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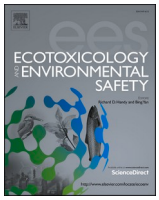

Acute toxicity of three alkylbenzene sulfonates in six freshwater aquatic species

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ABSTRACT

Alkylbenzene sulfonates (ABS) are surfactants widely used in residential and commercial products. To support the environmental risk assessment of these compounds, the acute toxicity of three ABS, linear (*n*-ABS), branched (BABS), and alkyl phenoxybenzene sulfonates (APBS), was evaluated using six aquatic organisms from different trophic levels (algae, daphnid, amphipod, mussel, snail, and fish). This approach allowed direct comparisons among species to provide insights into species sensitivity to these surfactants, and among compounds to provide information on those with a lack of ecotoxicity data (e.g., BABS, APBS). Endpoints related to survival, growth, and physiological changes were recorded. Comparisons among the three ABS were based on nominal concentrations due to the absence of pure analytical standards for APBS. However, analytical methods were developed for BABS and available for *n*-ABS, so effects of these compounds were also evaluated based on measured concentrations. Results showed differences in sensitivity among compounds for all species exposed to environmental concentrations of ABS, except for snails, which showed similar sensitivity to all surfactants and were among the most tolerant species. Based on nominal concentrations, the EC50/LC50 values for *n*-ABS, BABS, and APBS ranged, respectively, from 5.0 to 17.8 mg/L, 7.3 to 25.6 mg/L, and 3.5 to > 100 mg/L. The most sensitive species to *n*-ABS were fish, mussels, and amphipods, while amphipods and mussels were the most sensitive to BABS and APBS, respectively. Species sensitivity was also evaluated using measured concentrations of *n*-ABS and BABS. The results indicated that EC50/LC50 values varied from 1.24 to 13.13 mg/L and from 1.53 to 5.21 mg/L for *n*-ABS and BABS, respectively, and were in the range of concentrations reported in environmental surface waters. Amphipods and mussels could therefore be relevant sensitive model organisms for the environmental risk assessment of *n*-ABS and BABS.

1. Introduction

Alkylbenzene sulfonates (ABS) are anionic surfactants widely used in the formulation of household and industrial detergents (Gouda et al., 2022), such as laundry powders and liquids, dishwashing products and industrial cleaners (Cowan-Ellsberry et al., 2014), as they are efficient in grease and oil removal and play a pivotal role in the overall cleaning process (Scheibel, 2004). In addition to their utility in cleansing industries, ABS are used as emulsifying agents in agricultural herbicides, industrial paints and electric cable oils (Asok et al., 2015). Alkylbenzene sulfonates were introduced in 1947 in the form of branched compounds and used until the 1960s as surfactants in laundry detergents (Scheibel, 2004). Owing to their rapid expansion in use and their poor biodegradability, the presence of branched ABS in wastewaters led to environmental challenges (Cowan-Ellsberry et al., 2014), causing massive foaming problems in rivers and sewage treatment plants (Scheibel, 2004; Scott and Jones, 2000). As a result, regulations prohibited the use of branched ABS, first in the United States and then later in Western Europe (Scheibel, 2004). Linear alkylbenzene sulfonates (LAS) were then extensively used as alternative substances due to their decreased environmental persistence (Kaida et al., 2021; Scheibel, 2004). Global consumption of LAS reached 18.2 million tonnes in 2003 due to their low cost and high cleaning efficiency (Mungray and Kumar, 2009). Commercial LAS are mixtures of closely-related isomers and congeners

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containing a linear alkyl chain of C10–C14 (Cowan-Ellsberry et al., 2014), with most congeners being comprised of C11-C13. The linear alkyl group is in the *para*-position to the sulfonate on the benzene and attached at any carbon of the alkyl chain except for the terminus (see Table 1 for sample structures). Regional analyses in Canada, Europe, Japan, and the United States indicated a weighted average of 11.7–11.8 carbons (OECD, The Organisation for Economic Co-operation and Development, 2005). Because of this complexity in components, technical LAS formulations are often depicted using the short form, C12-ABS.

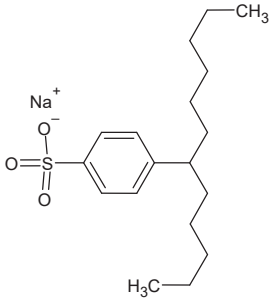
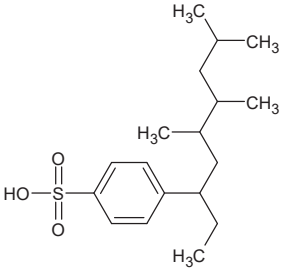
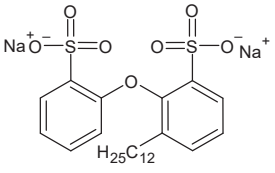
Linear alkylbenzene sulfonates can reach the environment via effluents from wastewater treatment facilities and municipal sludge applied as soil conditioner on agricultural lands (da Silva Coelho and Rocha, 2010; Freeling et al., 2019; Liu et al., 2019; Mungray and Kumar, 2009). LAS have been reported in surface water (Choque-Quispe et al., 2021; Sakai et al., 2017) and estuaries (León et al., 2002) as well as in coastal waters and marine sediments (Hampel et al., 2012; Petrovic et al., 2002) at concentrations ranging from μg/L to mg/L (Luo et al., 2023). Concentrations of LAS in coastal seawaters and near industrialized areas ranged from 1 to 20 mg/L (Rapaport and Eckhoff, 1990), while concentrations ranged from 0.05 to 0.5 mg/L in estuarine waters (León et al., 2002) and above 10 mg/L in surface waters (river basins, major lakes and reservoirs) of Malaysia and China (Luo et al., 2023; Sakai et al., 2017). Tubau et al. (2010) also reported that LAS were detected in groundwater in Barcelona, Spain, with maximum concentrations of 0.005 mg/L.

