Evaluating the Safety of Quaternary Ammonium Compounds (QACs)

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Introduction

Disinfectant QACs:

- Play a key role in human health
- Are used in a wide variety of disinfectant formulations to manage more than 140 different pathogens, many of which can be difficult to control
- Perform at very low concentrations
- Are both safe and effective when used as directed
- Are registered for use worldwide, based on robust datasets protective of human health and environmental fate

How Disinfectant QACs Are Used

- Food contact surface sanitizer uses are approved in the US with EPA allowances for total QACs of 400 parts per million (0.04%) without a water rinse
  - 400 ppm = 1 teaspoon (5 mL) of quat concentrate in 7 ½ gallons (~28 Liters) of water
- Disinfectant RTU QACs for consumer use on hard, non-porous surfaces are diluted, typically have less than 0.3% concentration (3000 ppm) in a water-based formulation
  - 3000 ppm = 1 teaspoon (5 mL) of quat concentrate in a 2-Liter bottle of water
- Some diluted disinfectant QACs are approved for use in healthcare, commercial and residential settings without requirements for PPE

Evaluating Disinfectant QACs

- Robust guideline-compliant studies meet a wide range of regulatory requirements for safety, efficacy, environmental fate, and other concerns
- GLP and regulatory guideline-compliant study results with large data sets are markedly consistent across multiple mammalian species that are predictive of human health endpoints
- Recent comprehensive reviews of disinfectant QACs have been conducted by EPA and ECHA with no restrictions on current uses

Human Health Effects Data for Disinfectant QACs are Comprehensive and Consistent

- Do not produce systemic toxicity
  - QACs are poorly absorbed orally and dermally and do not bioaccumulate
- Are point-of-contact irritants
  - Irritant effects are time- and concentration-dependent and follow known Adverse Outcome Pathways (AOP)
    - Irritation is only associated with concentrated products
  - No Observed Adverse Effect Levels (NOAELs) in animal tests exceed amounts found in dilute, end use and RTU products. These are exempt from listings and warnings
    - QACs do NOT appear on the CA Proposition 65 List

Environmental Fate and Effects of Disinfectant QACs

- QACs bind to wastewater biosolids, then undergo biodegradation and removal
- QAC partitioning in soil and sediment makes them immobile and unlikely to leach or accumulate in surface or ground water
- “Free QACs” (unbound) are not found in wastewater effluent in more than trace concentrations.
  - Based on low vapor pressures, they do not volatize from soil or water
- Acute and chronic aquatic ecotoxicity test results exist for dozens of freshwater species including algae, invertebrates, fish, and plants
  - Test results support all current uses in the US and worldwide
  - Ecotoxicity potential is mitigated by QAC characteristics

SB 1379 Criteria for Priority Chemicals

CRITERIA	DISINFECTANT QACS
Degree of potential exposure	Very Low US EPA: < 0.2 mg/kg/day Salamova et al.: < 0.001 mg/kg/day Arnold et al.: 1-60 micrograms/L WWTP effluent
Likelihood of chemical being a carcinogen or toxicant	Not carcinogenic, not developmental or reproductive toxicant, not neurotoxicant; Skin irritant in concentrate (occupational) settings where PPE is required
Limits of laboratory detection	Low concentrations detectable; validated and highly specific methods required, more than 500 potential mimics
Other criteria	Properties of Disinfectant QACs are not consistent with other compounds on the Biomonitoring California Priority List Long history of safe use, registered as effective against more than 140 existing pathogens of concern, efficacy against emerging pathogens

Comments on Recent Publications

Hora, P.I., Pati, S.G., McNamara, P.J., Arnold, WA. 2020

- QACs are challenging to assess analytically in environmental samples
  - Agree – methods must be validated to avoid artifacts and inaccuracies
- QACs are frequently detected in water and sediment samples
  - Agree – powerful analytical methods MAY detect QACs in water, but they are more commonly found in sediment.
- Sediment concentrations of QACs are declining
  - Agree – sound sampling methods are required for meaningful results
- QACs concentrations are typically low: ppm, ppb, or lower
  - Agree – concentrations are below levels of concern set by regulatory bodies worldwide

