

Incorporating Variability in Animal Studies into Regulatory Frameworks and NAM Assessment

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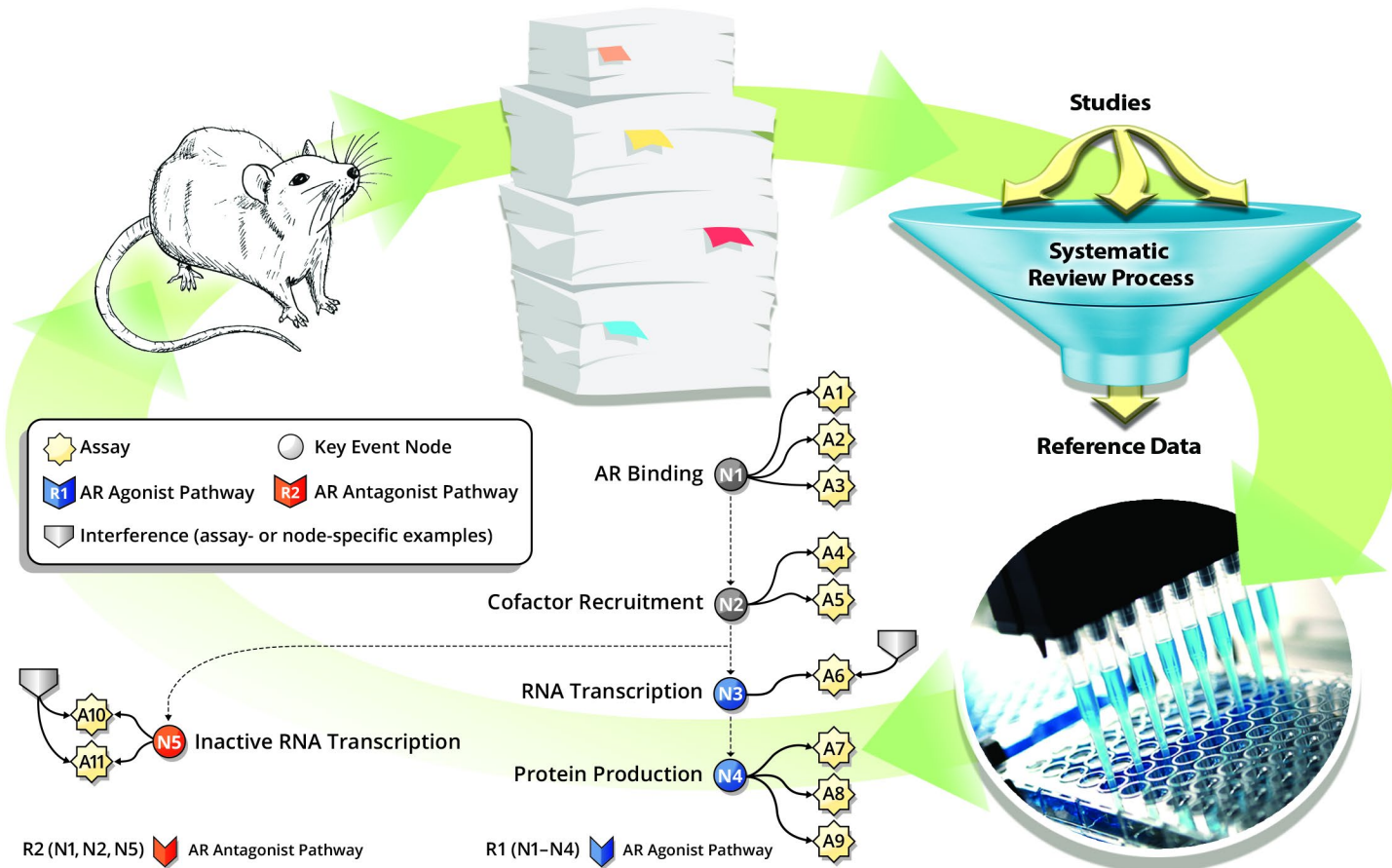


ICCVAM
*Advancing Alternatives
to Animal Testing*





Curating Reference Data



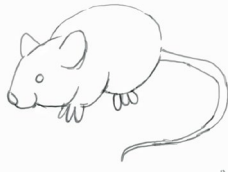


Reproducibility of Animal Data

Binary Hazard Classification



- Uterotrophic: ~74%
- Hershberger: ~72%
- Skin Sensitization: ~78%
- Acute Systemic: ~81%
- Skin Irritation: ~76%
- Eye Irritation: ~84%

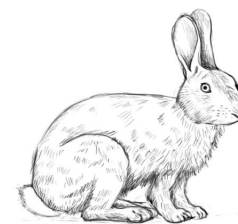




Reproducibility of the Draize Eye Test

Prior type	1	2A	2B	NC	Total
1	73%	16.1%	0.4%	10.4%	46
2A	4.2%	32.9%	3.5%	59.4%	138
2B	0.2%	4%	15.5%	80.2%	86
NC	1.1%	3.5%	1.5%	93.9%	400

