Data sharing and meta-analysis of *in vitro* toxicology assessment of diverse e-liquid and heat-not-burn platforms on the INTERVALS platform

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Introduction

- Extensive scientific studies are conducted to assess the relative risks of various candidate modified risk tobacco products (MRTP) compared with those of cigarettes.
- Aerosol characterization and standard *in vitro* toxicity testing are usually the first steps of any assessment strategy, and these type of data are already published for diverse heat-not-burn and e-cigarette platforms.
- In the context of tobacco harm reduction, it is important that the results obtained on new platforms are put in the context of smoking.
- As the scientific community conducts such assessments for diverse products and in a variety of laboratory models, knowledge on toxicity is spread across numerous scientific articles.
- We believe that by fostering the consolidation of data and knowledge gained from studies assessing novel tobacco/ nicotine-delivery products on a community platform, new hypotheses may be generated, and the weight of evidence may be increased.
- Therefore, we have created and are further developing INTERVALS™ (www.intervals.science) (Boue 2017), an online platform supporting independent, third-party collaboration by proactively sharing detailed protocols, tools, and data from assessment studies.

Conclusions

- Direct comparison of platforms tested in separate studies with different study designs (*e.g.*, different lists of chemicals quantified in the aerosols), and different methods of data reporting makes it difficult to compare results across all individual studies.
- Greater homogeneity in data reporting would allow for easier meta-analysis, and for contextualizing studies.
- It was possible to compare the results for aerosol chemistry, but not the direct results obtained in *in vitro* toxicity assays.
- However, the overall result is consistent in that all of the studies included in this analysis demonstrate lower levels and number of harmful or potentially harmful chemicals and of toxicity assessed *in vitro* for the tested platforms compared with cigarettes.
- As the scientific community integrates more studies and datasets into INTERVALS, it will become easier to conduct such meta-analyses and review results obtained across institutions, models, and platforms.

INTERVALS

The INTERVALS™ platform allows researchers to find all relevant information on studies, detailed protocols, and, most importantly, interoperable data files. This permits independent re-analysis of key findings, meta-analysis, and enables data reuse efficiency.

STUDIES

- Rationale and details of study design
- Detailed results
- Contact information of the reference scientist
- Links to publications
- Categorization

DATASETS

- Flexible data format incl. rich metadata
- Regulatory format (CDISC) & ISA-TAB accepted
- Analytics under development
- Categorization

