

# Repeating past mistakes: The banality and futility of nowadays cigarette smoke-related animal experimentation

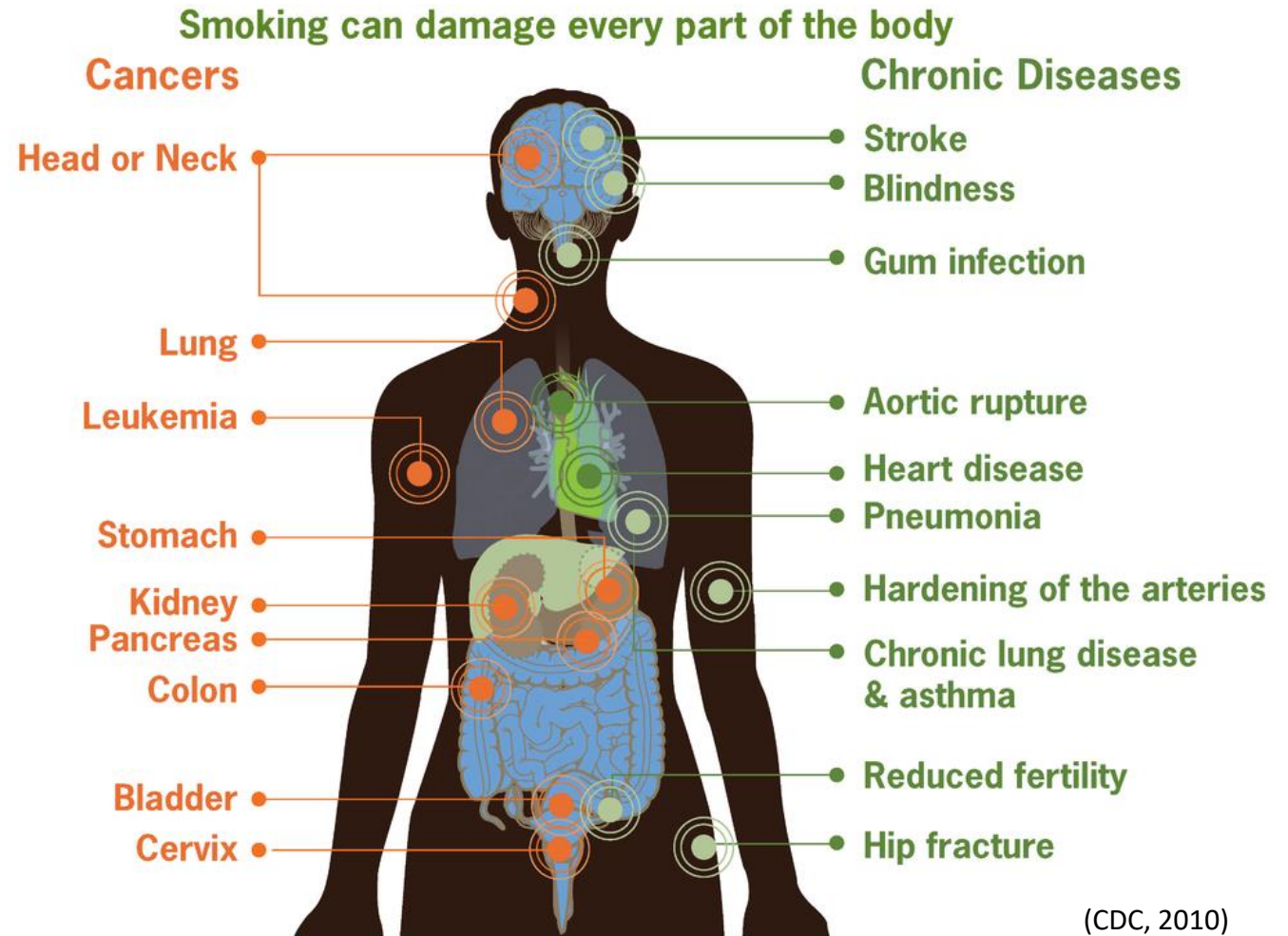


EUSAAT 2019, Linz, 13<sup>th</sup> September 2019

# Health hazards associated with cigarette smoke (CS)

Cigarette smoke:

- No. 1 cause of preventable deaths globally (6 Mio. deaths annually)
- A very wide array of chronic diseases and neoplasms
- The leading cause of lung cancer and chronic obstructive pulmonary disorder (COPD)
  - Demonstrated in humans by multiple epidemiological studies since 1930s



(CDC, 2010)

# CS and animal experiments: A historical overview

Animal experiments since 1930s in multiple species:

- Inconclusive evidence for CS-associated lung carcinogenesis
  - mice: inflammation, tar-induced skin cancer, no lung cancer
  - > 1000 rats: lung cancer only in females
  - 4440 hamsters: larynx tumors, no lung cancer
  - Dogs, monkeys, rabbits: variable results

