Negative										Drugs that are acceptable during pregnancy			
Testosterone	Negative										Hormone modifiers little relevant to <i>in vitro</i> DNT test			

Tetracycline	Negative								x		system targets	not tested	commonly used substances and/or drugs that are used during pregnancy without established adverse neurodevelopmental outcomes.	
Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Tiotropium	Negative										Drugs with low likelihood to affect DNT test systems due to their well characterized side effects and mode of action (may have direct effects on neural networks)			
Trolox	Negative										Water-soluble vitamin E analog;			

Ursodeoxycholic acid	Negative										Drugs with low likelihood to affect DNT test systems due to their well characterized side effects and mode of action (may have direct effects on neural networks)			
Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
										caspase inhibitor (usable at 100 µM)				

Compound	Positive or Negative	Rees et al (1990)	EPA Neurotox RA Guideline (1998)	Grandjean papers (2006, 2014)	Mundy et al List 1 (2015)	Aschner et al (2017)	Martin Negatives (2021)**	Harrill et al (2018)	Frank et al. (2017) & Shafer et al (2019)	Masjusthusman et al (2020)***	Masjusthusmann et al Rationale for Use as Negative (from Aschner et al., 2017)	Frank et al Rationale for Use as Negative	Shafer et al Rationale for Use as Negative	Harrill et al 2018 Rationale for Use as Negative
Verapamil	Negative										Drugs with low likelihood to affect DNT test systems due to their well characterized side effects and mode of action (may have direct effects on neural networks)			
Warfarin	Negative									x	Drugs with primary target only in stomach/live			

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