- ECHA database evaluation
- 491 substances with at least 2 Draize eye studies
- Conditional probabilities of Draize evaluations based on a previous test result
- Ex: 46 substances had multiple Draize test results that included at least one Category 1 response

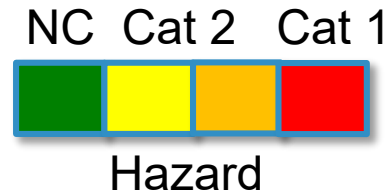




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- Some Category 1 could be NC in a subsequent test

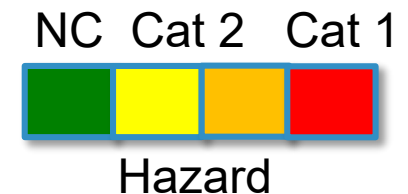




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- Category 2A and 2B more likely to be NC than Category 2 in a subsequent test
- Category 2B and NC very similar in response

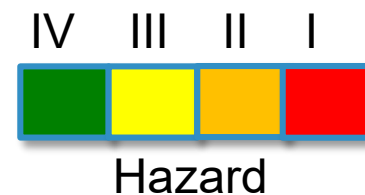




Reproducibility of the Rabbit Skin Irritation Test

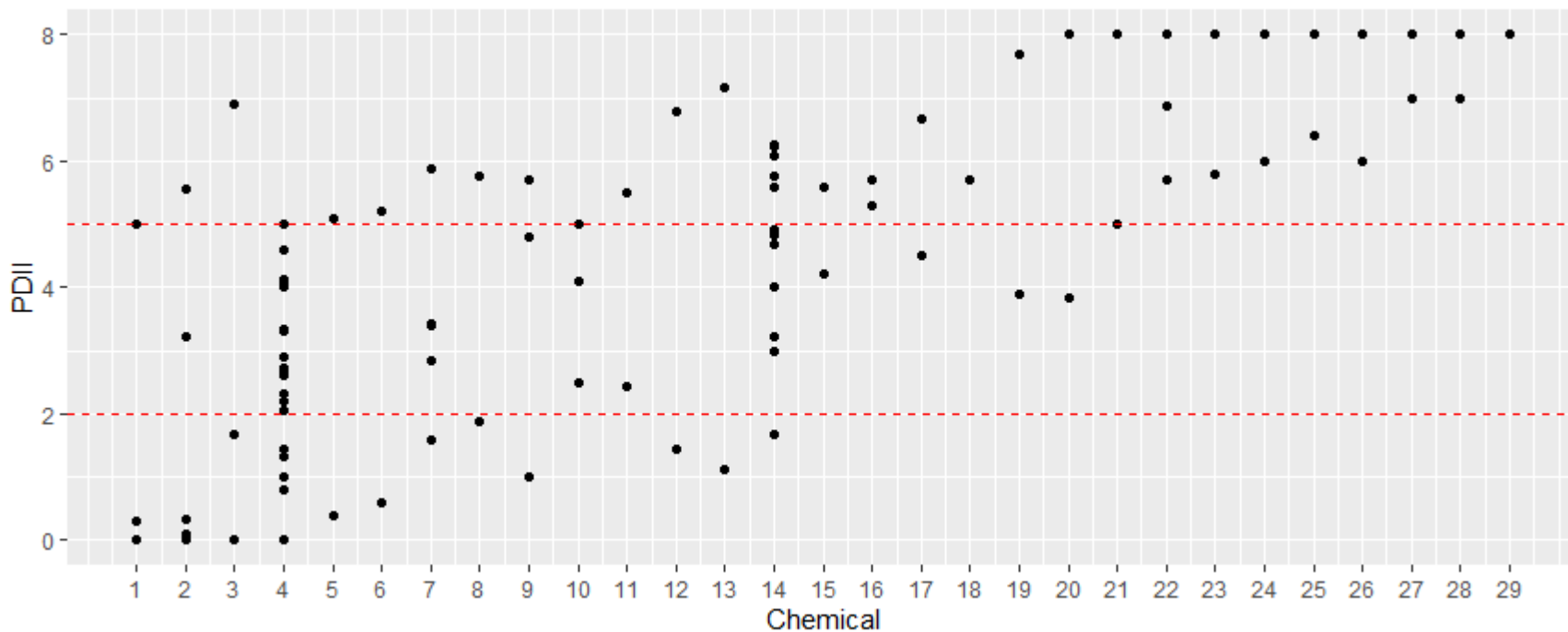
Prior type	I	II	III	IV	Total
I	86.5%	4.0%	7.2%	2.4%	217
II	10.4%	34.9%	31.1%	23.6%	37
III	4.5%	4.0%	43.5%	48.0%	174
IV	0.6%	1.5%	9.5%	88.4%	981