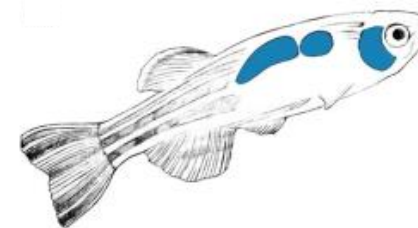
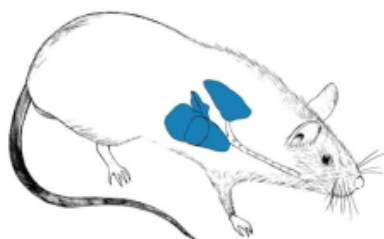
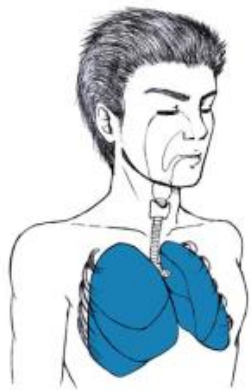
Hutt et al., 2005 – “the first example of a strong carcinogenic response in lungs of animals exposed to CS”

- No reliable animal models for studying CS effects except humans
- Vast public outcry against animal experiments



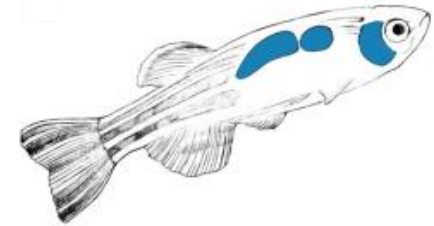
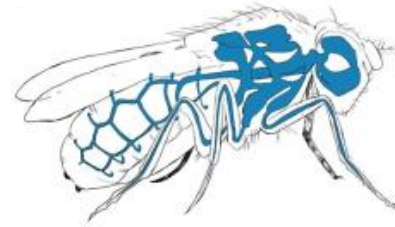
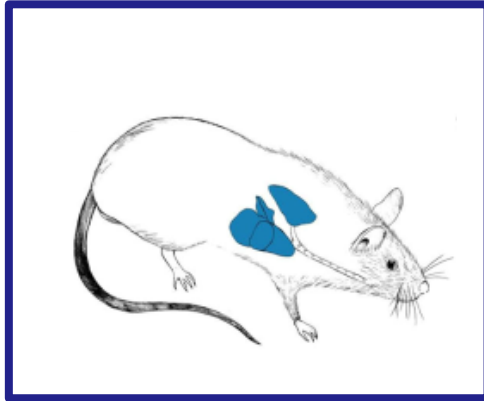
(Oden, 2018)

## Nowadays CS animal experiments



(Hammer et al., 2018)

# Nowadays CS animal experiments



(Hammer et al., 2018)

Food Res Int. 2019 Sep;123:697-703. doi: 10.1016/j.foodres.2019.06.001. Epub 2019 Jun 4.

## **Probiotic Prato cheese attenuates cigarette smoke-induced injuries in mice.**

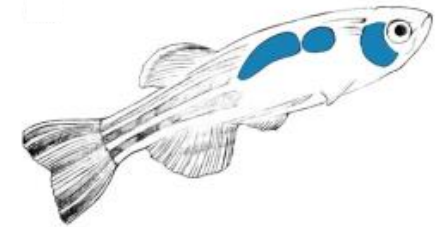
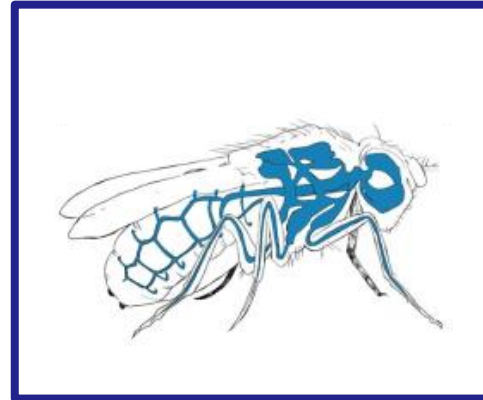
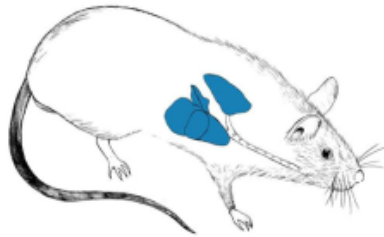
Vasconcelos FM<sup>1</sup>, Silva HLA<sup>2</sup>, Poso SMV<sup>1</sup>, Barroso MV<sup>1</sup>, Lanzetti M<sup>1</sup>, Rocha RS<sup>3</sup>, Graça JS<sup>4</sup>, Esmerino EA<sup>2</sup>, Freitas MQ<sup>2</sup>, Silva MC<sup>5</sup>, Raices RSL<sup>5</sup>, Granato D<sup>6</sup>, Pimentel TC<sup>7</sup>, Sant'Ana AS<sup>4</sup>, Cruz AG<sup>8</sup>, Valença SS<sup>9</sup>.

