# Quaternary Ammonium Compounds (QACs) found in sprayed disinfectants are sex-specifically pneumotoxic in micec

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University of California, Davis, 95616

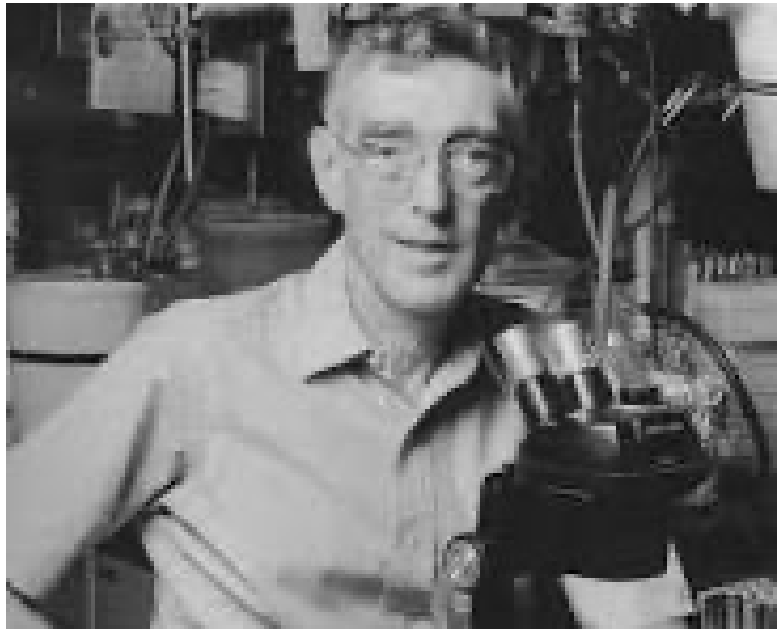
Other Authors: Adcock L, Montgomery CB, Barkhordari S, Datta S, Van Winkle LS, Cortopassi G

GETA

Bruce Ames. Developer of the Ames Test died Oct 5th, age 95

Developed the idea that one could develop a high-thrroughput test for Genetic Toxicity

‘Ames Test’ was developed with the concept that ‘mutagenic potency will predict carcinogenic potency’



Roadmap of the talk:

0. QACs structure and historical use as disinfectants, and why they've been presumed safe for 84 years.

--------some reasons QACs might not be as safe as previously presumed----------

1. QACs are dose-dependent mitochondrial toxins.

2. QACs are detectable in human blood and breastmilk.

3. Human in vivo QAC exposure dose-dependently alters mitochondrial function and inflammatory cytokines.

4. QACs as a chemical class are concentrated 2-27 fold in lung versus blood.

5. QACs are 100-fold more toxic when inhaled vs. eaten, in mice.

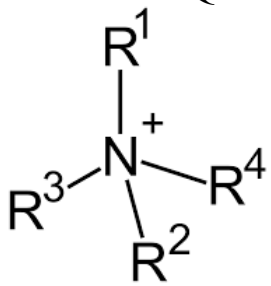
6. QACs are ~2X more toxic to male than female mice.

7. A few hypotheses for why females resist QAC exposure more than males.

# What Are Quaternary Ammonium Compounds (QACs)?

Branded Products containing this compound (from CPID)

## What is a QAC?




Quaternary Ammonium
has a positive charge

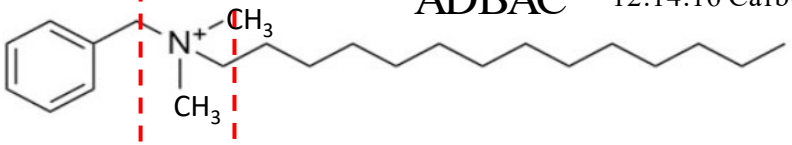
## Most common QACs

Benzalkonium Chloride (BAC)


BAC — 14 or 16 Carbon tail — 53

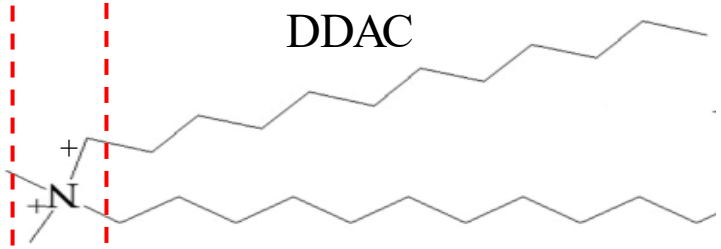


Alkyl dimethyl benzyl ammonium Chloride (ADBAC)




ADBAC — 12:14:16 Carbon tail mixture — 18

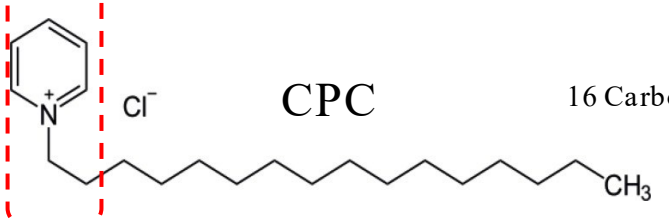

DDAC




Didecyldimethylammonium Chloride (DDAC)

210

Cetyl pyridinium chloride (CPC)




CPC — 16 Carbon tail — 33

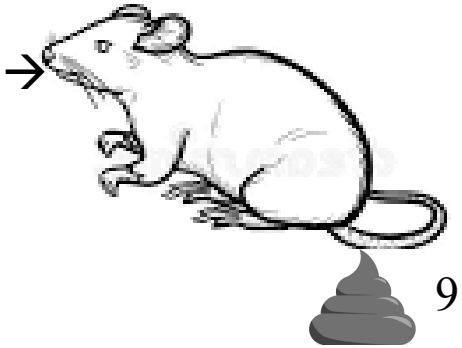

314 US branded products

QACs are usually listed as the Active Ingredient

# QACs have been presumed safe for ~80 years, for multiple reasons

Reason 1. Orally-dosed QACs do not penetrate the rodent or human body, except in cases of massive (suicidal) exposure:

100% QAC in→

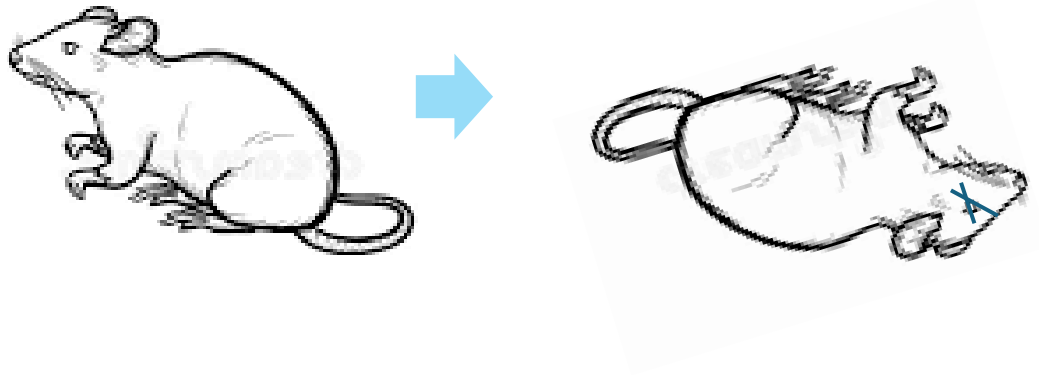


95% QAC out

Absorption, distribution and excretion of [14-C]CTAB, a quarternary ammonium surfactant, in the rat. Isomaa B. Food Cosmet Toxicol. 1975 Apr;13(2):231-7.

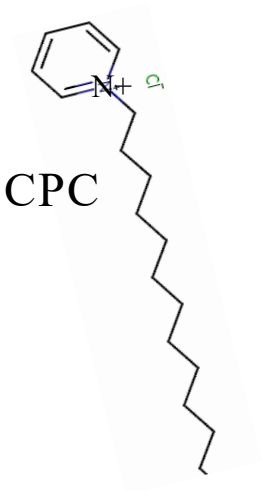
# Reason 2. Orally-Dosed QACs BAC and DDAC have high LD50s: 240-500 mgs/kg

(relative to other toxins, it takes about a half gram or more of oral QAC for lethality)

0.5 to 1gm QAC→ 

Reason 3. QAC's Mechanism of Action since 1941 was presumed to be 'soaplike', so QACs are 'safe as soap'.

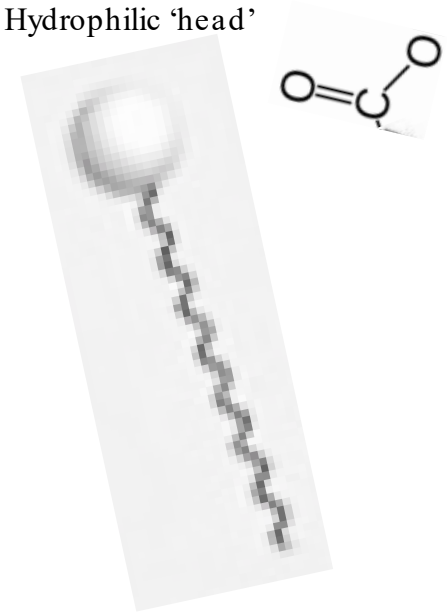
QAC




CPC

SOAP:

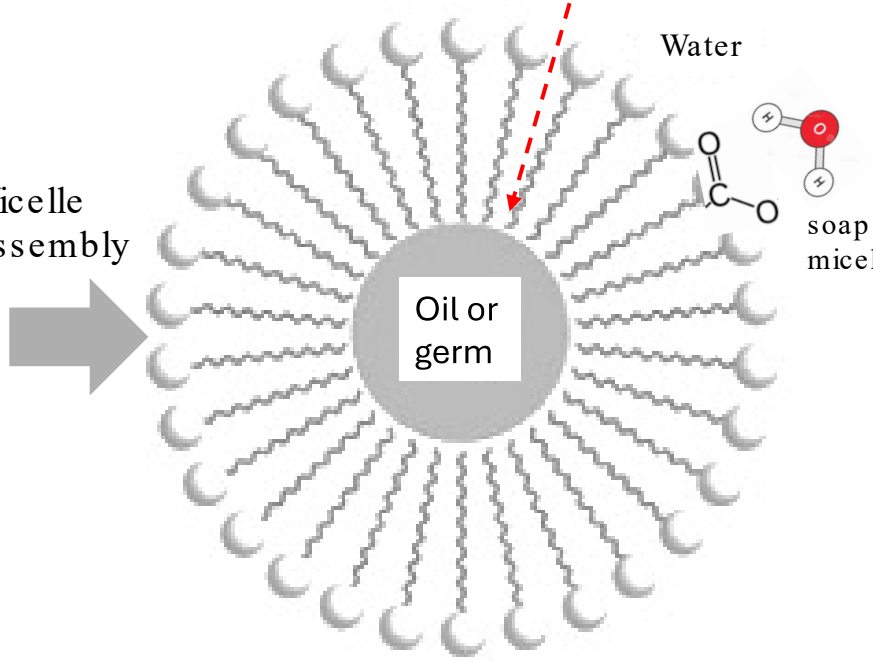
Hydrophilic 'head'



Hydrophobic 'tail'

Hydrophobic tails orient towards oil/germ, solvating it

Micelle Assembly




Water

soap carboxylate solvates micelle in water

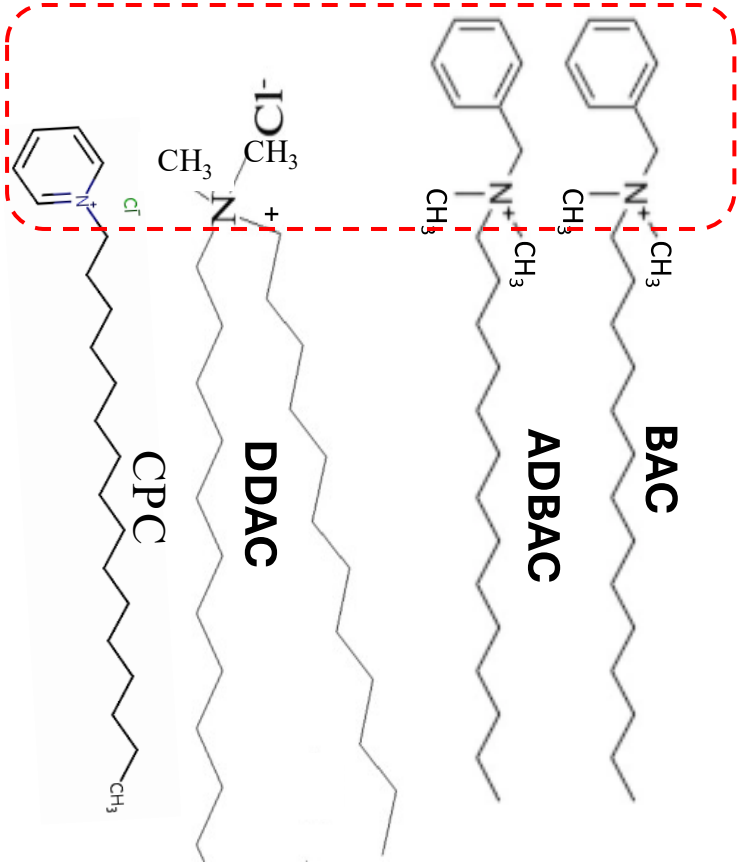
J Exp Med. 1941

ACTION OF SYNTHETIC DETERGENTS ON THE METABOLISM OF BACTERIA

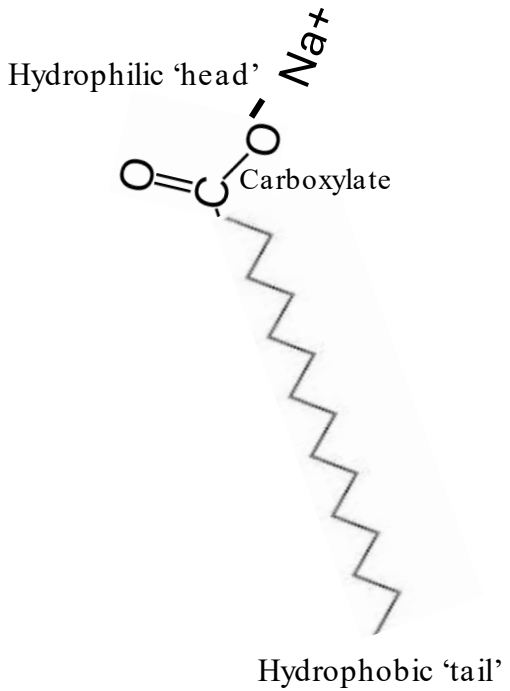
Z Baker [1], R W Harrison, B F Miller

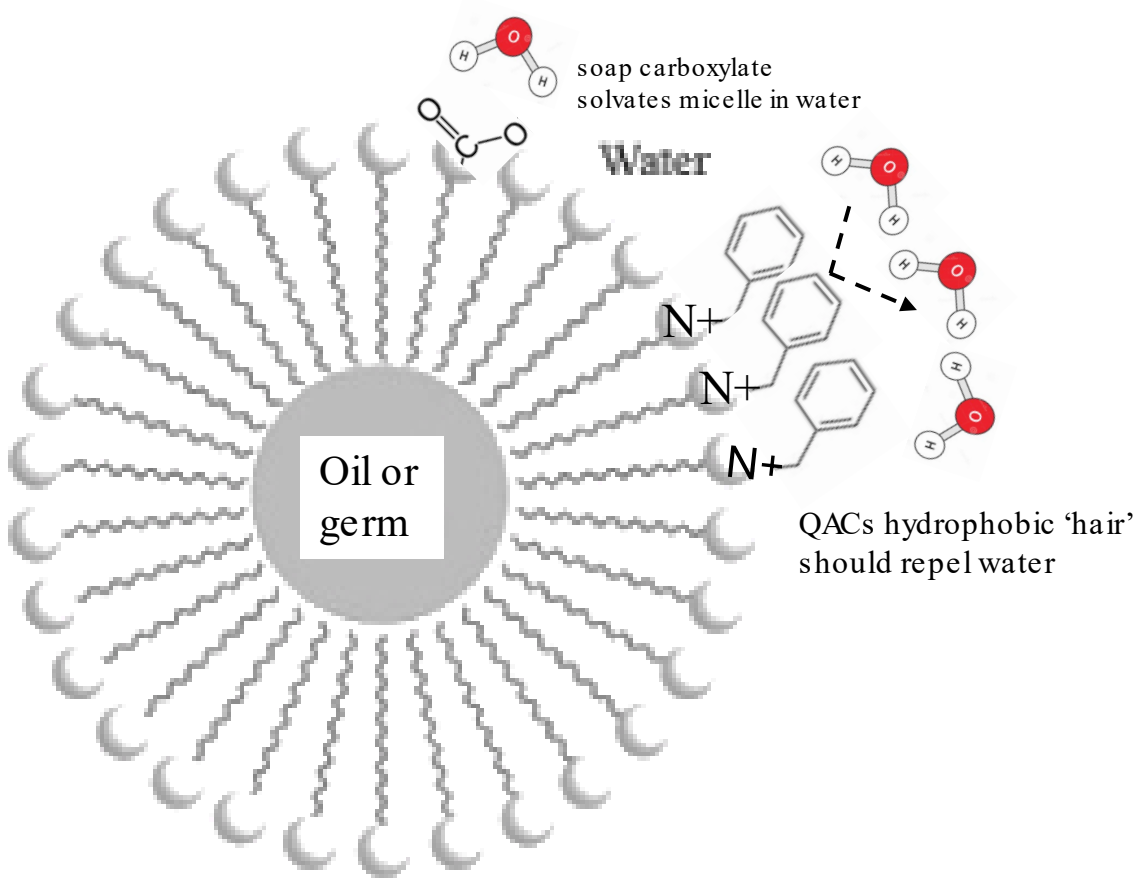
For the chemical nerds like me: QACs are not 'soaplike' --they contain hydrophobic 'hair' on top of the Ammonium (+) hydrophilic head, this hair should repel water, not attract it.

