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Quaternary ammonium disinfectants cause subfertility in mice by targeting both male and female reproductive processes

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ABSTRACT

Alkyl dimethyl benzyl ammonium chloride (ADBAC) and didecyl dimethyl ammonium chloride (DDAC) are common ingredients in household bathroom and kitchen cleaning sprays. ADBAC + DDAC cause reproductive toxicity in mice. The aim of the present study was to investigate gender-specific reproductive effects from ADBAC + DDAC. Female reproduction was assessed through ovulation, oocyte implantation, and estrus cycling. Male reproductive function was assessed by sperm concentration, motility, and viability. Numbers of corpora lutea were not different after 2 weeks, but decreased after 8 weeks of ADBAC + DDAC exposure. Dams exposed for 5 weeks to ADBAC + DDAC spent significantly less time in estrus. ADBAC + DDAC exposed males exhibited declines in both sperm concentration and motility, but not sperm viability. Subfertility in mice from ADBAC + DDAC exposure is, therefore, mediated through reproductive disturbances in both females and males. While the effect of ADBAC + DDAC exposure on human health is unclear, widespread exposure necessitates further consideration of their potential reproductive toxicity.

1. Introduction

Quaternary ammonium compounds (QACs) are antimicrobial agents commonly found in cleaning solutions used in residential, commercial and medical settings. Mono-alkyl benzalkonium chloride compounds constituted the first generation of antimicrobial QACs, and were routinely used for disinfection of eating utensils and medical instruments, and as active ingredients in cleaning products for floors and walls. The ability to optimize alkyl benzalkonium chloride structures for specific functions has increased the utilization of these compounds and, therefore, several generations exist. Bactericidal action of QACs is mediated through alkyl chain length. The cationic portion of the QAC molecule is attracted to negatively charged proteins on the bacterial cell membrane. Once proximate, the long alkyl chains of the QAC molecule pierce the bacterial lipid bilayer, causing membrane disruption and leakage of cellular contents. Modifications to alkyl chain length have been used to enhance antimicrobial effectiveness of QACs. QACs exhibit a wide spectrum of biocidal activity, and are effective against many bacteria, fungi, and protozoa. Over time, the applications of QACs have gone beyond simple disinfectants. QAC compounds are currently the most common preservatives in ophthalmic solutions and nasal sprays [1,2].

The addition of QACs in products ranging from algaecides in swimming pools, lumber treatments, anti-static laundry products, to preservatives in cosmetics, has likely resulted in widespread human exposure [3–8]. Incidences of QAC toxicity from consumer products are well documented, and include corneal cytotoxicity, allergic rhinitis, contact dermatitis, and occupational asthma [9–14]. Nevertheless, QACs remain prevalent in consumer and industrial products and are considered relatively safe; however, few peer-reviewed studies have evaluated the toxicity of these compounds.

Between 2001 and 2010 the number of assisted reproductive technology (ART) procedures performed in the United States increased from 107,587 to 147,260 per year [15]. This increased reliance on ART suggests that infertility rates may be increasing. Additionally, global sperm numbers in males have decreased significantly over the last 70 years, from an estimated 113–49.9 million average sperm per mL [16,17]. Increases in reproductive dysfunction have been attributed to several environmental contaminants.

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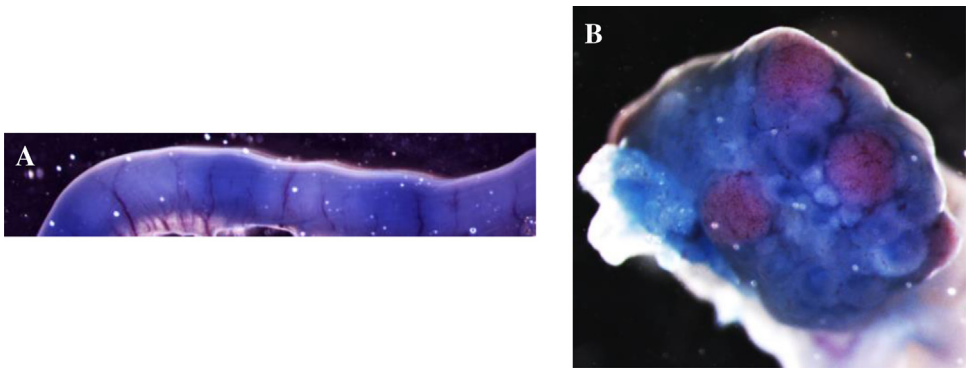

Fig. 1. Control mouse uterus. (A) Blue bands are indicative of implantation sites, visualized by intravenous Pontamine blue injection. (B) Pink follicles represent corpora lutea or the number of oocytes ovulated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Endocrine disruptors (ED) are a class of hormonally active compounds that imitate or interfere with hormone homeostasis and alter body function including reproduction. Several prevalent compounds present in the environment and in consumer products have been identified as EDs [18–24]. Many of these compounds, such as bisphenol-A and the triclosan, have been used in consumer products for decades before being identified as EDs [25]. EDs are associated with a number of human health problems, including cancer, metabolic disorders, and infertility [18–24]. For this reason, public concern over potential negative health effects from chronic low-dose exposure to chemicals has risen substantially.