These surfactants can undergo biodegradation in water under aerobic conditions, producing sulfophenyl alkyl acids and further chain shortening intermediates followed by ring opening to complete mineralization (Marin et al., 1991). In river water and under aerobic conditions, the half-life of C12-LAS was 0.23 days for the parent compound, mineralization half-lives were less than 3 days (Larson and Payne, 1981), and biodegradation was estimated to be more than 99 % after 40 days at 7 °C (Perales et al., 1999). However, LAS are persistent under anaerobic conditions (Ying, 2006).

LAS are some of the most prevalent surfactants, and therefore their biological effects have been the primary focus of research on ABS, with several studies published regarding effects on aquatic organisms (Van Stempvoort et al., 2020). Studies reported that LAS induced growth inhibition of the freshwater algae, *Scenedesmus obliquus* (Cheng et al., 2021). Exposure at the maximum LAS concentration permitted in Brazil freshwaters (0.5 mg/L) affected the cardiac function and impaired the physiological performance of bullfrog tadpoles, *Lithobates catesbeianus* (Jones-Costa et al., 2018). Exposure to LAS inhibited the activity of several enzymes in hepatocytes of spotted snakehead fish, *Channa punctatus* (Gupta et al., 1989), and induced an inflammatory response and histopathological anomalies in the testes of Asian stinging catfish, *Heteropneustes fossilis* (Kumar et al., 2007). This surfactant also negatively impacted the reproduction of zebrafish, *Danio rerio* (Akbulut and Yon, 2019) and induced various symptoms in zebrafish larvae, such as body rupture and bent tails (Han and Jung, 2021). A significant decrease in the fecundity of cladocerans *Ceriodaphnia dubia* and *Ceriodaphnia silvestrii* exposed to LAS was also reported (da Silva Coelho and Rocha, 2010). Because of their continuous discharge into aquatic ecosystems (Sobrino-Figueroa, 2018), additional toxicity information is warranted for the risk assessment of these compounds.

Another ABS group of interest used as detergents, the alkyl phenoxybenzene sulfonates (APBS), has been in production since the 1950s. The chemical structure of APBS is different from other ABS in that the base structure contains a diphenyl ether (Table 1). There is very little information available on the environmental presence and fate of APBS, likely due to the absence of pure standards and the complexity of technical mixtures. Commercial mixtures of APBS contain four subclasses of constituents, including monoalkyldiphenylether sulfonates, monoalkyldiphenylether disulfonates, dialkyldiphenylether sulfonates, and dialkyldiphenylether disulfonates (US Patent 6743764), with the alkyl substituents being linear or branched and ranging from 6 to 16 carbons. It has been suggested that the double charge stemming from the pair of sulfonates on the diphenyl ether increases the water solubility of these surfactants and therefore their suitability for applications in textile dyeing, polymer emulsion processing, agricultural chemical manufacturing, and cleaning fluids (US Patent 6743764). Using semi-quantitative analyses, Van Stempvoort et al. (2020) reported

Table 1
Specifications of the three alkylbenzene sulfonates (ABS) investigated.

Acronym	*n*-ABS	BABS	APBS
Commercial name	Sodium dodecylbenzene sulfonate	Benzenesulfonic acid Mono-C11–13-branched alkyl derivatives	Benzenesulfonic acid. Dodecyl (sulfophenoxy)-sodium salt
Supplier	BOC Sciences	BOC Sciences	BOC Sciences
CAS #	25155–30–0	68608–88–8	28519–02–0
Category	Linear ABS	Branched ABS	Sulfophenoxy-ABS
Molecular weight	348.48	326.49	542.62
Representative structure			
% actives (measured)	94 ± 4.4 %	18 ± 3.4 %	Not measured
% composition by weight (measured)	ND: C8 linear 0.38 % C9 linear 10 % C10 linear 38 % C11 linear 29 % C12 linear 16 % C13 linear 0.046 % C14 linear	0.79 % branched C8 0.84 % branched C9 1.6 % branched C10 2.4 % branched C11 8.9 % branched C12 1.8 % branched C13 1.1 % branched C14	Not measured

ND = Not detected

concentrations of quantifiable APBS with possible chain lengths ranging from C9 to C18 corresponding to 0.9–14 μg/L in municipal wastewater influent from various locations across Canada and < 0.04 μg/L (limit of detection) to 4.0 μg/L in final treated wastewater effluent. Van Stempvoort et al. (2020) were unable to quantify monoalkldiphenylether disulfonates and dialkyldiphenylether monosulfonates due to analytical challenges.