### **Author information**

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# Nowadays CS animal experiments



(Hammer et al., 2018)

[BMC Dev Biol.](#) 2018 Jun 14;18(1):13. doi: 10.1186/s12861-018-0172-6.

**Developmental nicotine exposure affects larval brain size and the adult dopaminergic system of *Drosophila melanogaster*.**

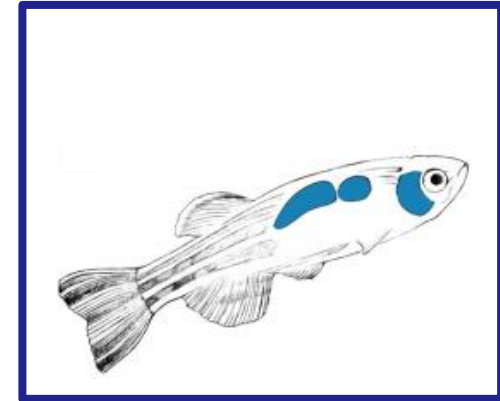
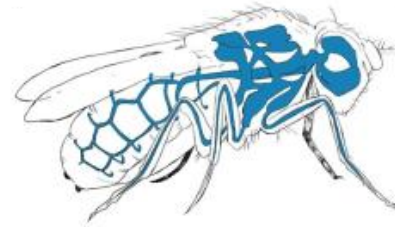
[nato](#)

[Morris M](#)<sup>1</sup>, [Shaw A](#)<sup>2</sup>, [Lambert M](#)<sup>3</sup>, [Perry HH](#)<sup>3</sup>, [Lowenstein E](#)<sup>3</sup>, [Valenzuela D](#)<sup>4</sup>, [Velazquez-Ulloa NA](#)<sup>5</sup>.

⊖ [Author information](#)

<sup>1</sup> School of Medicine, University of Washington, Seattle, USA.

# Nowadays CS animal experiments



(Hammer et al., 2018)

Front Biosci (Elite Ed), 2019 Jan 1;11:109-120.

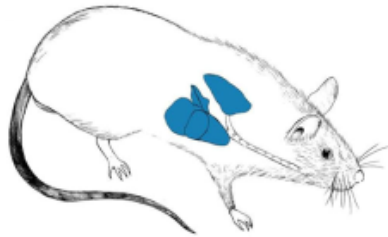
## **Curcumin prevents cigarette smoke extract induced cognitive impairment.**

Muthuraman A<sup>1</sup>, Thilagavathi L<sup>2</sup>, Jabeen S<sup>2</sup>, Ravishankar SB<sup>2</sup>, Ahmed SS<sup>2</sup>, George T<sup>2</sup>, Rishitha N<sup>2</sup>, Paramakrishnan N<sup>2</sup>.

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- 1 JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru-570 015, Karnataka, India, arunachalammu@gmail.com.

# Nowadays CS animal experiments



(Hammer et al., 2018)

- Genetically 'predisposed' GMOs
- 'Assisted' CS symptoms
- Alone the mouse background influences the CS effects (Enzmann et al., 2019)

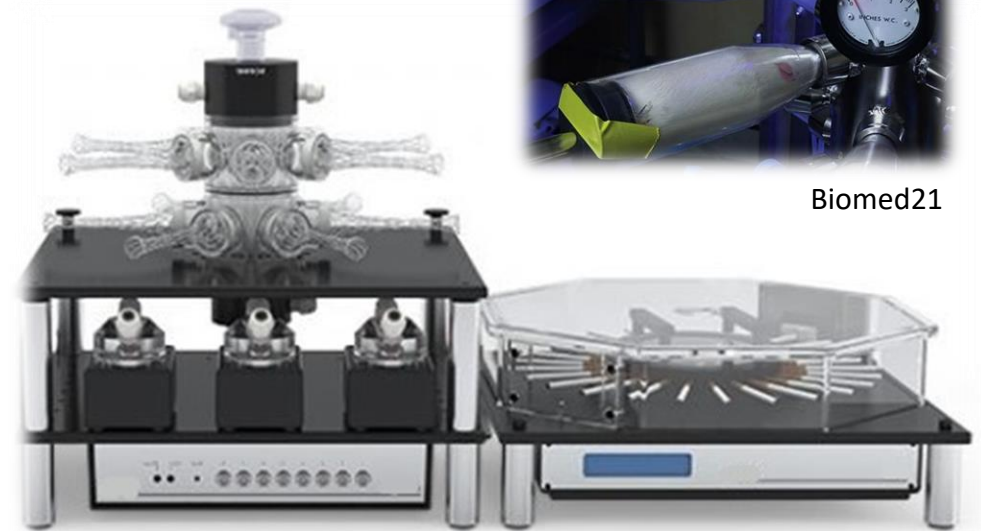
Diverse CS exposure systems:

