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# Exposure to common quaternary ammonium disinfectants decreases fertility in mice

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ABSTRACT

Quaternary ammonium compounds (QACs) are antimicrobial disinfectants commonly used in commercial and household settings. Extensive use of QACs results in ubiquitous human exposure, yet reproductive toxicity has not been evaluated. Decreased reproductive performance in laboratory mice coincided with the introduction of a disinfectant containing both alkyl dimethyl benzyl ammonium chloride (ADBAC) and didecyl dimethyl ammonium chloride (DDAC). QACs were detected in caging material over a period of several months following cessation of disinfectant use. Breeding pairs exposed for six months to a QAC disinfectant exhibited decreases in fertility and fecundity: increased time to first litter, longer pregnancy intervals, fewer pups per litter and fewer pregnancies. Significant morbidity in near term dams was also observed. In summary, exposure to a common QAC disinfectant mixture significantly impaired reproductive health in mice. This study illustrates the importance of assessing mixture toxicity of commonly used products whose components have only been evaluated individually.



## 1. Introduction

The impetus for this investigation of the effects of quaternary ammonium compounds (QACs) on reproductive performance in the laboratory mouse was changes in breeding performance in mouse colonies used by the Hunt and Hrubec laboratories. Both groups noted abrupt changes in colony productivity and reductions in maternal and fetal health that coincided with the introduction of disinfectants containing QACs, Alkyl (60% C14, 25% C12, 15% C16) dimethyl benzyl ammonium chloride (ADBAC) and didecyl dimethyl ammonium chloride (DDAC).

QACs are commonly found in cleaning solutions used in residential, commercial and medical settings, as well as in restaurants and food production facilities. The ability to adapt and optimize QAC structure for specific functions has increased the utilization of these compounds in consumer products [1,2] and, as a result, several generations of QACs exist. The earliest QACs were benzalkonium chloride compounds that were developed as antimicrobial agents. All QACs are permanently charged ions with four alkyl side chains, and biocidal activity is conferred through alkyl chain length [3–5]. Modifications to alkyl chain length have been used to optimize cleaning and antimicrobial properties. Specifically, through substitution of aromatic ring hydrogen with chlorine, methyl, and ethyl groups to increase antimicrobial efficiency and improve detergent strength, different generations of QAC compounds have been generated. Twin-chain or dialkyl quaternary QACs represent the newest generation and exhibit a wide spectrum of activity. These new synthetic polymeric QACs contain multiple positively charged amine centers that confer antimicrobial, anti-static, and surfactant properties in solution.

QACs are often used in shampoos and laundry products to neutralize negative static charges and in cosmetics to preserve products

*Abbreviations:* QAC, quaternary ammonium compound; ADBAC, alkyl dimethyl benzyl ammonium chloride; DDAC, didecyl dimethyl ammonium chloride; DDA, dimethyl didecyl ammonium; HWS-256, disinfectant mixture of alkyl (60% C14, 25% C12, 15% C16) dimethyl benzyl ammonium chloride (ADBAC) and didecyl dimethyl ammonium chloride (DDAC); GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; CWRU, Case Western Reserve University; WSU, Washington State University; VPI, Virginia Polytechnic Institute and State University.

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from microbial contamination [6–9]. The increased reliance on QAC mixtures in consumer products has likely resulted in significant human exposure. For instance, products applied directly to and left on the skin, such as body lotions, often contain QACs [10]. In the health care industry QACs have replaced many alcohol-based hand sanitizers, being more effective at reducing bacterial contamination and limiting the spread of nosocomial infections [11]. In addition, elementary students are provided instant hand sanitizers containing QACs to decrease the spread of illness and reduce rates of absenteeism [12]. QACs are being increasingly incorporated into contemporary products that are utilized orally, such as mouth wash, applied to the skin or eyes or administered as a nasal spray [13–20].

QACs have been in use for approximately 50 years and are considered relatively safe. Despite the duration and prevalence of their use in commercial and consumer products, few studies have assessed the toxicity of single QACs. The majority of studies investigating the toxicity of single QACs are unpublished company reports which indicate weight reduction as the main effect in mice [21–23]. Moreover, no peer-reviewed studies have examined the toxicity of newer QAC combinations. Since chemical mixtures can act synergistically to produce greater toxic effects than the sum of the individual components, evaluation of common mixtures is essential in the evaluation of chemical risk [24–27].

Formulation HWS-256 is a commercial mixture containing a combination of two QACs: Alkyl (60% C14, 25% C12, 15% C16) dimethyl benzyl ammonium chloride (ADBAC, benzalkonium chloride) and didecyl dimethyl ammonium chloride (DDAC). Combinations of ADBAC and DDAC are common in disinfectant and cleaning solutions that are widely used in clinical and residential settings. We proposed that a disinfectant solution containing both ADBAC and DDAC caused severe reproductive defects in exposed mice [28]. This assertion is supported by the results of a six-month breeding study, wherein mice exposed to this QAC mixture demonstrated significant declines in fertility and fecundity. Our results show that the ADBAC + DDAC mixture not only significantly impaired reproduction in breeding pairs, but also contributed to dam morbidity.