Alkyl dimethyl benzyl ammonium chloride (ADBAC) and didecyl dimethyl ammonium chloride (DDAC) are among the most common QACs utilized in household bathroom and kitchen cleaners. Additionally, ADBAC + DDAC are routinely used as sanitizers in medical settings, as well as in restaurants and food production facilities. Previously, we reported a decline in breeding performance of mice chronically exposed to 120 mg/kg/day of ADBAC + DDAC [26]. Mice took longer to get pregnant, demonstrated significantly fewer pregnancies and reduced litter sizes. The current study evaluated the gender-specific reproductive toxicity of ADBAC + DDAC.

2. Materials and methods

2.1. Animal husbandry

CD-1 mice were initially purchased from Charles River Laboratories (Raleigh, NC) and were maintained in disposable caging (Innovive, San Diego, CA) on a 12-h light/dark cycle at 20–25 °C with 30–60% relative humidity. Harlan 7013 rodent chow (Harlan Teklad, Madison, WI) and distilled water were provided ad libitum. Mice were reared in a facility free of QAC contaminants (QF) for two generations. Control mice were housed in the QF facility, while QAC-exposed mice were transferred prior to dosing and were housed in the facility utilizing the ADBAC + DDAC disinfectant. For breeding, all mice were paired two females to one male and co-housed for a maximum of two estrus cycles.

2.1.1. ADBAC + DDAC dosing

Mice were dosed by adding ADBAC + DDAC (Sanitation Strategies, Holt, MI) into Nutra-gel diet (purified dry mix formula, Bio-Serv, Frenchtown, NJ), which was prepared following manufacturer instructions, or dosed by adding the ADBAC + DDAC into distilled water provided for the mice. Doses of ADBAC + DDAC/kg body weight/day were calculated based on the sum of ADBAC + DDAC (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 [26]. Food consumption was recorded daily and was not significantly different between treatment groups (data not shown). For water dosing, ADBAC + DDAC/kg body weight/day were calculated based on the sum of ADBAC + DDAC (6.76% ADBAC (60% C-14, 25% C-12, 15% C-16) and 10.1% DDAC), with an average daily water consumption of 10% body weight and provided fresh daily. Control mice were kept in a QF facility and provided undosed distilled water and treated mice were moved to the QAC facility and provided 120 mg/kg/day ADBAC + DDAC in distilled water. Additionally, the effects of ADBAC + DDAC on male mouse sperm concentration and motility were assessed following ambient exposure or oral gavage. Male mice were maintained in a housing facility and exposed for 7 weeks to ADBAC + DDAC through routine disinfectant use by husbandry staff or received an 8 day oral gavage of 7.5 mg ADBAC + DDAC/kg body weight.

2.2. Pontamine blue assessment of female ovulation and implantation

Percentages of successful implantations were evaluated in mice dosed with ADBAC + DDAC for 2 and 8 weeks in gel food and distilled water, respectively. Both males and females were maintained on treatments for 2 or 8 weeks and throughout breeding. Females were bred to unrelated males and the presence of a copulatory plug designated gestational day (GD) 0. On GD 6, females were given an intravenous injection of Pontamine sky blue dye (Sigma–Aldrich, St. Louis, MO) in the tail vein [27]. One minute after injection, females were euthanized using CO_2 inhalation. Gravid uteri were then assessed for blastocyst attachment sites on the uterine epithelium indicated by bands of pontamine blue staining. The number of pink corpora lutea in the ovary was recorded in 6 females per treatment for the 2 week exposure and 9–10 females per treatment for the 8 week exposure (Fig. 1).

2.3. Quantification of estrus cycling

Mice raised in the QF facility were divided into control and treated groups at 6–8 weeks of age (n = 9–10 per group). Control mice were kept in the QF facility and provided undosed gel food. Treated mice were moved to the QAC facility and provided 120 mg/kg/day 6.76% ADBAC + 10.1% DDAC in gel food for 2 weeks prior to evaluating vaginal cytology and throughout a 20 day evaluation period (5 weeks exposure total). Vaginal cytology was collected and transferred to slides, air dried, and submerged in methanol fixative followed by eosin and methylene blue staining (Harleco Hemacolor, Philadelphia, PA). Cells were then enumerated by counting the differential percentage of each cell type out of 100 cells. Estrus cycle stage was identified according to specifications reported in Byers et al. [28].