QACs:
hydrophobic 'hair'




SOAP:




So if QACs are not killing microbes like soap, what is their true anti-microbial mechanism?

Some Possible reasons QACs might Not be as safe as Previously presumed:

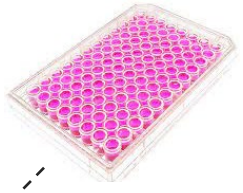
# Discovery that QACs are dose-dependent mitochondrial toxins:

We screened 1600 active ingredients in household products and drugs for their impact on mitochondrial functions

Household products

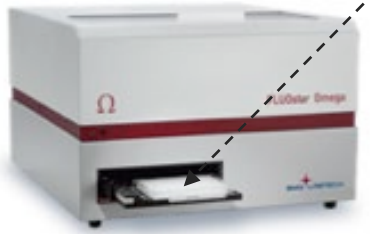
FDA-approved drugs used in people

Multi-well format



Mitochondrially-Measured Parameters

BMG LabTech Plate Reader



Mito-**Oxidative stress**

Mito-**$O_2$ consumption**

Mito-**ATP synthesis**

Mito-**Membrane Potential**

Sunil Sahdeo

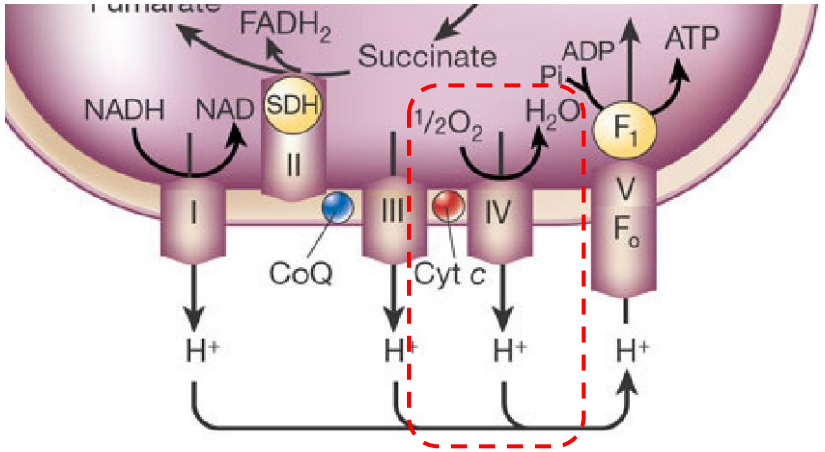
Sandipan Datta

Anabela Rolo

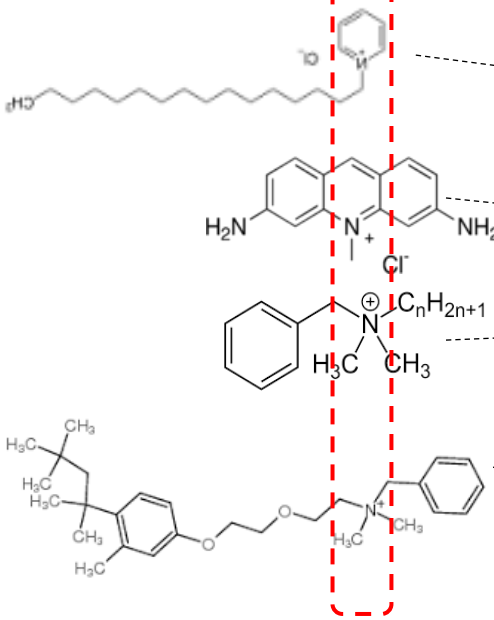
Which compounds make mitochondria sick?

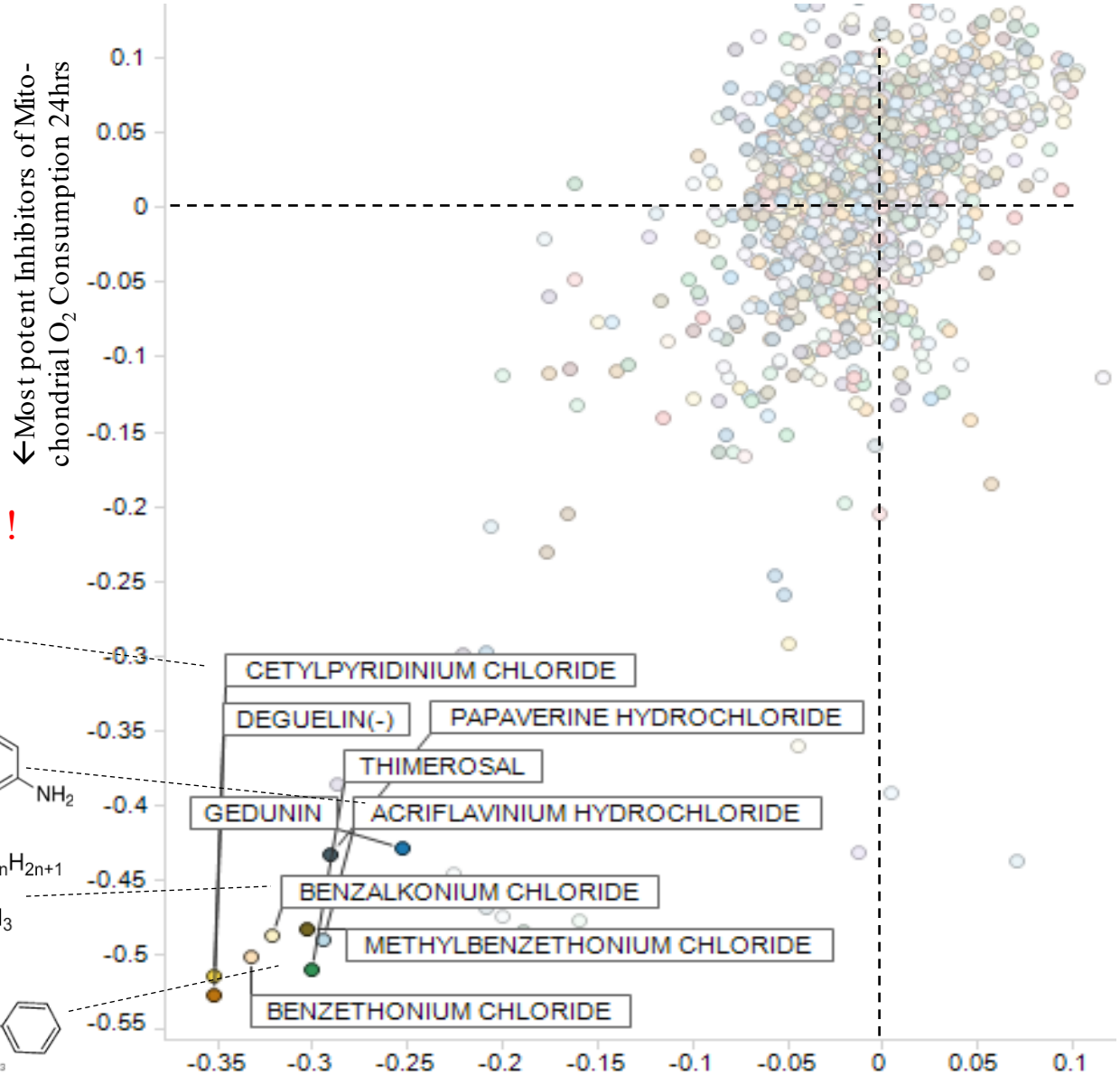
Quaternary Ammonium Compounds (QACs) inhibit mitochondrial $O_2$ consumption the *most* of 1600 household products and drugs

## Assay of mitochondrial Oxygen Consumption




QACs!