- Whole body
- Nose only

**Price > 100,000 €**



Porsolt



Scireq®



Biomed21

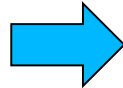
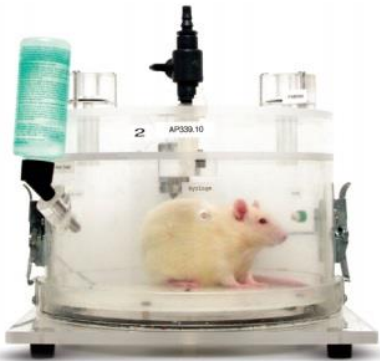


# Absurd animal experiments: CS is bad in lung trauma and blood loss

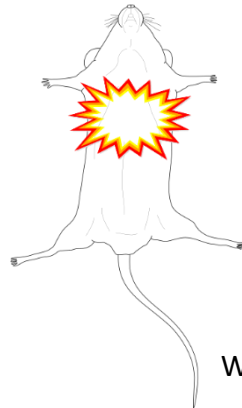
Hartmann et al., 2018, University hospital of Ulm, Germany  
Animals: C57BL/6J mice (11/53 died during the procedures)

➡ **Unnecessary, trivial findings**

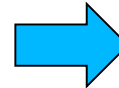
CS exposure:  
4-8 cigarettes / day



Blunt chest trauma:  
A blast to the chest



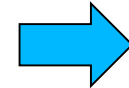
Wikimedia



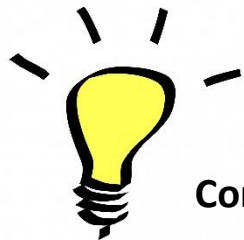
Hemorrhagic shock for 1 h:  
ca. 50 % blood vol., 30 % arterial pressure



Kohut et al., 2018



Killed,  
Analysis



Results: CS ↑ inflammation; CS + trauma ↑ mortality

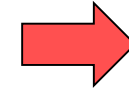
Conclusion: Smoking negatively influences the outcome of lung trauma and blood loss in mice



Similar CS-trauma studies since at least 2011

## Absurd animal experiments: CS is bad in lung trauma and blood loss

Hartmann et al., 2018, University hospital of Ulm, Germany  
Animals: C57BL/6J mice (11/53 died during the procedures)

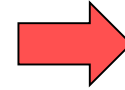


**Unnecessary, trivial findings**



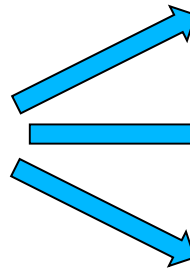
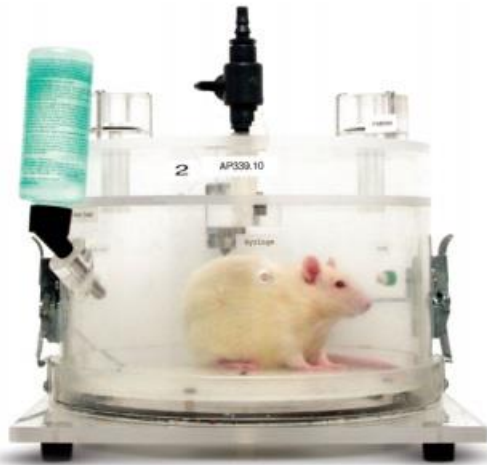
# Absurd animal experiments: Repetition and contradiction of clinical data

**Bucher et al., 2017**, Boehringer Ingelheim Pharma, Germany  
Animals: BALB/c mice



**Unnecessary, meaningless results  
for humans**

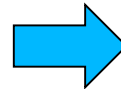
CS exposure:  
4 cigarettes/day; 4 days



CS

CS + H1N1

CS + H1N1  
+ Antibodies  
(IL-1R1, IL-1 $\alpha\beta$ )



Killed,  
Analysis

Results:

CS + H1N1 – worst outcome

Abs – some protective function

**However, the same target Abs  
previously failed in clinical trials**

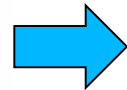
**Conclusion: Abs not working in humans, reduce inflammation in mice**



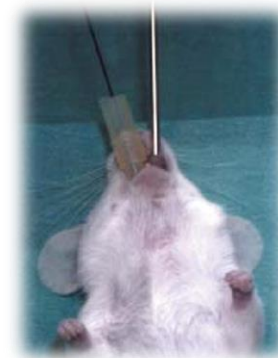
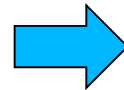
# Absurd animal experiments: COPD in a highly artificial environment

**Jia et al., 2018**, Helmholtz Zentrum Munich, Germany  
Animals: C57Bl/6J mice (different KOs)

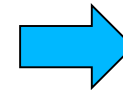
CS exposure: 100 % CS  
2 x 50 min/day; 5 days/week; 4-6 months