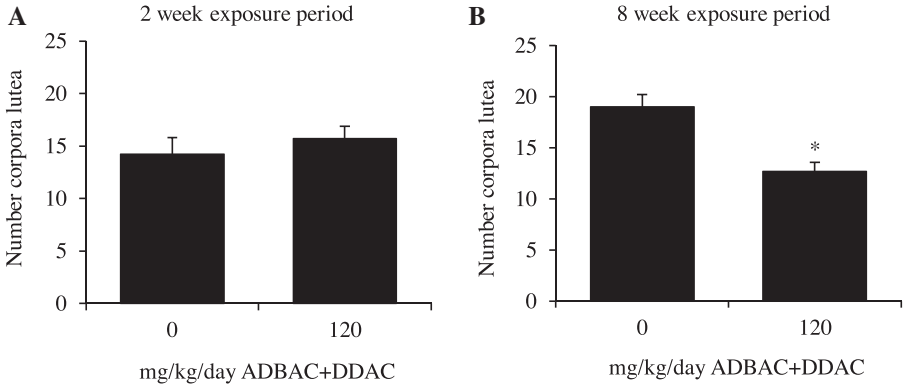

Fig. 2. Numbers of corpora lutea. Mice were exposed for 2 or 8 weeks and throughout breeding, up to the day of evaluation on GD6. (A) The number of corpora lutea in females exposed to ADBAC + DDAC did not significantly differ from controls after 2 weeks of ADBAC + DDAC exposure ($p > 0.05$; t-test; $n = 6$). (B) After 8 weeks of exposure to ADBAC + DDAC, however, the number of corpora lutea was significantly lower compared to controls ($p < 0.001$; t-test; $n = 9–10$).

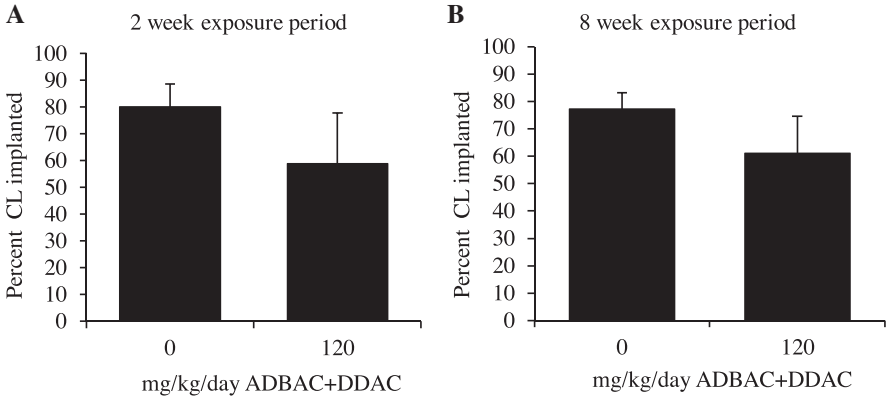

Fig. 3. Percent of corpora lutea implanted. Mice were exposed for 2 or 8 weeks and throughout breeding, up to the day of evaluation on GD6. The number of corpora lutea and implantation sites were visualized on gestational day six by intravenous injection of Pontamine blue dye. (C) The percent of successful implantations, or proportion of ovulated oocytes that implanted, was not significantly different between controls and females exposed to 120 mg/kg/day ADBAC + DDAC for 2 weeks ($p > 0.05$, t-test; $n = 6$). (D) The percent of successful implantations was not significantly different between controls and females exposed to 120 mg/kg/day ADBAC + DDAC for 8 weeks ($p > 0.05$, t-test; $n = 9–10$).

2.4. Evaluation of post-implantation losses

Mice raised in the QF facility were divided into control and treated groups ($n = 9–10$ per group). Control mice were kept in the QF facility and provided undosed gel food. Treated mice were moved to the QAC facility and provided 120 mg/kg/day 6.76% ADBAC + 10.1% DDAC in gel food for 8 weeks prior to breeding and throughout gestation. On GD10, females were euthanized by CO_2 inhalation. Mid-gestational embryos were dissected out of the uterus and evaluated. Embryonic resorptions were characterized by the absence of normal embryonic tissue and abnormally sized and shaped decidual tissue. All viable embryos were staged by GD, somite count, branchial arches, extent of heart and limb bud formation, and deepening of the lens pit.