PROTOCOLS

- Short description
- Step-by-step instructions
- Versioning
- Categorization

DISEASES & PATHWAYS

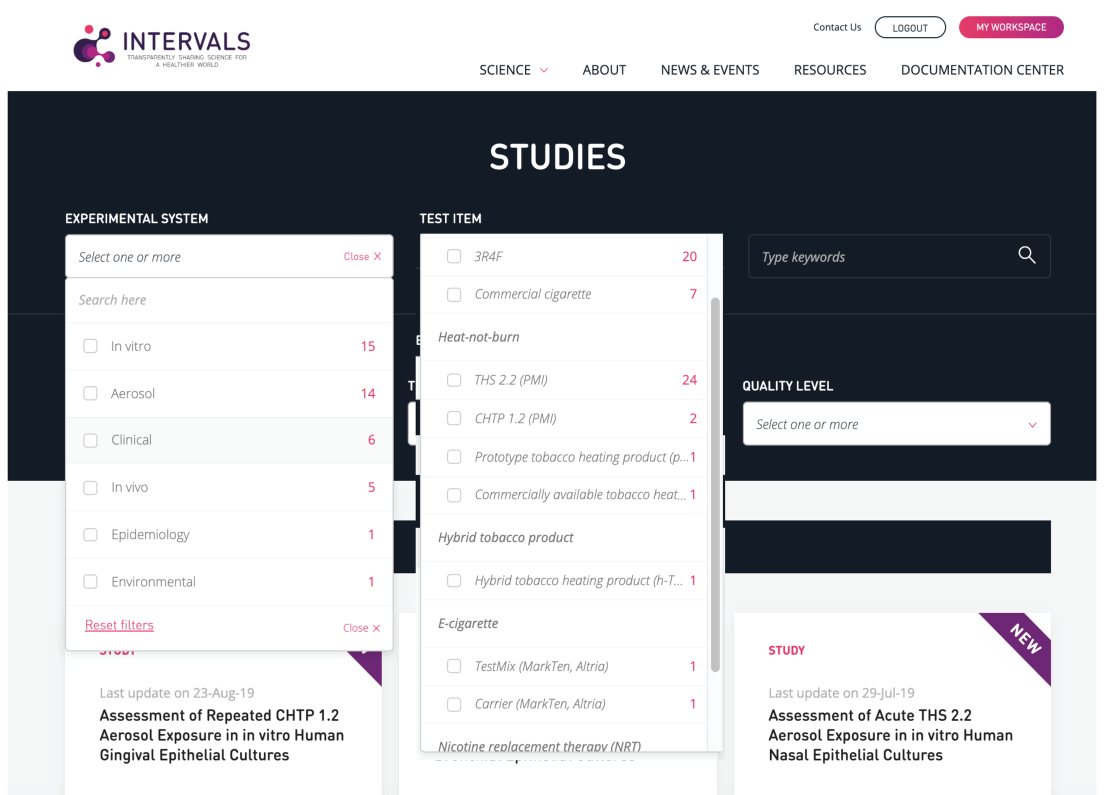
- Explore studies and results by pathway or disease of interest
- Gain mechanistic understanding to formulate new hypotheses

Initiated by Philip Morris International, most studies hosted on INTERVALS to date were assessing PMI's platforms.

It is crucial, however, that diverse contributions are added to the platform, so that the scientific credibility and robustness of information on categories of MRTPs are further enhanced.

The deposition in INTERVALS of the data for endpoints that are classically not deposited in databases can facilitate the comparison of outcomes across studies.

The figure on the right indicates the classification of the studies published on INTERVALS as of August 28th, 2019.

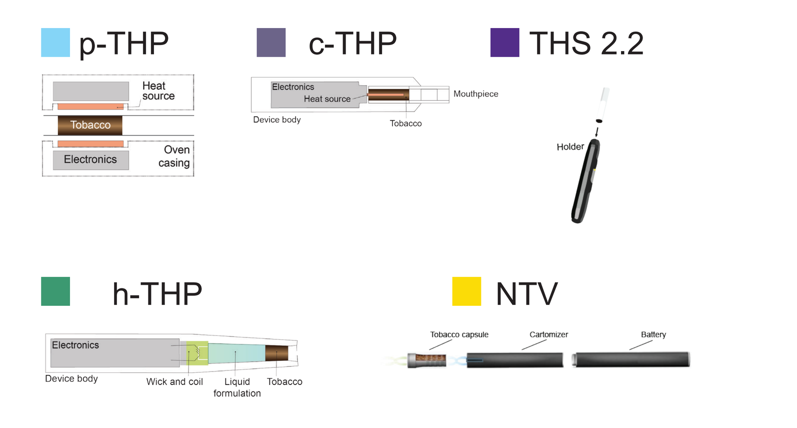


Methods

We summarize below the studies included in the meta-analysis of *in vitro* toxicology assessment studies. We concentrate on aerosol characterization, neutral red uptake assay, and mouse lymphoma assay, which are reported in all three publictions for various platforms compared with the 3R4F reference cigarette.
Numerical data from graphs were extracted by using WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/).