- Highest variability in the moderate (II) and mild (III) irritant categories





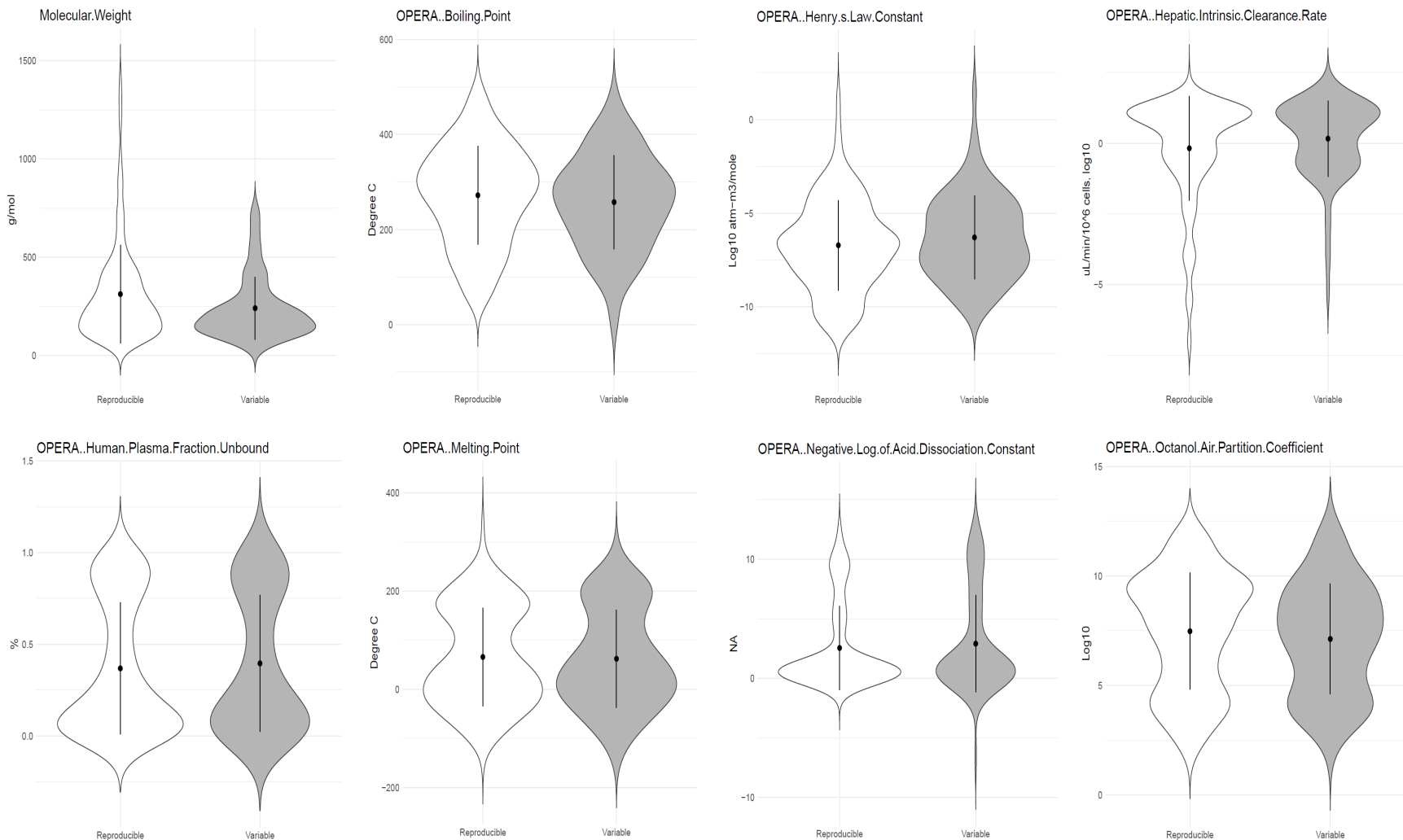
PDII analysis of Curated Dataset



PDII's of chemicals that have at least 1 category II classification do not cluster around the PDII=5.0 cutoff.