2.5. Sperm collection

Epididymal sperm were collected following Wang [29]. Briefly, males were euthanized by CO_2 inhalation, both cauda epididymes excised and placed in a 35 × 10 mm Petri dish (Falcon, Oxnard, CA) containing 1 mL of pre-warmed Dulbecco's Modified Eagle's Medium (DMEM: sterile filtered 1000 mg/L glucose and sodium bicarbonate, pyridoxine HCl with no L-glutamine or phenol red) supplemented with 1% fetal bovine serum (GIBCO, Grand Island, NY). Cauda epididymes were minced with a sterile surgical blade (Feather Safety Razor Co., Ltd., Osaka, Japan), mixed, and incubated at 37 °C with 5% CO_2 for 10–15 min to release contents. A 500 μL aliquot of cauda epididymal extract was transferred to a 1.5 mL conical tube (Falcon, Oxnard, CA) and diluted to 1.5 mL with pre-warmed DMEM supplemented with 1 % fetal bovine serum.

2.5.1. Hemocytometer sperm counts

A 500 μL aliquot of cauda epididymal extract was diluted 10× with distilled deionized water to inhibit sperm motility. A 10 μL aliquot of the sperm suspension was loaded onto both sides of a Neubauer hemocytometer (American Optical, Buffalo, NY) and counted twice. Fully intact sperm within five primary squares of the counting chamber were totaled. Duplicate counts were averaged for each sample.

2.5.2. Computer-automated sperm analysis (CASA)

Duplicate 3 μL sample extracts were analyzed using 20 μm-deep 4-chamber Leja slides (Minitube of America, Verona, WI). Slides were allowed to settle for 2–3 min before analysis. Eight random fields were analyzed with 20× phase-contrast at 30 frames per second (Model BX41, Olympus Optical, Tokyo, Japan) using Sperm Vision 3.5 (Minitube of America, Verona, WI) to calculate sperm concentration and motility. Sperm following a non-linear, linear, or curvilinear swimming pattern were classified as motile, while non-swimming sperm were classified as immotile.

2.5.3. Sperm viability

SYBR-14 and propidium iodide (PI) were used to assess sperm membrane integrity and viability. Cauda epididymal extracts

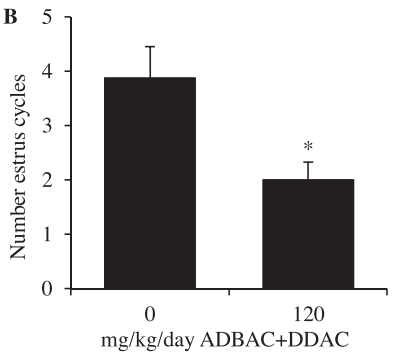

Fig. 4. Average time spent in estrus as determined by vaginal cytology with ADBAC + DDAC exposure. Females exposed to ADBAC + DDAC for 2 weeks progressed through fewer estrus cycles compared to controls ($p = 0.009$, t-test; $n = 8$/treatment).

(300 μL) were treated with 2 μL of 2.4 mM PI and 2 μL of 10 μM SYBR-14 [30]. Samples were incubated for 15 min prior to flow cytometric analysis with a Coulter EPICS XL-MCL benchtop analyzer with a 488 nm excitation source (Indianapolis, IN). Gates were set according to forward and side scatter characteristics of mouse sperm, as published in Garner and Johnson [30]. Proportions of PI (dead), dual (moribund), and SYBR-14 (live) stained sperm were used to characterize membrane integrity and viability.

2.6. Statistics

Statistical analysis was conducted using Statistix (Tallahassee, FL). Data are expressed as the mean ± SEM. Data normality was determined using normal probability plots and compared using the Student t-test for normally distributed data or two-sample rank testing for data not distributed normally. Significance was set at $p \leq 0.05$).

3. Results

3.1. Ovulation and implantation

The number of CL in females exposed to ADBAC + DDAC for 2 weeks (15.7 ± 1.2) was not significantly different from controls (14.2 ± 1.6; Fig. 2A). A subsequent study with increased sample size and a longer 8 weeks dosing period, found decreased numbers of CL in mice exposed to 120 mg/kg/day ADBAC + DDAC. Females exposed to ADBAC + DDAC for 8 weeks had 12.7 ± 0.9 CL while controls had 19 ± 1.2CL ($p < 0.001$; Fig. 2B).