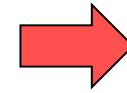
+ elastase-induced  
emphysema



(Vergari et al., 2004)



Killed,  
Analysis



**Unnecessary, repetitive, highly  
artificial**

Results: Specific genes and immunological reactions are dysregulated in mice and COPD patients

**Why show it in mice, if shown in patients?**

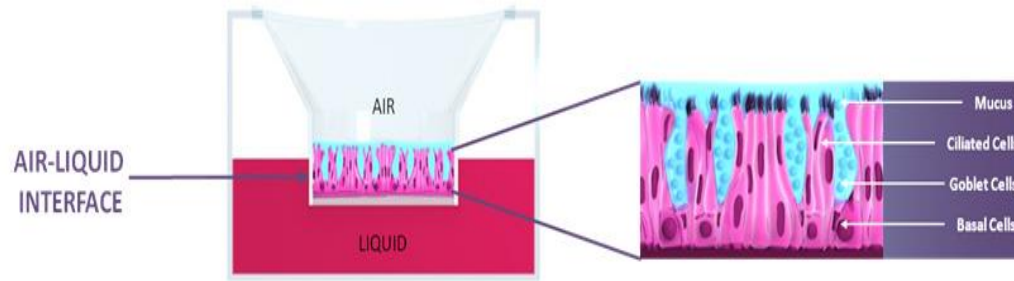
# *In vitro* and human-based systems

## Regulatory accepted *in vitro* methods

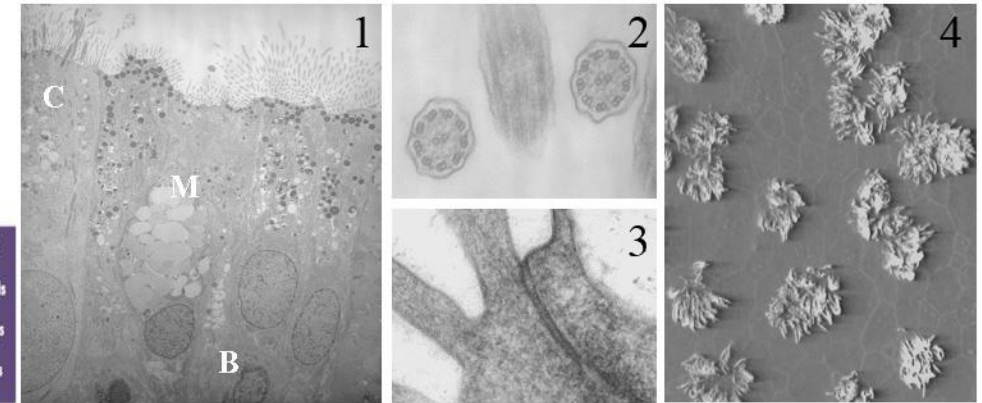
- Acute (inhalation) toxicity: NRU cytotoxicity assay
- Carcinogenicity: Bhas 42 cell and three cell transformation assays

## 3D airway cultures

- Normal
- Smoker
- Allergic Rhinitis
- Asthma
- COPD
- Cystic Fibrosis



Viable > 1 year



(epithelix.com)