Comments on Recent Publications

Zheng, G. Filippelli, GM, and Salamova, A. 2020

- Approach – Dust from vacuum cleaner bags analyzed
  - Comment: Unclear how QACs identified in dust samples relate to "dust ingestion"
- Results – Mean levels of QACs in dust samples have apparently increased during the pandemic
  - Comment: Not unexpected; QAC levels in dust, < 60 ppm, are not of concern to regulatory agencies: Acceptable Daily Ingestion = 0.44 mg/kg/day
    - Salamova exposure estimate (toddler) is **~ 700-times lower:** 0.000615 mg/kg/day
- Conclusion – Results "call for urgent research on risks associated with the increased exposure" to QACs
  - Comment: Decades of research have already been conducted and ruled out risk associated with low level exposures to QACs; this conclusion appears unfounded and unnecessarily alarmist

Disinfectant QAC Evaluation Summary

- QACs have been extensively evaluated in high quality studies for acute, developmental, reproductive, and chronic toxicity/carcinogenicity endpoints
- High-dose, short-term studies as well as subacute, subchronic, and long-term toxicity studies in multiple, guideline-recommended species, from all relevant routes of exposure - inhalation, oral, and dermal - have been conducted, submitted, reviewed and accepted by worldwide regulatory agencies
- All current uses of QACs are supported globally by robust, consistent, and relevant data sets

Toxicology Endpoints – European Chemicals Agency (ECHA) Review of ADBAC (example)

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Carcinogenicity (OECD 453)	
Species/type of tumour	Rat/none, Mouse/none
Reproductive toxicity	
Developmental toxicity **(OECD 414)**	
Species / Developmental target / critical effect	Rabbit/maternal toxicity
Relevant maternal NOAEL	Rabbit: 4 mg/kg bw; No specific concern for developmental toxicity. Maternal NOAELs consistently lower than developmental NOAELs. Maternal effects mostly due to g.i. distress, not relevant to systemic toxicity
Relevant developmental NOAEL	Rabbit: 12 mg/kg/bw; No specific concern for developmental toxicity

Disinfectant QACs Investigated as Potential Developmental and Reproductive Toxicants

- A Panel of Experts has been convened to conduct a rigorous and systematic review of published and unpublished reports addressing this concern
- Panel Members
  - John DeSesso, PhD, DABFM, DABFE, FACFEI, DABCHS, Fellow ATS
  - Anthony R. Scialli, MD
  - Stephen B. Harris, PhD, FATS, FRSB
  - Amy Williams, PhD, DABT

Objective of the Systematic Review

- Recently, a series of papers allege that ADBAC and DDAC cause reduced fertility and neural tube defects (NTDs) in mice and rats
- ADBAC and DDAC
  - Used for more than half a century
  - Evaluated by multiple regulatory bodies
  - Considered safe when used as directed
- Consequently, a Teratology Working Group was empaneled
  - Assess all developmental and reproductive data for ADBAC and DDAC
  - Ascertain whether disinfectant quats are reproductive or developmental toxicants

What is a Systematic Review?

- An assessment of scientific evidence related to a clearly formulated question
- Uses explicit and transparent, well-defined methods to:
  - Identify
  - Select
  - Critically appraise the scientific quality of relevant primary research
- Extracts and analyzes data from the identified high-quality studies to be used by decision makers

Identification and Selection

- An automated search of published literature was conducted using the following search terms
  - ADBAC, DDAC, benzalkonium chloride (BAC), quaternary ammonium compounds, their CAS numbers
  - Gestation, pregnancy, prenatal, fetal, maternal developmental toxicity, reproduction, neural tube defects
- The literature search identified 789 potential articles
  - Review of titles and abstracts culled the list
    - 8 *in vivo* laboratory studies in mammals investigating ADBAC, DDAC or BAC

Identification and Selection - *(concluded)*

- A dissertation was identified and available online
  - 2 chapters were published (captured in literature search)
  - 2 additional chapters, written in manuscript format but not published, were included among the 8 studies evaluated
- Safety studies for ADBAC and DDAC (unpublished) were supplied to Working Group
  - 6 definitive safety assessment studies for regulatory submissions
    - Also 4 dose range finding studies (considered in combination with the definitive studies)

Assessing Quality of Studies

- Objective evaluation of study quality *for the purpose of assessing potential risk*
- The European Centre for the Validation of Alternative Methods (ECVAM) developed and published "ToxRTool" (Schneider et al, Toxicol. Lett. 189:138–144, 2009)
  - "ToxRTool" (Toxicological Data Reliability Assessment Tool) is a software-based tool that provides comprehensive criteria and guidance for evaluating the inherent quality of toxicological data, thereby making the decision process of assigning reliability categories more transparent and harmonized. (https://ec.europa.eu/jrc/en/scientific-tool/toxrtool-toxicological-data-reliability-assessment-tool)
- ToxRTool asks a series of yes-no (scored 1 or 0) questions relating to study design and execution
  - The results are summed and quality categories are determined by score
    - Score 18 or greater — **Category 1** – Reliable without restrictions
    - Score 13 – 17 — **Category 2** – Reliable with restrictions
    - Score 12 or lower — **Category 3** – Not reliable