Mice exposed to ADBAC + DDAC for 2 weeks implanted 58.8 ± 19.1% of ovulated oocytes, while 80.1 ± 8.5% of oocytes implanted in the control group. After 8 weeks of exposure, embryos of mice exposed to ADBAC + DDAC implanted at 61.1 ± 13.6%, compared to 77.2 ± 5.9% in controls (Fig. 3A–B). There were no significant differences in the percentage of successful implantations between control, and ADBAC + DDAC exposed mice in either the 2, or 8 week exposure periods ($p > 0.05$).

3.2. Estrus cycling

Vaginal cytology was evaluated in mice maintained on ADBAC + DDAC for 2 weeks and throughout the 20 day evaluation period (5 weeks total). The 20 day monitoring period translates to approximately four estrus cycles. Females exposed to ADBAC + DDAC progressed through fewer estrus cycles compared to controls ($p = 0.009$; Fig. 4).

3.3. Post-implantation loss

To determine if pre-natal and in-utero exposure to ADBAC + DDAC induced post-implantation embryonic death, numbers of resorptions and viable embryos were assessed mid-gestation. Significantly fewer viable mid-gestation embryos were observed in mice exposed for 8 weeks to 120 mg/kg/day ADBAC + DDAC compared to controls ($p = 0.002$); however, there was no significant difference between resorptions with ADBAC + DDAC exposure ($p = 0.6$, Fig. 5A–B).

3.4. Sperm assessment

The effect of ADBAC + DDAC on male reproductive parameters was assessed through sperm counts, motility, and viability analysis.

3.4.1. Hemocytometer counts

Hemocytometer counts from males administered an 8 day oral gavage of 7.5 mg/kg/day ADBAC + DDAC exhibited significantly reduced sperm numbers ($30.1 \pm 1.2 \times 10^6$) compared to controls ($38.3 \pm 1.7 \times 10^6$). Exposure to ambient levels of ADBAC + DDAC for 7 weeks through routine husbandry disinfection procedures also significantly decreased sperm concentration ($29.0 \pm 0.8 \times 10^6$) relative to controls (Fig. 6).

3.4.2. CASA

Computer-automated sperm analysis of epididymal extracts from males exposed ambiently and directly to ADBAC + DDAC

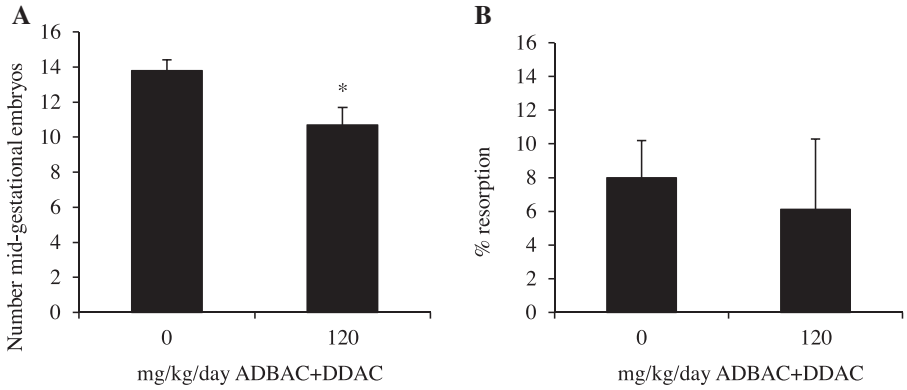

Fig. 5. The average number of mid-gestational embryos in control (0 mg/kg/day) and mice treated with a QAC disinfectant at 120 mg ADBAC + DDAC/kg/day for 8 weeks. A. ADBAC + DDAC exposure significantly reduced the number of viable embryos ($p = 0.002$, t-test); B. The average percent per litter of resorbed embryos in control (0 mg/kg/day) and mice treated with a QAC disinfectant at 120 mg ADBAC + DDAC/kg/day for 8 weeks ($n = 10$/group). The number of resorptions was not significantly different between groups ($p = 0.6$, t-test; $n = 10$/treatment).

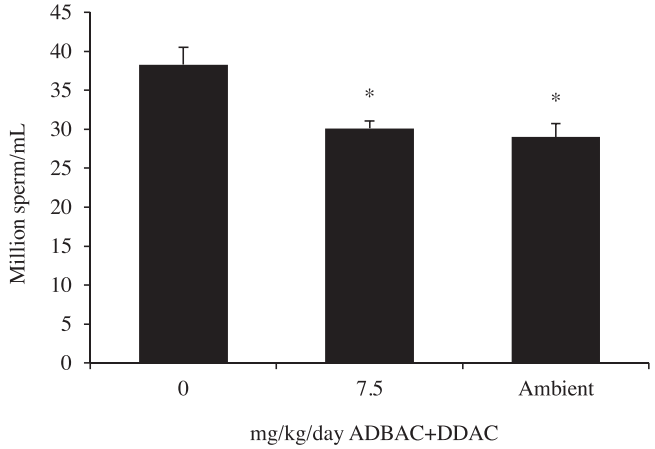

Fig. 6. Hemocytometer counts of epididymal extracts in males exposed to ADBAC + DDAC. Compared to controls, sperm concentration was significantly reduced in males that received an 8 day oral gavage of 7.5 mg/kg/day ADBAC + DDAC, as well as males exposed ambiently for 7 weeks to ADBAC + DDAC through routine disinfection of the animal room ($p < 0.01$, Kruskal–Wallis; $n = 4$–7/treatment).

