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Assessment of alternative human derived cell lines for adoption with the OECD TG 129 for cytotoxicity determination

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Outline and aims

- The Neutral Red Uptake assay (NRU), part of the in vitro CORESTA test “battery”, is one of the most adopted cytotoxicity assay for the evaluation of combustible cigarettes and Next Generation Products (NGPs)
- OECD TG129 guideline for the NRU assay was originally adapted for mouse fibroblasts (BALB/c 3T3) and normal human epidermal keratinocytes (NHK), and was validated in the *In Vitro Basal Cytotoxicity Validation Study* with 72 reference substances (ICCVAM, 2006a)
- At IB the cell lines Beas-2B and HepG2 are routinely used, as they are of human origin and have physiological relevance for NGP/ Tobacco assessment
- The aim of this study was to compare the cytotoxic response of cell lines BEAS-2B and HEPG2 to that of the OECD TG129 recommended cell line BALB/c 3T3 to ensure appropriate sensitivity using a selection of nine substances from the ICCVAM Reference list



Interagency Coordinating Committee on
the Validation of Alternative Methods

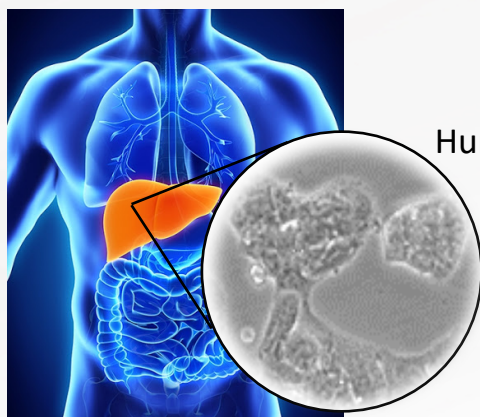


Study design



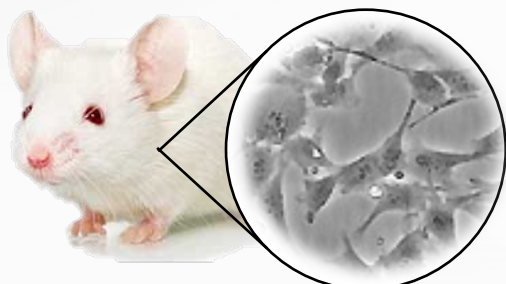
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BEAS-2B
Human bronchial
epithelium cell line



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HepG2
Human hepatoma
cell line



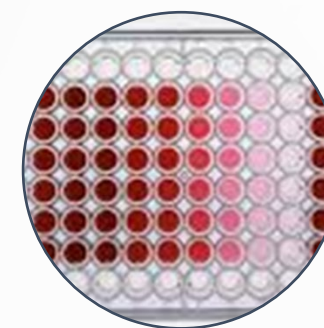
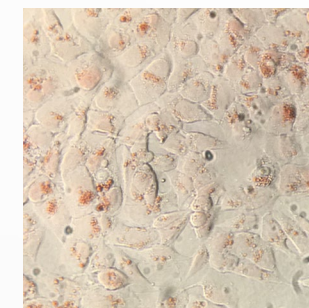
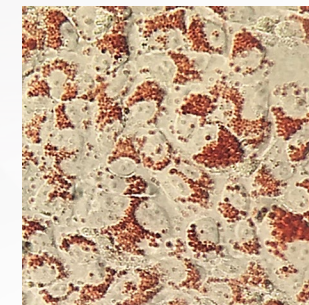
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BALB/c 3T3
Mouse fibroblast
cell line

Organic compound	CAS number	Chemical Class
Caffeine	58-08-2	Heterocyclic compound
Ethylene glycol	107-21-1	Alcohol
Glycerol	56-81-5	Alcohol
Lactic Acid	50-21-5	Carboxylic acid
Phenylthiourea	103-85-5	Sulphur compound; Urea
Verapamil	152-11-4	Amine
Ethanol	64-17-5	Alcohol
2-Propanol	67-63-0	Alcohol
Nicotine	54-11-5	Heterocyclic compound

Value of comparison: EC50
(concentration to achieve 50% growth inhibition)