The Chemicals Management Plan is an initiative of the Government of Canada aiming to reduce the risks posed by chemicals in commerce to human and environmental health. A review of the available scientific data on alkylbenzene sulfonates identified several knowledge gaps regarding the aquatic toxicity of this group of compounds, particularly for BABS and APBS. Understanding of relative toxicity among these three distinct classes of surfactants contributes to predictive structure-activity relationships and viability of chemical alternatives in an ecotoxicological context. Thus, the aim of the present study was to provide toxicological information on these data-poor compounds for use in risk assessment. The acute toxicity of three technical mixtures of distinct classes of alkylbenzene sulfonates (linear ABS (*n*-ABS), branched ABS (BABS), and APBS) was evaluated using six freshwater species from different trophic levels: algae (*Chlamydomonas reinhardtii*), crustacea (water flea, *Daphnia magna* and amphipod, *Hyalella azteca*), freshwater mollusks (wavy-rayed lampmussel, *Lampsilis fasciola* and ramshorn snail, *Planorbella pilsbryi*), and fish (fathead minnow, *Pimephales promelas*). This comprehensive, multi-species approach allowed direct comparisons among species and compounds. Multiple endpoints such as survival, growth, mobility, and physiological changes for fish (e.g., abnormalities, length) were recorded during exposures to determine species sensitivity to the selected products and to support risk assessment activities for this group of chemicals.

2. Material and methods

2.1. Stock solution of surfactants

Three alkylbenzene sulfonate mixtures were selected based on an initial screening conducted by the Ecological Assessment Division of Environment and Climate Change Canada (ECCC). These sulfonates were included on a list of priority compounds because they are used in commerce in Canada and toxicity data are needed for risk assessment. All three were technical mixtures consisting of homologous series of chain lengths and positional isomers. Linear (*n*-ABS, CAS 25155–30–0), branched (BABS, CAS 68608–88–8) and sulfophenoxy (APBS, CAS 28519–02–0) benzyl sulfonates (Table 1) were purchased from BOC Sciences (London, UK) and tested for toxicity in six aquatic species (*C. reinhardtii*, *D. magna*, *H. azteca*, *L. fasciola*, *P. pilsbryi*, and *P. promelas*). The stock solutions were prepared gravimetrically by weighing the substance and adding it to deionized water, municipal tap water (Burlington city water, sourced from Lake Ontario, that was dechlorinated, charcoal filtered, particulate filtered, and UV sterilized), moderately hard reconstituted water, or algae media, as appropriate. The stock solutions were then diluted with the specific media to obtain the desired nominal concentrations determined based on the results of range-finding tests specific to each species. Details on the nominal concentrations tested for each species are described in the acute toxicity section.

2.2. Acute toxicity testing

2.2.1. Chlamydomonas reinhardtii

The short life cycle and generation time (5–6 h) of the unicellular alga *C. reinhardtii* allowed the testing of ABS over 96-h exposure periods. A *Chlamydomonas reinhardtii* CC124 strain was obtained from the Canadian Phycological Culture Centre (University of Waterloo, Waterloo, ON). Cultures and exposures of *C. reinhardtii* were maintained following methods published by Sanchez et al. (2015) and Esperanza et al. (2017), and algal culture inoculation was carried out under aseptic conditions. Algal cells were maintained in 250-mL Erlenmeyer flasks containing 125 mL of sterile Sueoka's high salt medium (HSM) in a controlled temperature incubator (22 ± 1 °C) under a 12 h light:12 h dark cycle with continuous shaking (100 rpm).

To assess the acute effects of ABS, the microalgal species was aseptically inoculated and cultured for at least two cycles and grown to the exponential growth phase. *C. reinhardtii* cultures with density of 2.5×10^5 cell/mL were exposed to increasing nominal ABS concentrations (0, 0.001, 0.01, 0.1, 1, 10 and 100 mg/L) in 125-mL Erlenmeyer flasks and incubated for 96 h. The untreated algal suspension served as a control group and the experiments were conducted using 3 replicates per treatment. ABS stock solutions (0.5 g/L) were prepared by dissolving ABS in 200 mL of algae media followed by filtration through a 0.22-μm acetate membrane. Stock solutions were held in the refrigerator at 4 °C. To reduce differences in photon radiance during the exposure, the flasks were randomly arranged in the incubator. The pH of media was recorded throughout the test. At the end of the exposure period, the growth rate was determined by flow cytometry following the procedures described by Esperanza et al. (2017). A suspension of fluorochrome-containing micro-spheres was used for calibration. Growth rates (μ) expressed as day^{-1} were calculated as described by Samadani and Dewez (2018) via the following formula:

$$\mu = \frac{[\ln(N_t) \quad - \ln(N_0)]}{\Delta t};$$

where N_t is the cell density at the end of the exposure time; N_0 is the cell density at time 0 and Δt is the length of the time interval ($t_t - t_0$). Samples of media were collected at t = 0 and t = 96 h for chemical analysis.