indicated significantly reduced sperm concentrations and motility. Control males had significantly higher sperm concentrations ($40.8 \pm 0.77 \times 10^6$ sperm/mL) compared to males exposed ambiently for 7 weeks and those given an 8 day oral gavage of ADBAC + DDAC ($30.4 \pm 1.4 \times 10^6$ sperm/mL and $31.7 \pm 0.4 \times 10^6$ sperm/mL, respectively; $p < 0.001$, Fig. 6). The average motility in males exposed to ADBAC + DDAC ambiently and by oral gavage was $77.6 \pm 3.0\%$ and $72.4 \pm 3.8\%$, respectively. Control males exhibited $86.1 \pm 2.7\%$ motility ($p < 0.001$, Fig. 7).

3.4.3. Sperm viability

Flow cytometric analysis of dual-stained sperm did not identify a significant difference in the proportions of in live, dead and moribund sperm between control and mice dosed with 120 mg ADBAC + DDAC/kg/day for 8 weeks; $p > 0.05$, Fig. 8A–B).

4. Discussion

QACs are registered as pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). As antimicrobials, QACs are extremely effective and are among the most common disinfectants used to prevent microbial growth and contamination in commercial and industrial settings. A survey investigating cleaning activities in commercial food production, storage, and preparation facilities reported that QAC sprays were the most heavily used disinfectants [31]. Additionally, QACs are common preservatives in a wide range of personal care products such as shampoos, cosmetics, and baby wipes. Despite being in use for over 50 years, little is known about QAC toxicity. Mouse breeding pairs exposed over a 6-month period to ADBAC + DDAC exhibited significantly reduced fertility and fecundity; ADBAC + DDAC mice had fewer pregnancies, took longer to get pregnant, and had smaller litter sizes compared to controls [26]. Although these results clearly demonstrate that exposure to ADBAC + DDAC affected reproduction in mouse breeding pairs, the study design did not distinguish between toxic effects on the dam, sire, fetus or a combination of effects.

The current study demonstrates that ADBAC + DDAC exposure is toxic to both male and female fertility. Female mice exposed to ADBAC + DDAC exhibited decreased reproductive capacity with reduced ovulation and fewer estrus cycles. Male mice exposed to ADBAC + DDAC exhibited significantly decreased sperm concentration and motility. While the reproductive effects of ADBAC + DDAC have been characterized in this study, the specific mechanism(s) by which ADBAC + DDAC disrupt female and male reproduction remains to be identified.

Female reproduction is dependent on normal ovarian function, since estrus cyclicity and oocyte maturation and release are regulated primarily by hormones secreted by the ovary. Gonadotropic luteinizing hormone (LH) and follicle stimulating hormone (FSH) regulate ovarian prolactin, estradiol, and progesterone secretion and all are required for follicular maturation and subsequent ovulation. Agonism/antagonism of endogenous hormone receptors by exogenous chemicals results in an excess of hormonally active compounds, leading to abnormal production, transport, metabolism, or secretion of endogenous hormones. Hormonal contraceptives, typically a combination of estrogen and progesterone, deliberately disrupt the endocrine axis to inhibit follicular maturation and ovulation; hormonal contraceptives suppress production of FSH and LH through negative feedback inhibition to prevent ovulation. The efficacy of hormonal contraceptives is dependent on the fact that estrogen-mediated processes are sensitive to perturbation from exposure to exogenous hormones or hormonally active ED chemicals.

