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Evaluating the Safety of Quaternary Ammonium Compounds (QACs)

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Presentation to the Toxics Use Reduction Science Advisory Board
14 January 2021

Introduction

- The ADBAC & DDAC Issues Steering Committees are consortia that jointly address toxicology and regulatory requirements for quaternary ammonium compounds (QACs) in the US, Canada, and Europe
- Member companies make sanitizing and disinfecting products, and also manufacture the active ingredients
- Toxicology Regulatory Services (TRS)* has been providing toxicology and regulatory support for the ADBAC and DDAC consortia continuously since 1991

  * TRS is now part of SafeBridge® Regulatory & Life Sciences Group, a Trinity Consultants Company

How QACs Are Used

- Disinfectant QACs are effectively used in health care settings, schools, commercial buildings, food service, food processing, and hospital environments
- Food contact surface sanitizer uses are approved in the US with allowances for total QAC of 400 parts per million (0.04%) without a water rinse
- QACs are non-volatile; inhalation exposures are negligible because spray products deliver droplets that fall immediately to the surface
- QAC concentrates, packaged to limit weight and bulk, have appropriate labeling for industrial use
- QACs intended for consumer use on hard, non-porous surfaces are dilute and typically have less than 0.3% in a water-based formulation
- QACs have demonstrated efficacy against more than 140 different pathogens, including SARS-CoV-2

Evaluating QACs

- QACs have been extensively evaluated for safety
  - Robust guideline-compliant studies conducted to meet regulatory requirements confirm the safety associated with biocide uses
  - Mechanistic work to identify mechanisms of action and adverse outcome pathways has also added to the understanding of QAC effects on biological systems
- Uses are supported by large consistent data sets in multiple species
- The list of GLP-compliant studies supporting their safety will be summarized
- In the US, EPA published Reregistration Eligibility Decision Documents for ADBAC and DDAC in 2006 and issued Final Work Plans in 2017
- In Europe, ECHA and the Biocidal Products Committee recently completed reviews of veterinary products and food/feed area biocides with no restrictions or requirements for new data

Human Health and Environmental Fate Reflect the Physico-Chemical Properties of QACs

- QACs are readily biodegradable
  - Strongly adsorptive and rapidly partition to soil and sediment making them immobile and unlikely to leach or accumulate in surface or ground water
- QACs do not produce systemic toxicity
  - QACs are poorly absorbed orally and dermally
  - QACs do not bioaccumulate
  - No adverse effects in target tissues distant from point of contact
- QACs are point-of-contact irritants
  - Irritant effects are time- and concentration-dependent and follow known Adverse Outcome Pathways (AOP)
  - No observed effect concentrations in animal tests are far greater than amounts found in dilute, end use products, typically by a factor of 100 or more
- Results are markedly consistent across multiple species

Summary of Environmental Fate and Effects of QACs

- QACs are heavily regulated for human and environmental safety around the world; robust datasets have been developed to evaluate potential ecological hazards and environmental fate
- A recent publication summarizes published and unpublished data reviewed by regulatory authorities to support biocidal product registrations (DeLeo et al. 2020)
- Acute and chronic aquatic ecotoxicity data exist for freshwater species including algae, invertebrates, fish, and plants
- The lower limit of the range of ecotox data for disinfectant QACs tends to be lower than that for other surface-active agents

Summary of Environmental Fate and Effects of QACs, cont.

- Ecotoxicity is mitigated by environmental fate characteristics
  - QACs are highly biodegradable
  - QACs have a strong tendency to adsorb to wastewater biosolids, sediment, and soil
- QACs are largely removed during wastewater treatment, around 95%
- Residues discharged in treated effluent are likely to rapidly bind to suspended solids or sediments, thus mitigating their toxicity
- Lab tests in clean water overestimate toxicity under actual environmental conditions because QACs adsorb to suspended solids and tend to form complexes with anionic surfactants in natural waters

Summary of Environmental Fate and Effects of QACs, cont.

- QAC vapor pressures are extremely low; they do not volatilize from soil or water
- QACs have strong affinity for sewage sludge, sediments, and soils and undergo rapid biodegradation in conventional wastewater treatment systems, aquatic systems, and soils.
- QACs strongly adsorb to soils, and thus, migration into subsurface environments and potential leaching into surface water and groundwater is negligible
- ADBAC is readily biodegradable, mostly broken down in 10 days and completely degraded in 28 days
- DDAC is 70% degraded in 28 days, and 90% degraded in 28 days
- The dataset is extensive
  - ADBAC has been tested in 29 different freshwater species
  - DDAC has been tested in 16 freshwater species

QAC Toxicology Regulatory Dataset Overview

- Environmental Fate and Effects
- Mammalian ADME (absorption, distribution, metabolism, excretion)
- Acute Toxicity tests for oral, dermal, and inhalation exposures
- Skin and Eye Irritation
- Skin Sensitization
- Genotoxicity
- Subchronic/Repeat Dose Toxicity (multiple exposure routes, multiple species)
- Chronic/Long Term Toxicity (multiple species)
- Carcinogenicity (multiple species)

QAC Toxicology Regulatory Dataset Overview, cont.

- Reproductive/Developmental Toxicity
  - Prenatal Developmental Toxicity
  - Fertility
- Neurotoxicity
- Developmental Neurotoxicity
- Immunotoxicity
- Developmental Immunotoxicity

Developmental and Reproductive Toxicology Evaluations

- As required by Good Laboratory Practice (GLP) regulations, careful attention must be paid to dose range-finding and definitive studies
  - Study design must meet criteria to allow for use in human health risk assessment
  - GLP studies are subject to strict oversight and auditing by independent quality control experts
- Critical study features
  - Guideline-recommended species and strains
  - Adequate sample sizes
  - Full characterization of test substance
  - Confirmation of accuracy of doses administered
  - Documentation of overt toxicity, evidenced by changes in body weight, food consumption, clinical signs, etc.