Comparison of Physchem Properties



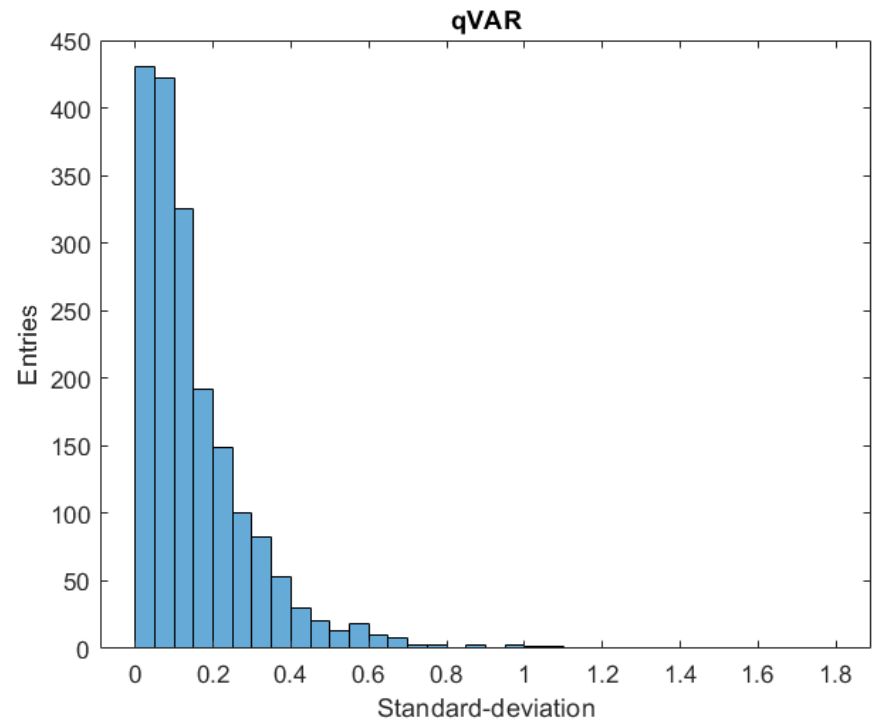
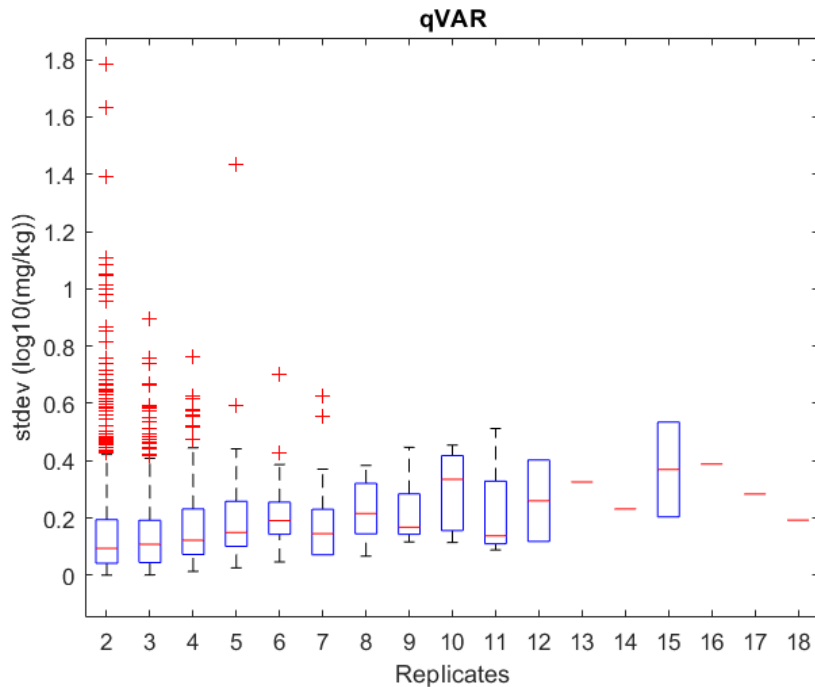
*Generated using ICE Chemical Characterization Tool