	Breheny et al. 2017	Schaller et al. 2017	Takahashi et al. 2018
Publication DOI	10.1016/j.fct.2017.05.023	10.1016/j.yrtph.2016.10.001	10.1016/j.yrtph.2017.11.009
INTERVALS DOI	10.26126/intervals.6t2ejz.1	aerosol (THS 2.2): 10.26126/intervals.82hxcs.1 tox (THS 2.2): 10.26126/intervals.msx63a.1 aerosol (THS 2.2M): 10.26126/intervals.bwhuts.1 tox (THS2.2M): 10.26126/intervals.25g5qb.1	10.26126/intervals.v2ubz6.1
Test item category	HNB	HNB	Tobacco vapor
Test items	c-THP, p-THP, h-THP	THS 2.2, THS 2.2M	NTV
Reference item	3R4F	3R4F	3R4F
Smoking regimen reference item	HCI: 55 mL puff volume, 2 s puff duration, 30 s puff interval, bell-shaped puff profile, 100% vent blocking	HCI: 55 mL puff volume, 2 s puff duration, 30 s puff interval, bell-shaped puff profile, butt length set to 35 mm	HCI: 55 mL puff volume, 2 s puff duration, 30 s puff interval, bell-shape puff profile, butt length set to 35 mm
Smoking regimen test items	c-THP and p-THP: 55mL puff volume, 2 s puff duration, 30 s puff interval, no vent blocking h-THP: 55 ml puff volume, 3 s puff duration. 30 s puff interval, rectangular flow profile puff	THS 2.2, THS 2.2M: 55 mL puff volume, 2 s puff duration, 30 s puff interval, 12 puff	NTV: 55 mL puff volume, 2 s puff duration, 30 s puff interval, 70 puff
Tested fractions	WA, TPM, AqE	TPM, GVP	TPM, GVP
Level of nicotine tested?	yes	yes	yes
Endpoints reported (aerosol chemistry and in vitro regulatory assays)	Aerosol chemistry (WA)	Aerosol chemistry (WA)	Aerosol chemistry (WA)
	Ames assay (TPM)	Ames assay (TPM, GVP)	Ames assay (TPM)
	NRU assay (TPM)	NRU assay (TPM, GVP)	NRU assay (TPM, GVP)
	Carbonyls in AqE	MLA assay (TPM, GVP)	*in vitro* micronucleus assay
	DNA damage assessment (TPM)		
	Tumour-promoting potential (WA, TPM)		
Notes	Reports in addition the followng *in vitro* models of disease: the DCF assay, GSH:GSSG, and ARE-reporter assays measuring oxidative stress, the Apolive-Glo assay measuring apoptosis, and the scratch wound assay measuring endothelial wound repair.	Different smoking regimen and climatic conditions tested for the aerosol chemistry. Physical measurement of the aerosol included.	Reports in addition the temperature inside the NTV tobacco capsule

Test items



Reference

Abbreviations:

- c-THP: commercially available tobacco heating product, as defined in Breheny et al., 2017.
- HCI: Health Canada Intense smoking regimen
- h-THP: hybrid tobacco heating product, as defined in Breheny et al., 2017.
- p-THP: prototype tobacco heating product, as defined in Breheny et al., 2017.
- HNB: heat-not-burn tobacco product
- WA: whole aerosol
- TPM: total particulate matter
- AqE: aqueous extract
- NTV: novel tobacco vapor product

Aerosol chemistry

Different lists of chemicals have been characterized in the aerosols tested in each study. Because they have been reported in different ways (*e.g.*, per mg nicotine, per stick, and per puff), in the table below, we show the percent change relative to each 3R4F control in order to demonstrate the reduction in harmful and potentially harmful compounds, which is clearly demonstrated in each study and for each test item.

While the table below only reports the chemicals measured in all studies, it is worthwhile keeping in mind that some studies report additional chemicals that might warrant further investigation (*e.g.*, triacetin in Takahashi et al. [which was increased in concentration in the test item relative to 3R4F, but not measured in the other studies] or NDEA in Breheny et al.).

Aerosol chemistry

	Breheny et al.				Schaller et al.								Takahashi et al.	
					D2		FR1		D1M		FR1M			
	3R4F	p-THP	c-THP	h-THP	3R4F	THS2.2	3R4F	THS 2.2	3R4F	THS 2.2M	3R4F	THS 2.2M	3R4F	NTV
Ammonia	0.0%	-26.3%	-73.0%	-96.4%	0.0%	-60.2%	0.0%	-63.9%	0.0%	-64.5%	0.0%	-64.9%	0.0%	-58.3%
1-aminonaphthalene	0.0%	-98.4%	-100.0%	-100.0%	0.0%	-99.7%	0.0%	-99.6%	0.0%	-100.0%	0.0%	-99.6%	0.0%	-100.0%
2-aminonaphthalene	0.0%	-98.3%	-99.2%	-100.0%	0.0%	-100.0%	0.0%	-99.6%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%
3-aminobiphenyl	0.0%	-96.3%	-100.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-99.2%	0.0%	-100.0%
4-aminobiphenyl	0.0%	-95.0%	-100.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%
Hydrogen cyanide	0.0%	-99.1%	-99.1%	-99.8%	0.0%	-99.2%	0.0%	-99.0%	0.0%	-98.8%	0.0%	-99.0%	0.0%	-100.0%
Mercury	0.0%	-75.0%	-62.5%	-100.0%	0.0%	-75.5%	0.0%	-75.6%	0.0%	-73.1%	0.0%	-72.1%	0.0%	-100.0%
Nitric oxide	0.0%	-100.0%	-97.6%	-100.0%	0.0%	-95.8%	0.0%	-96.6%	0.0%	-96.3%	0.0%	-97.5%	0.0%	-100.0%
Nitrogen oxides	0.0%	-100.0%	-97.6%	-100.0%	0.0%	-95.8%	0.0%	-96.8%	0.0%	-96.4%	0.0%	-97.7%	0.0%	-100.0%
Benzo(a)pyrene	0.0%	-69.1%	-93.5%	-99.3%	0.0%	-91.3%	0.0%	-100.0%	0.0%	-92.1%	0.0%	-90.9%	0.0%	-100.0%
Catechol	0.0%	35.8%	-87.9%	-100.0%	0.0%	-81.5%	0.0%	-82.2%	0.0%	-85.6%	0.0%	-81.3%	0.0%	-100.0%
Hydroquinone	0.0%	-53.4%	-94.2%	-100.0%	0.0%	-90.7%	0.0%	-90.3%	0.0%	-92.6%	0.0%	-89.2%	0.0%	-100.0%
m-cresol	0.0%	-84.8%	-100.0%	-100.0%	0.0%	-98.9%	0.0%	-99.0%	0.0%	-99.1%	0.0%	-98.9%	0.0%	-100.0%
o-cresol	0.0%	-72.7%	-97.7%	-100.0%	0.0%	-97.8%	0.0%	-98.5%	0.0%	-98.8%	0.0%	-97.9%	0.0%	-100.0%
p-cresol	0.0%	-90.0%	-100.0%	-100.0%	0.0%	-99.1%	0.0%	-99.2%	0.0%	-99.5%	0.0%	-99.1%	0.0%	-100.0%
Phenol	0.0%	83.3%	-92.8%	-99.3%	0.0%	-88.6%	0.0%	-91.5%	0.0%	-92.4%	0.0%	-88.2%	0.0%	-100.0%
Resorcinol	0.0%	-83.3%	-94.4%	-100.0%	0.0%	-97.2%	0.0%	-97.8%	0.0%	-98.2%	0.0%	-97.4%	0.0%	-100.0%
Acetaldehyde	0.0%	-75.9%	-90.3%	-100.0%	0.0%	-86.6%	0.0%	-85.9%	0.0%	-86.2%	0.0%	-86.8%	0.0%	-100.0%
Acetone	0.0%	-91.9%	-96.0%	-99.9%	0.0%	-95.4%	0.0%	-94.5%	0.0%	-94.2%	0.0%	-94.6%	0.0%	-99.0%
Acrolein	0.0%	-87.8%	-95.8%	-99.6%	0.0%	-95.1%	0.0%	-92.7%	0.0%	-94.3%	0.0%	-94.1%	0.0%	-100.0%
Butyraldehyde	0.0%	-69.9%	-81.2%	-99.9%	0.0%	-75.6%	0.0%	-70.5%	0.0%	-74.6%	0.0%	-69.8%	0.0%	-100.0%