# *In vitro* and human-based systems

## Regulatory accepted *in vitro* methods

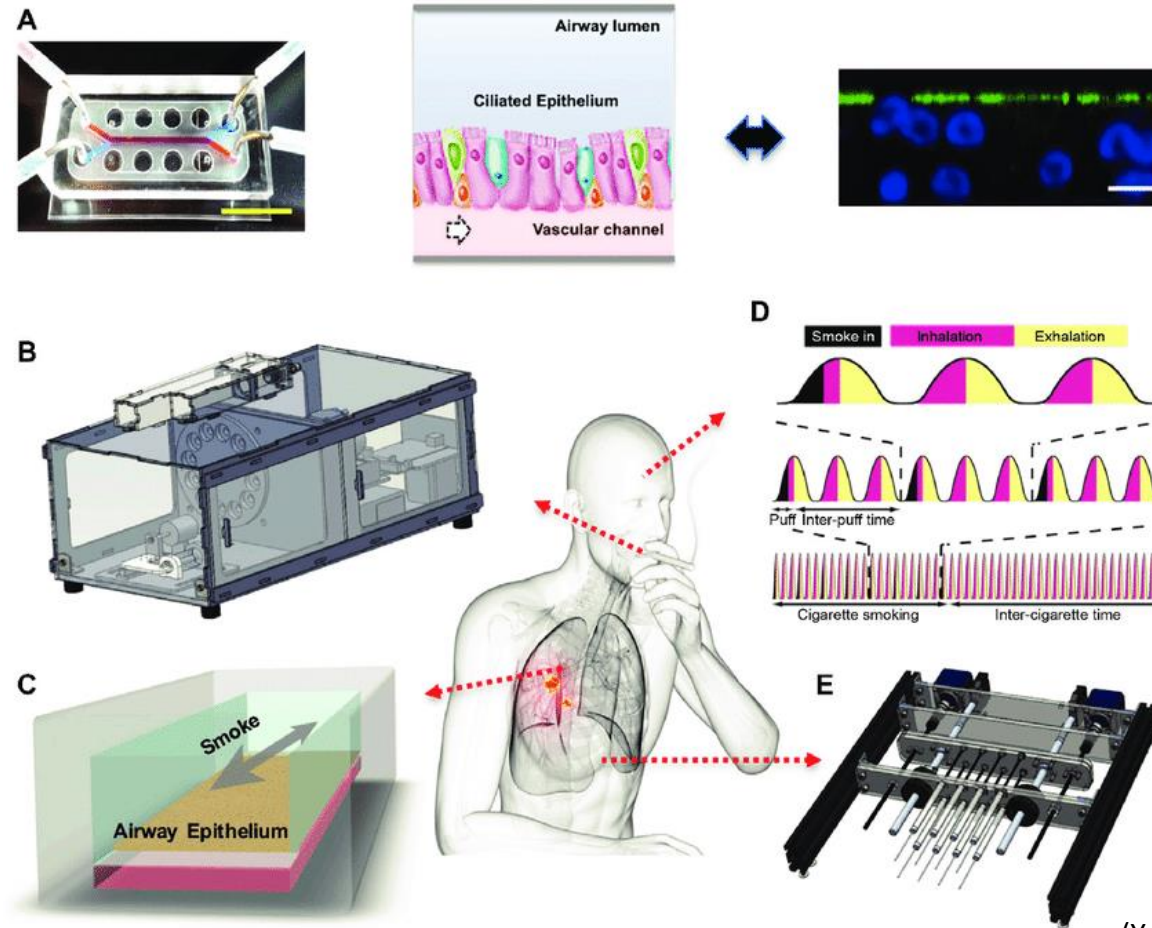
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## 3D airway cultures

- Normal
- Smoker
- Allergic Rhinitis
- Asthma
- COPD
- Cystic Fibrosis

## (Smoking-)Lung-on-a-chip models

- Multiple human models
  - Injury
  - Inflammation
  - Pulmonary fibrosis
  - cancer
- Personalized solutions



(Yesil-Celiktas et al., 2018)

# Repeating past mistakes: e-cigarettes and waterpipe



[PLoS One](#). 2019 Feb 27;14(2):e0211645. doi: 10.1371/journal.pone.0211645. eCollection 2019.

## Waterpipe smoke and e-cigarette vapor differentially affect circadian molecular clock gene expression in mouse lungs.

[Khan NA](#)<sup>1</sup>, [Yogeswaran S](#)<sup>1</sup>, [Wang Q](#)<sup>1</sup>, [Muthumalage T](#)<sup>1</sup>, [Sundar IK](#)<sup>1</sup>, [Rahman I](#)<sup>1</sup>.

### Author information

<sup>1</sup> Department of Environmental Medicine, University of Rochester Medical Center, Rochester, New York, United States of America.

[Sci Rep](#). 2019 Sep 20;9(1):13671. doi: 10.1038/s41598-019-50223-y.

## The Effect of Flavored E-cigarettes on Murine Allergic Airways Disease.

[Chapman DG](#)<sup>1,2,3</sup>, [Casey DT](#)<sup>4</sup>, [Ather JL](#)<sup>5</sup>, [Aliyeva M](#)<sup>5</sup>, [Daphtary N](#)<sup>5</sup>, [Lahue KG](#)<sup>4</sup>, [van der Velden JL](#)<sup>4</sup>, [Janssen-Heininger YMW](#)<sup>4</sup>, [Irvin CG](#)<sup>5</sup>.

### Author information

<sup>1</sup> Department of Medicine, University of Vermont College of Medicine, Burlington, Vermont, United States. [David.Chapman@uts.edu.au](mailto:David.Chapman@uts.edu.au).

[Inhal Toxicol](#). 2018 Nov - Dec;30(13-14):553-567. doi: 10.1080/08958378.2019.1576807. Epub 2019 Mar 8.

## Biological changes in C57BL/6 mice following 3 weeks of inhalation exposure to cigarette smoke or e-vapor aerosols.

[Lee KM](#)<sup>1</sup>, [Hoeng J](#)<sup>2</sup>, [Harbo S](#)<sup>3</sup>, [Kogel U](#)<sup>2</sup>, [Gardner W](#)<sup>1</sup>, [Oldham M](#)<sup>1</sup>, [Benson E](#)<sup>3</sup>, [Talikka M](#)<sup>2</sup>, [Kondylis A](#)<sup>2</sup>, [Martin F](#)<sup>2</sup>, [Titz B](#)<sup>2</sup>, [Ansari S](#)<sup>2</sup>, [Trivedi K](#)<sup>2</sup>, [Guedj E](#)<sup>2</sup>, [Elamin A](#)<sup>2</sup>, [Ivanov NV](#)<sup>2</sup>, [Vanscheeuwijck P](#)<sup>2</sup>, [Peitsch MC](#)<sup>2</sup>, [McKinney WJ Jr](#)<sup>1</sup>.