NRU Assay

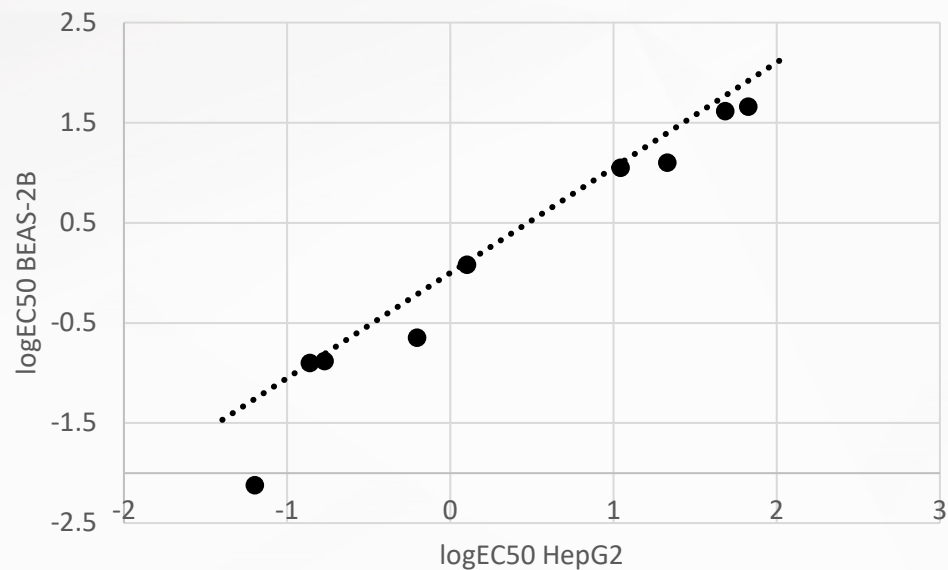
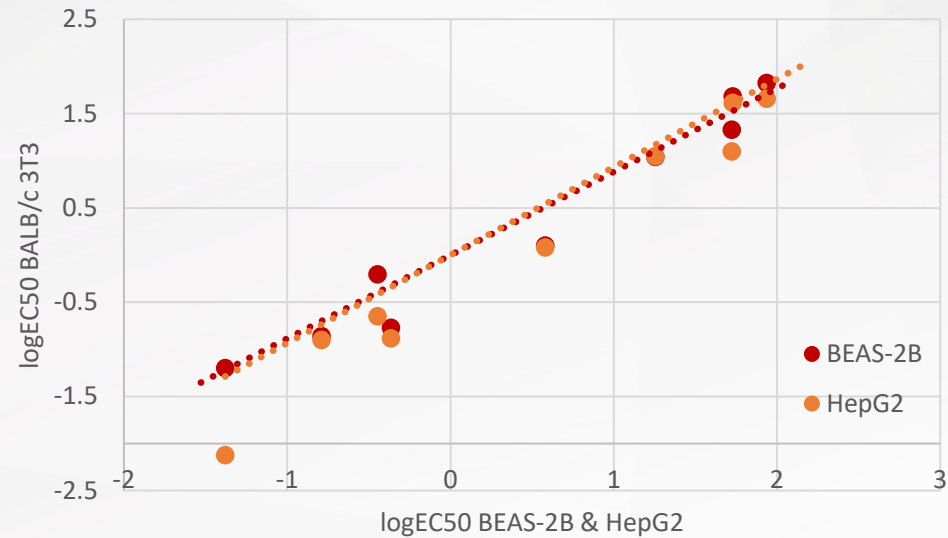


Study design

	BALB/c 3T3	BEAS-2B	HepG2
Medium Culture	DMEM low glucose/ 10% NCS/ 4mM Glutamine	Promocell AEGM	MIS/ 2mM Glutamine/ 4mM Glutamax/ 1% Insulin/ 1% Ultrosor G
Medium Treatment	DMEM low glucose/ 5% NCS/ 4mM Glutamine	Promocell AEGM	MIS/ 2mM Glutamine/ 4mM Glutamax/ 1% Insulin/ 0.2% Ultrosor G
Cell number per well	5e3	8e3	2e4
Pre-treatment incubation [h]	24	24	24
Treatment [h]	48±1	65±2	
NR staining solution	DMEM/ 5% NCS/ 4mM Glutamine/ 2% HEPES/ 0.76% NR dye 250µl per well	MIS/ 10% FBS/ 2% HEPES/ 2% NR dye 200µl per well	