Acute Oral Toxicity Data: Variability of LD50s

Quantitative analysis: point estimates

- Standard-deviation distribution



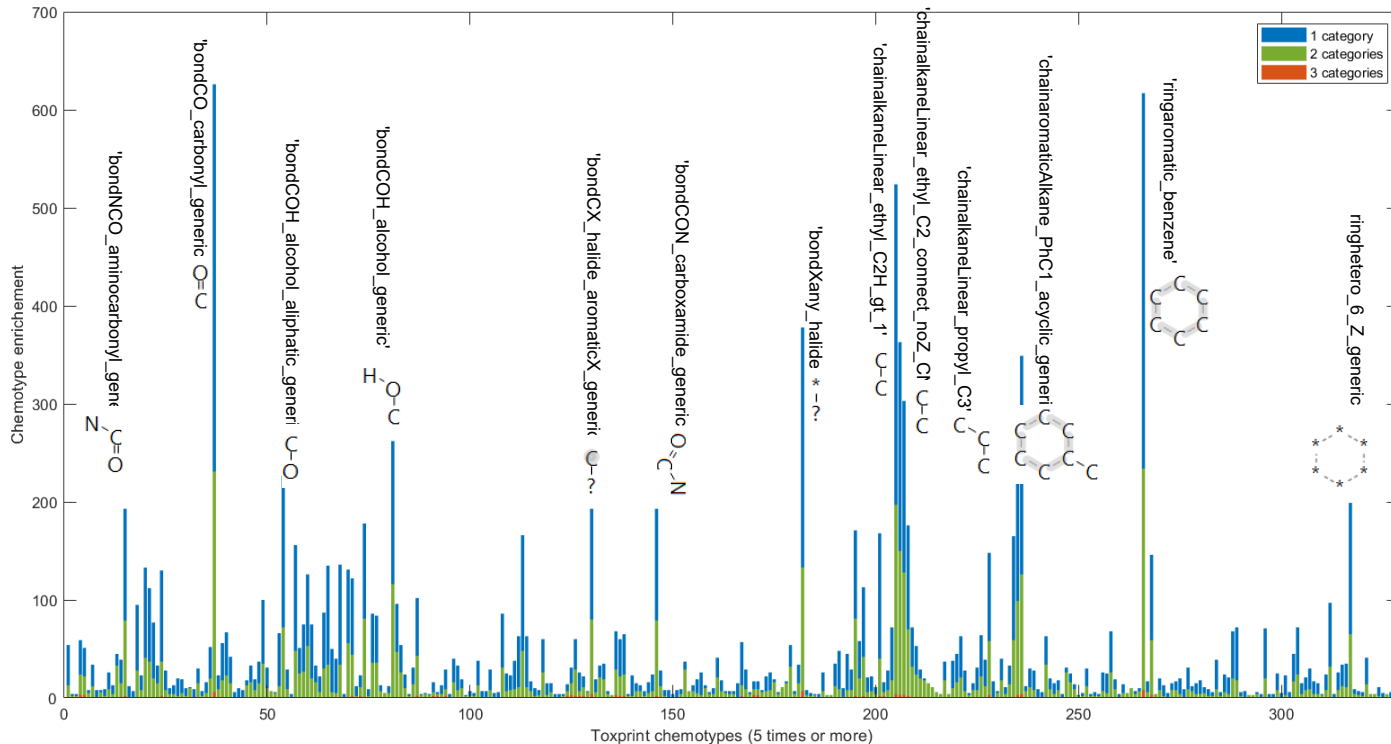
Bootstrapping of standard deviations leads to confidence interval of
~0.3 log(mg/kg)



Acute Oral Toxicity Data: Variability of LD50s

Qualitative analysis: point estimates + limit test

Toxprint Chemotypes enrichment (based on EPA categories)



Variability classes

- 1 category: low variability
- 2 categories: medium variability
- 3 categories: high variability

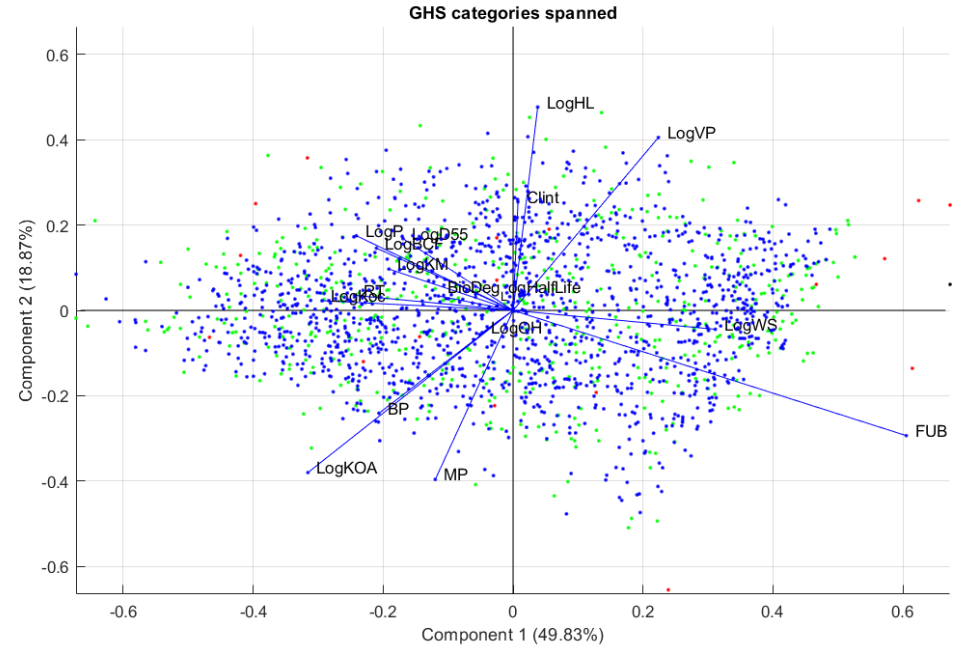
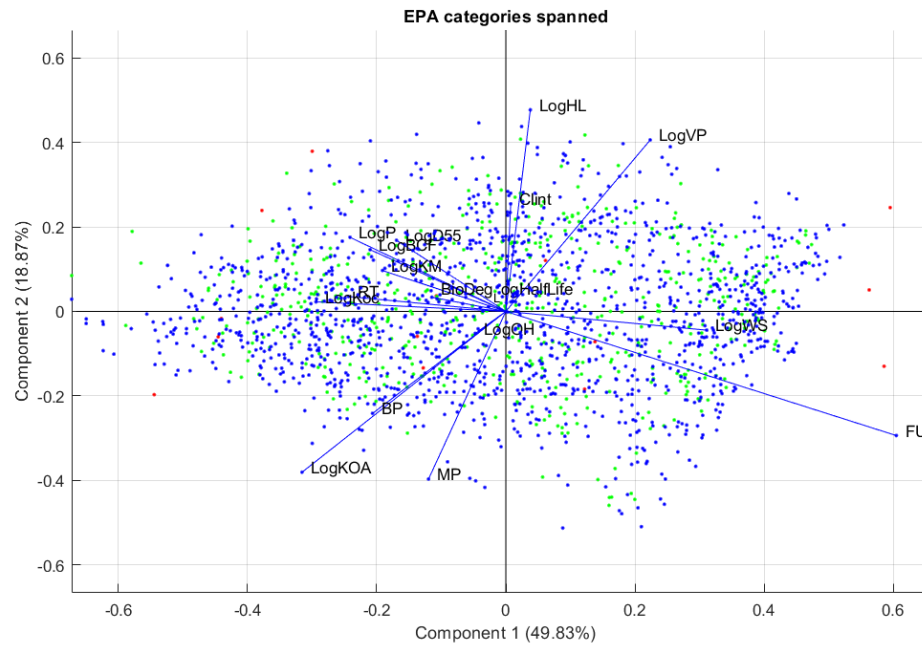
Enrichment is proportional to the number of chemicals per variability class. No significant differentiators