2.2.2. Daphnia magna

D. magna is a widely distributed freshwater microcrustacean and is a model organism used in ecotoxicology. Under healthy conditions, the females reproduce by parthenogenesis and generate individual clones. Culture maintenance and acute toxicity tests with *D. magna* were performed in moderately hard reconstituted water according to standardized methods (Environment Canada, 1990). Cultures were held at 20 ± 1 °C with a 16 h light:8 h dark cycle and fed daily with a 3.85×10^5 cells/mL green algae *Pseudokirchneriella subcapitata* and 0.0125 g/L YCT (yeast-cerophyll-trout chow) preparation. All experiments were conducted under constant temperature and light conditions.

Neonates (< 24 h) were transferred to polypropylene beakers filled with 150 mL of culture medium alone (control) or containing 0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg/L (nominal) of ABS for 48 h. Ten daphnids were used for each exposure treatment and assays were conducted in duplicate without feeding. The conductivity, temperature, and pH were recorded during testing (Table S1). At the end of the 48- h exposure, endpoints of immobilization and death were recorded. Samples of media were collected at t = 0 and t = 48 h for chemical analysis.

2.2.3. Hyalella azteca

H. azteca is an epibenthic freshwater amphipod commonly used in toxicity testing. Cultures were maintained according to Borgmann et al. (1989), and both cultures and experiments used dechlorinated municipal tap water at 25 °C under a photoperiod of 16 h light:8 h dark. Physicochemical parameters of the culture water were as follows: hardness 120–160 mg/L, alkalinity 88–114 mg/L, pH 8.3–8.4. Amphipods were fed with finely ground TetraMin fish food flakes (Tetra GMBH, Melle, Germany).

Seven-day acute amphipod toxicity tests were performed in duplicate using 2-to-9-day-old neonates (N = 15 for each replicate, three replicates per treatment group per test). Organisms were transferred to glass beakers, each containing 1 piece of 2.5 cm× 2.5 cm cotton gauze and filled with 200 mL of dechlorinated tap water (controls) or increasing concentrations of ABS (0.2, 0.5, 1.3, 3.2, 8.0, 20, and 50 mg/L,

nominal). Amphipods were fed 2 times with 2.5 mg TetraMin, at the beginning and in the middle of the exposure. Water quality characteristics (dissolved oxygen, conductivity, pH, and total ammonia) for *H. azteca* were measured at the beginning and the end of each test (Table S1). At the end of the 7-d exposure, the number of survivors was recorded. Samples of media were collected at t = 0 and t = 7 d for chemical analysis.

2.2.4. *Lampsilis fasciola*

Gravid wavy-rayed lampmussels (*Lampsilis fasciola*) were collected in September 2021 from a reference site on the Speed River (ON, Canada) with a stable population (Gillis et al., 2017). Mussels were held at ECCC's Aquatic Life Research Facility (ALRF; Burlington, Ontario), and maintained in a flow-through system with dechlorinated municipal tap water at 11 ± 2 °C to prevent glochidia (i.e., larvae) release. Mussels were fed with an algal mixture (Instant Algae Shellfish Diet 1800® and Nanno 3600®, Reed Mariculture, Campbell, CA, USA) twice daily. Glochidia were collected by flushing the brooding chambers with a water-filled syringe.

Acute (48- h) toxicity tests with glochidia were conducted according to standard methods (ASTM, American Society of Testing Materials, 2013) as described by Gillis (2011). All exposures were conducted at 20 ± 2 °C under a photoperiod of 16 h light:8 h dark. Glochidia with viability (ability to close valves, see below) > 90 % were collected from at least three gravid females and transferred to a mixture (50/50) of reconstituted moderately hard water (20 °C) and dechlorinated municipal tap water (11° C) to reduce any potential temperature or osmotic-related stress. Glochidia (~500–1000) were then exposed in 250-mL glass beakers for 48 h to various concentrations (N = 4 replicates/treatment) of each of the three tested ABS compounds. The control group (N = 5 replicates) consisted of glochidia exposed to dechlorinated municipal tap water which was used to prepare the different ABS dilutions (0, 0.8, 1.6, 3.1, 6.2, 12.5, 25, 50, and 100 mg/L, nominal). Physicochemical parameters of the exposure water (pH, conductivity, dissolved oxygen, chloride, total ammonia, and temperature) were assessed in each treatment at the beginning (t = 0 h) and at the end of each exposure (t = 48 h) (Table S1). After 24 and 48 h of exposure, glochidia viability was assessed in a sub-sample (≥ 100) of exposed specimens using the sodium chloride response test (ASTM, American Society of Testing Materials, 2013). Since glochidia are obligatory parasites, for practical purposes, non-responsive glochidia should be considered functionally 'dead' since they would be unable to attach to a host and complete their life cycle. Viability was calculated using the following equation:

$$\%\,\text{viability} = 100\,x\,\frac{\text{Number of closed glochidia after NaCl addition} - \text{Number of closed glochidia before NaCl addition}}{\text{Number of closed glochidia after NaCl addition} + \text{Number of open glochidia after NaCl addition}}$$

2.2.5. *Planorbella pilsbryi*

Testing with juvenile snails was completed using a continuous culture of File Ramshorn snails (*Planorbella pilsbryi*) established in the ALRF (described in Gilroy et al., 2025). Briefly, the culture was maintained in 62-L aquaria filled with dechlorinated municipal tap water (pH 7.62 ± 0.39, hardness 127.0 ± 5.8 mg/L; alkalinity 95.3 ± 4.6 mg/L; calcium 36.2 ± 1.8 mg/L) further enriched in calcium carbonate (7 g $CaCO_3$ per tank per week), and kept at approximately 22 °C with a photoperiod of 16 h light:8 h dark, and snails were fed twice a week with 9 g of shrimp pellets (Omega One, Blacksburg, VA, USA) per tank and organic spinach leaves *ad libitum*.