ISO 17025 accredited

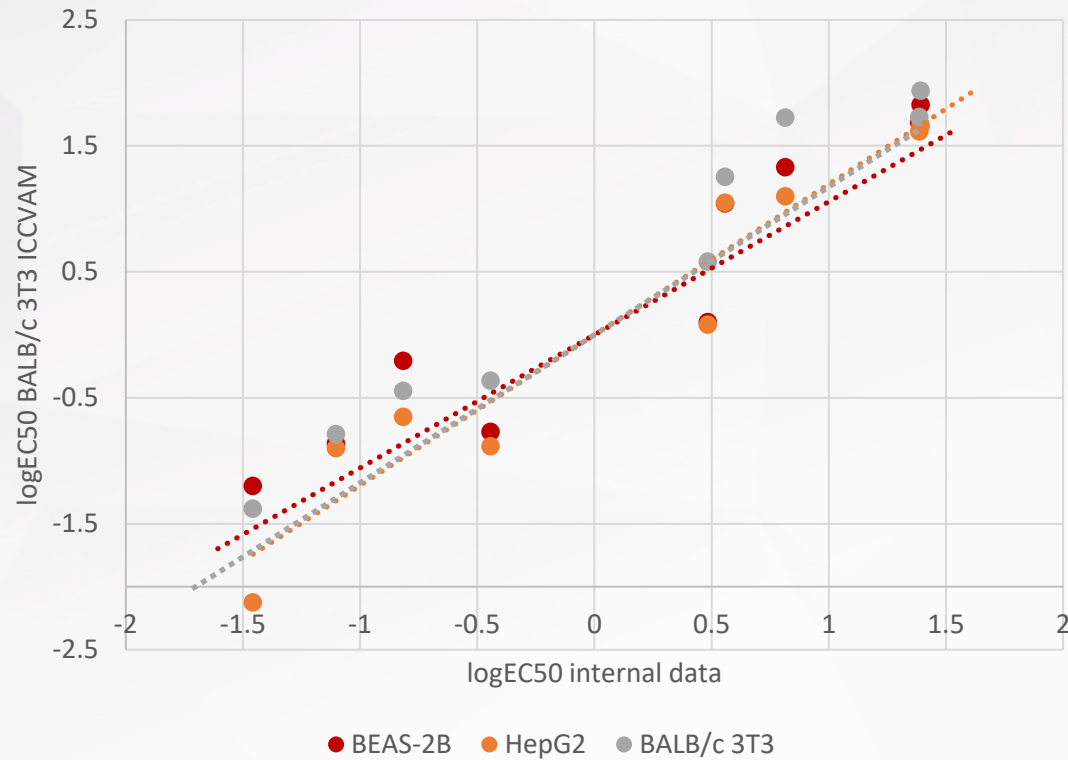
Results: Correlation between internal data



	BALB/c 3T3 vs. BEAS-2B	BALB/c 3T3 vs. HepG2
Pearson r	0.9801	0.9853
95% confidence interval	0.9050 to 0.9959	0.9294 to 0.9970
R squared	0.9605	0.9709
P value	<0,0001	<0,0001
P value summary	****	****
Significant? (alpha = 0.05)	Yes	Yes
Number of XY Pairs	9	9