## 2. Materials and methods

### 2.1. Animals and experimental design

#### 2.1.1. Case Western Reserve (CWRU) and Washington State University (WSU)

C57BL/6J (Jackson Laboratory, Bar Harbor, ME) mice were housed in ventilated rack caging (Thorin caging (CWRU) or Lab Products (WSU)) in a pathogen-free facility. Breeding stocks were maintained by brother to sister trio mating of two females with one male. To obtain timed pregnancies, six week-old C57BL/6J female pups born in the new facility were placed with adult males, checked each morning for the presence of a copulation plug, and separated from the male on the morning a plug was found. All animal breeding experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at CWRU and WSU; both institutions are fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

#### 2.1.2. Virginia Polytechnic Institute (VPI)

All CD-1 mice (Charles River Labs, Raleigh, NC) were bred for two generations in a room free of QAC disinfectants in order to eliminate any potential effects from previous QAC exposure. Mice were housed in disposable caging (Innovive, San Diego, CA). Exposed mice were maintained in a room utilizing an ADBAC + DDAC disinfectant (HWS-256, Sanitation Strategies, Holt, MI) while control mice were in an adjacent room utilizing ethanol as a disinfectant. Both rooms were climate-controlled with a 12-h light/dark cycle, 20–25 °C, and 30–60% relative humidity. Ethanol washes were used to remove ADBAC + DDAC contaminants from equipment and personnel prior to entering the room housing the control mice. Personnel also donned hair bonnets, face masks, disposable gowns, gloves, and dedicated footwear prior to entering the control room to reduce potential ADBAC + DDAC contamination. Mice were dosed by adding HWS-256 into Nutra-gel diet (purified dry mix formula, Bio-Serv, Frenchtown, NJ) which was prepared following manufacturer instructions. Doses of ADBAC + DDAC/kg body weight/day were calculated based on the sum of active ingredient in the disinfectant (6.76% ADBAC (60% C-14, 25% C-12, 15% C-16) and 10.1% DDAC), with an average daily food consumption of 28% body weight and provided daily in a 25 g Nutra-gel diet cube. Fresh gel cubes were added and food consumption monitored each day. In all experiments, mice were acclimated to the gel diet for one week prior to dosing. A short-term dose finding study was performed in unbred mice to identify the lowest observable adverse effect limit (LOAEL). Gel food was dosed with 0, 60, 120, 240, and 480 mg ADBAC + DDAC disinfectant/kg/day and provided to 5 mice per dose group for two weeks. Mice were monitored daily and evaluated against 16 different health parameters for physical appearance, activity, physiology, and body weight loss. Signs of toxicity, such as inappetance, lethargy, and rough haircoat, were observed in animals receiving the 240 and 480 mg doses; thus, the LOAEL was identified as 240 mg ADBAC + DDAC disinfectant/kg/day. Two dose levels below the LOAEL (i.e., 60 and 120 mg ADBAC + DDAC/kg/day) were selected to evaluate the long-term effects of exposure to QACs. At 5 weeks of age, all mice were provided undosed gel diet. At 6 weeks of age, males and females were combined into breeding pairs and randomly assigned to 0 (control), 60, or 120 mg ADBAC + DDAC/kg body weight/day treatment. Ten dedicated breeding pairs per group were subsequently dosed for a total of six months. Mice were monitored daily and evaluated against 16 different health parameters for physical appearance, activity, physiology and feed consumption. Male mouse weight was recorded weekly. Female body weight was not recorded as body weight fluctuated with their stage of pregnancy. At birth, delivered pups were counted, weighed, evaluated for gross malformations, and then euthanized by IP injection of sodium pentobarbital (0.05 mL/g). This experimental design allowed a multi-tier assessment of chronic toxicity from a QAC mixture directly to adult breeding pairs and indirectly to pups. All animal experiments were approved by the IACUC at the College of Veterinary Medicine at VPI, an AAALAC accredited facility.

### 2.2. Assessing cage contamination

To obtain extracts for chemical analysis, five cages were washed with a small volume of methanol using the following procedure: approximately 40 mL of methanol (JT Baker, Phillipsburg, NJ) was used to thoroughly rinse the inside walls of one polysulfone microisolator cage. The methanol was transferred to a second cage, the rinsing procedure was repeated, and the methanol was transferred to the third cage. This procedure was repeated for two additional cages, thus combining the chemical residue from five cages into one methanol sample. The resultant methanol extracts were collected and stored in glass vials that had been previously tested and demonstrated to be free of exogenous chemical residue (i.e., as for cages, test vials were washed with methanol and extracts run to assess contamination). Analysis of cage extracts was performed with an Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to an API-4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX,