The effect of charcoal filters on gas vapour phase in vitro toxicity tested in different air/liquid interface exposure systems

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Imperial Tobacco

Objective and experimental approach

- To compare different fresh smoke exposure systems.

- For this purpose, contribution of Gas Vapour Phase (GVP) to fresh Whole Smoke (WS) toxicity *in vitro* was determined for cigarettes with different filters.

Methods - *in vitro*

Cell line **Hep-G2** (ATCC HB-8065)

Human hepatoma cells were incubated in mineral medium supplemented with Insulin-Transferrin-Selenium and 1% serum substitute (Ultroser-G; BioSepra S.A.).

Cytotoxicity assay

NRU - *after smoke exposure the cells were incubated in mineral medium with 0.1% serum substitute for 65 hours.*

Methods - exposure of cells

Fresh Smoke Exposure Systems

Bt020 smoke aerosol exposure system; Burghart GmbH; Wedel, Germany

Ø Exposure vessels / chamber

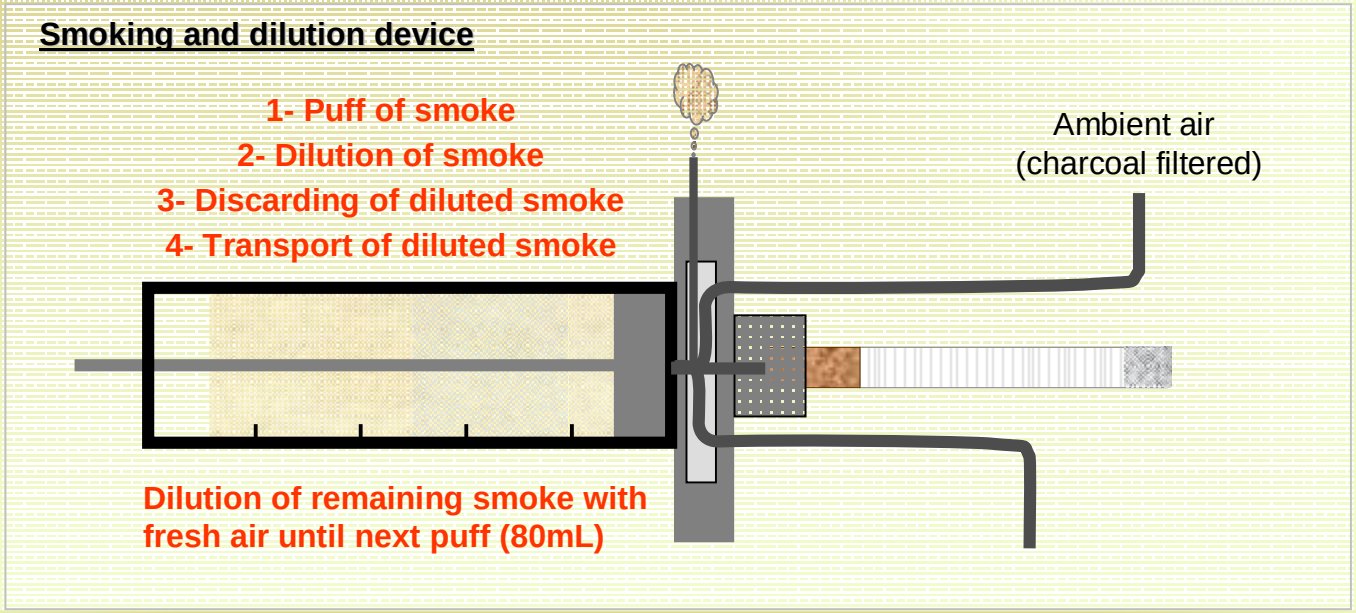
- in round bottom wells of 96 Multiwell Plate (96MWP)

- in inserts (24MWP)

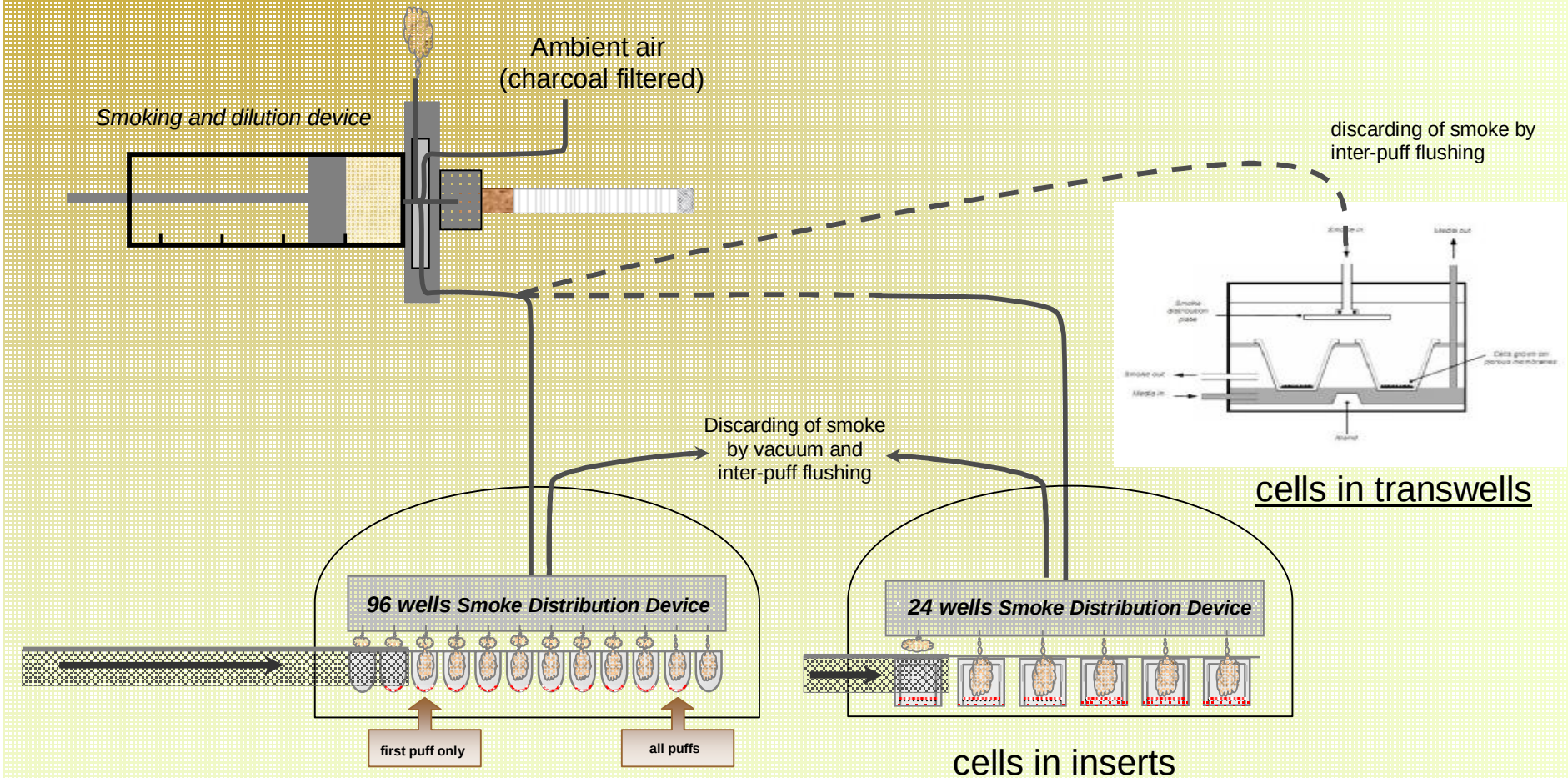
- BAT exposure chamber with transwells (PCT WO 03/100417)

Methods - smoke generation and dilution

- Each puff of smoke is diluted, if necessary, directly in syringe by partly rejecting and refilling with charcoal filtered ambient air.
- The volume of smoke transported to the exposure chamber is constant and independent of dilution factor.
- Smoke dilution and transport after puff till to contact with cells takes less than 6 seconds.
- Excess smoke actively removed by inter-puff flushing cycles with 80 ml charcoal filtered air.



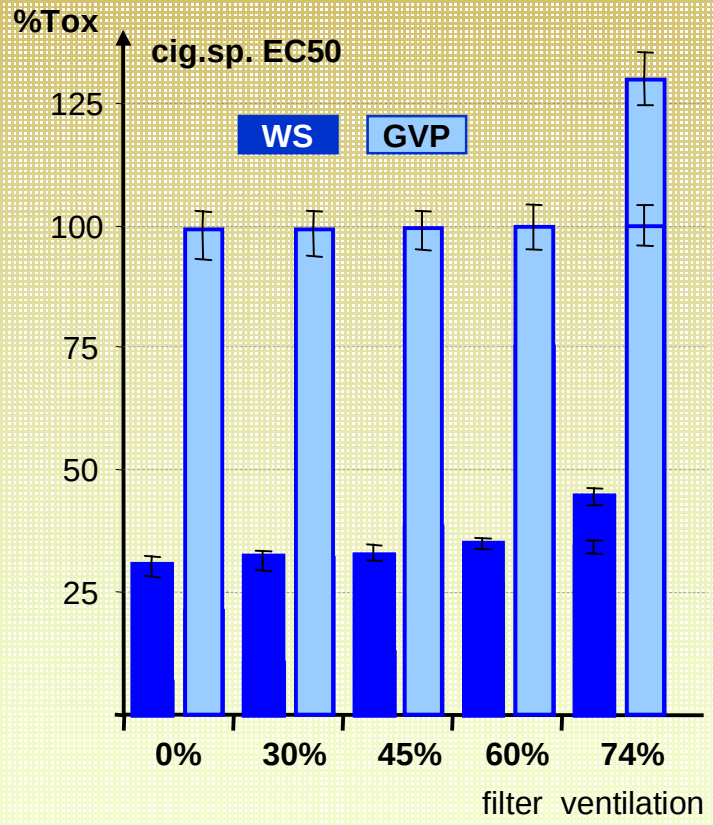
Methods - used exposure systems



Contribution of GVP to WS - Cellulose Acetate filter

Toxicity of Fresh Whole Smoke
Test in 96 MWP (2/2/35)

higher EC50 à lower toxicity



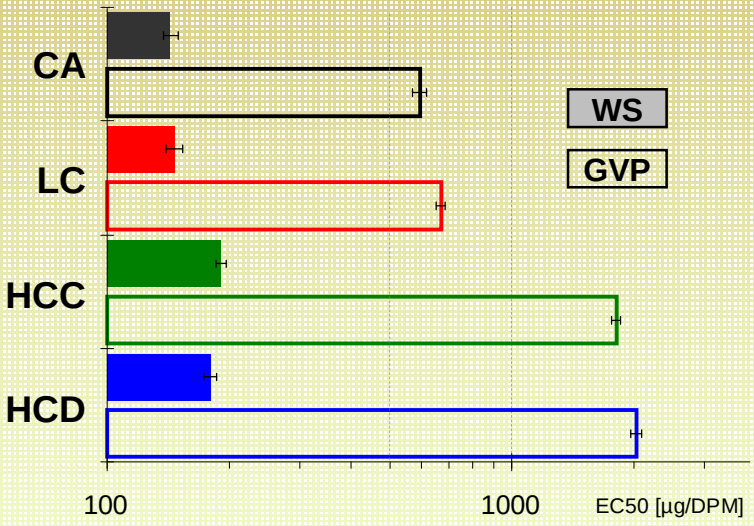
Ø The contribution level of GVP to WS toxicity in cigarettes with cellulose acetate filter stayed roughly the same at about 33%, independent of puff volume and filter ventilation

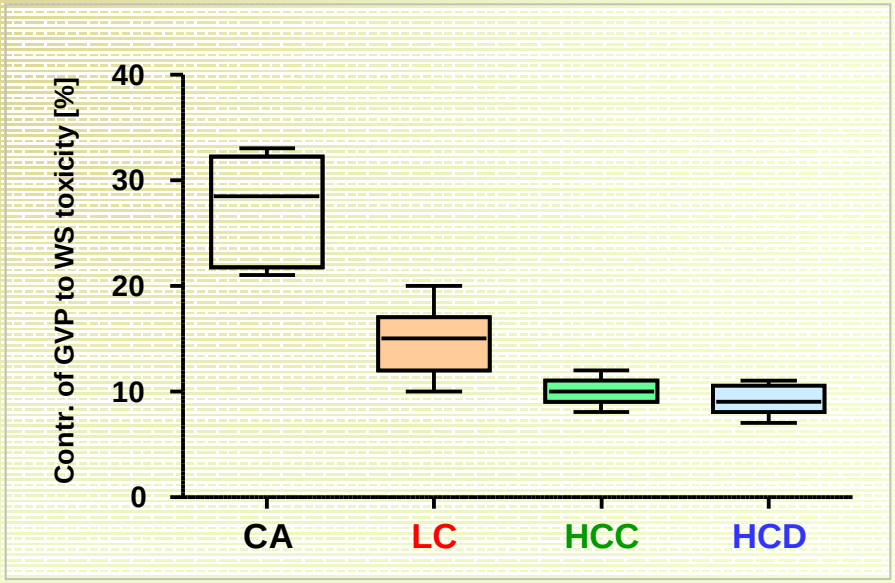
2010 CORESTA, Edinburgh, Scotland (SSPT32) The effect of puff volume on in vitro toxicity of mainstream cigarette smoke

Contribution of GVP

Screening of cigarette toxicity using 96 MWP with 4µl buffer per well

Code	Filter	Dry Particulate Matter **DPM** [mg/cig]	**Charcoal** [mg/filter]
CA	acetate	**13.9**	**0.0**
LC	acetate, carbon, cellulose	**15.7**	**19.0**
HCC	acetate, carbon / **cavity**	**14.6**	**85.0**
HCD	acetate, carbon / **dalmatian**	**14.3**	**82.5**

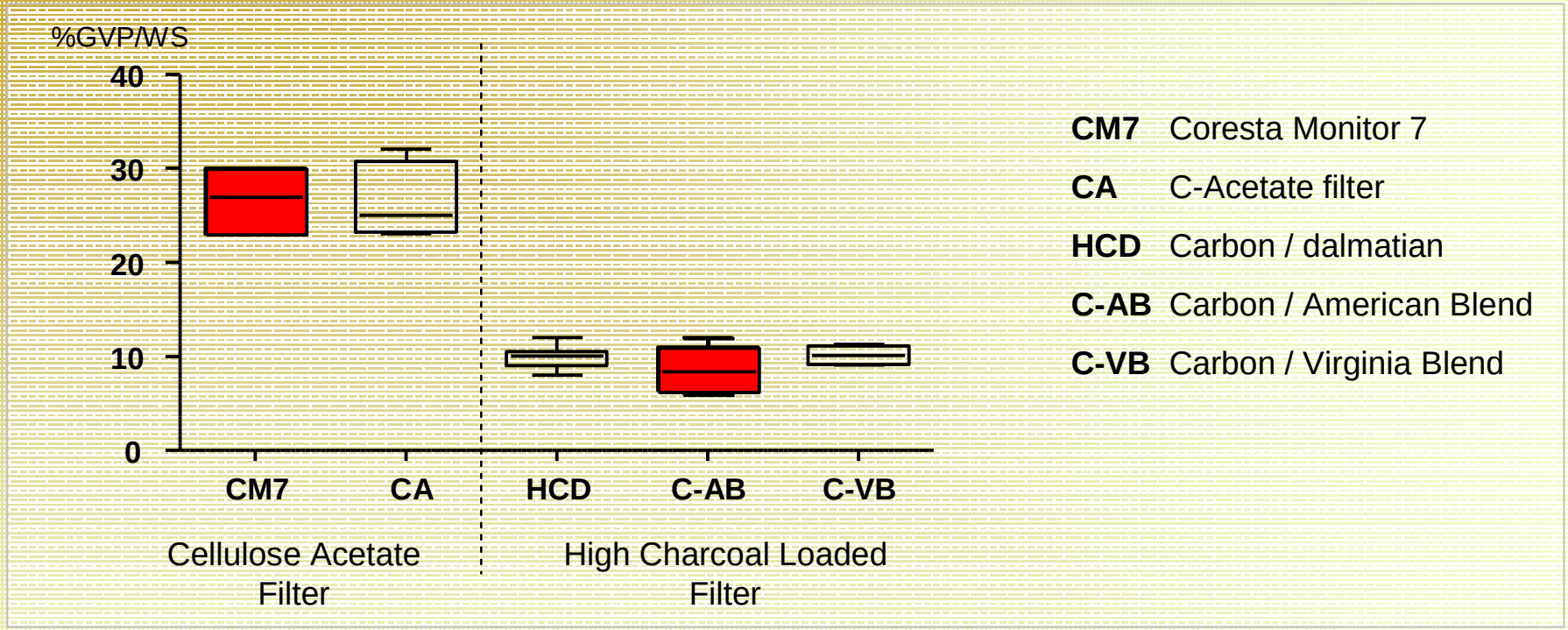




Ø The contribution of GVP to WS toxicity correlates with the amount of charcoal in filter

Contribution of GVP (contd.)

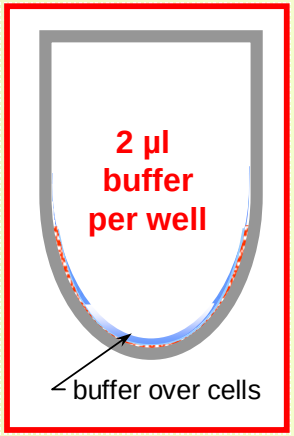
Cigarette toxicity using 96 MWP with 2µl buffer per well

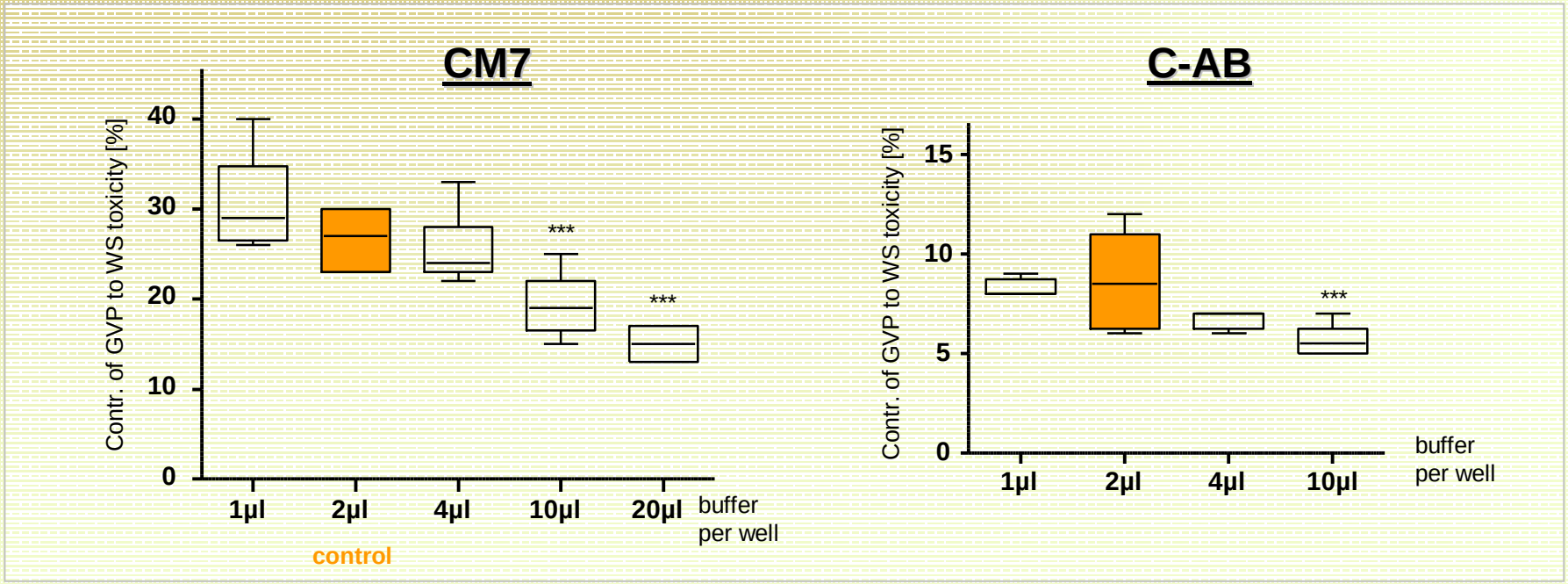


- HCLF decreased the contribution GVP to WS toxicity by roughly 15-20%
- Tests with 2 µl and 4 µl buffer per well do not change significantly the contribution level of GVP.

Influence of fluid layer thickness on GVP effect in 96MWP exposure system

- HEPES buffered PBS in wells protects cells against drying during smoke exposure procedure.
- The buffer covers the cells on the bottom of well but the cells in edge region are directly exposed to smoke.
- The lower the buffer volume in wells the higher the air/liquid interface area.
- Overlaid cells in round bottom wells are less sensitive to GVP than to WS.



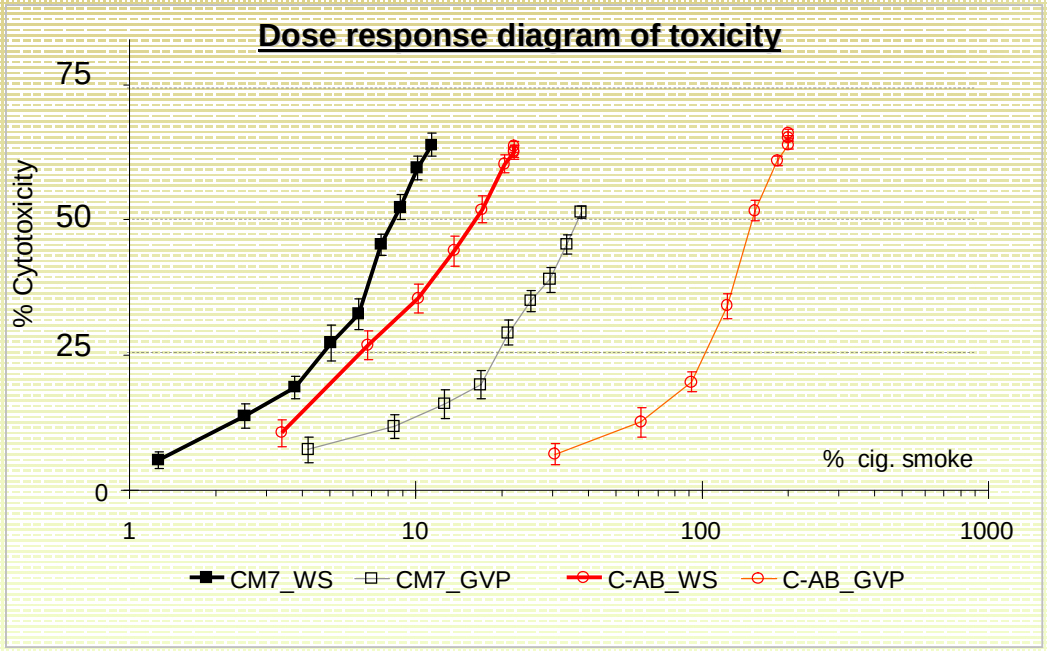


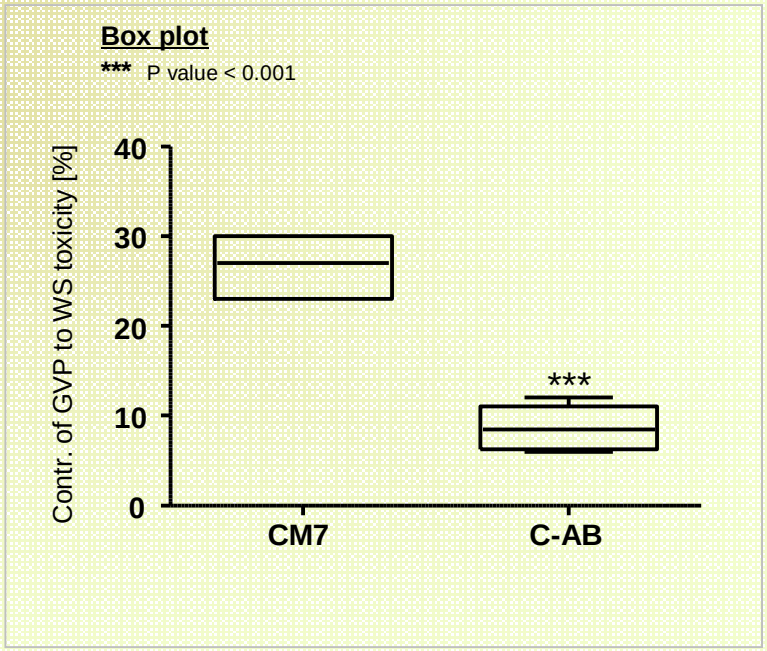
Comparison of methods

Contribution of GVP to WS toxicity in 96MWP with 2 µl buffer

Code	Filter	TPM [mg/cig]	Gas Vapour Phase* [µg/cig]
CM7	Acetate Filter	16.0	2282
C-AB	High Charcoal Loaded Filter	13.4	1175

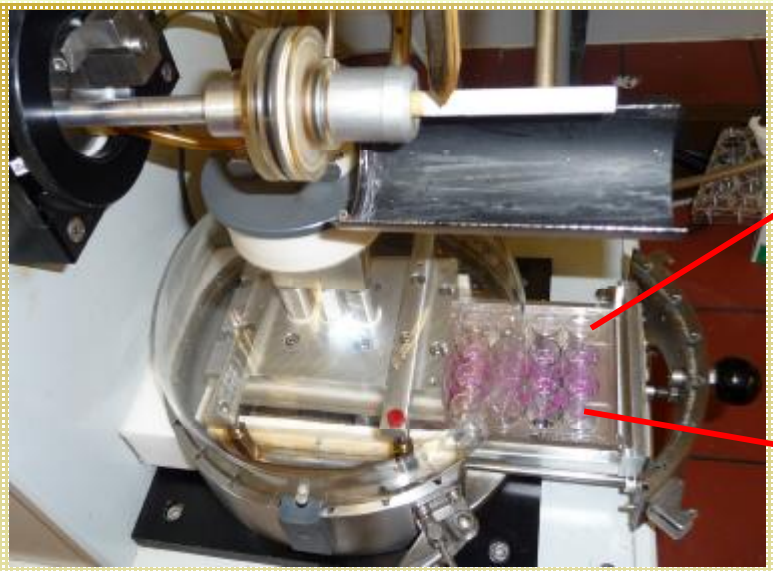
* Sum of 12 selected analytes

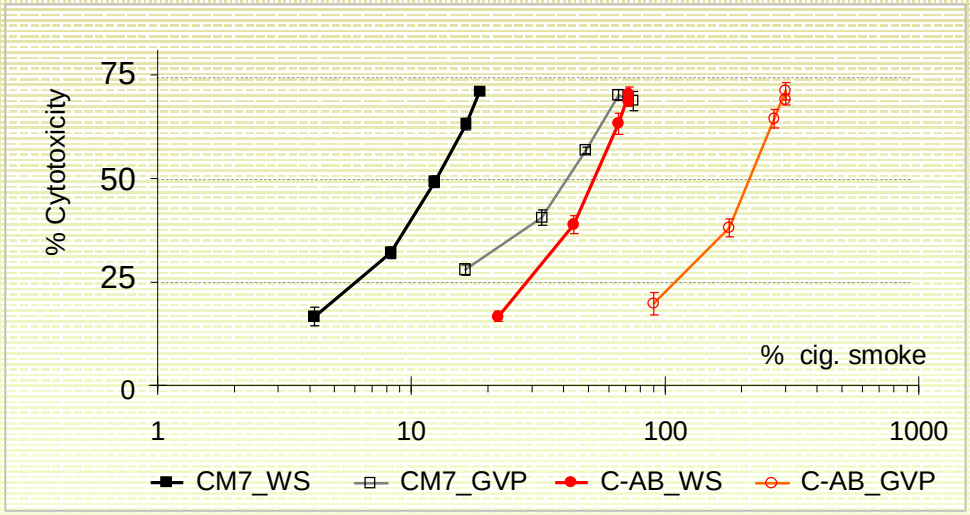


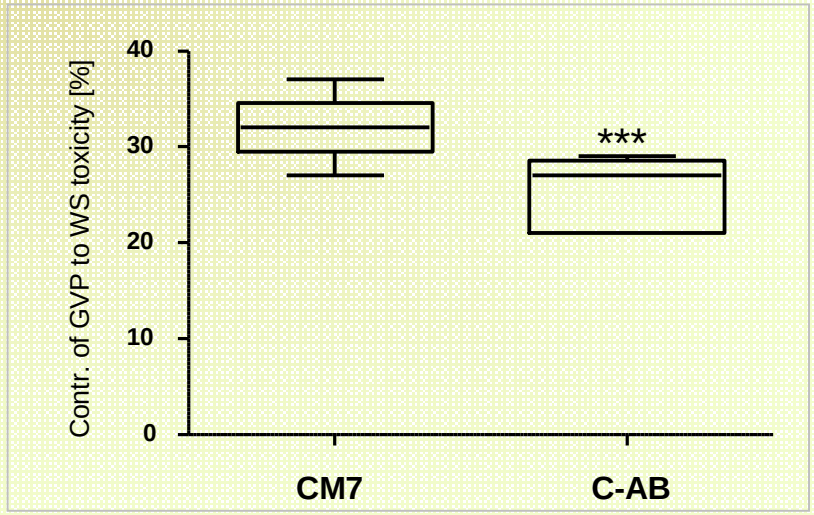


Comparison of methods

Contribution of GVP to WS toxicity in inserts (24MWP)