Five mature *P. pilsbryi* (between 11 and 17 mm) were added to each of 6–8 aerated polystyrene jars filled with 800 mL of dechlorinated, charcoal-filtered and UV-sterilized calcified tap water (0.16 mg $CaCO_3$/L) and capped with a polypropylene lid to prevent evaporation. The mature snails were fed with 0.1 g of ground shrimp pellets and one organic spinach leaf and were removed after 72 h; the egg masses were left in the jars to hatch. After 11 d, the juvenile snails (approximately 96 h old) were transferred into a crystallization dish for transfer into exposure vessels. The toxicity of ABS on the survival of juvenile *P. pilsbryi* was assessed in 7-d static renewal tests at nominal concentrations of 0.1, 0.32, 1, 3.2, 10, 32 and 100 mg/L. Juvenile snails were randomly transferred into each well of 24-well culture plates (5 specimens/well, N = 6 replicates per concentration) filled with 2 mL of dechlorinated, calcified municipal tap water. Once all snails were transferred, the water was removed and replaced with 2 mL of exposure solution using a randomized complete block design. Each well was fed with 20 μL of *Nannochloropsis* (68 million cells/mL; Nanno 3600™, Reed Mariculture, Campbell, CA, USA). Culture plates were covered with a lid to prevent evaporation and kept at 22 °C with a 16 h light: 8 h dark cycle. After 96 h of exposure, the exposure solutions were removed from each well, replaced with fresh solution, and the wells were fed again. Samples of media were collected at t = 0 and t = 96 h for chemical analysis. At the end of the exposure, survival was assessed. Given the small sample volumes (2 mL), standard water quality parameters could not be measured.

2.2.6. *Pimephales promelas*

Embryo-to-hatch fathead minnow (*P. promelas*) testing methods are preferred over the standard larval fish test due to ethical concerns around vertebrate testing. Before the three ABS for the study were selected, two tests were performed on a different linear ABS (CAS # 121–65–3) to see if the 6-d embryo test (Marentette et al., 2015) in fathead minnows (which is more humane) was similar in sensitivity to the standard 7-d larval growth test in fathead minnows. The embryo test proved to be similar in sensitivity to the larval test (LC50 of approximately 2 mg/L for both tests; Table S2), so it was used to assess the three ABS selected for the study. Detailed information concerning the larval exposure is described in the Supplementary Material.

Bioassays of fathead minnow embryos were conducted at the ALRF under Animal Use SOP GWACC-119 and AUP 2184, approved by ECCC's Animal Care Committee. The stock solutions (1 g/L) were further diluted 2 h prior to the exposure or to the daily exposure solution renewal to obtain an ABS nominal stock concentration at 100 mg/L. Exposure solutions of ABS were prepared in dechlorinated municipal tap water (0, 0.18, 0.32, 0.56. 1.00, 1.78, 3.16 5.62, 10 mg/L for the three ABS and 17.78 mg/L for BABS only). Newly fertilized embryos were purchased from Aquatox Laboratories (Guelph, ON). The eggs used in testing had been fertilized < 18 h before the start of the exposure. Eggs from ≥ 4 breeding groups were used to begin each replicate, with three replicate plates per treatment, and 6 replicate plates for water controls. Embryos were exposed using daily static renewal methods in 24-well cell culture plates (one embryo per 2-mL well), following the method of Marentette et al. (2015). Solutions for chemical analyses of ABS were sampled before and after the daily solution changes on three separate days during the exposure. The embryo tests were performed under controlled conditions at 25 °C, 16 h light:8 h dark, and 60 % humidity, and viable embryos were moved daily to new plates containing fresh test solutions. At 2 days post-fertilization, 5 embryos per plate were videotaped in their respective wells to count heart rates. Embryos began to hatch at 4–5 days post-fertilization, and time of hatch was noted for each. Newly hatched fry were assessed for abnormalities, hatch success, and length (measured on a dissecting microscope), and then humanely euthanized. Abnormalities assessed in fry included edemas and circulatory problems (necrosis, cardiac edema, yolk edema, bubbles under skin, hemorrhages, and others such as tube heart), craniofacial abnormalities (small face, eye edema, or other jaw deformities), and spinal abnormalities (lordosis or "belly out", kyphosis or "belly in", scoliosis or "bent to the side", bent tail fin, or others).

2.3. Analysis of ABS in exposure media

The technical mixtures of *n*-ABS and BABS were characterized by liquid chromatography tandem mass spectrometry (Vanquish-H TSQ Altis Plus, ThermoFisher Scientific) using negative electrospray ionization. Both were found to be a homologous series of different chain lengths, centered around C11 and C12. However, the shorter retention times observed in BABS relative to *n*-ABS confirmed that BABS was indeed a branched mixture of ABS and that *n*-ABS corresponded to the linear ABS. For both classes, the components were quantified using an authentic standard of linear decyl benzene sulfonate (Fujifilm Wako Chemicals, Tokyo, Japan). The distribution of substances in *n*-ABS and BABS is presented in Table 1 along with the purity of the technical mixtures.

Exposure solutions were sampled depending on the experimental design of each species (Table S3) and stored at −20 °C until analysis. A subset of samples for each test species was selected for chemical analysis that included controls and concentrations approximating those at which effects were observed. For each subset of samples, one sample was diluted using HPLC grade water (Fisher Scientific) and analyzed in duplicate to determine precision based on % difference. Targeted analyses were performed for C8, C9, C10, C11, C12, C13, and C14 using the instrumental parameters specified in Table S4. Blanks consisted of HPLC grade water used for dilutions. Quantification was based on calibration curves consisting of the pure standard of linear C10 ABS and homologues were assumed to have equal response to the C10 ABS. Limits of detection (LOD) ranged from 0.84 to 1.7 ng/mL (Table S4) based on the concentration of each analyte in a low-level standard, and corresponded to the average concentration plus three times the standard deviation. The concentrations of ABS in exposure media were expressed in mg/L and are the sum of homologues C8, C9, C10, C11, C12, C13, and C14. APBS could not be measured due to the complexity of the compound and the absence of pure standards required for quantification.

2.4. Statistical analysis

Data were expressed as mean ± standard deviation (SD) and statistical analyses were performed using STATISTICA (version 7, Statsoft Inc., 1995). Normality and homogeneity of variance were checked using the Shapiro-Wilk and Bartlett's tests, respectively. When data fulfilled the requirements of parametric tests, the significance of difference between the control and the treatment groups was examined by one-way analysis of variance followed by post-hoc tests. However, nonparametric alternatives (Kruskal-Wallis and Mann-Whitney U tests) were used when the data violated parametric assumptions. Significance from the control was set at $p < 0.05$. The concentrations causing 10, 25, and 50 % mortality (LC10, LC25, and LC50) and/or effect concentrations causing 10, 25, and 50 % effect (EC10, EC25, and EC50) were determined using the best-fitting model among 3- or 4-parameter log-logistic or Weibul models (constrained between 0 and 100, as needed) using the statistical package *drc* v.1.1.456 (Ritz et al., 2015) in R v.4.1.0 (R Core Team, 2021).

3. Results

3.1. ABS chemical analyses

Results showed a strong association between the measured concentrations of *n*-ABS and BABS and the gravimetric nominal concentrations (Fig. 1). At the end of the 7-d experiments, our data indicated that *n*-ABS content measured in the exposure media of amphipods and snails showed a marked decline from the concentration measured at time 0, with decreases ranging from 78 % to 91 % in the amphipod exposure (for treatment 3.2 and 8 mg/L, 7 d) to more than 99 % in the snail exposure (for 10 and 32 mg/L, 4 d), respectively (Table S5). This agrees with the reported rapid degradation half-life of *n*-ABS. The decrease in *n*-ABS concentrations was less pronounced in exposure media of the 48-h mussel (4–30 %) and *Daphnia* (2–12 %) tests, and the 96-h algae