Crotonaldehyde	0.0%	-91.5%	-96.6%	-99.8%	0.0%	-95.9%	0.0%	-94.0%	0.0%	-95.5%	0.0%	-95.3%	0.0%	-100.0%
Formaldehyde	0.0%	-84.0%	-90.8%	-98.6%	0.0%	-92.4%	0.0%	-90.2%	0.0%	-91.0%	0.0%	-91.9%	0.0%	-93.6%
Methyl Etyl Ketone	0.0%	-92.0%	-96.6%	-99.5%	0.0%	-96.7%	0.0%	-96.2%	0.0%	-95.8%	0.0%	-96.3%	0.0%	-100.0%
Propionaldehyde	0.0%	-80.3%	-92.0%	-99.9%	0.0%	-90.7%	0.0%	-88.4%	0.0%	-89.2%	0.0%	-88.9%	0.0%	-100.0%
Pyridine	0.0%	-59.8%	-84.8%	-100.0%	0.0%	-81.9%	0.0%	-79.1%	0.0%	-80.5%	0.0%	-80.0%	0.0%	-100.0%
Quinoline	0.0%	-75.0%	-100.0%	-100.0%	0.0%	-96.4%	0.0%	-100.0%	0.0%	-97.4%	0.0%	-100.0%	0.0%	-100.0%
Styrene	0.0%	-92.9%	-93.5%	-100.0%	0.0%	-97.7%	0.0%	-97.5%	0.0%	-97.8%	0.0%	-97.7%	0.0%	-100.0%
CO	0.0%	-94.9%	-98.9%	-97.8%	0.0%	-98.1%	0.0%	-98.4%	0.0%	-98.0%	0.0%	-98.2%	0.0%	-100.0%
Nicotine	0.0%	-29.4%	-47.1%	-88.2%	0.0%	-39.7%	0.0%	-30.2%	0.0%	-36.8%	0.0%	-36.0%	0.0%	-45.3%
NAB	0.0%	2.5%	-93.0%	-100.0%	0.0%	-89.7%	0.0%	-100.0%	0.0%	-90.4%	0.0%	-100.0%	0.0%	-100.0%
NAT	0.0%	-48.9%	-95.1%	-99.9%	0.0%	-92.6%	0.0%	-93.6%	0.0%	-93.8%	0.0%	-93.8%	0.0%	-100.0%
NNK	0.0%	-60.7%	-97.5%	-99.9%	0.0%	-96.1%	0.0%	-97.5%	0.0%	-96.9%	0.0%	-97.8%	0.0%	-100.0%
NNN	0.0%	-23.6%	-96.8%	-99.9%	0.0%	-96.2%	0.0%	-94.4%	0.0%	-97.1%	0.0%	-95.6%	0.0%	-100.0%
Arsenic	0.0%	-100.0%	-67.0%	-78.4%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%
Cadmium	0.0%	-100.0%	-99.2%	-99.1%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%
Chromium	0.0%	-100.0%	-11.1%	7.4%	0.0%	-100.0%			0.0%	-83.7%				
Lead	0.0%	-100.0%	-92.3%	-90.7%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%
Nickel	0.0%	-100.0%	-10.5%	-33.3%	0.0%	-100.0%			0.0%	-32.3%				
Selenium	0.0%	-100.0%	-81.3%	-75.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-100.0%	0.0%	-51.9%		
1,3-butadiene	0.0%	-99.7%	-99.8%	-100.0%	0.0%	-99.7%	0.0%	-99.5%	0.0%	-99.6%	0.0%	-99.6%	0.0%	-100.0%
Acrylonitrile	0.0%	-99.0%	-99.5%	-100.0%	0.0%	-99.4%	0.0%	-99.2%	0.0%	-99.4%	0.0%	-99.3%	0.0%	-100.0%
Benzene	0.0%	-98.7%	-99.3%	-100.0%	0.0%	-99.4%	0.0%	-99.3%	0.0%	-99.4%	0.0%	-99.3%	0.0%	-100.0%
Isoprene	0.0%	-99.9%	-99.8%	-100.0%	0.0%	-99.7%	0.0%	-99.7%	0.0%	-99.7%	0.0%	-99.7%	0.0%	-100.0%
Toluene	0.0%	-98.9%	-98.4%	-99.8%	0.0%	-99.2%	0.0%	-98.6%	0.0%	-99.2%	0.0%	-98.7%	0.0%	-100.0%