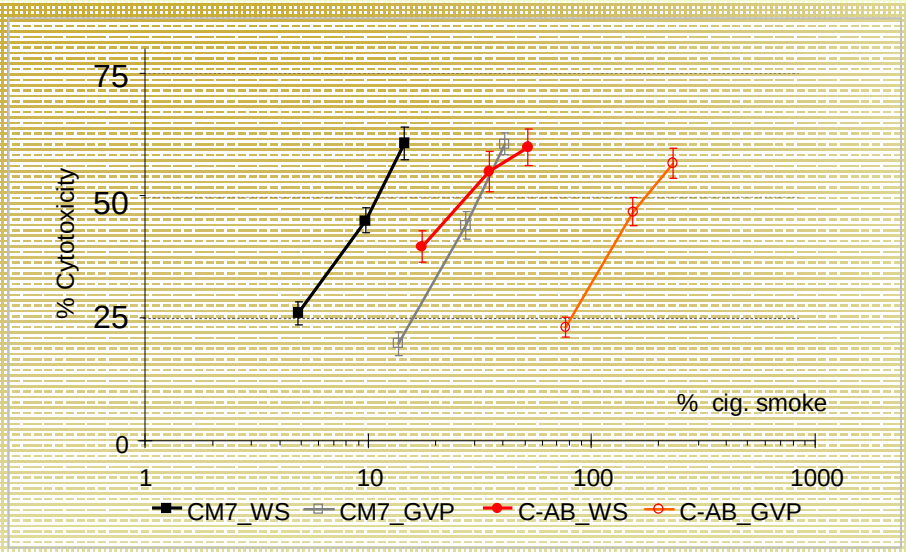


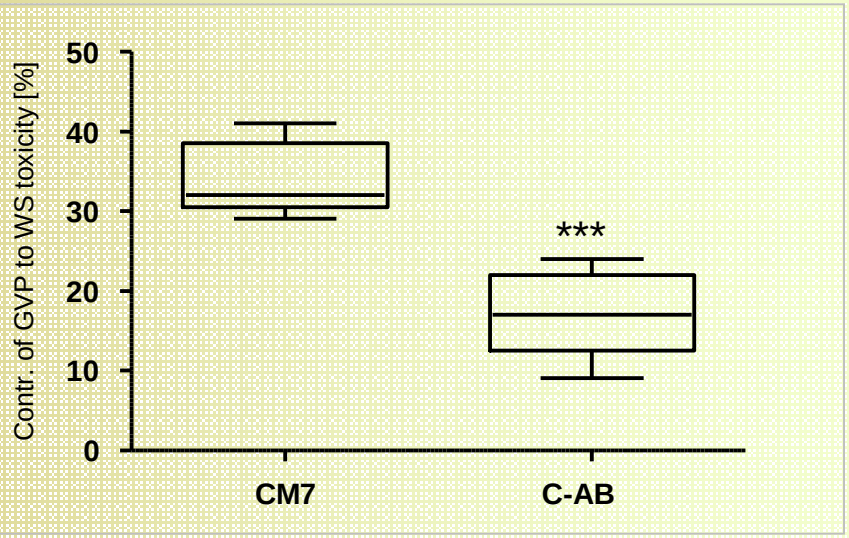


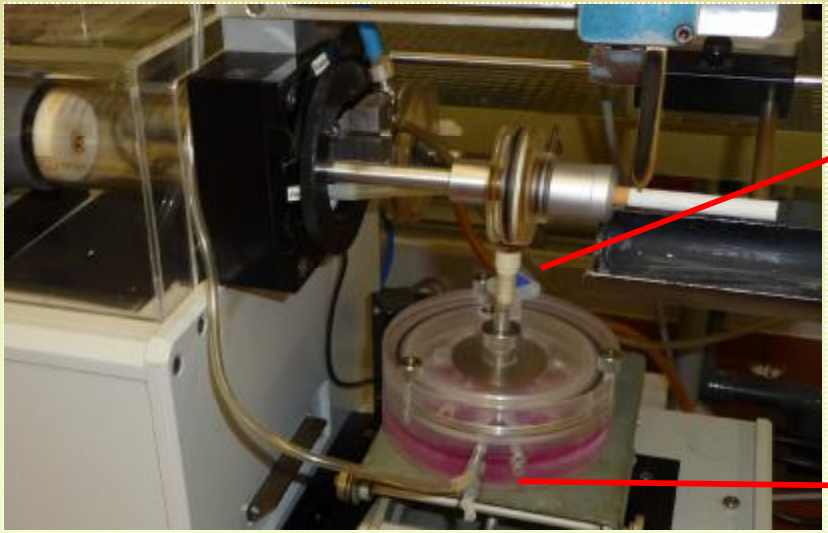


Comparison of methods

Contribution of GVP to WS toxicity in transwell system







BAT exposure chamber
(PCT WO 03/100417)

Comparison of methods - summary

Exposure system	CM7 [%]	C-AB [%]
96MWP-U wells	27	8.5
24MWP-Inserts	32	27
BAT-Transwell	32	17

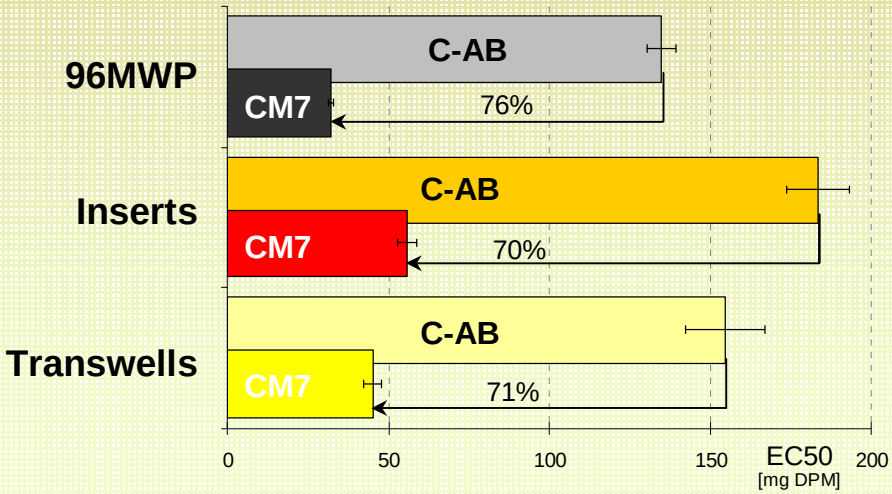
The contribution of GVP to WS toxicity:

- **constant for CM7 cellulose acetate filter at 30%**
- **variable for charcoal filter cigarettes**