A number of environmentally ubiquitous compounds have been identified as estrogenic EDs. Inadvertent exposure to EDs through environmental contamination has increased concern

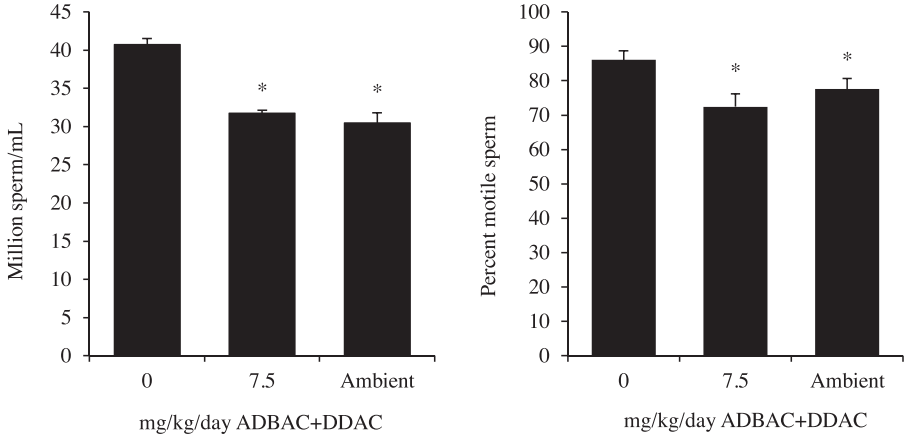

Fig. 7. CASA determination of sperm parameters in control mice, mice administered oral gavage of ADBAC + DDAC, and mice exposed ambiently to ADBAC + DDAC. (A) Sperm concentration was significantly reduced in males administered 7.5 mg ADBAC + DDAC/kg body weight for 8 days ($p < 0.001$, Kruskal–Wallis; $n = 7$) and males exposed ambiently to ADBAC + DDAC for 7 weeks ($p < 0.001$, Kruskal–Wallis; $n = 4$) compared to controls ($n = 4$). (B) Significantly fewer sperm were motile in males exposed ambiently and administered oral gavage of ADBAC + DDAC compared to controls ($p = 0.05$, Kruskal–Wallis; $n = 3$–4/treatment).

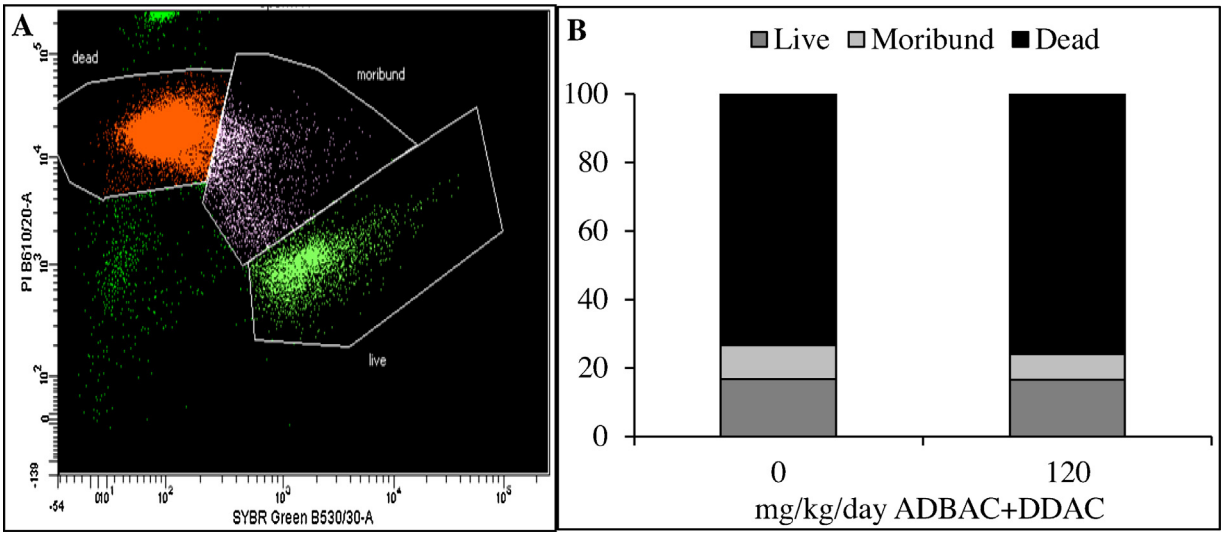

Fig. 8. Sperm viability in control and ADBAC + DDAC treated males. (A) Flow cytometric gating parameters using dual-staining (PI, SYBR-14) to evaluate sperm viability. (B) There were no significant differences in sperm viability between control and mice dosed with 120 mg ADBAC + DDAC/kg/day for 8 weeks ($p < 0.05$, Kruskal–Wallis; $n = 8$–12/treatment).

over the effects that these compounds may have on human reproductive function. Estrus cycle irregularities and decreased ovulatory capacity have been observed in rodents administered common ED compounds such as bisphenol A, nonylphenol, genistein, and zearalenone [32–35]. In the current study, females exposed to ADBAC + DDAC demonstrated significantly decreased ovulatory capacity, spent less time in estrus, and progressed through fewer estrus cycles compared to controls. It is, therefore, conceivable that ADBAC + DDAC reduced estrus length and frequency through disruption of estrogen-regulated processes. ADBAC + DDAC treated mice had significantly fewer estrus cycles over the evaluation period. This correlates directly with our 6 month breeding trial which observed significantly fewer litters produced in ADBAC + DDAC treated mice [26]. These two findings reinforce that ADBAC + DDAC disrupt reproductive function in female mice.