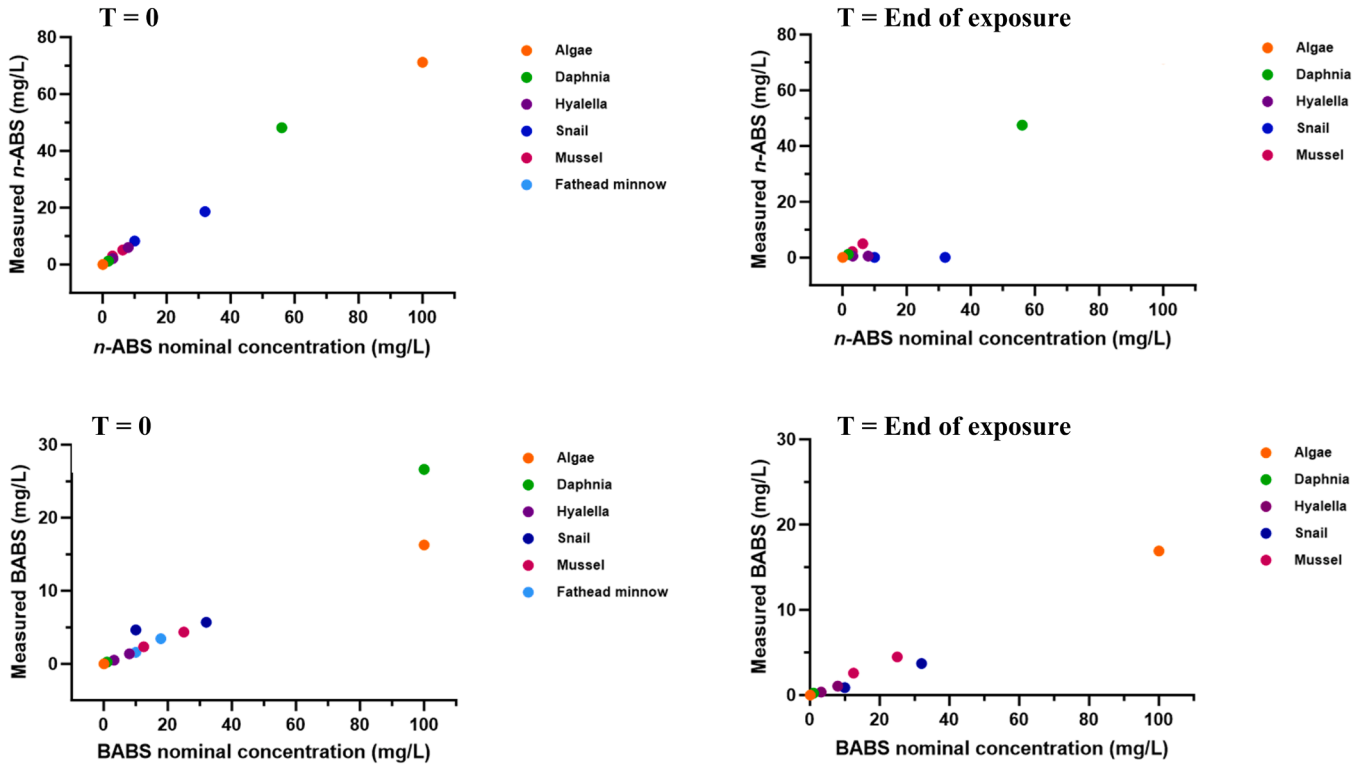


Fig. 1. Comparison of measured concentrations (mg/L) for *n*-ABS and BABS with nominal concentrations at the beginning and the end of the exposure (T). Exposures ranged from 48 h to 7 d for the various species tested (Table S3).

(0–25 %) test. In comparison to *n*-ABS, the measured BABS did not show a decline for several experiments (Table S5). However, the measured concentrations were much lower than the nominal concentrations. The measured concentrations at t = 0 for BABS were generally 13–46 % of the gravimetric nominal concentration. These results suggest the chemical purity may have been low or that degradation occurred in the bottle that the neat chemical was stored in, although it is possible that quantifying BABS against a pure standard of C10 *n*-ABS resulted in under-estimation of BABS measurements. However, in other linear versus branched alkyl isomers, electrospray ionization factors were not grossly different. For example, nine branched isomers of perfluorooctane sulfonate (PFOS) were 70–110 % of the response factor of linear PFOS (Riddell et al., 2009). Adsorption to container walls, food particles, etc. may have also played a role in decreasing concentrations of *n*-ABS and BABS during the tests (Könnecker et al., 2011).

3.2. Toxicity in the different species

The results of the current study are reported based on nominal concentrations due to the absence of pure standards for APBS chemical analysis and to compare with the toxicity of ABS published in the literature. However, estimated LC_x/EC_x values were calculated for both nominal and measured concentrations for *n*-ABS and BABS and are reported in Table 2 to support new knowledge on the behaviour of these compounds in exposure media and provide information for the regulatory context. Estimated LC_x/EC_x based on the measured concentrations were determined using the linear regression of measured exposure concentrations at t = 0 for all species.

3.2.1. Chlamydomonas reinhardtii growth rate

Growth rate (GR) of algae was determined based on the change in cell density after 96 h of exposure (Table 3). In comparison with the control group, algae exposed to the highest concentration (100 mg/L) of *n*-ABS and BABS displayed negative GRs of $-0.31\ d^{-1}$ and $-0.49\ d^{-1}$ ($p < 0.001$), respectively, while APBS at this concentration induced a moderate toxicity (GR decreased by 17 %, $p < 0.05$). The lowest concentration at which ABS induced a significant decrease in GR differed among compounds: 0.1 mg/L of BABS ($p < 0.05$), 1 mg/L of *n*-ABS ($p < 0.01$), and 10 mg/L of APBS ($p < 0.05$). The relative toxicity ranking based on 96-h EC50 values in order of most to least toxic was as follows: *n*-ABS > BABS > APBS (Table 2).

3.2.2. Daphnia magna immobilization and survival

Little to no immobilization was observed in *D. magna* exposed to concentrations < 10 mg/L for the three ABS tested (Fig. 2 A). However, immobilization increased steeply at 10 mg/L and greater, reaching 100 % at 10 mg/L for BABS ($p < 0.001$), 18 mg/L for *n*-ABS ($p < 0.001$) and 32 mg/L for APBS ($p < 0.001$). The results also showed that the three ABS tested significantly reduced *D. magna* survival at concentrations of 10 mg/L and greater for *n*-ABS and APBS, and 18 mg/L and greater for BABS (Fig. 2 A). Complete mortality was induced by the two highest concentrations (32 and 56 mg/L) of *n*-ABS and APBS ($p < 0.001$), but only by the highest concentration (56 mg/L) of BABS ($p < 0.001$). The lowest concentration causing a significant decline in survival was 10 mg/L for *n*-ABS (10 %, $p < 0.05$) and APBS (25 % $p < 0.01$), and 18 mg/L for BABS (15 %, $p < 0.01$). The relative toxicity of the ABS differed between endpoints. Although the 48-h EC50 of BABS was almost two times lower than that estimated for APBS, the obtained 48-h LC50 for BABS was nearly two times greater than APBS and *n*-ABS (Table 2). For both endpoints, the toxicity of *n*-ABS to *D. magna* was similar to that of APBS.