Differences in GVP of C-AB in comparison to CM7 testpiece

Selected GVP substances of CM7 in comparison to C-AB

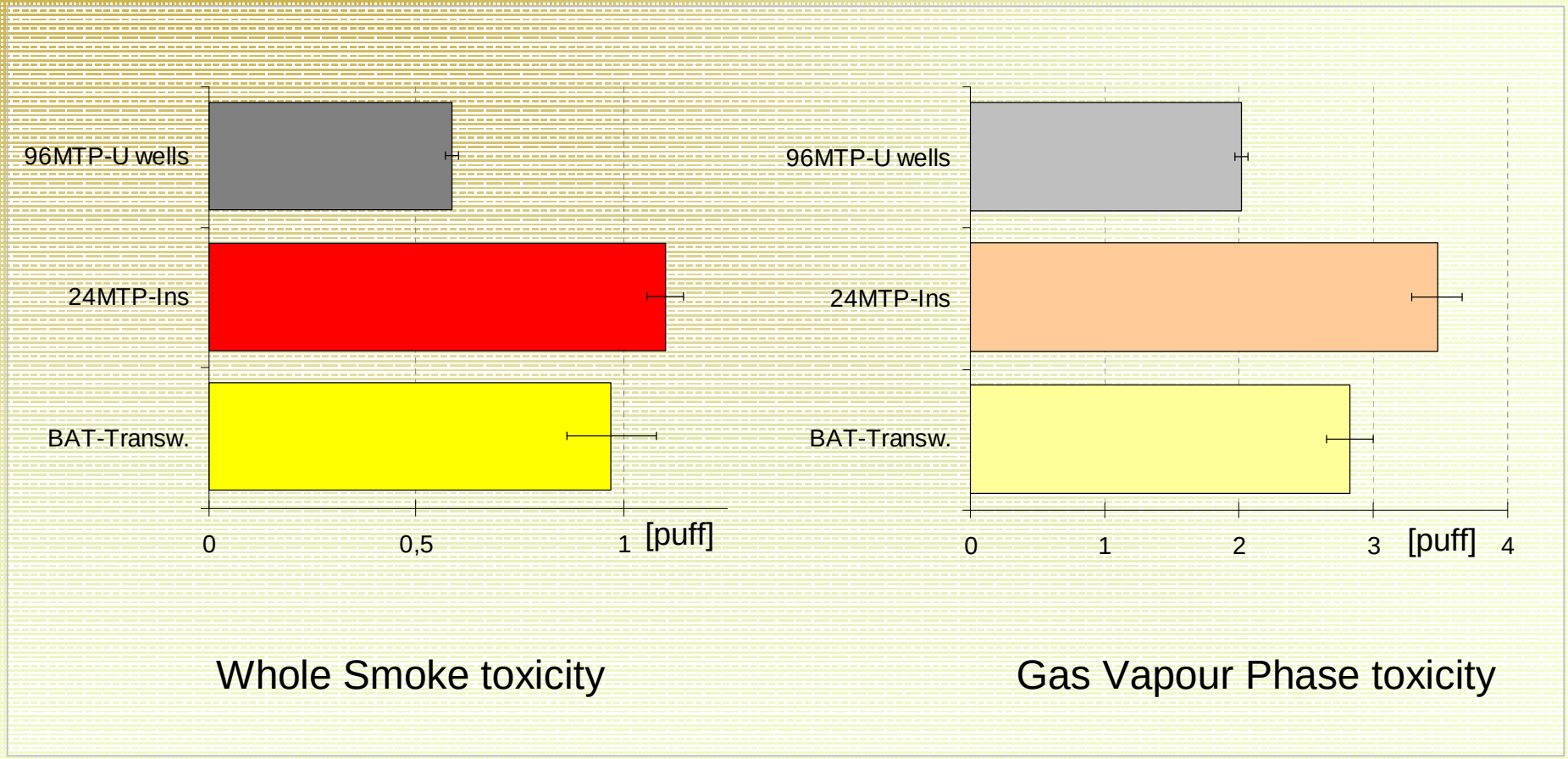
Substance	CM7 [µg/cig]	C-AB [µg/cig]
Acrolein	81.9	31.1
HCN	127.4	46.9
Acetaldehyde	803	556
Acetonitrile	114.2	42.4
Benzene	53.4	16.8



Direct comparison of GVP toxicity in NRU *in vitro* assay (EC50) between CM7 and C-AB

Comparison of sensitivity of the exposure systems

Puff specific EC50 (CM7)



Whole Smoke toxicity

Gas Vapour Phase toxicity

Conclusions

- **All three tested air/liquid exposure systems are able to show:**
  - **dose response effects with WS and GVP.**
  - **significantly higher WS and GVP toxicity of CM7 testpiece compared to charcoal filter cigarette.**
  - **similar decrease of GVP toxicity.**
  - **significantly higher contribution level of GVP to WS toxicity of CM7 testpiece compared to charcoal filter cigarette.**
- **The contribution of GVP to WS toxicity amounts to 30% for cellulose acetate filter. For charcoal filter cigarettes different effects were observed.**
- **The level of medium in the round bottom wells had a direct influence on the sensitivity of the cells. Up to a volume of 4 µl, the contribution of GVP to WS toxicity seems to be constant.**

Conclusions (contd.)

- It was observed, that the level of medium below the growth membrane of insert/transwell affects the liquid meniscus at the edge of the membrane. As a consequence, the exposure conditions can change due to different covering of the cells with fluid.
- The exposure system with 96MWP in round bottom wells and growth membrane delivers “meaningful” data.
- Nevertheless, further tests for better comparability of results are needed.

Thanks to

ITG tox and analytical team

&

BAT for providing their smoke exposure chamber

… and for your kind attention