Acute Oral Toxicity Data: Variability of LD50s

Qualitative analysis: point estimates + limit test

Unsupervised PCA using OPERA predictions





Importance of Data Quality: LD50 Data Curation

Issues in acute oral tox data revealed by model predictions

C	L	M	T	V	BH	BI	BJ	BK	BL	BM	BO	BP	BQ
RML.CAS.r	Count	Original_LD50 (Concatenate)	ld50_mea	log(LD50)	ECHA_log(LD50) (Median)	CATMoS_LD50_data	CATMoS_LD50_pred	AD_LD50	AD_index	Conf_inde	Curated LD50 (mg/kg unless otherwise specified)	New LD50(mg/kg)	ECHA dossier
106-88-7	2	>1<1.58, ca.900	635.4839	2.010766	1.53241611	2.698970004	2.853029628	1	1	0.916667	900, 1100uL/kg (so ~1100 mg/kg)	1100	https://www.e
107-83-5	1	ca.15.84			1.199755177		3.448749354	1	1	0.725	15.84, 15.840 from analog - hexane?	15840	https://www.e
109-99-9	1	1.65			0.217483944	3.217483944	3.187110886	1	1	0.95363	1.65 g/kg	1650	https://www.e
111-66-0	15	>5, >2000, >2000<5000, >5000	2841.763	0.640297			3.45444881	1	1	0.835565	5ml/kg, 10ml/kg (so ~5000mg/kg and 5600	5600	https://www.e
111-67-1	8	>5, >5000, >5000, >5000, >500	3152.287	0.871083			3.492481795	1	1	0.829743	> 10,000 mg/kg	10000	https://www.e
111-90-0	10	<5, >5000, 5600, 6300, 6429, 7	4053.38	1.120322		3.745855195	3.65968502	1	1	0.96	6031mg/kg	6031	https://www.e
112-41-4	15	>5, >2000, >2000<5000, >5000	2841.763	0.640297			3.544496936	1	1	0.818182	> 5 600 mg/kg bw	5600	https://www.e
112-88-9	30	>5, >5, >2000, >2000, >2000<5	2792.337	0.62916			3.635710211	1	1	0.818182	>5600 mg/kg	5600	https://www.e
1120-36-1	30	>5, >5, >2000, >2000, >2000<5	2792.337	0.62916			3.596186376	1	1	0.818182	>5600 mg/kg	5600	https://www.e
120657-54	1	>5					3.666120933	1	0.939981	0.800223	>5000mg/kg based on methods septic	5600	https://www.e
15290-77-	1	>2					2.753248503	1	1	0.928571	>2000	2500	https://www.e
15708-41-	2	ca.10, >2000	2467.803	1.798928	2.272034022	3.699056855	3.542618212	1	1	0.826087	>2000, 10000	6750	https://www.e
2082-81-7	1	1066			1.002856926	1.002856926	3.519759531	1	0.925145	0.857464	10.066 listed, but dose groups were n	10066	https://www.e
27689-12-	1	>17					3.199754313	1	0.819989	0.820274	16 mL/kg (17,600 mg/kg).	17600	https://www.e
39255-32-	3	>5, >5, >2000	2004.849	1.21517			3.706432708	1	1	0.75	>2000, >5000(MALES), >5000(FEMALE)	3500	https://www.e
4499-91-6	7	>33, >300, >655, >2000, >2000	2447.428	0.74583			3.83929336	1	1	0.755952	2000, 2000, 5000, 5000, 2000, >5<15g	3500	https://www.e
543-39-5	1	5.3			0.72427587	0.72427587	3.290357289	1	0.95565	0.898544	5.3g/kg	5300	https://www.e
56-81-5	3	>20<39800, 27, 18300	11044.07	1.645202			3.958324932	1	1	0.68	27260 mg/kg	18300	https://www.e
592-41-6	15	>5, >2000, >2000<5000, >5000	2841.763	0.640297			3.296929233	1	0.955175	0.823902	read-across source >5600 mg/kg.	5600	https://www.e
629-73-2	30	>5, >5, >2000, >2000, >2000<5	2792.337	0.62916			3.60464617	1	1	0.818182	5ml/kg, 10ml/kg, 5g/kg, >2000<5000	3500	https://www.e
75-50-3	11	ca.2, 396.9, 397, 460, 500, 512	496.477	0.783002	2.823474229	2.662757832	2.657059529	1	1	0.806983	2.0g/kg	666	https://www.e
76114-73-	4	<2, ca.1000, >1000<2000, >=1:	744.9386	1.568433	3.08804563		2.692073541	1	1	0.761905	ECHA typo lists 2mg/kg, but test dose: 1250	1250	https://www.e
7620-77-1	7	>33, >300, >655, >2000, >2000	2447.428	0.74583			3.722889223	1	1	0.794444	5g/kg, >5<15g/kg, 3g/kg, 15g/kg, 300(:	3500	https://www.e
77-98-5	11	12.575, >12.5<125, 43.75, 47,	423.6832	0.5267	2.235528447		2.963016785	1	1	0.791173	>300<2000, >12.5<125, 43.75, 12.5-75	175	https://www.e
872-05-9	15	>5, >2000, >2000<5000, >5000	2841.763	0.640297			3.514069783	1	1	0.826087	5ml/kg, 10ml/kg, 5gm/kg, >2000<5000	3500	https://www.e