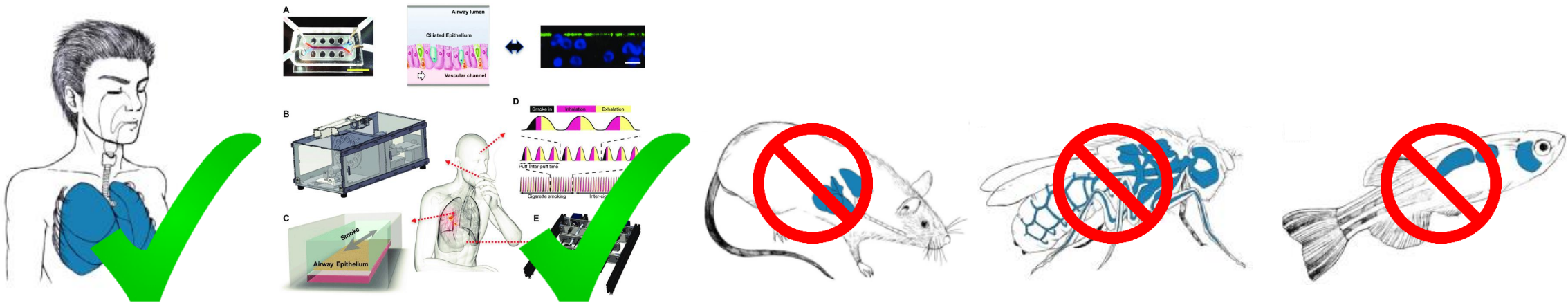
### Author information

<sup>1</sup> a Altria Client Services LLC, Richmond, VA, USA.



## Is history repeating itself over and over?

# Conclusions and outlook: Humans are the only reliable model for CS effects



- Unnecessary, ineffective CS-related animal experiments are still performed
- A regulatory ban is needed for such experiments
- CS research: a focus on prevention and human-based methods

# Doctors Against Animal Experiments Germany (DAAE)

Charitable organization founded in 1979

~3000 members, 1/3 are doctors & scientists working in the medical field

Provides scientific information against animal experiments for scientists and the public

Organizes campaigns, actions, lectures, congresses etc.



The Herbert Stiller Research Grant for  
outstanding animal-free, human-based  
scientific projects

Congress  
“Science Instead of Animal Experiments”  
Cologne, 27th Oct. 2018  
[www.wist-kongress.de/](http://www.wist-kongress.de/)



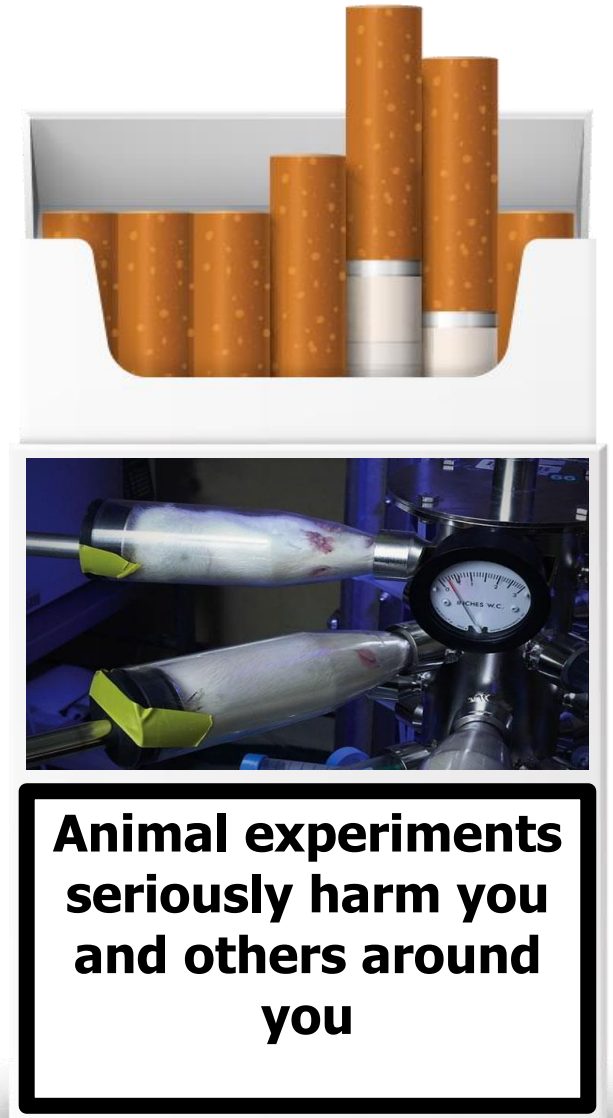
Abolition of all animal experiments for ethical and scientific reasons