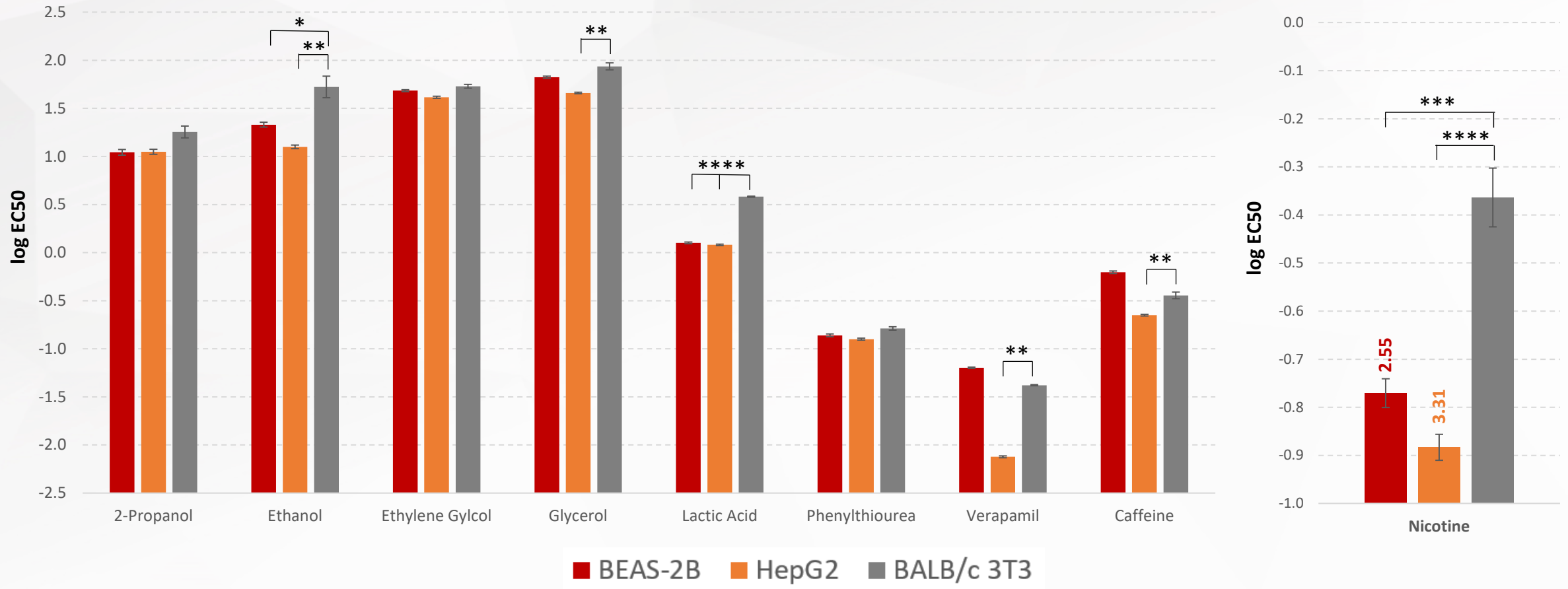
	BEAS-2B vs. HepG2
Pearson r	0.9801
95% confidence interval	0.9050 to 0.9959
R squared	0.9605
P value	<0.0001
P value summary	****
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	9

Results: Correlation between internal and ICCVAM data



	BALB ICCVAM vs. BEAS-2B	BALB ICCVAM vs. HepG2	BALB ICCVAM vs. BALB/c 3T3 internal
Pearson r	0.9523	0.9654	0.9801
95% confidence interval	0.7842 to 0.9902	0.8394 to 0.9929	0.9053 to 0.9960
R squared	0.9069	0.9319	0.9607
P value	<0.0001	<0.0001	<0.0001
P value summary	****	****	****
Significant? (alpha = 0.05)	Yes	Yes	Yes
Number of XY Pairs	9	9	9

Results: Sensitivity of the cell lines



EC50 = concentration to achieve 50% growth inhibition
→ lower EC50 = higher sensitivity

Conclusions

- Imperial Brands Biological and Toxicology Lab performs NRU assays with the human cell lines BEAS-2B and HepG2 for cytotoxicity assessments for combustible tobacco products, non-combustible NGPs, neat ingredients and OND/ snus extractions
- The methods are internally validated and ISO 17025 accredited, and therefore strictly controlled and standardized to ensure a high reproducibility of the results
- The presented study shows a high correlation between the EC50 values of nine different tested compounds with the BEAS-2B, HepG2, the OECD recommended cell line BALBc and the externally obtained data from the ICCVAM Validation study
- BEAS-2B and HepG2 cell lines display a higher sensitivity especially to nicotine
- These findings confirm the applicability of human-relevant cell lines for the cytotoxicity determination following the OECD TG 129 method
- Moreover, the superior sensitivity of BEAS-2B and HepG2 cell lines over BALB/c 3T3 especially to nicotine is particularly beneficial for human-relevant assessments of tobacco or NGP ingredients

Thank you

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