% Difference in Avg.. (-100% to 1,000%)

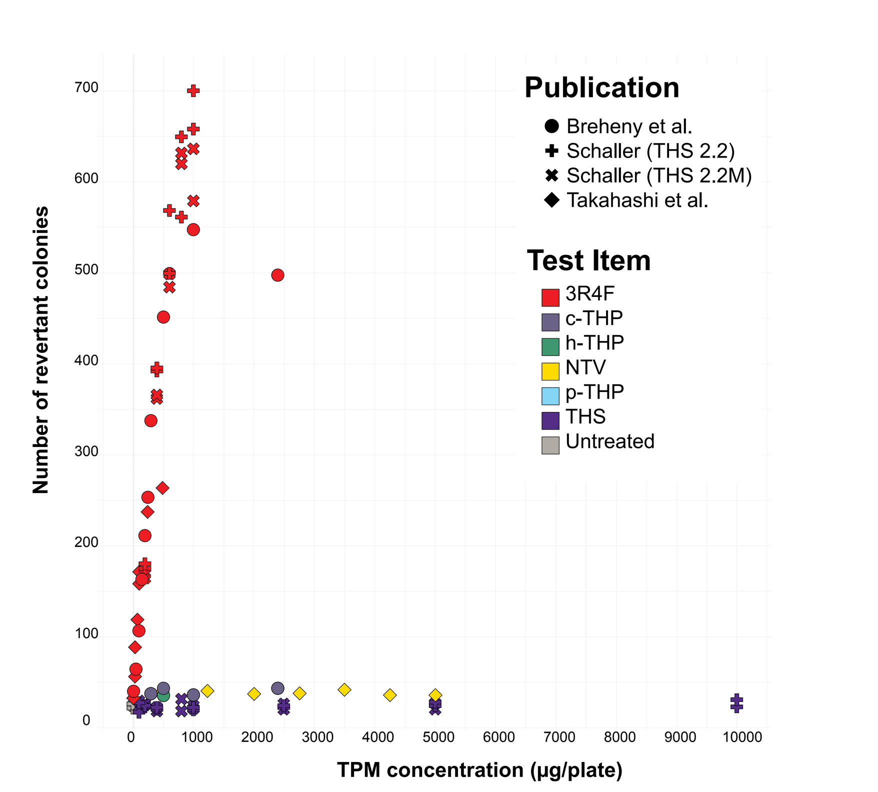
Ames Assay

The Ames assay may be conducted with different strains and with or without metabolic activation by the S9 fraction.

All publications report the assessment with the TA98 strain with metabolic activation.

The results are aggregated in the figure on the right.

Across all studies, the results obtained with 3R4F are remarkably similar. In addition, for all new test items (c-THP, p-THP, h-THP, NTV, and THS), the mutagenic potential of is much reduced relative to 3R4F, even at very high TPM concentrations.