Increased support of animal-free research

Increased support of disease prevention



**Thank you for the attention!**



**Animal experiments  
seriously harm you  
and others around  
you**



# Literature

- Biomed21, <https://biomed21.org/2019/11/in-copd-awareness-month-time-to-stop-smoking-cigarettes-whether-youre-a-mouse-a-dog-or-a-human/>, accessed on 02. 01. 2020
- Bucher, H., Mang, S., Keck, M., Przibilla, M., Lamb, D.J., Schiele, F., Wittenbrink, M., Fuchs, K., Jung, B., Erb, K.J., et al. (2017). Neutralization of both IL-1 $\alpha$ /IL-1 $\beta$  plays a major role in suppressing combined cigarette smoke/virus-induced pulmonary inflammation in mice. *Pulm Pharmacol Ther* 44, 96–105.
- CDC: Tobacco Use-CDC Vital Signs-September 2010.pdf, Public Domain, <https://commons.wikimedia.org/w/index.php?curid=20852937>
- Enzmann, G., Adelfio, R., Godel, A., Haghayegh Jahromi, N., Tietz, S., Burgener, S.S., Deutsch, U., Wekerle, H., Benarafa, C., and Engelhardt, B. (2019). The Genetic Background of Mice Influences the Effects of Cigarette Smoke on Onset and Severity of Experimental Autoimmune Encephalomyelitis. *Int J Mol Sci* 20.
- Hammer, B., Wagner, C., Divac Rankov, A., Reuter, S., Bartel, S., Hylkema, M.N., Krüger, A., Svanes, C., and Krauss-Etschmann, S. (2018). In utero exposure to cigarette smoke and effects across generations: A conference of animals on asthma. *Clin Exp Allergy* 48, 1378–1390.
- Hartmann, C., Gröger, M., Noirhomme, J.-P., Scheuerle, A., Möller, P., Wachter, U., Huber-Lang, M., Nussbaum, B., Jung, B., Merz, T., et al. (2019). In-Depth Characterization of the Effects of Cigarette Smoke Exposure on the Acute Trauma Response and Hemorrhage in Mice. *Shock* 51, 68–77.
- Hutt, J.A., Vuilleminot, B.R., Barr, E.B., Grimes, M.J., Hahn, F.F., Hobbs, C.H., March, T.H., Gigliotti, A.P., Seilkop, S.K., Finch, G.L., et al. (2005). Life-span inhalation exposure to mainstream cigarette smoke induces lung cancer in B6C3F1 mice through genetic and epigenetic pathways. *Carcinogenesis* 26, 1999–2009. <http://www.epithelix.com/>
- Jia, J., Conlon, T.M., Sarker, R.S., Taşdemir, D., Smirnova, N.F., Srivastava, B., Verleden, S.E., Güneş, G., Wu, X., Prehn, C., et al. (2018). Cholesterol metabolism promotes B-cell positioning during immune pathogenesis of chronic obstructive pulmonary disease. *EMBO Mol Med* 10.
- Kohut, L.K., Darwiche, S.S., Brumfield, J.M., Frank, A.M., and Billiar, T.R. (2011). Fixed Volume or Fixed Pressure: A Murine Model of Hemorrhagic Shock. *JoVE* 2068.
- Oden F, 2018, <https://www.quora.com/Why-do-monkeys-smoke>, accessed on 04.10. 2019
- Porsolt, [https://www.porsolt.com/file\\_manager\\_download.php?id=215](https://www.porsolt.com/file_manager_download.php?id=215), , accessed on 02. 01. 2020
- Scireq, <https://www.scireq.com/2016/05/19/inexpose-featured-in-recent-nature/>, , accessed on 02. 01. 2020
- Vergari, A., Gunnella, B., Rodolà, F., Frassanito, L., Musumeci, M., Palazzesi, S., and Casalnuovo, I.A. (2004). A new method of orotracheal intubation in mice. *Eur Rev Med Pharmacol Sci* 8, 103–106.
- Wikimedia, [https://commons.wikimedia.org/wiki/File:Vector\\_diagram\\_of\\_mouse\\_lying\\_on\\_its\\_back.svg](https://commons.wikimedia.org/wiki/File:Vector_diagram_of_mouse_lying_on_its_back.svg), accessed on 04.10. 2019
- Yesil-Celiktas, O., Hassan, S., Miri, A.K., Maharjan, S., Al-kharboosh, R., Quiñones-Hinojosa, A., and Zhang, Y.S. (2018). Mimicking Human Pathophysiology in Organ-on-Chip Devices. *Adv. Biosys.* 2, 1800109.