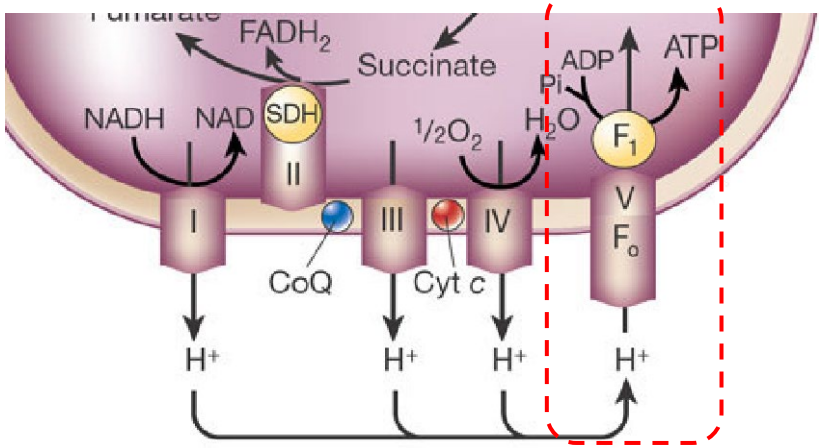



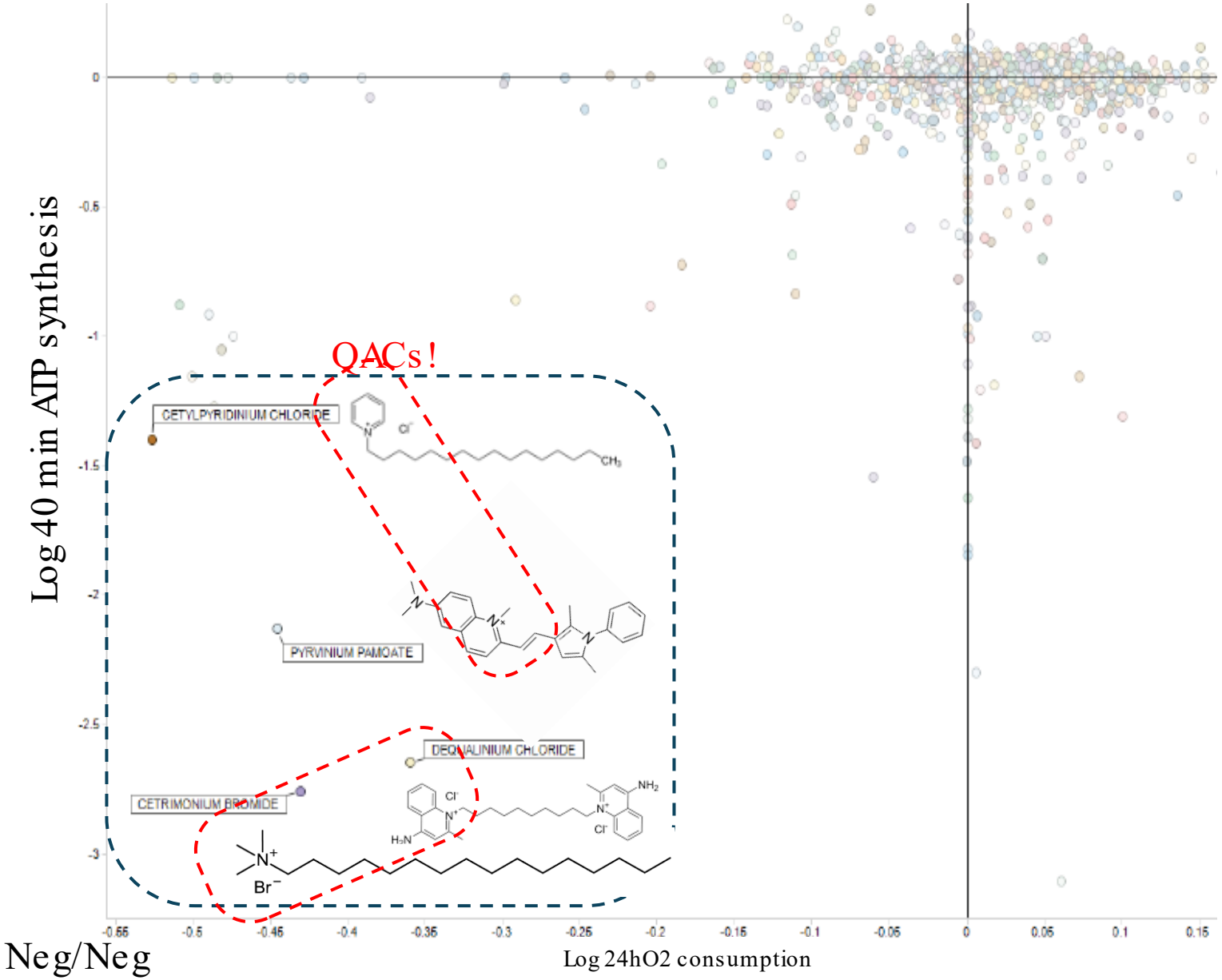

Sunil Sahdeo

# Quaternary Ammonium Compounds (QACs) decrease Mitochondrial ATP synthesis Most of ~1600 household products

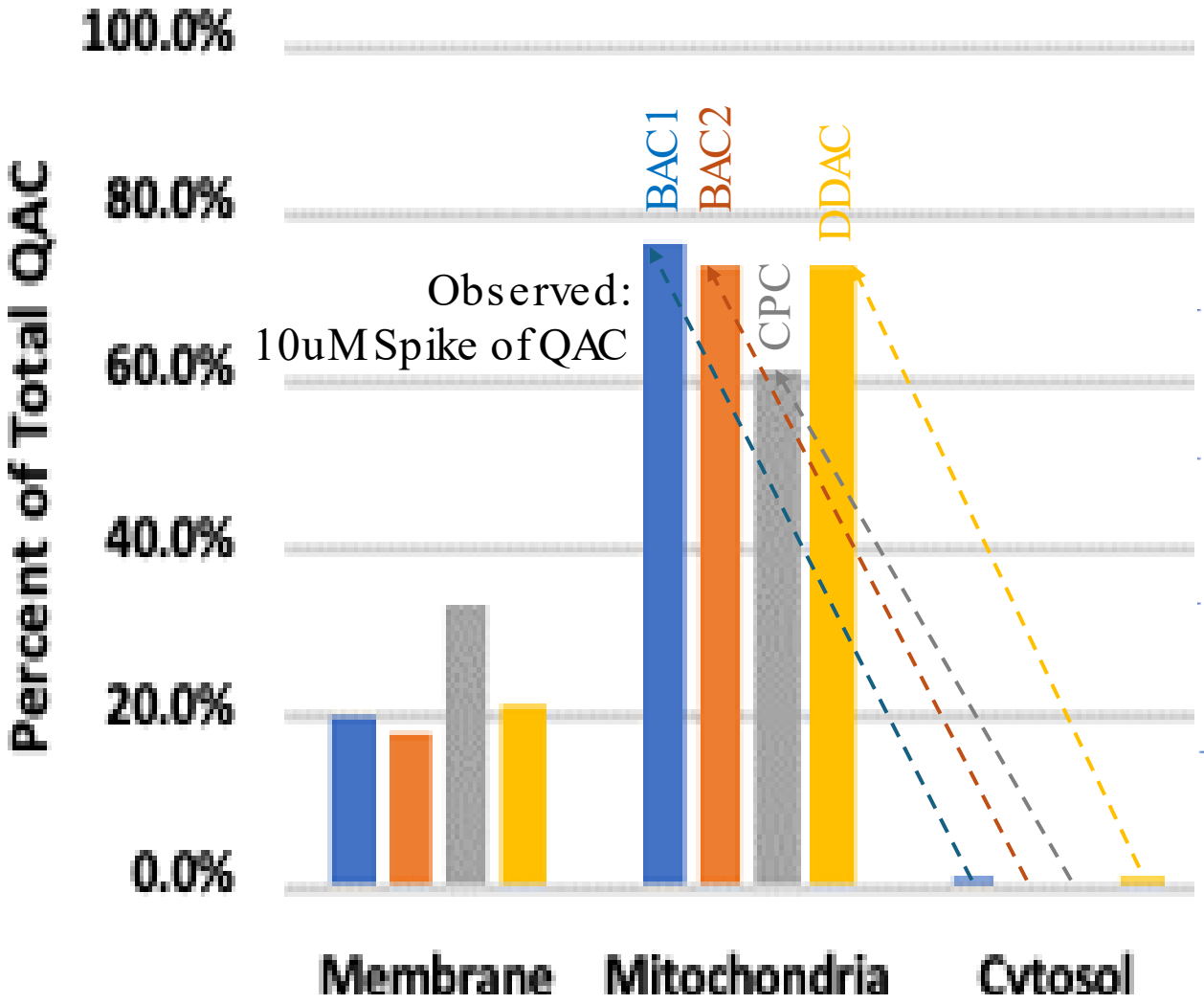
Sandipan Datta

Assay of mitochondrial ATP synthesis



# QACs are concentrated about ~100X in Mitochondria vs. Cytosol and media in cells in vitro




Total BAC 1

Mitochondria / Cytosol = 81.54

Total BAC 2

Mitochondria / Cytosol = 159.83

CPC

Mitochondria / Cytosol = 93.59

DDAC

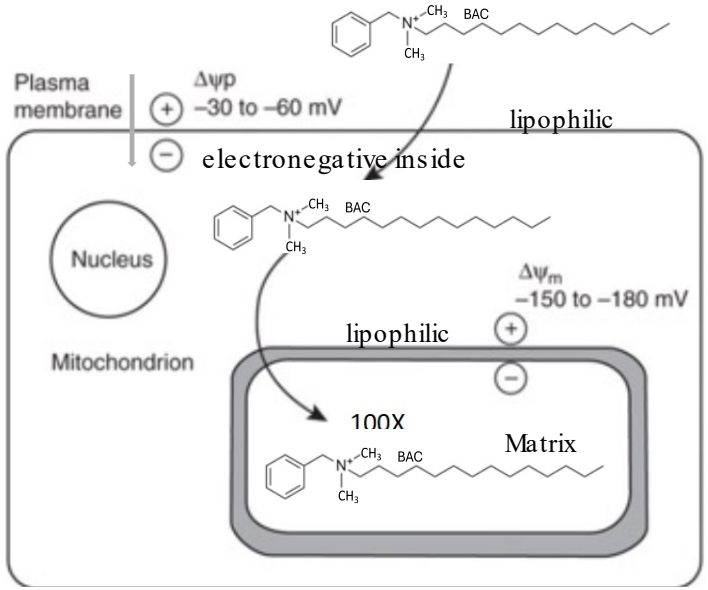
Mitochondria / Cytosol = 87.76

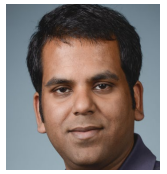
Mean= 106:1

QAC mito:cyto

## Why are QACs taken up by Mitochondria?

Mitochondria are an electronegative compartment that loves to take up lipophilic cations (+)

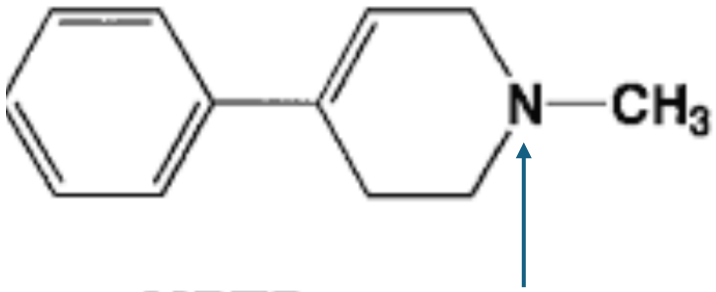




Sandipan Datta


Clayton Bioszies


Oliver Fiehn

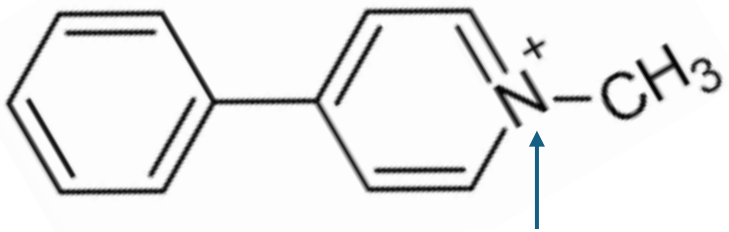
# Other Famous Toxins that are QACs: MPP+ and Paraquat




MPTP

Non-QAC,

less toxic



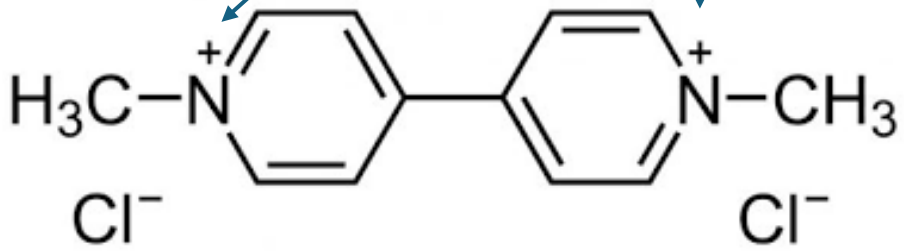

MPP+

QAC (Toxic)

Neurotoxic,

Parkinsonism

William Langston
1980s




ParaQuat—'Para-substituted QACs

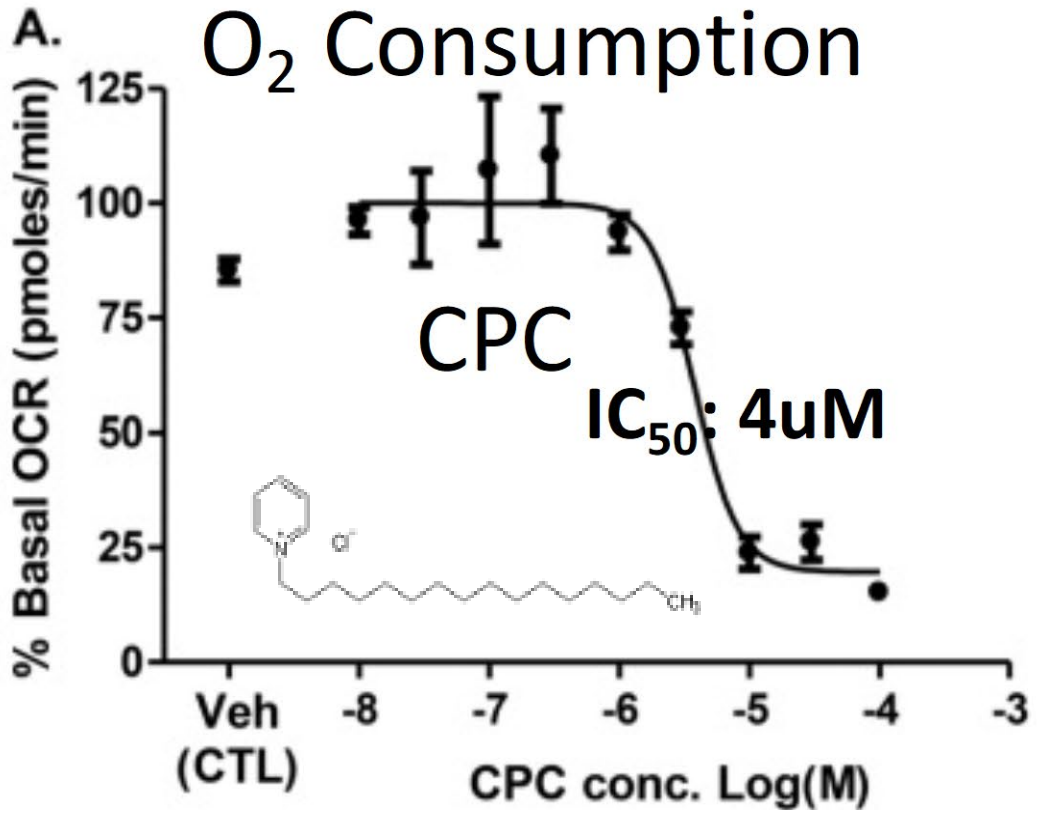
Pneomotoxic,

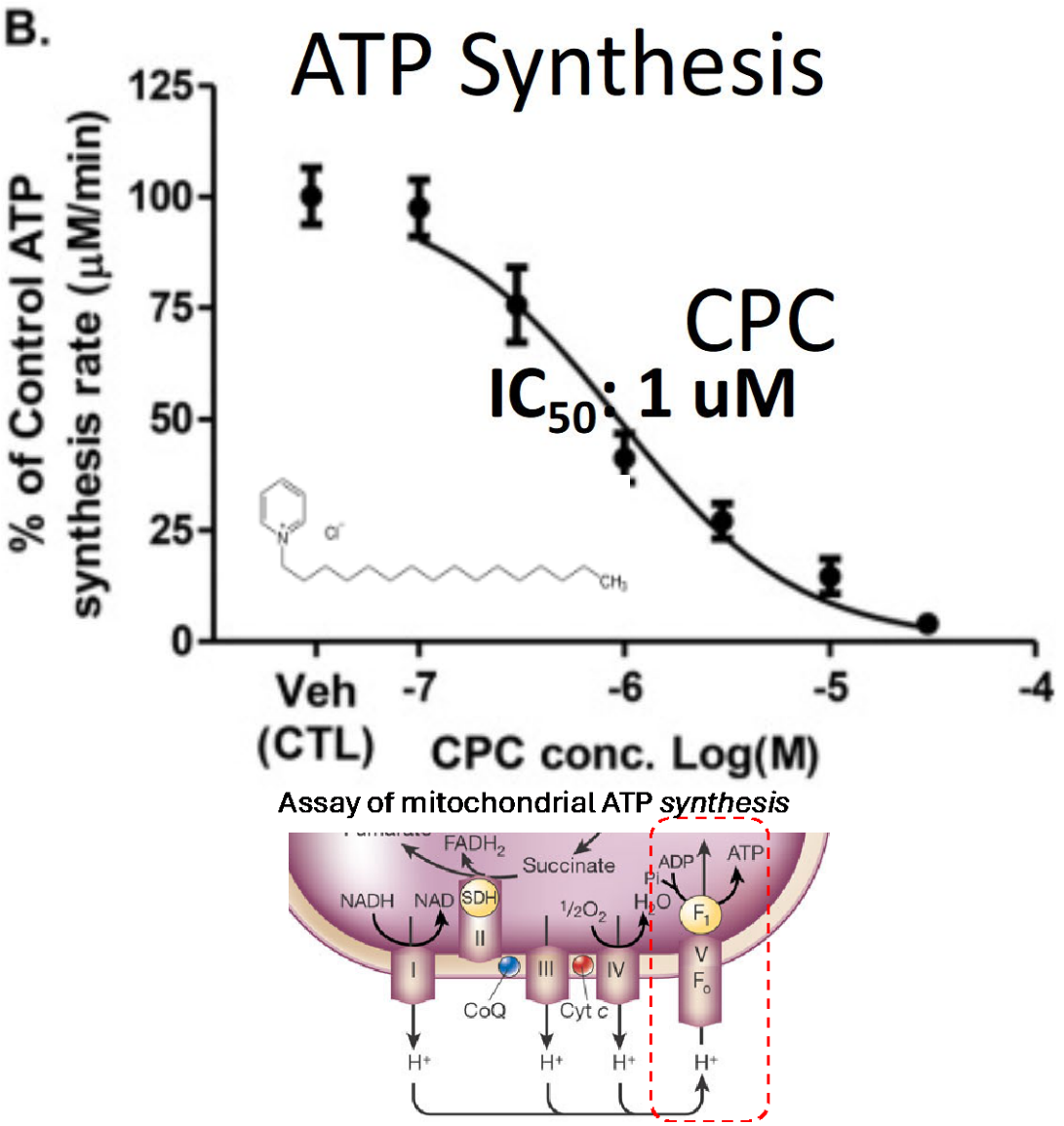
Concentrated ~3-10Xin human and animal lung vs. blood.

Respiratory Distress Syndrome

# QACs are Dose-dependent Mitochondrial Inhibitors

# QAC CPC Dose-dependently inhibits Mitochondrial $O_2$ Consumption & ATP Synthesis in the low micromolar range



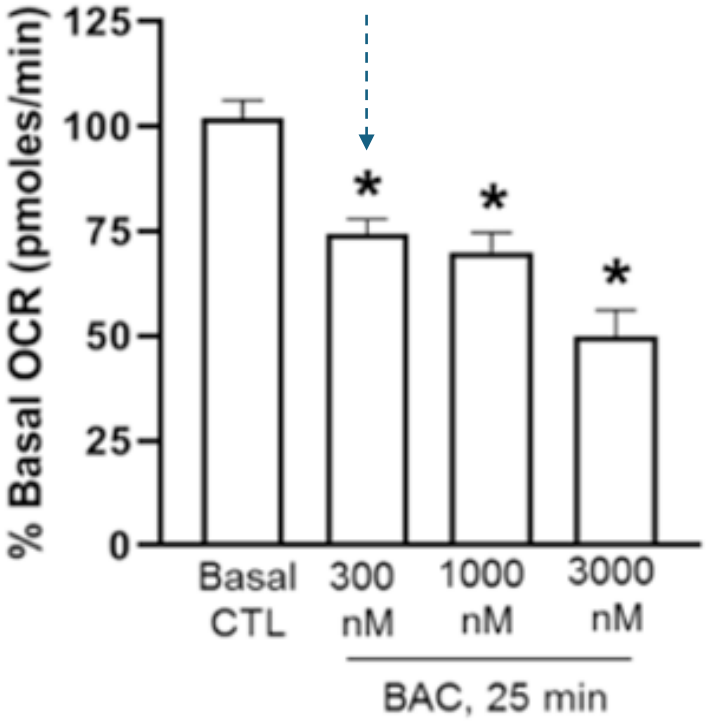

Sandipan Datta

The Eye Drop Preservative Benzalkonium Chloride Potently Induces Mitochondrial Dysfunction and Preferentially Affects LHON Mutant Cells. Datta S, Baudouin C, Brignole-Baudouin F, Denoyer A, Cortopassi GA. Invest Ophthalmol Vis Sci. 2017a Apr 1;58(4):2406-2412. doi: 10.1167/iovs.16-20903..