Examples where the 5 models (VT, NT, EPA, GHS, LD50) are in agreement with high confidence levels, with high margin between predictions and ECHA data



Data Curation – is the LC50 really this variable?

CASRN	LC50	LC50 unit	source
79-11-8	1268	mg/L	eChemPortal
79-11-8	0.18	mg/L	ChemIDplus

Results and discussion

Effect levels

Sex:	male/female
Dose descriptor:	LC50
Effect level:	> 1 268 mg/L air (analytical)
Based on:	test mat.
Exp. duration:	4 h

Acute Toxicity: inhalation

Currently viewing: 001 Key | Experimental result

Administrative data Data source Materials and methods Results and discussion Ap

Duration of exposure:	ca. 4 h
Concentrations:	512 (± 150 mg/m ³) and 1268 (± 77 mg/m ³)
No. of animals per sex per dose:	5 animals per sex per dose

NIH U.S. National Library of Medicine TOXNET TOXICOLOGY DATA NETWORK

TOXNET > ChemIDplus > Substance

Registry Number equals 79-11-8 Search

Download Start New Query Modify Query Search History

Switch to Summary View

Substance Name: Chloroacetic acid [BSI:ISO]
RN: 79-11-8
UNII: 5GD84Y125G
InChIKey: FOCAUTSVDIKZOP-UHFFFAOYSA-N

Note
Urinary metabolite of vinyl chloride.

Molecular Formula
C₂H₃ClO₂

Molecular Weight
94.4967

Chemical structure: ClCC(=O)O

All Classifications Links to Resources Names & Synonyms Registry Numbers Structure Descriptors Toxicity Physical Properties