The numbers of mid gestation embryos were significantly reduced by ADBAC + DDAC exposure. Reductions in embryo number can be caused by decreased ovulatory frequency, decreased implantation rate or increased post-implantation embryo loss. As we observed no differences in implantation or resorption rates, but did observe significant differences in ovulatory rate, the reduction in embryo number is likely due to impaired ovulation. Changes in ovulatory frequency can be caused by disruption of the hypothalamic pituitary ovarian axis, or other endocrine and metabolic disorders [36–39]. Disruption of the hypothalamic pituitary ovarian axis is frequently observed following exposure to endocrine disrupting compounds [18].

Male germ cells are susceptible to damage from environmental exposures. Over the last 70 years, the average sperm concentration in males has decreased from 113 million sperm per mL to 49.9 million [16,17]. More than 90% of male infertility cases are due to low sperm counts and/or poor sperm quality [40]. EDs, such as synthetic estrogenic compounds, are suspected of playing a large role in the rising rates of male infertility [41–43]. Zearalenone-induced reduction in sperm parameters has been observed in male rodents and included decreased sperm concentration and abnormal sperm morphology [44]. Epidemiological studies evaluating the effects of occupational exposure to bisphenol-A on male reproduction indicated that men with elevated blood/urinary levels of bisphenol-A had abnormal sperm/semen parameters [45]. Urinary concentrations of genistein have also been correlated with idiopathic male infertility and reduced sperm parameters in Chinese men [46].

In our study, male mice had significantly reduced sperm concentrations and motility at low dose exposure and when exposed to ambient concentrations of ADBAC + DDAC. Toxicity studies routinely include a high dose exposure in order to ensure a biologic response. Viability was not affected when dosed at 120 mg/kg/day indicating that this parameter is not a target of ADBAC + DDAC reproductive toxicity. The observed declines in male sperm parameters seen at the low and ambient exposures are particularly significant and troubling with respect to potential toxicity to humans. Humans are exposed regularly through occupational use and common consumer products such as cleaners and toiletries.

Reproductive toxicants represent a diverse class of both synthetic and natural compounds such as plasticizers, organochlorine pesticides, flame retardants, industrial solvents, detergents, and phytoestrogens. A molecule's structure can be used to predict its potential to bind androgen and estrogen receptors and estimate potential ED behavior. EDs are difficult to identify simply based on structure, since EDs may disrupt endocrine systems through mechanisms other than receptor binding. It is possible that ADBAC + DDAC decrease mouse fertility through an ED mechanism; however, other mechanisms may also be involved. Chemically reactive compounds or compounds that emulate endogenous molecules are capable of interfering with enzyme systems or signaling pathways which can result in altered cellular homeostasis. Additionally, reproductive toxicants can alter the metabolism or excretion of compounds required for reproductive system maintenance. For example, the plasticizing agent mono-(2-ethylhexyl) phthalate suppresses aromatase action through activation of peroxisome proliferator-activated receptors, ultimately leading to related changes in gene expression in cultured granulosa cells [47]. Reproductive toxicants can also be metabolically activated and change pharmacodynamic and/or pharmacokinetic responsiveness of the exposed individuals, as exemplified by the toxic metabolite of molinate which induces severe spermatogenic and testicular damage [48]. Lastly, variations in the individual responsiveness to toxicants may be due to dose-time differences, differences in endocrine status, and/or differences in metabolism and excretion of chemicals [36,39–41].

5. Conclusions

Results of this study support previous observations that QACs ADBAC + DDAC reduce fertility in mice. Exposure to ADBAC + DDAC QACs reduced breeding capacity through disturbances in ovulatory capacity and estrus cyclicity in females and reduction in sperm concentration and motility in male mice. Further experiments are underway to clarify the mechanisms by which ADBAC + DDAC reduce reproductive function in both male and female mice. Importantly, this study demonstrates reproductive toxicity following exposure to ambient concentrations of these disinfectants. This finding is particularly relevant if ADBAC + DDAC exert toxicity in humans as there is extensive exposure to these compounds. These results indicate that the potential risk of reproductive toxicity to humans should be evaluated further.

Transparency document

The Transparency document associated with this article can be found in the online version.

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