Results of ToxRTool Scoring to Date

- Results found:
  - **Category 1**: 4 studies (reliable without restriction)
  - **Category 2**: 2 studies (reliable with restrictions)*
  - **Category 3**: 8 studies (not reliable)

*Both studies used rabbits and met contemporary (early 1990's) guidance

- Score sheet evaluated as Reliable without restriction (Category 1)
- Working Group classified as Reliable with restriction (Category 2) because numbers of animals per group are lower than current guidance

Comments on Published Studies Alleging Reproductive Harm

- Five studies that allege reproductive and developmental effects of ADBAC and DDAC are among the not reliable (Category 3) studies
- Numerous issues with these studies
  - Many findings are anecdotal and not based on rigorous scientific data
  - Used nonstandard, poorly described methods
- As examples, the following concerns will be addressed briefly
  - Exposure considerations in experiments using “ambient” conditions
  - Computation of doses
  - Terminology used to describe findings

Comments on Published Studies Alleging Reproductive Harm - *(continued)*

- "Ambient" conditions
  - Cleaning/disinfectant containing ADBAC and DDAC was used in animal rooms
  - Neither substance was measured or identified in maternal tissues (blood, liver, placenta) or embryos
- Mode of exposure was not identified
  - Neither substance has an appreciable vapor pressure compared to water

Vapor Pressures at 1 Atm and 25°C	
Water	**23.8 mm Hg**
ADBAC	**3.53×10^{-12} mm Hg**
DDAC	**$< 4.3 \times 10^{-5}$ mm Hg**

  - Disposable cages had lids – how did animal contact occur?

Comments on Published Studies Alleging Reproductive Harm - *(continued)*

- Dose calculations
  - Female mice were not weighed during gestation
  - Dietary dosing by means of 25 g feed-gel cubes placed in cages "as needed"
    - No indication how daily food consumption was monitored
    - Stated mice consumed 28% of body weight per day – but they were not weighed
    - Number of mice per cage not stated (caging designed to hold 5 mice)
  - Reported doses appear to be based on a series of estimates

Comments on Published Studies Alleging Reproductive Harm - *(continued)*

- Brief introduction to “Neural Tube Defects”
  - A loosely defined set of structural malformations observed at the end of gestation that
    - Always affect the protective coverings (skull/vertebrae and meninges) of the brain/spinal cord
    - May (or may not) include malformations of brain and/or spinal cord
      - Arise during gestation as failure of neural tube to close or as rupture of closed neural tube

Comments on Published Studies Alleging Reproductive Harm - *(concluded)*

- Terminology is misused
  - Findings in gestational day (GD) 10.5 mouse embryos mischaracterized as “neural tube defects”
    - Gestational period in mice is ~20 days
    - Neural tube typically closes on late GD 9 to early GD 10 in mice
    - Meninges and bones of skull have not yet formed at GD 10.5
    - Photographs show open neural tubes in treated embryos
      - Some embryos appear to be at a younger developmental stage than controls, indicating possible developmental delay rather than a neural tube defect
- Importantly, when pregnancy allowed to continue to term there were <u>no cases of neural tube defects</u>
  - Suggests that GD 10.5 findings were either developmental delays that were corrected or resorbing embryos

Interim Results – Reliable Studies

- No test article-related developmental or reproductive endpoints in rats or rabbits among the six (6) combined reliable (Category 1 & 2) studies
- US EPA and ECHA (European Chemicals Agency) approved ADBAC and DDAC based on studies the Working Group scored as reliable (Categories 1 & 2)
  - ECHA's latest assessment also considered and rejected the data from studies in Category 3
  - ECHA specifically approved QAC use in animal facilities when used following directions
    - Publication on neural tube defects "does not meet any of the requirements of ECHA Guidance on information requirements in terms of quality and reporting and therefore should not be considered in the risk assessment of the (QAC) substance."
- At this point in the analysis, the Working Group concurs with the regulatory agencies

Interim Conclusions of Systematic Review

- The dataset consisting of reliable studies (Categories 1 & 2) for assessing the potential reproductive and developmental toxicity of ADBAC and DDAC is robust and extensive
- The Working Group's ongoing, thorough review of all available published and unpublished studies focused on the most reliable science
  - The currently reviewed data indicate that ADBAC and DDAC are not developmental and reproductive toxicants
- Our interim conclusion aligns with those of the US EPA and ECHA
- We will publish our findings in a peer-reviewed journal once our research is complete

Conclusion

Disinfectant QACs:

- Play a key role in protecting human health
- Are used in a wide variety of disinfectant formulations to manage more than 140 different pathogens, many of which can be difficult to control
- Perform at very low concentrations
- Are both safe and effective when used as directed
- Are registered for use worldwide, based on robust datasets protective of human health and environmental fate

Appendix

QACs and Antibiotic Resistance

- Laboratory studies with disinfectant active ingredients suggesting elevated Minimal Inhibitory Concentrations (MICs) are of questionable relevance and have limited predictive value in real world applications
- Non-GLP / University studies are rarely conducted on EPA registered formulated products
- There is no conclusive evidence linking the use of QAC-based disinfectants with the development of antibiotic-resistant organisms under actual use conditions
- Impacts of antibiotics in waste streams are more alarming

Toxicology Endpoints – ADBAC

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Absorption, distribution, metabolism and excretion in mammals (OECD 417)	
Rate and extent of oral absorption	Based on data on urine excretion (5-8%) and tissue residues (<1%), and on the highly ionic nature of the a.s., it is expected that the oral absorption is around 10% at non-corrosive concentrations
Rate and extent of dermal absorption	the value for dermal absorption of the a.s. is 8.3% at non-corrosive concentrations
Distribution	Most radioactivity was confined to the intestines. Levels in central organs (liver and kidney) were low and decreased rapidly over time
Potential for accumulation	None noted
Rate and extent of excretion	Following oral administration in rats: 87 –99% excreted in feces as unabsorbed material, 5 – 8% excreted in urine
Toxicologically significant metabolite(s)	None

Toxicology Endpoints – ADBAC, cont.

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Acute Toxicity	
Rat LD_{50} oral **(OECD 420)**	344 mg/kg bw
Rat LD_{50} dermal **(OECD 402)**	2848 mg/kg bw
Skin corrosion/irritation (OECD 404)	Corrosive NOAEC = 0.3% in water at 2.0 mL/kg bw per day (2 wk-treatment)
Eye irritation (OECD 405)	Corrosive
Skin sensitisation (test method used and species) (OECD 406)	Not Sensitizing (Buehler Test on guinea pig)
Subchronic Toxicity	Local effects (irritation/corrosivity) at the site of contact in all species tested. Non specific systemic effects (e.g. reduced body weight and body weight gain), secondary to local effects.
Relevant oral NOAEL / LOAEL **(OECD 452)**	13.1 mg/kg bw/day (1 year, Dog)
Relevant dermal NOAEL / LOAEL **(OECD 411)**	20 mg/kg bw/day (highest dose tested)

Toxicology Endpoints – ADBAC, cont.

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Carcinogenicity (OECD 453)	
Species/type of tumour	Rat/none, Mouse/none
Relevant NOAEL/LOAEL	No carcinogenic effects were observed (Rat and mice)
Reproductive toxicity	
Developmental toxicity **(OECD 414)**	
Species/ Developmental target / critical effect	Rabbit/maternal toxicity
Relevant maternal NOAEL	Rabbit: 4 mg/kg bw; No specific concern for developmental toxicity. Maternal NOAELs consistently lower than developmental NOAELs. Maternal effects mostly due to g.i. distress, not relevant to systemic toxicity
Relevant developmental NOAEL	Rabbit: 12 mg/kg bw; No specific concern for developmental toxicity

Toxicology Endpoints – ADBAC, cont.