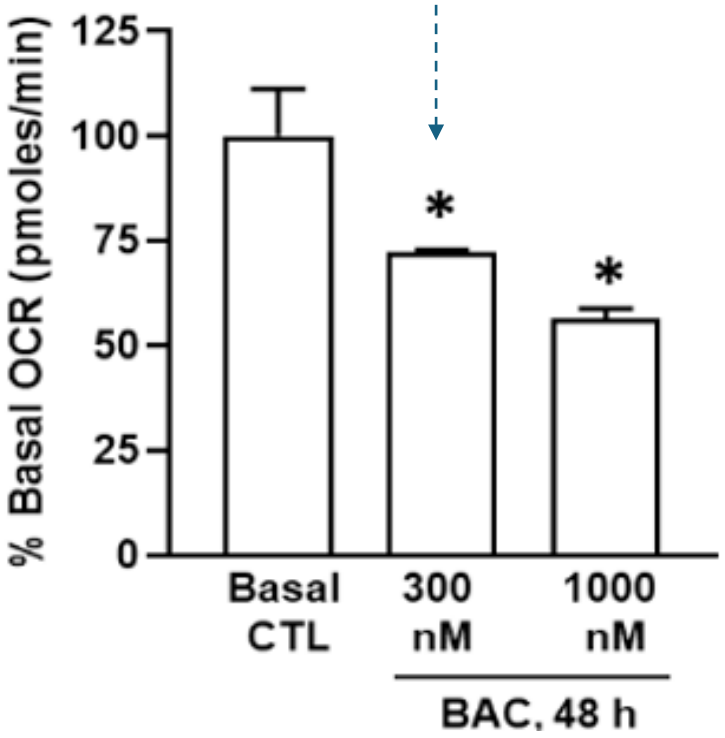
In Vitro Evaluation of Mitochondrial Function and Estrogen Signaling in Cell Lines Exposed to the Antiseptic Cetylpyridinium Chloride. Datta S, He G..Denison MS, Cortopassi G. Environ Health Perspect. 2017b Aug 22;125(8):087015. doi: 10.1289/EHP1404

Primary Cells are 3-10 fold more sensitive to QACs than cancer cell lines—300nM is sufficient for inhibition.

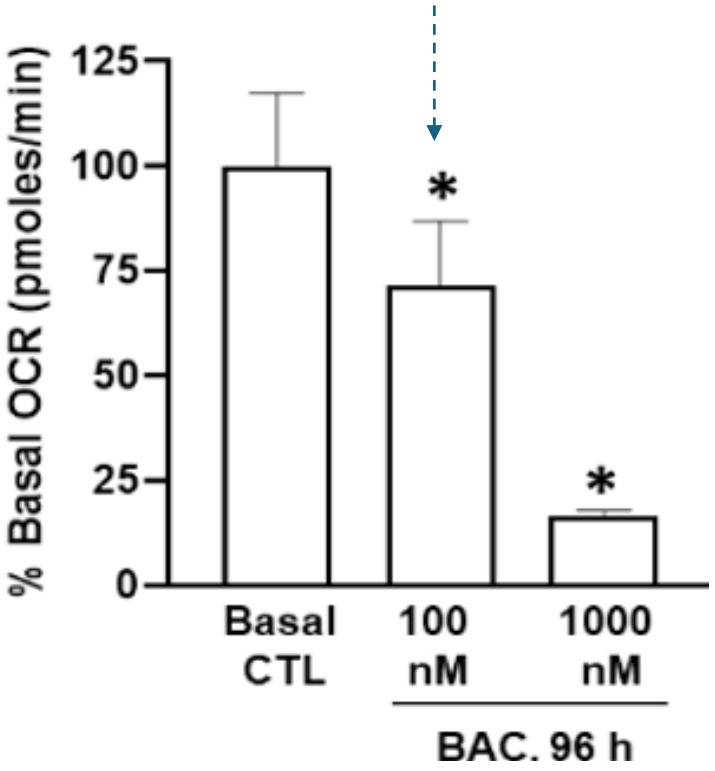

Primary Human Corneal




Primary Human Skeletal Muscle

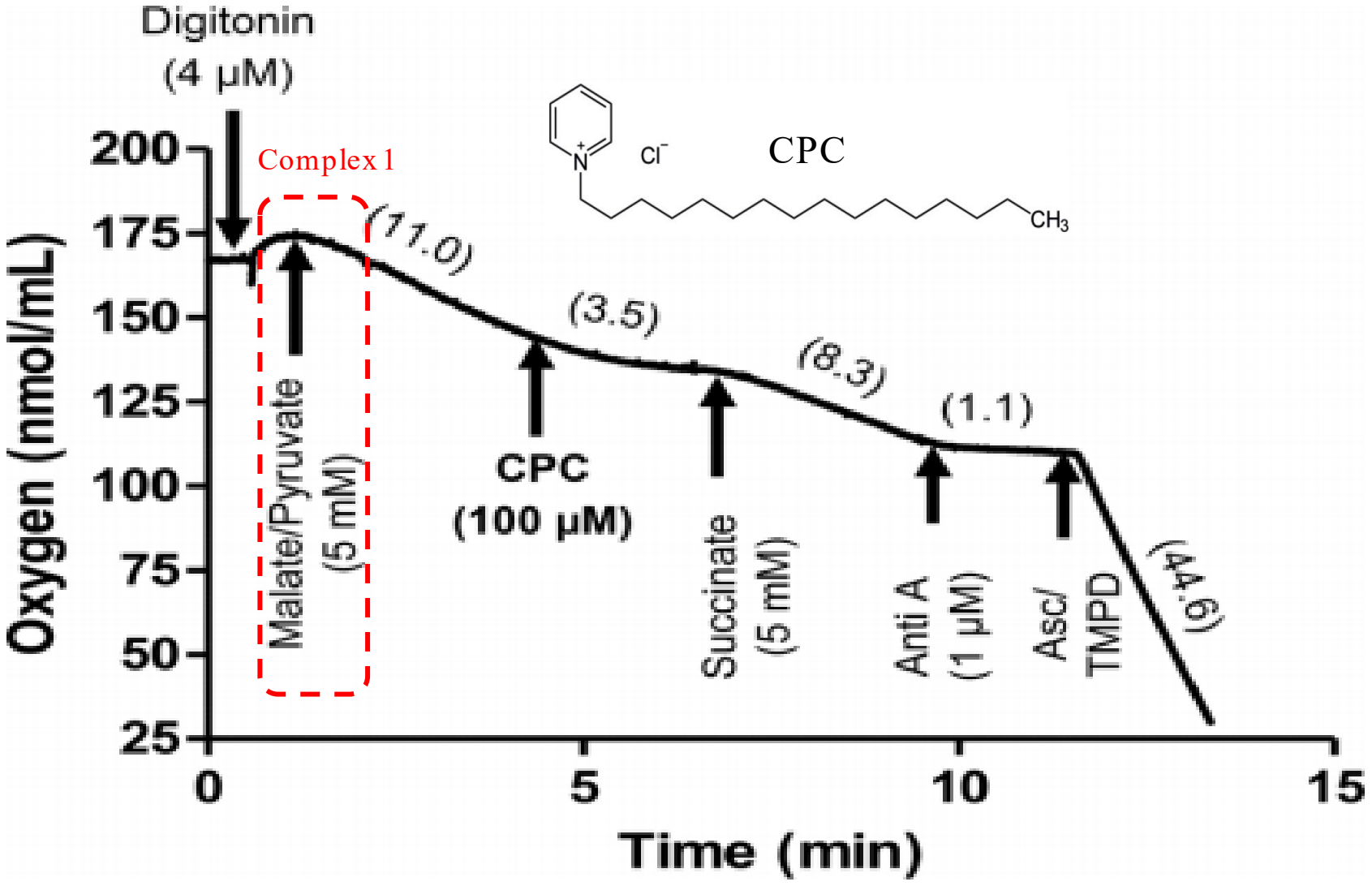



Primary Mouse living hepatocyte




Sandipan Datta

# CPC QAC Specifically Blocks Mitochondria at Mitochondrial Complex I

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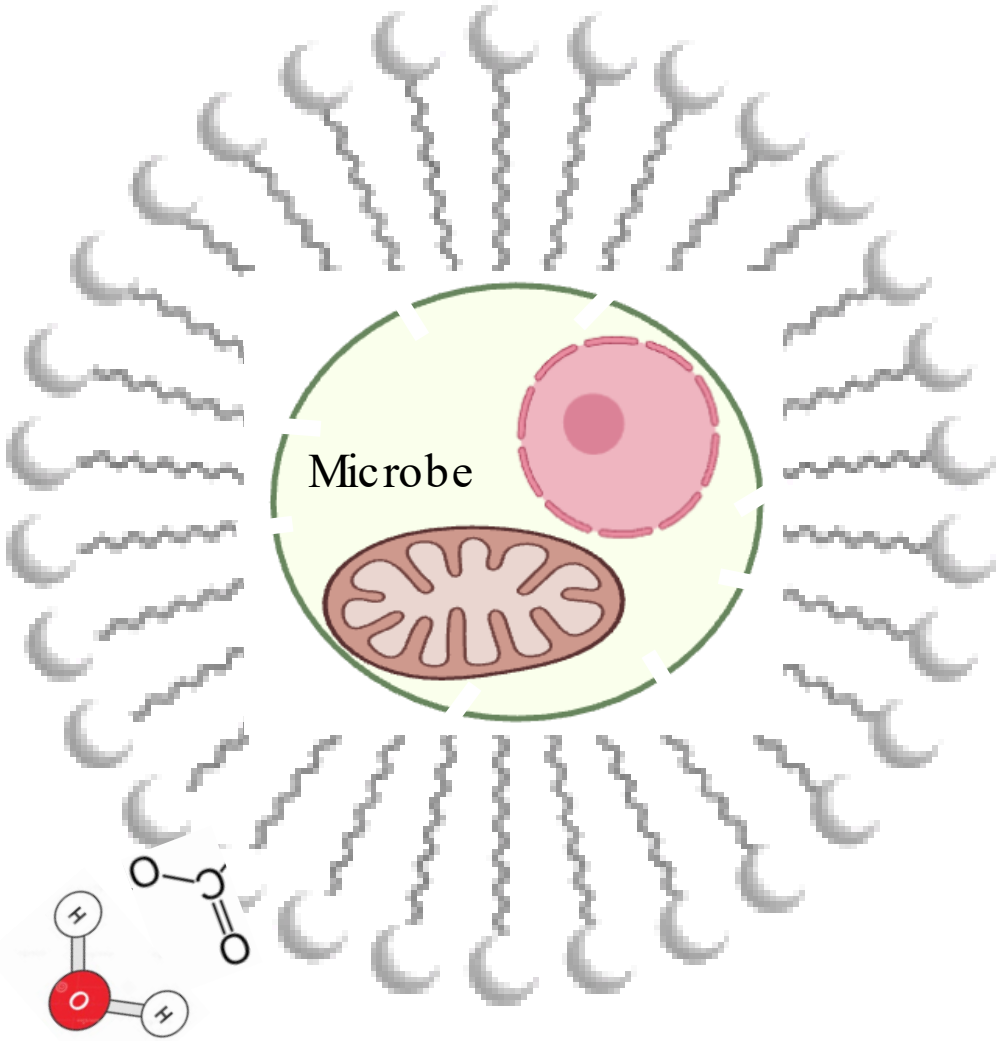
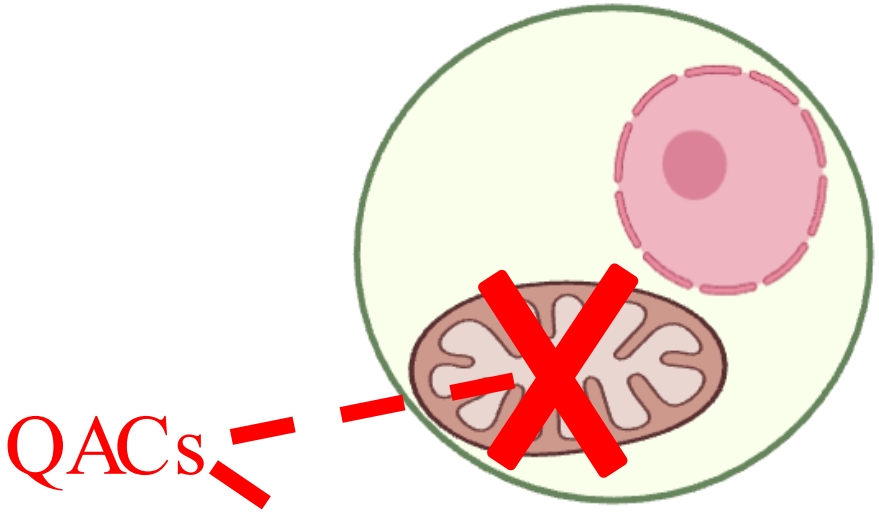
# Our hypothesis for why QACs are more potent antimicrobials than soap.

## Soap solubilizes microbial membranes to kill microbes

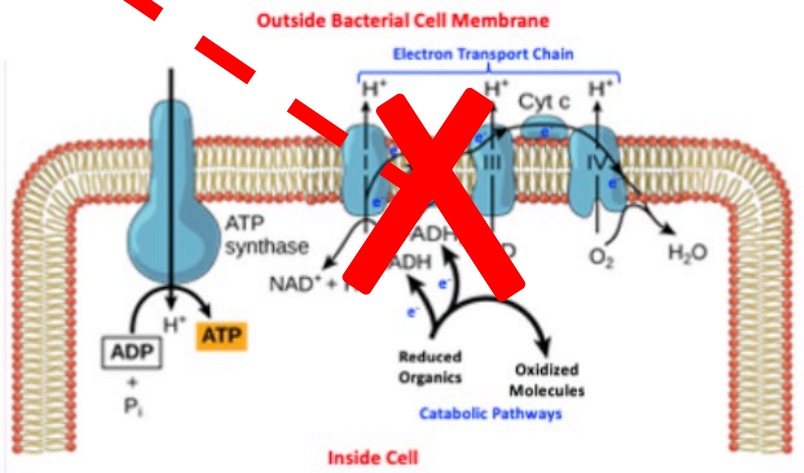



## QACs kill the electron transport chain to kill microbes



QACs

Eukaryotic microbes




Bacterial microbes

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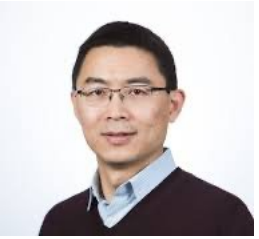
Prof. Terry Hrubec
UVA & VTCom



Libin Xu, UW
School of Pharmacy

# 2021: Virginia Tech QAC human Study: 35/43 volunteers have QACs in blood, 2-40nM

43 Students, faculty and personnel on VT campus

Bloods Taken, QACs level determined by LC MS Libin Xu lab. UW

Mitochondrial Activity of PBMCs Measured in Cortopassi Lab

Surprise! ~35/43=80% of human volunteers walking around VT campus had total PBMC QAC level that was measurable in the 2-40nM range.

Hokie Bird

This was the first study to show that QACs penetrated inside the human body.

It was previously thought that QACs never entered the body.

Toxicology Reports 8 (2021) 646–656

Contents lists available at ScienceDirect

Toxicology Reports

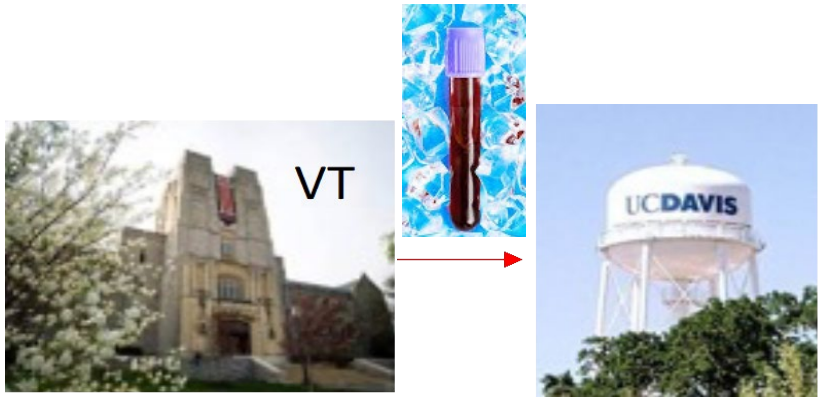
journal homepage: www.elsevier.com/locate/toxrep

## Altered toxicological endpoints in humans from common quaternary ammonium compound disinfectant exposure

Terry C. Hrubec [a,b,*], Ryan P. Seguin [c], Libin Xu [c], Gino A. Cortopassi [d], Sandipan Datta [d], Alexandra L. Hanlon [e], Alicia J. Lozano [e], Valerie A. McDonald [a], Claire A. Healy [a], Tyler C. Anderson [a], Najaha A. Musse [a], Richard T. Williams [a]

[a] Department of Biomedical Science, E. Via College of Osteopathic Medicine – Virginia, Blacksburg, VA, 24060, USA
[b] Department of Biomedical Science and Pathobiology, VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, 24061, USA
[c] Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle, WA, 98195, USA
[d] Department of Molecular Biosciences, School of Veterinary Medicine, University of California – Davis, Davis, CA, 95618, USA
[e] Center for Biostatistics and Health Data Science, Department of Statistics, College of Science, Virginia Tech, Riverside Circle, Roanoke, VA, 24016, USA

Bloods were overnighted from Virginia Tech to UC Davis for Mitochondrial analysis:

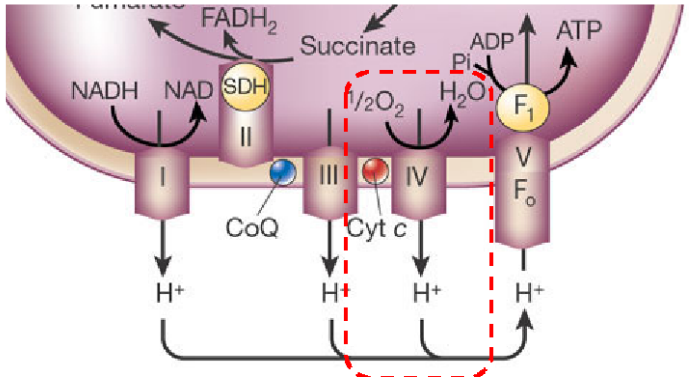








Assay of mitochondrial *Oxygen Consumption*

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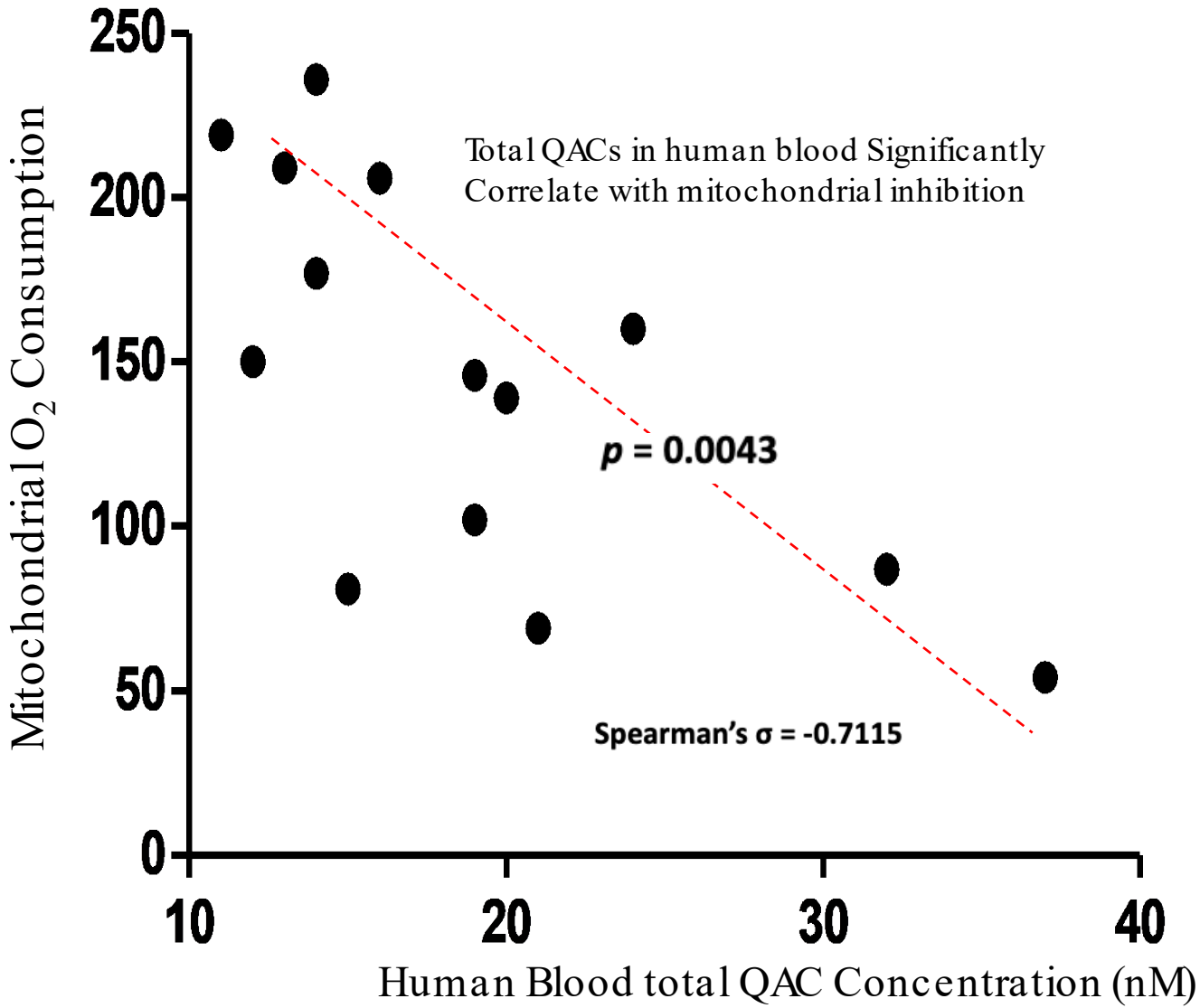
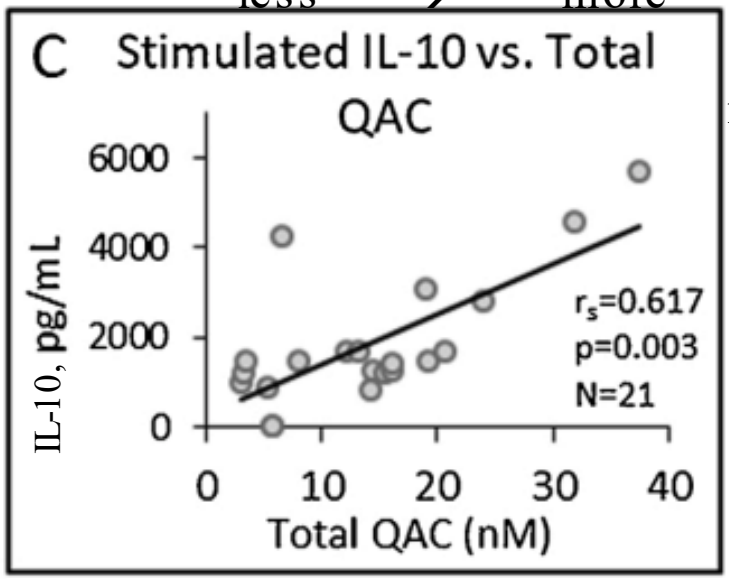
More QAC measured in human blood, less mitochondrial function



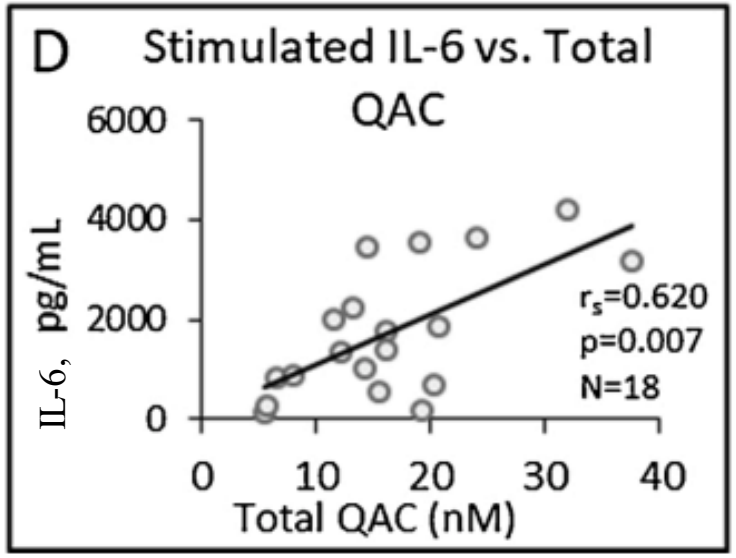
Prof. Terry Hrubec
UVA & VTCom

# Higher blood total QAC levels significantly correlate with higher blood inflammatory cytokines

less → more inflammation




More QAC in blood, more IL-10 inflammatory cytokine in blood




More QAC in blood, more IL-6 inflammatory cytokine in blood

Toxicology Reports 8 (2021) 646–656

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## Altered toxicological endpoints in humans from common quaternary ammonium compound disinfectant exposure

Terry C. Hrubec [a,b,*], Ryan P. Seguin [c], Libin Xu [c], Gino A. Cortopassi [d], Sandipan Datta [d], Alexandra L. Hanlon [e], Alicia J. Lozano [e], Valerie A. McDonald [a], Claire A. Healy [a], Tyler C. Anderson [a], Najaha A. Musse [a], Richard T. Williams [a]

[a] Department of Biomedical Science, E. Via College of Osteopathic Medicine – Virginia, Blacksburg, VA, 24060, USA
[b] Department of Biomedical Science and Pathobiology, VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, 24061, USA
[c] Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle, WA, 98195, USA
[d] Department of Molecular Biosciences, School of Veterinary Medicine, University of California – Davis, Davis, CA, 95618, USA
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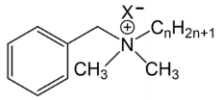
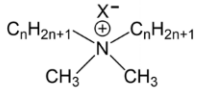
# 2022: Salamova's group shows QACs are measurable in ~50% of breast milk samples in Washington.

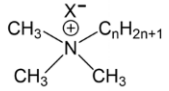
BACs



DDACs



ATMACs



Salamova study, 50% of milk samples were QAC positive, the lowest quartile of All QACs was ~3nM, and sample with highest sum of all QACs was ~24nM, range 3-24nM.

In the Hrubec study, 35/43=80% of subjects bloods were QAC positive, and the range of all QACs was 2-40nM.

Thus in the two human QAC studies that have been done, 50-80% of samples are QAC-positive, and the overlapping total QAC range in blood and breastmilk is 3-24nM.

Article | Published: 18 April 2022

## The first detection of quaternary ammonium compounds in breast milk: Implications for early-life exposure

Guomao Zheng, Erika Schreder, Sheela Sathyanarayana & Amina Salamova

*Journal of Exposure Science & Environmental Epidemiology* **32**, 682–688 (2022) | Cite this article

Toxicology Reports 8 (2021) 646–656

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## Altered toxicological endpoints in humans from common quaternary ammonium compound disinfectant exposure

Terry C. Hrubec [a,b,*], Ryan P. Seguin [c], Libin Xu [c], Gino A. Cortopassi [d], Sandipan Datta [d], Alexandra L. Hanlon [e], Alicia J. Lozano [e], Valerie A. McDonald [a], Claire A. Healy [a], Tyler C. Anderson [a], Najaha A. Musse [a], Richard T. Williams [a]

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# BAC is concentrated 3.5 to 24-fold in lung versus blood in rats (Xue et al, 2004a,b).

**IV administration**

1 minute after BAC IV administration, lung concentrates BAC 3.5X vs. blood

Table 4
BAC levels in blood and tissues
rats[a]

| BAC 15 mg/kg | | | |
|---|---|---|---|
| | Sampling time (min) | BAC (μg/ | |
| Serum | 1 | 119nM | 44.4 |
| | | ~3.5X | A |
| Brain | 1 | | 4.8 |
| Lung | 1 | 420nM | 154.8 |

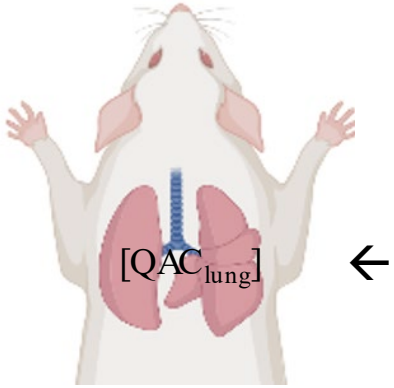
30 minutes after BAC IV administration, lung concentrates BAC ~6.8X vs. blood.

6.2nM
~6.8X

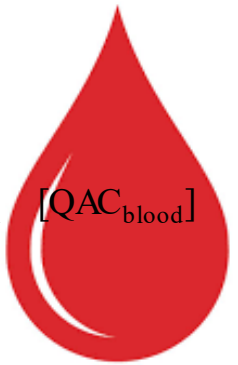
Lungs concentrate BAC 7-24X vs. blood after oral administration.

| Route | Time | N | Concentrations of BAC (μg/g) | |
|---|---|---|---|---|
| | | | Blood | Lung |
| PO | 1 h | 6 | 0.06 ± 0.02 | 0.84 ± 0.32 |
| | | | 14X-> | |
| | 2 h | 6 | 0.08 ± 0.02 | 1.94 ± 0.85 |
| | | | 24X-> | |
| | 4 h | 6 | 0.06 ± 0.01 | 0.50 ± 0.12 |
| | | | 8X-> | |
| | 8 h | 6 | 0.09 ± 0.05 | 0.39 ± 0.14 |
| | | | 4X-> | |
| | 24 h | 6 | 0.34 ± 0.13 | 2.75 ± 1.17 |
| | | | 7X-> | |

Thus, blood concentrations underestimate lung BAC concentrations by 3.5 to 24-fold in rats.




←3.5-24X higher than→



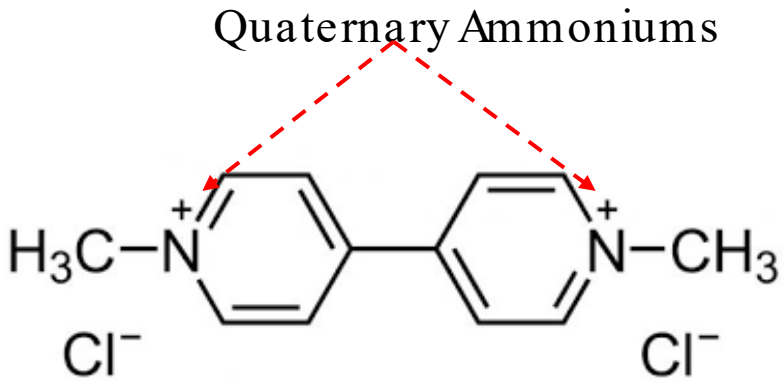

The average of 3.5X and 24X is 15.

15-fold concentration of the blood QAC concentrations to observed in the VT study translates to predicted range of 30-600nM QAC in lung.

Kinetic characteristics and toxic effects of benzalkonium chloride following intravascular and oral administration in rats. Xue Y, Hieda Y, Kimura K, Takayama K, Fujihara J, Tsujino Y. J Chromatogr B Analyt Technol Biomed Life Sci. 2004 Nov 5;811(1):53-8. doi: 10.1016

Distribution and disposition of benzalkonium chloride following various routes of administration in rats. Xue Y, Hieda Y, Saito Y, Nomura T, Fujihara J, Takayama K, Kimura K, Takeshita H. Toxicol Lett. 2004 Mar 14;148(1-2):113-23. doi: 10.1016/ j.toxlet.2003. 12.068.

There is evidence for other QACs being concentrated in human lung vs. blood: example Paraquat.




Para Quat (i.e. 2 Quaternary Ammoniums in Para position) is a QAC.

Paraquat is also toxic to lung[1,2,3].

Lung paraquat concentration is 10–20 times greater than blood in humans...refs, 1,2,3

1.Mechanism of paraquat toxicity in lung and its relevance to treatment. Smith LL.Human Toxicology 1987 Jan;6(1):31-6. doi: 10.1177/096032718700600105. PMID: 3546084.

2. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML, Carvalho F. Crit Rev Toxicol. 2008;38(1):13-71. doi: 10.1080/ 10408440701669959.PMID: 18161502

3. > Indian J Crit Care Med. 2019 Dec;23(Suppl 4):S263-S266.
doi: 10.5005/jp-journals-10071-23306.