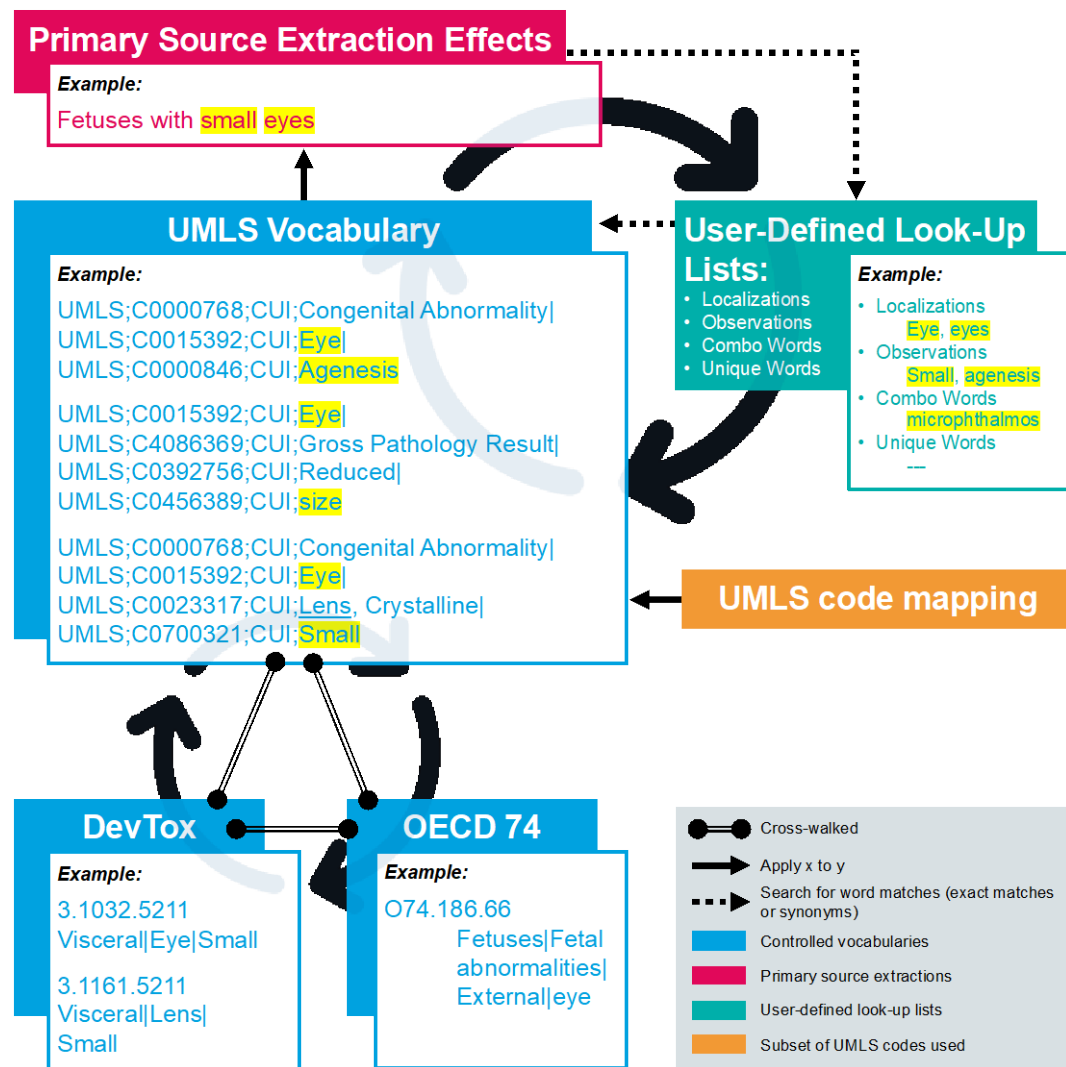
Toxicity					
Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
mouse	LD50	subcutaneous	250mg/kg (250mg/kg)		Archives Internationales de Pharmacodynamie et de Therapie. Vol. 116, Pg. 154, 1958.
rat	LC50	inhalation	180mg/m ³ (180mg/m ³)		Gigiena Truda i Professional'nye Zabolevaniya. Labor Hygiene and Occupational Diseases. Vol. 18(9), Pg. 32, 1974.
rat	LD50	intraperitoneal	166000µg/kg (16.6mg/kg)		Russian Pharmacology and Toxicology Vol. 41, Pg. 113, 1978.
rat	LD50	oral	55mg/kg (55mg/kg)		Gigiena Truda i Professional'nye Zabolevaniya. Labor Hygiene and Occupational Diseases. Vol. 18(9), Pg. 32, 1974.
rat	LD50	subcutaneous	5mg/kg (5mg/kg)		Toxicology and Applied Pharmacology. Vol. 22, Pg. 303, 1972.

LC50 = 1.268 and 0.18 mg/L instead?



Study Extractions and Endpoint Mapping

- Extract study details from prenatal developmental toxicity guideline studies
 - NTP legacy studies
 - ECHA submissions (expert reviewed for quality)
- Programmatically map results to controlled vocabularies/ontologies
 - UMLS (ToxRefDBv2.0)
 - EPA/BfR DevTox DB
 - OECD Harmonized Templates





- Large databases of *in vivo* toxicology study data have been compiled and curated from numerous resources
- Chemicals with multiple results were used to evaluate the performance of the *in vivo* assays and characterize variability
- In each case, sources of variability were investigated:
 - Number of experiments, potency, chemical use category, or physchem properties do not correlate with increased variability
- It is essential that the variability of current *in vivo* test methods be considered in the context of establishing confidence in NAMs that replace the use of animals



Benchmarking Alternative Models



Human data and
human biology as the
gold standard

Using the AOP
framework to develop
testing strategies





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