Toxicology Endpoints – ADBAC

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
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
Reproductive toxicity	
Fertility ***(OECD 416)***	
Species/critical effect	Rat/ cortical adrenal hypertrophy in F0 females, lower weight gain and higher spleen weights in F1
Relevant parental NOAEL	608 mg/kg food (≥ 30 mg/kg bw/day); Conclusion: No specific concern for reproductive toxicity. Parental NOAELs related to general toxicity
Relevant offspring NOAEL	608 mg/kg food (≥ 30 mg/kg bw/day); Conclusion: No specific concern for reproductive toxicity. NOAELs in F1 related to general toxicity and equal to the parental ones
Relevant fertility NOAEL	1620 mg/kg food (≥ 52 mg/kg bw/day)

Developmental and Reproductive Toxicology Study Findings

- In dozens of studies in mice, rats, rabbits, and dogs, there has been no evidence of changes to endocrine-responsive tissues following short-term or long-term exposure to QACs by oral, dermal, or inhalation routes
  - No developmental or reproductive effects were seen in properly conducted guideline studies
- US EPA and ECHA independently concluded that developmental and reproductive studies are complete and meet all requirements for data quality and study reliability
  - ADBAC and DDAC have never been classified as developmental or reproductive toxicants
  - Two papers are to be published soon in the peer-reviewed journal *Birth Defects Research* describing the developmental and multigenerational studies
- Wide safety margins exist
- A Safety Margin is the ratio of the dose that caused no effects in definitive animal studies and the estimated dose or exposure in humans

Developmental and Reproductive Toxicology Study Findings
Example of a Wide Safety Margin

- EPA Target (i.e. minimum) Safety Margin = 100
- ADBAC (rat developmental study)
  - No Observable Adverse Effect Level = 100 mg/kg;
- Human exposure: EPA estimate from dietary ingestion from food contact exposure = 0.0159 mg/kg/day
- **Safety Margin = 6,289; more than 60-times the EPA target or minimum**
- In some Hrubec studies, mice were administered QACs, known membrane contact irritants, at doses of 120 mg/kg/day for up to 8 weeks
  - This is the equivalent to a 150-pound person drinking 1.5 quarts of disinfectant solution daily for two months

Guideline Studies vs. Non-Regulatory / Non-Guideline Research

- Academia-based research or other investigations on endpoints including altered cellular function and effects on energy metabolism are more commonly of interest *in the absence of* comprehensive guideline studies
- The mechanism of action of QAC effects, common to the inactivation of microbes and on contact-related irritation of mammalian membranes, has been extensively studied and is well established
- The Adverse Outcome Pathway (AOP) for QACs describes the molecular initiating events responsible for molecular, cellular, and tissue level adverse outcomes

Summary

- QACs have been extensively evaluated for acute, developmental, reproductive, and chronic toxicity/carcinogenicity endpoints
- GLP-compliant studies show there is no evidence of cellular changes in tissues distant from the point of contact
  - These include high-dose, short-term studies as well as subacute, subchronic, and long-term toxicity studies in mice, rats, rabbits, and dogs, from all routes of exposure – inhalation, oral, and dermal
- Broad uses of QACs are supported by large consistent data sets
- Adverse effects in robust guideline studies (point of contact irritation, reduced body weight gain in feeding studies, etc.) occur at doses that greatly exceed human exposure to QACs
- Approvals demonstrate confidence in the strength of the QAC data sets
- QACs are approved and widely used because of their safety profiles, their effectiveness at low concentrations, and their importance in protecting human health in the face of existing and emerging pathogens

Appendix

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/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; 90-day, rat)

Toxicology Endpoints – ADBAC, cont.

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Chronic / Long term Toxicity (OECD 453)	
Species/ target / critical effect	Rat: 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	44 mg/kg/day (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 474)	Micronucleus assay – negative

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
Reproductive toxicity	
Fertility ***(OECD 416)***	
Species/critical effect	Rat/ cortical adrenal hypertrophy in F0 females, lower weight gain and higher spleen weights in F1
Relevant parental NOAEL	608 mg/kg food (≥ 30 mg/kg bw/day); Conclusion: No specific concern for reproductive toxicity. Parental NOAELs related to general toxicity
Relevant offspring NOAEL	608 mg/kg food (≥ 30 mg/kg bw/day); Conclusion: No specific concern for reproductive toxicity. NOAELs in F1 related to general toxicity and equal to the parental ones
Relevant fertility NOAEL	1620 mg/kg food (≥ 52 mg/kg bw/day)

Toxicology Endpoints – ADBAC, cont.

Type of Study (OECD Guideline equivalent)	ECHA / Biocidal Products Committee Assessment
Neurotoxicity	
Species/ target/critical effect	Study not required/ 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/gi 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 activation) Chromosomal aberration test – negative (with and without metabolic activation) Mammalian cell gene mutation assay – negative (with and without metabolic activation)
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
Reproductive toxicity	
Fertility ***(OECD 416)***	
Species/critical effect	Rat /NOEL/reduced body weight and food consumption in parental and F1-F2 animals; available studies do not indicate any specific potential for reproductive toxicity. Observed effects concern solely general toxicity.
Relevant parental NOAEL	750 mg/kg food (≥ 31 mg/kg bw/day)
Relevant offspring NOAEL	750 mg/kg food (≥ 31 mg/kg bw/day)
Relevant fertility NOAEL	≥ 750 mg/kg food (≥ 31 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 not required
Developmental Immunotoxicity	
Species/ target/critical effect	Not applicable
Medical data	No medical reports on the manufacturing personnel have been submitted