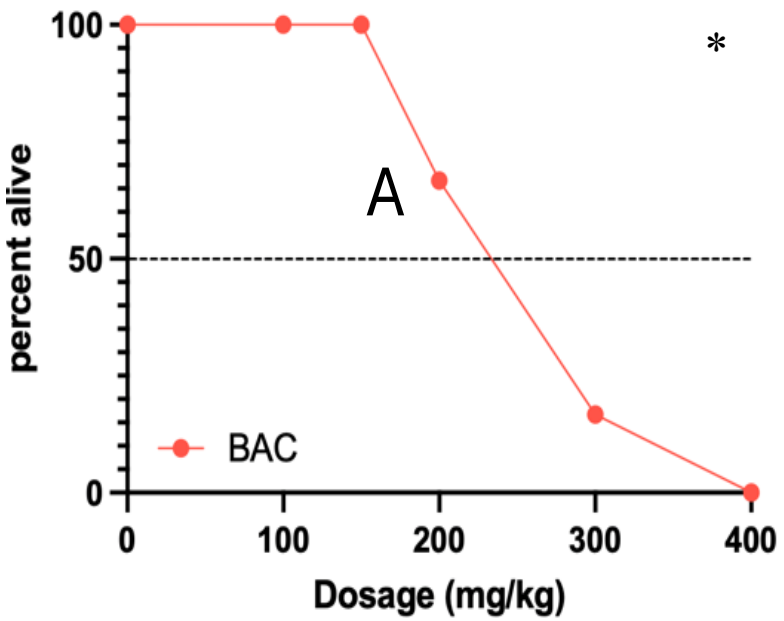
**Paraquat: The Poison Potion**

Cynthia A Sukumar [1], Vishal Shanbhag [2], Ananthakrishna B Shastry [1]

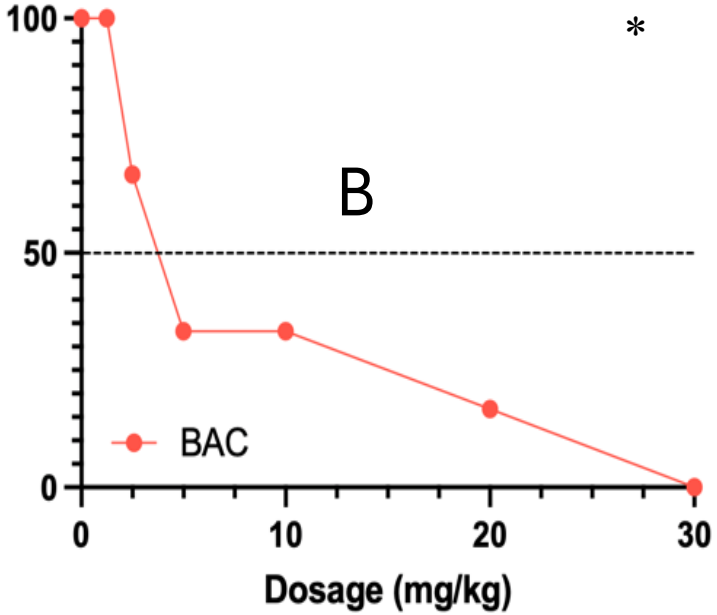
# 5. QACs are 100-fold more toxic when inhaled vs. eaten, in mice.

LD50s of QAC Exposure by different Routes of Administration

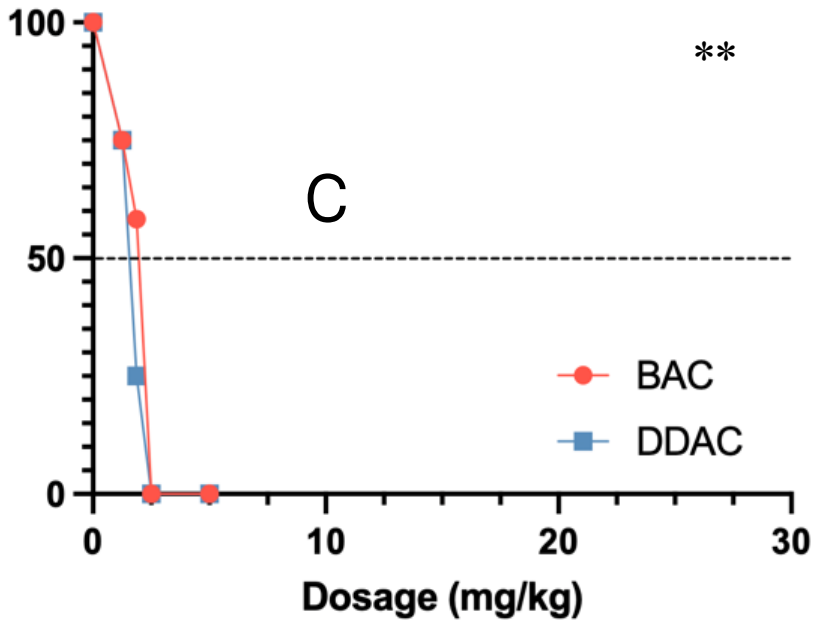
Oral LD50: 241 mpk

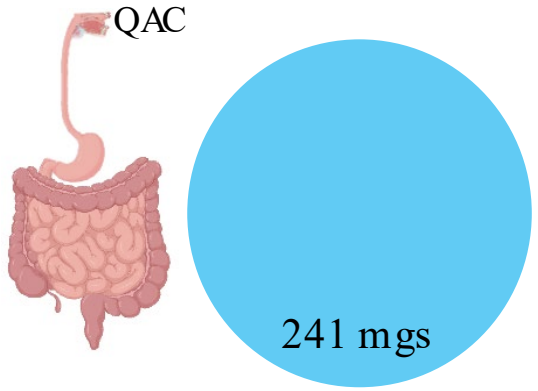


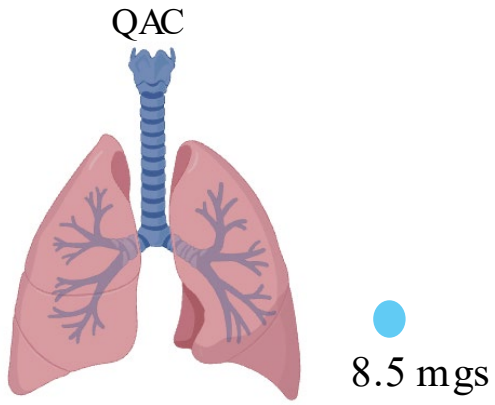

Intratracheal LD50: 8.5 mpk

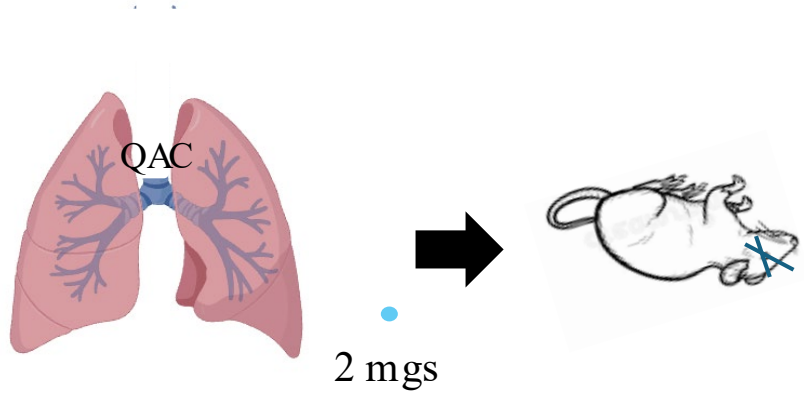



Oropharyngeal Aspiration (OPA) LD50: ~2 mpk



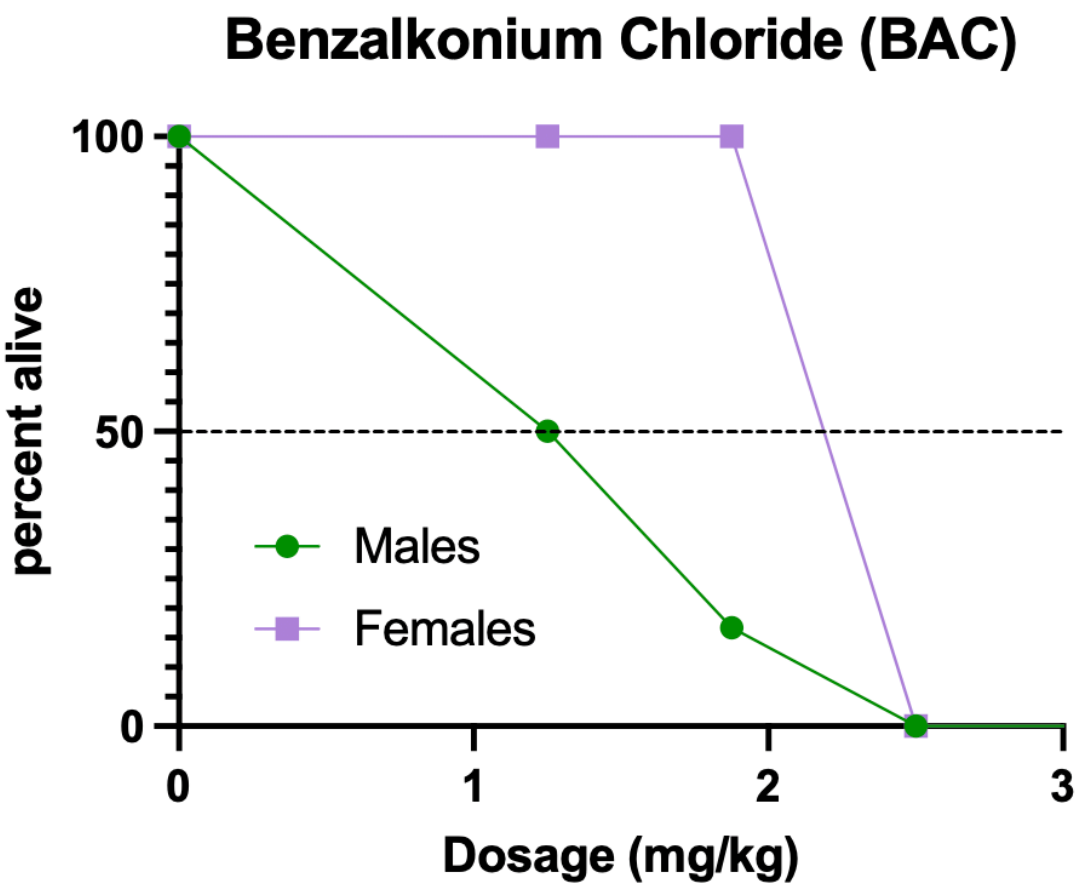



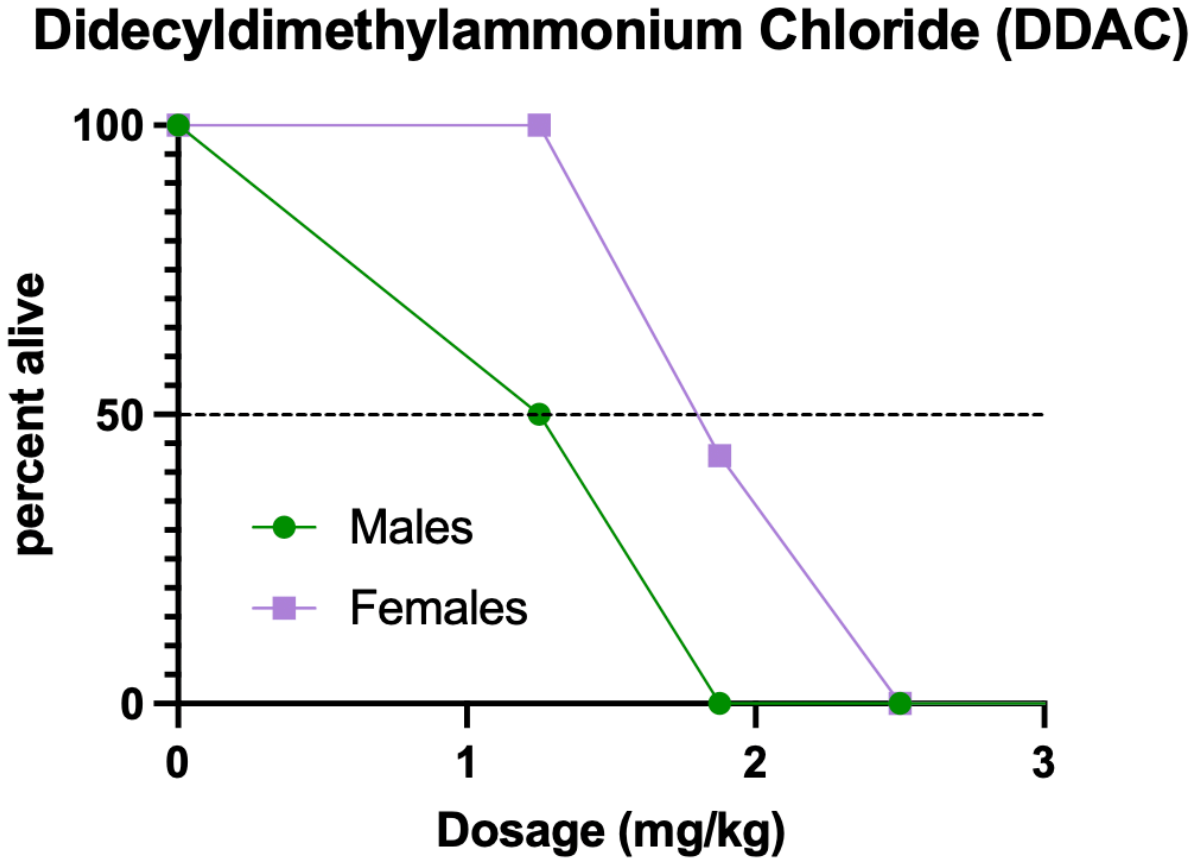



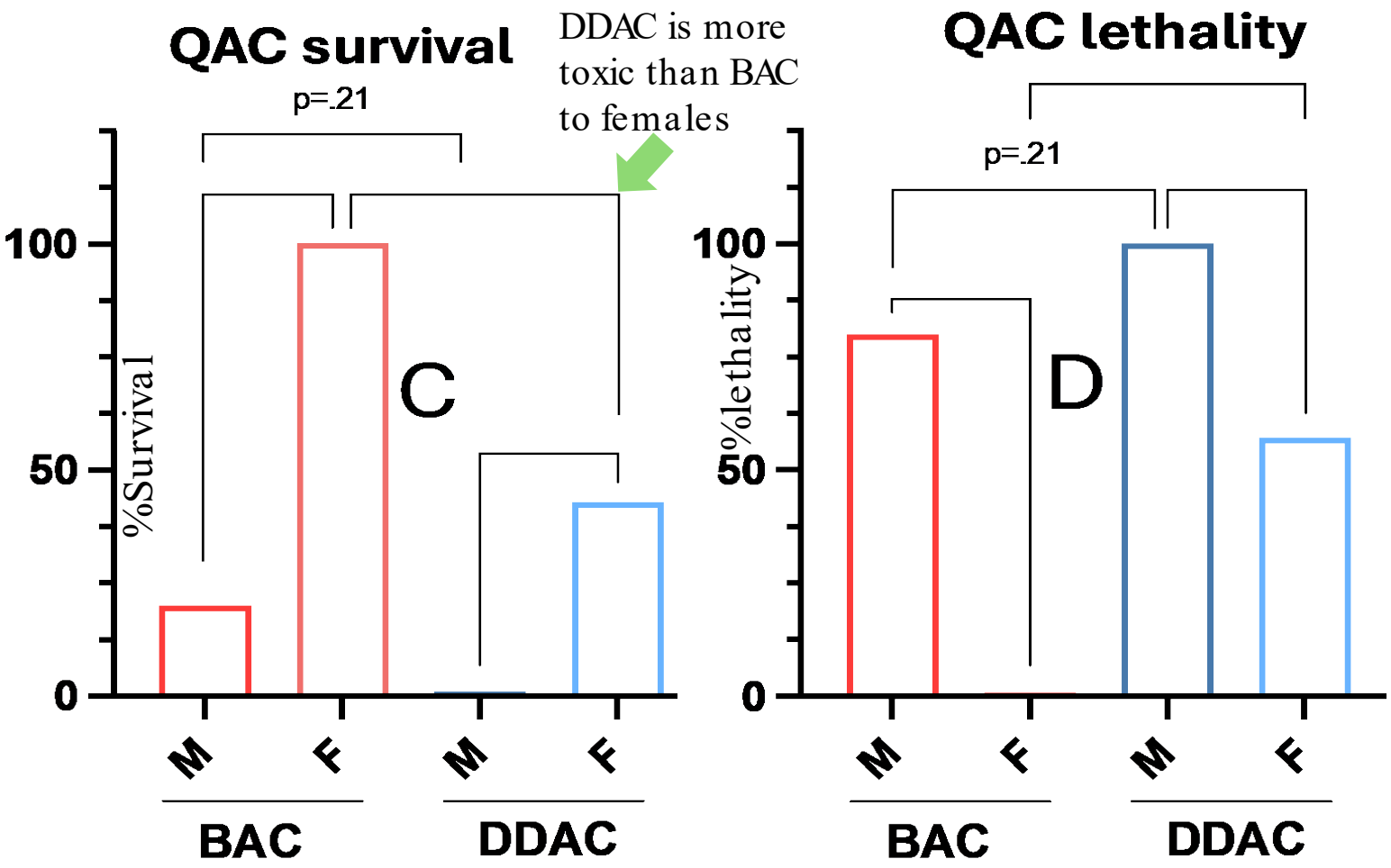

*Acute toxicity of benzalkonium chloride in Balb/c mice following intratracheal instillation and oral administration. Lee H, Park K.Environ Anal Health Toxicol. 2019 Sep;34(3):e2019009. doi: 10.5620/ eaht. e2019009. Epub 2019 Sep 19.PMID: 31771318. ** Adcock et al., in preparation.

6. QACs are more toxic to male than female mice.

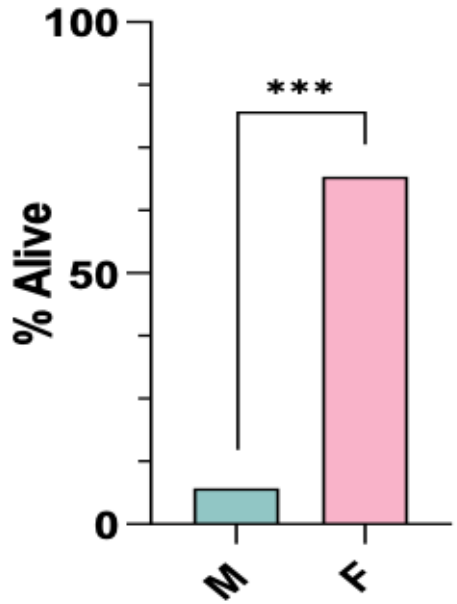


At a single OPA dose of 1.9mpk BAC and DDAC, significantly more Females survive than Males




(BAC + DDAC) survival events @1.9mpk are 10-fold more in females than males




Also, DDAC is more toxic than BAC to females (significant in females, trending in males)

# Males appear to have more lung damage than Females at the same QAC dose, 24hr

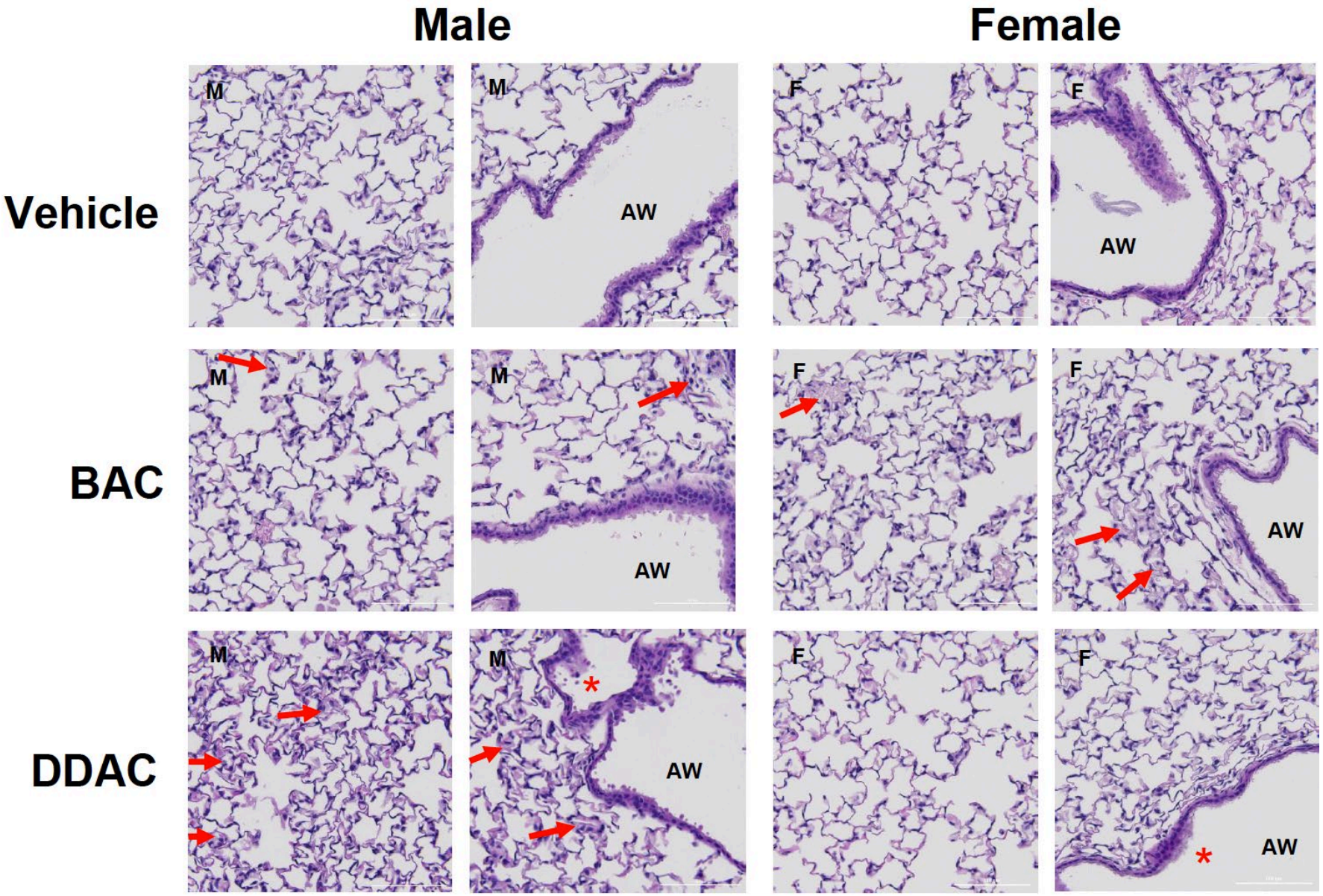
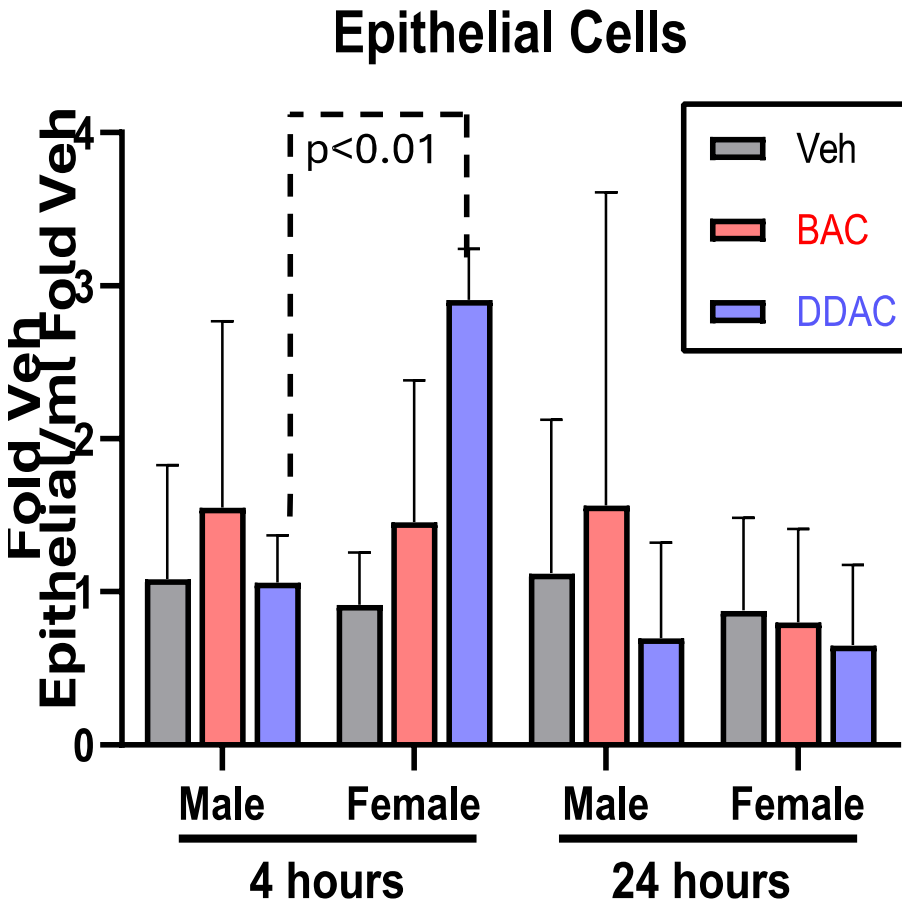
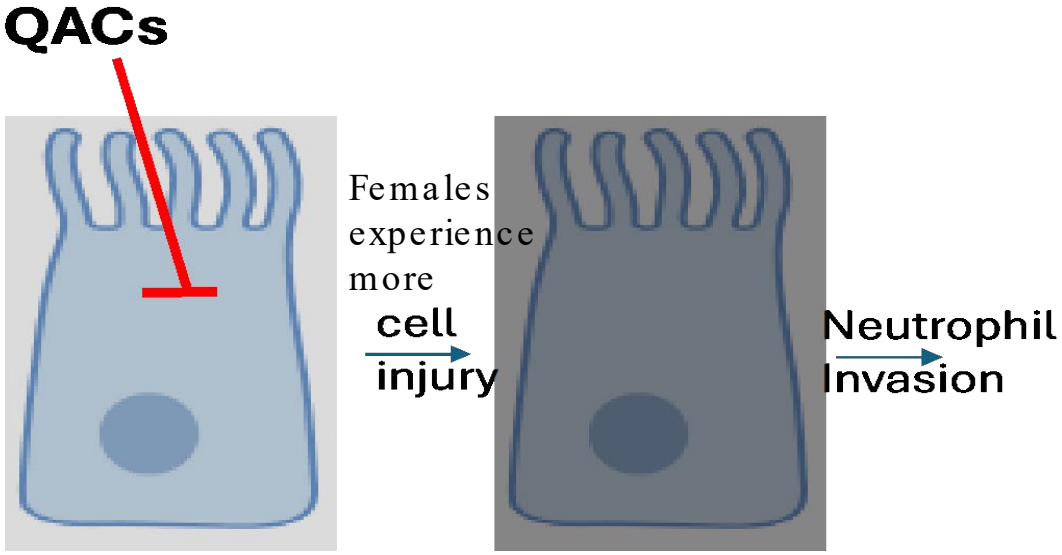



Figure legend: QAC or Vehicle control exposed male or female mouse lungs 24 hrs post OPA exposure. Alveoli (left panels) and bronchiolar AW (right panels) for each sex and exposure group are shown. Animals were exposed to vehicle control , BAC or DDAC at ¼ the LD50 ~0.45 mpk. Arrowheads point to areas of edema and cellular infiltration in the alveoli that are especially prominent in the BAC/DDAC exposed groups and bronchiolar epithelial disruption in the DDAC exposed animals *. It can also be appreciated that male mice in the DDAC exposed groups have more edema than females at this timepoint and exposure.

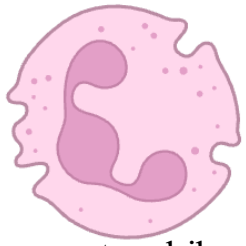
7. Differences in the Female response to QACs that may explain why females are (relatively) protected from QACs.

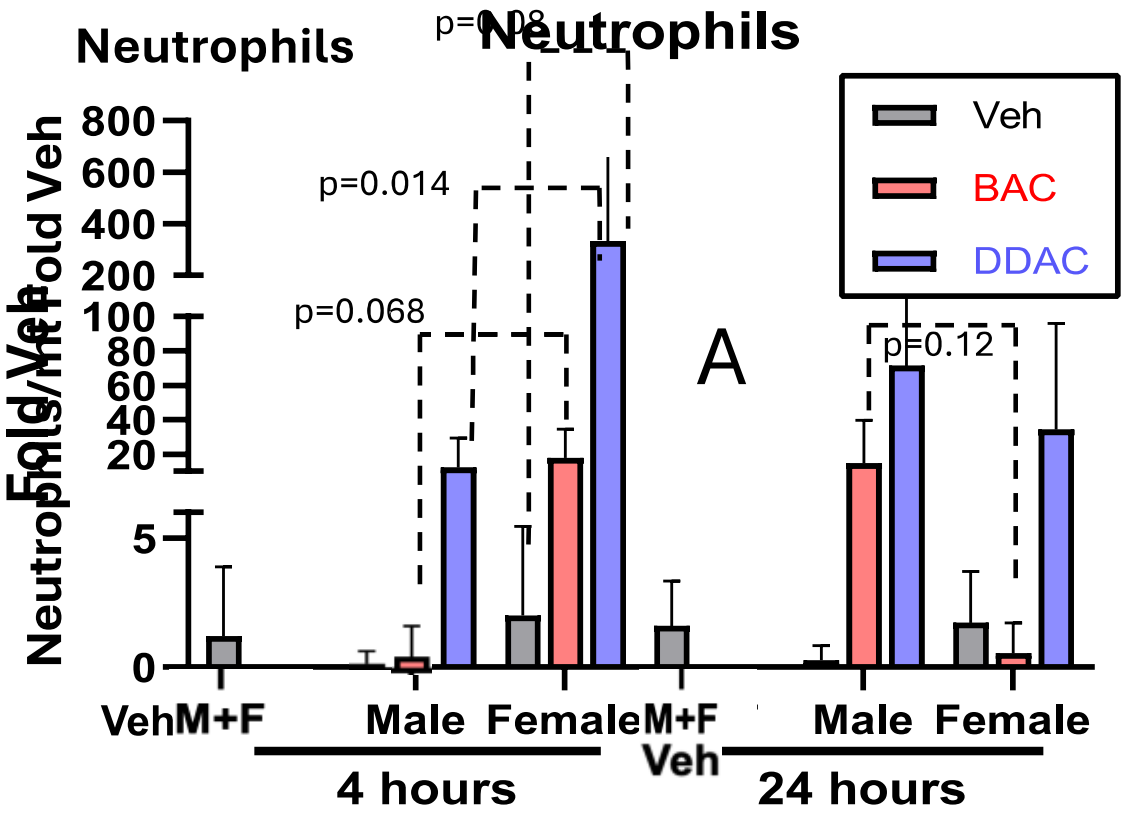
Females experience more lung epithelial cell injury than males at the 1.9mpk DDAC dose: BronchoAlveolar lavage (BAL).