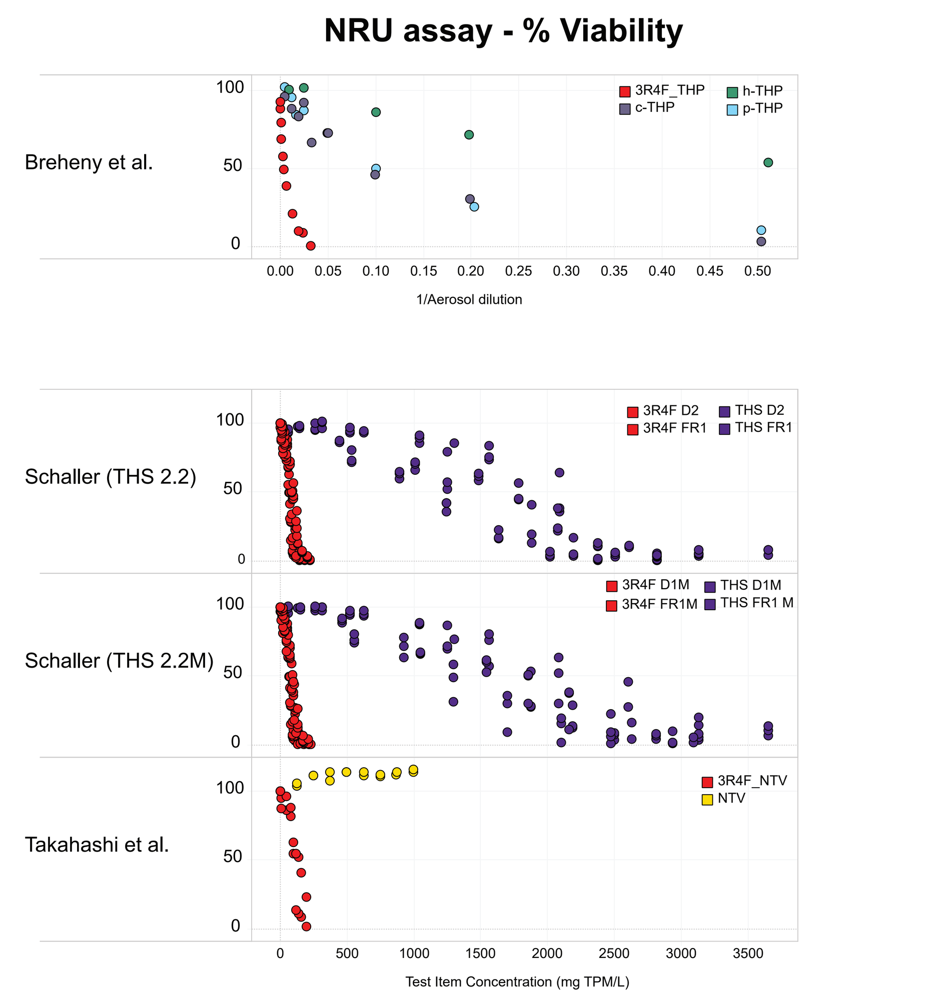


NRU assay

The NRU cytotoxicity assay was conducted slightly differently in each institution. For example, the cell type used varied among the institutions: H292 lung epithelial cells in Breheny et al., Balb/c 3T3 cells in Schaller et al., and CHO-WBL cell line in Takahashi et al.

The manner in which the results were reported in the different publications is very heterogeneous, and an attempt as harmonizing the presentation of the results is given in the figure on the right. We would consider here the relative toxicity of the different test items to the same reference, 3R4F, within each study but it would not be prudent to attempt extrapolating results across-studies.

While 3R4F is cytotoxic at rather small concentrations or TPM levels, the concentration of aerosols of the diverse test items in the three studies needed to be considerably increased to observe a heighten cytotoxicity.



EUROTOX 2019, HELSINKI

Competing Financial Interest – Philip Morris International is the sole source of funding and sponsor of this research and platform.