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Neurotoxicity	
Species/ target/critical effect	Study not conducted/ not relevant; Conclusion: No specific concern for neurotoxicity
Developmental Neurotoxicity	
Species/ target/critical effect	No indication from available studies: Conclusion: No specific concern for developmental neurotoxicity
Immunotoxicity	
Species/ target/critical effect	No indication of such an effect in the available toxicity studies; Conclusion: No specific concern for immunotoxicity
Developmental Immunotoxicity	
Species/ target/critical effect	No indication from available studies; Conclusion: No specific concern for developmental immunotoxicity
Medical data	No substance-specific effects have been noted. No specific observations or sensitivity/allergenicity have been reported

Toxicology Endpoints – DDAC

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Absorption, distribution, metabolism and excretion in mammals (OECD 417)	
Rate and extent of oral absorption	Urinary excretion (≈3%), tissue residues (<1%), and 90% recovery of radioactivity in feces as unabsorbed material DDAC oral indicate absorption is < 10% at non-corrosive concentrations
Rate and extent of dermal absorption	0.1% of a DDAC dose delivered as aqueous solution fully penetrated human skin in vitro in 24 h; mean total absorbable DDAC was approximately 10% at non-corrosive concentrations
Distribution	Mainly in the g.i. tract, tissue residues (<1%)
Potential for accumulation	None. Tissue residues (<1%)
Rate and extent of excretion	The majority (>90%) of orally administered DDAC is excreted, very likely unabsorbed, via the feces. Urine excretion ≈3% in 24-48 hours
Toxicologically significant metabolite(s)	None

Toxicology Endpoints – DDAC, cont.

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Acute Toxicity	
Rat LD_{50} oral **(OECD 420)**	238 mg/kg bw
Rat LD_{50} dermal **(OECD 402)**	3342 mg/kg bw
Skin corrosion/irritation (OECD 404)	Corrosive
Eye irritation (OECD 405)	Corrosive
Skin sensitisation (test method used and species) (OECD 406)	Not a skin sensitiser (Magnusson and Kligman procedure)
Subchronic Toxicity	Rat and dog/g.i. tract/ irritation corrosivity leading to body weight reduction
Relevant oral NOAEL / LOAEL **(OECD 452)**	NOAEL for local effects: 3 mg/kg bw/day (1 year, dog) NOAEL for systemic effects: 10 mg/kg bw/day (1 year, dog)
Relevant dermal NOAEL / LOAEL **(OECD 411)**	Local effects NOAEL = 2 mg/kg bw/day (90-day, rat) Systemic NOAEL = 12 mg/kg bw/day (90-day, rat; highest dose tested)

Toxicology Endpoints – DDAC, cont.

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Chronic / Long term Toxicity (OECD 453)	
Species/ target / critical effect	Rat/mice /gi tract/ irritation corrosivity leading to body weight reduction
Relevant oral NOAEL / LOAEL	32 mg/kg/day, non-neoplastic effects (2-year, rat)
Relevant dermal NOAEL / LOAEL	Study not required – not relevant
Relevant inhalation NOAEL / LOAEL	Study not required – not relevant Active substance is not volatile and corrosive
Genotoxicity	
In vitro: - Ames **(OECD 471)** - Chromosome aberration **(OECD 473)** - Gene Mutation **(OECD 476)**	Ames test – negative (with and without metabolic activity) Chromosomal aberration test – negative (with and without metabolic activity) Mammalian cell gene mutation assay – negative (with and without metabolic activity)
In vivo: (OECD 475)	Chromosomal aberration test in rat bone marrow – negative

Toxicology Endpoints – DDAC, cont.

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Carcinogenicity (OECD 453)	
Species/type of tumour	Rat/none, Mouse/none
Relevant NOAEL/LOAEL	No carcinogenic effects were observed (Rat and mice)
Reproductive toxicity	
Developmental toxicity **(OECD 414)**	
Species/ Developmental target / critical effect	1) Rat / NOAEL / maternal toxicity 2) Rabbit / NOAEL /maternal toxicity No specific concern for developmental toxicity; prenatal effects only seen as unspecific consequence of maternal distress
Relevant maternal NOAEL	1) 0.8 mg/kg bw/day (local effects) 2) 1.0 mg/kg bw/day (local effects)
Relevant developmental NOAEL	1) ≥ 16.2 mg/kg bw/day 2) ≥ 3 mg/kg bw/day

Toxicology Endpoints – DDAC, cont.

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Neurotoxicity	
Species/ target/critical effect	Study not required/ not relevant; Conclusion: No structural similarity to known neurotoxin; no alert for neurotoxic effects; no sign of neurotoxicity found in sub-chronic/chronic study
Developmental Neurotoxicity	
Species/ target/critical effect	Not applicable
Immunotoxicity	
Species/ target/critical effect	Study no required
Developmental Immunotoxicity	
Species/ target/critical effect	Not applicable
Medical data	No medical reports on the manufacturing personnel have been submitted