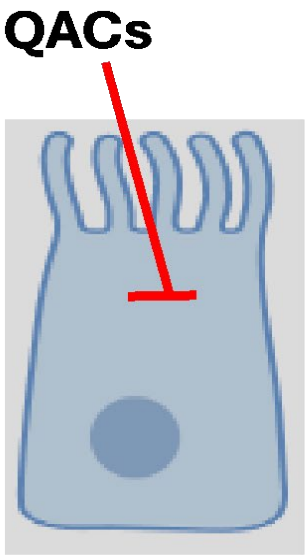


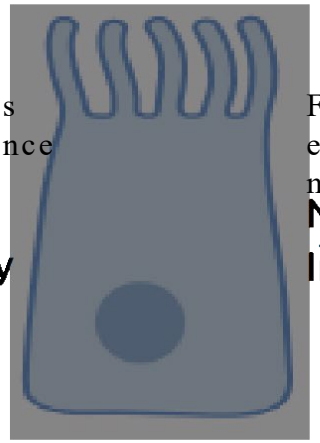
Females experience earlier and greater neutrophil recruitment to lung but not other cell types



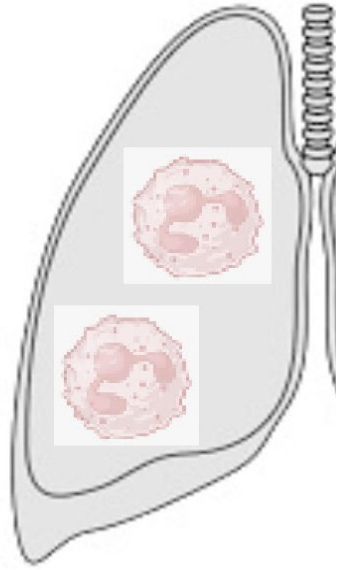



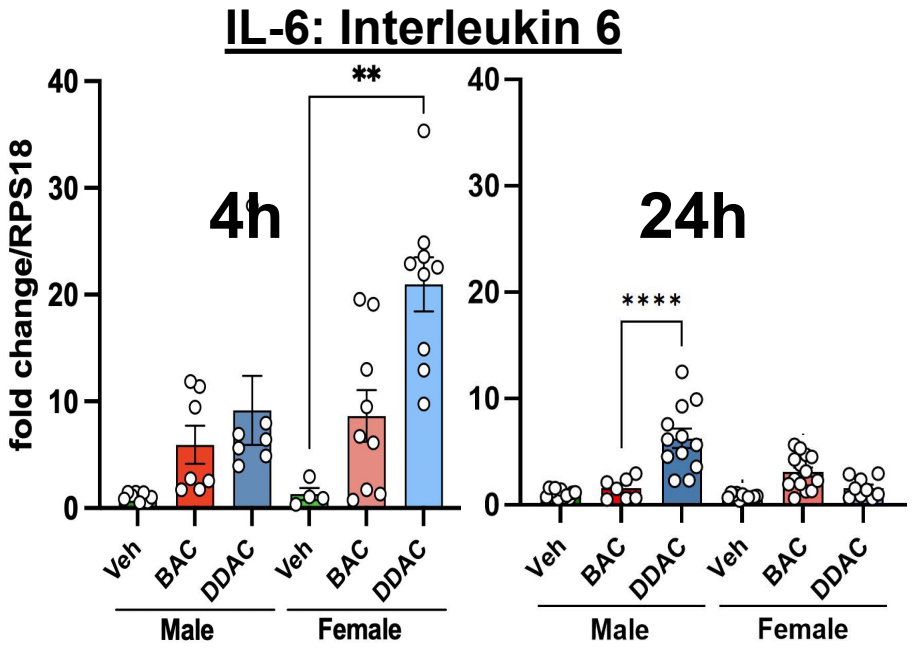

Females experience more cell injury

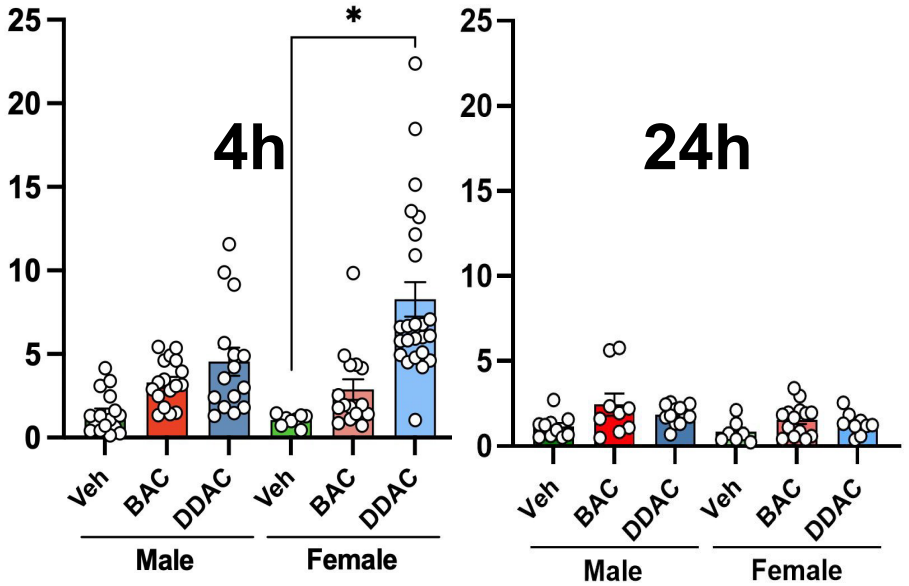


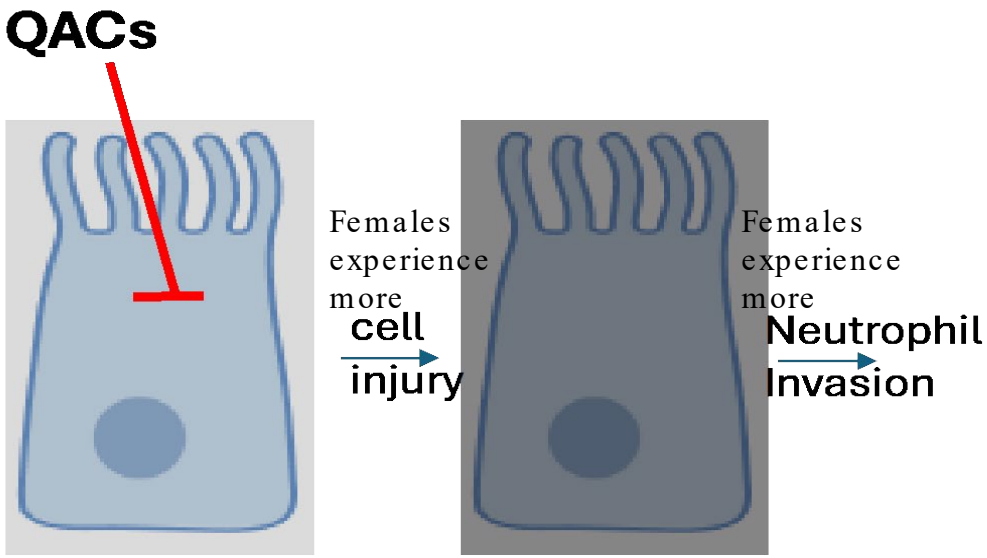
Females experience more Neutrophil Invasion

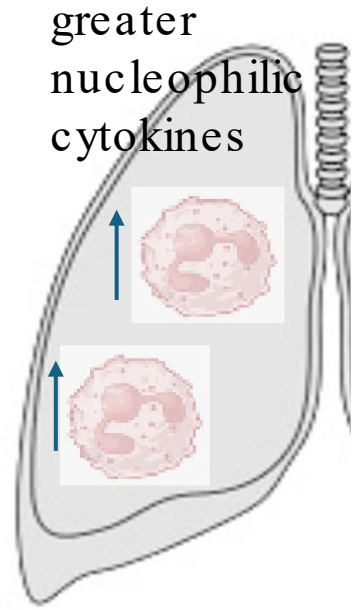


Females experience earlier and greater activation Neutrophilic cytokine transcripts than males (Lung QRTPCR)

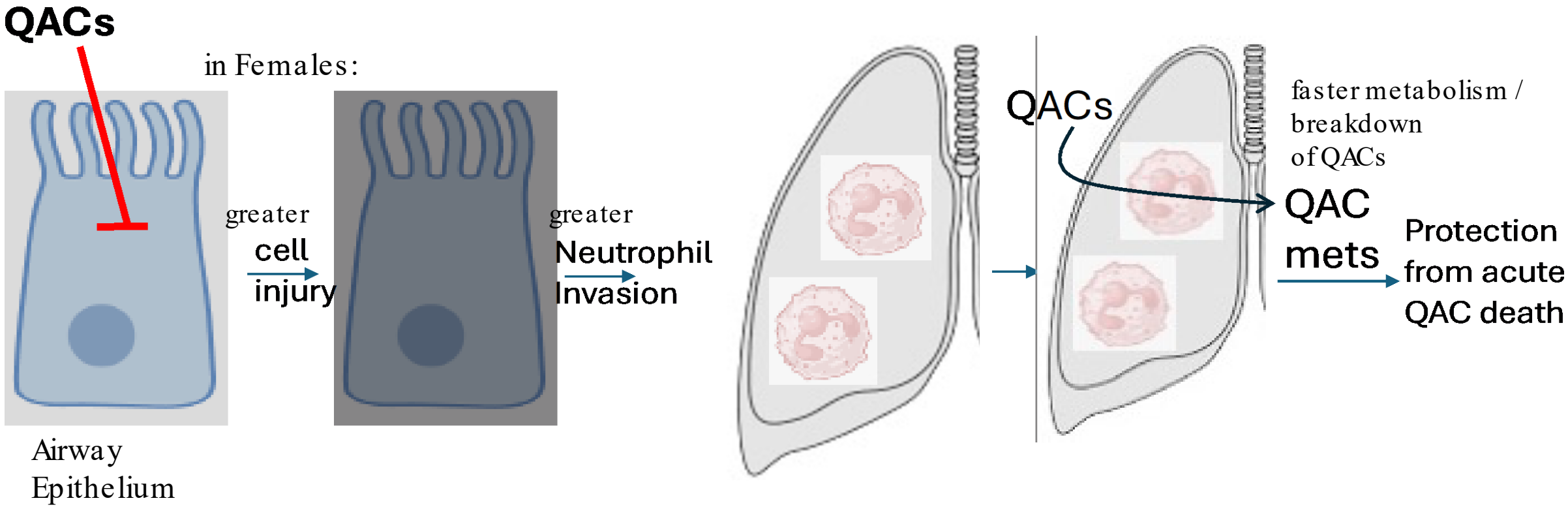


One hypothesis for female protection from QAC lung injury: lung neutrophils metabolize/detoxify QACs, reducing burden



## Summary/ conclusions

0. QACs structure and historical use as disinfectants, and why they've been presumed safe for 84 years.

--------some reasons QACs might not be as safe as previously presumed----------

1. QACs are dose-dependent mitochondrial toxins, and are concentrated about 100X in mitochondria.

2. QACs are detectable in human blood and breastmilk at levels of 2-40nM.

3. Human in vivo QAC exposure dose-dependently alters mitochondrial function and inflammatory cytokine production.

4. QAC (BAC) is concentrated 2-27 fold in mouse lung versus blood, QAC (paraquat) is concentrated 10X in human lung vs. blood.

5. QACs are 100-fold more toxic when inhaled vs. eaten, in mice.

6. QACs are ~2X more toxic to male than female mice.

7. Multiple hypotheses for why females resist QAC exposure more than males exist; one is that DDAC elicits more injury in female lung epithelia, that elicits an earlier neutrophilic response, resulting in earlier detoxification in females.

# People who did the work and acknowledgements

Lau(Ren) Adcock

Claire Montgomery

Sepehr Barkhordari

Sandipan Datta

Laura Stewart van Winkle

Oliver Fiehn

Ameer Taha

Collaborators:

Prof. Terry Hrubec
UVA & VTCom

Libin Xu, UW
School of Pharmacy

NIH National Institute of Environmental Health Sciences

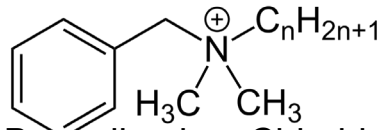
UCDAVIS VETERINARY MEDICINE

This work was supported by a Pilot Project grant from NIEHS-funded UC Davis Environmental Health Sciences Center

This work was supported by an R21 award ES033089 from NIEHS, 'Mitochondrial-mediated lung injury mechanisms of QACs in vivo'.

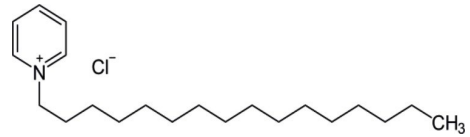
END

Quats Benzalkonium Chloride and Cetylpyridinium Chloride are dosed in millions of people as the active ingredient of the composition




$Cl^{\ominus}$

Benzalkonium Chloride

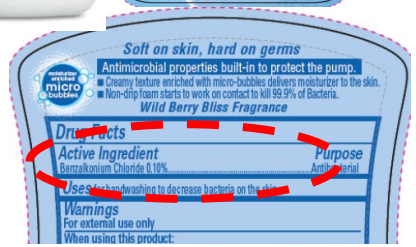



Cetylpyridinium Chloride: As mouthwash, losenge, toothpaste

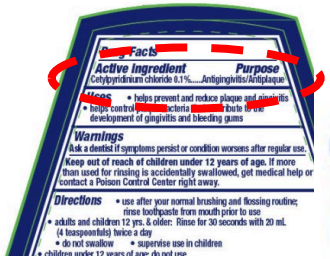

in eyedrops:


Disinfectants






in antibacterial soaps and cleaners:

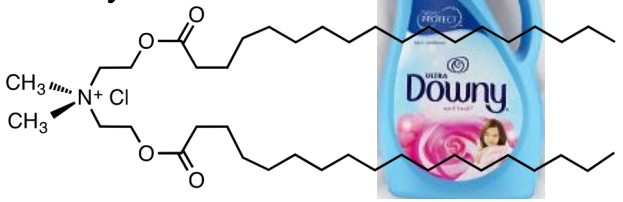






As spermicides, intravaginal sponges



Downy fabric softener:



# Quaternary Ammonium Compounds (QACs) found in sprayed disinfectants are sex-specifically pneumotoxic in mice

**Quaternary Ammonium Compounds are acutely toxic when delivered directly to lung**

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Quaternary Ammonium Compounds (QACs) have been in use since the 1940's as potent biocides and disinfectants and cleaning supplies. The two most-used QACs in the USA are Benzalkonium Chloride (BAC) and Didecyl-dimethylammonium Chloride (DDAC). QACs effectiveness in killing a variety of microbes led to their increased use as aerosolized sprays, foams, and wipes, and aerosolized QACs are widespread, Clorox alone sells 23 branded products containing BAC as a pump spray.

QACs have always been presumed to be safe, for three main reasons: 1) QACs mechanism of action was thought to be soaplike; 2) orally-dosed QACs do not penetrate the body to a significant extent; and 3) the LD50s of orally dosed QACs to rodents were relatively high, 240-500mg/kg. Our finding in 2017 that QACs are dose-dependent mitochondrial toxins began to challenge the notion that QACs were 'safe as soap'. Also, two studies of human populations measured QACs at 2-40nM in blood of 80% of participants from Virginia Tech (Hrubec 2021), and 2-24nM in majority of breast milk samples tested from lactating mothers (Salamova, 2022). So if QACs aren't a natural compound, and pass through the GI tract unabsorbed, how do they get into the blood and breastmilk of human subjects?

The above findings motivated us to test the toxicity of aerosol- and lung-delivered QACs. Mice were dosed at ascending dosages of BAC and DDAC QACs by OroPharyngeal Aspiration (OPA). We found OPA-dosed QACs are 100-fold more toxic (LD50 ~2mg/kg) than orally dosed QACs (~≥240mg/kg). We discovered a sexual dimorphism in response to QACs, significantly more males die than females exposed to OPA-dosed BAC and DDAC. Conversely, females are significantly protected from OPA-dosed BAC and DDAC death. Females had increased neutrophilic infiltration to the lungs, which we hypothesize may underlie their protection.

Our data suggest a new hypothesis for how QACs come to be at 1-40nM in 80% of blood of human subjects--they are likely to enter the body as aerosols through the lung. If this is correct route of exposure and administration, the finding that lung exposure raises QAC's toxicity by 100-fold seems impactful. These new findings might support alternative rational and informed regulation of aerosolized QACs whose potent anti-microbial disinfectant action could have unintended side effects.