3.2.3. Hyalella azteca survival

Survival of *H. azteca* decreased rapidly at concentrations of 8.0 mg/L and above, dropping to 0 % at the two highest concentrations of *n*-ABS and BABS (20 and 50 mg/L) and 17 % at the highest concentration of APBS (50 mg/L; Fig. 2B). A strong and similar decrease in survival relative to controls occurred with *n*-ABS and BABS, while survival decreased more gradually with APBS. The 7-d LC50s for *H. azteca* were similar between *n*-ABS and BABS, but two to three times greater for APBS (Table 2).

3.2.4. Lampsilis fasciola glochidia viability

The first significant decrease in glochidia viability (standard method survival surrogate) was observed after 24 h of exposure to ≥ 6.2 mg/L of n-ABS (> 69 %, $p < 0.001$), APBS (> 20 %, $p < 0.01$), and ≥ 25 mg/L of BABS (0 %, $p < 0.001$; Fig. S1). Results also showed that all three ABS tested induced 100 % mortality at concentrations ≥ 25 mg/L and did not affect mussel viability at low concentrations (≤ 3.1 mg/L) (Fig. S1). Similar effects on viability were observed after 48 h of exposure, with significant decreases at concentrations ≥ 3.1 mg/L for APBS (> 30 %), ≥ 6.2 mg/L for *n*-ABS (80 %), and ≥ 25 mg/L for BABS (> 95 %) (Fig. 2 C). The toxicity ranking of the three ABS for *L. fasciola*, in order of

Table 2
Estimated ECx/LCx (and 95 % confidence intervals) of the three ABS tested in six aquatic species based on nominal and measured exposure concentrations. All data are expressed as mg/L.

		ECx /LCx based on nominal concentrations (mg/L)			ECx /LCx based on measured concentrations (mg/L)	
Species	**Endpoint**	***n*-ABS**[#]	**BABS**[#]	**APBS**	***n*-ABS**	**BABS**
C. reinhardtii	96h-EC10	0.51 (−0.06–1.08)	5.39 (−1.01–11.79)		0.46 (0.04–0.89)	1.7 (0.51–2.89)
	96h-EC25	2.31 (0.70–3.92)	12.36 (3.17–21.56)		1.95 (0.85–3.05)	2.54 (1.19–3.89)
	96h-EC50	8.69 (4.79–12.60)	25.56 (11.54–39.58)	> 100	6.85 (4.10–9.59)	3.80 (−1.21–8.82)
D. magna	48h- EC10	9.76 (7.75–11.76)	7.04 (6.76–7.33)	7.84 (6.79–8.89)	7.24 (6.99–7.50)	1.71 (1.7–1.72)
	48h-EC25	10.34 (7.43–13.26)	7.12 (6.84–7.40)	10.08 (9.17–11.00)	7.54 (7.12–7.97)	1.79 (1.78–1.80)
	48h-EC50	10.97 (2.55–19.39)	7.23 (6.96–7.50)	12.97 (12.01–13.92)	7.98 (6.56–9.4)	1.86 (1.85–1.87)
	48h- LC10	10.03 (9.35–10.72)	16.58 (15.78–17.39)	7.20 (6.19–8.22)	7.34 (6.74–7.94)	3.49 (3.29–3.68)
	48h-LC25	12.60 (12.06–13.13)	20.27 (19.57–20.97)	10.69 (9.80–11.58)	9.24 (8.77–9.71)	4.26 (4.10–4.43)
	48h-LC50	15.38 (15.02–15.74)	24.78 (24.11–25.45)	15.11 (14.31–15.91)	11.30 (10.99–11.60)	5.21 (5.06–5.37)
H. azteca	7d- LC10	3.78 (3.03–4.54)	4.56 (2.97–6.15)	3.61 (1.41–5.74)	2.78 (2.23–3.34)	0.96 (0.63–1.30)
	7d- LC25	4.62 (3.92–5.31)	5.85 (4.69–7.00)	7.81 (4.93–10.70)	3.40 (2.88–3.91)	1.23 (0.99–1.47)
	7d- LC50	5.63 (5.02–6.24)	7.26 (6.76–7.77)	16.92 (13.17–20.66)	4.14 (3.70–4.59)	1.53 (1.42–1.64)
L. fasciola	48h- EC10	5.15 (−0.16–10.46)	14.57 (7.63–21.50)	2.09 (1.68–2.49)	1.07 (0.03–2.12)	3.07 (1.61 – 4.53)
	48h- EC25	5.55 (1.93–9.16)	15.58 (6.30–24.86)	2.71 (2.31–3.10)	1.16 (0.45–1.88)	3.28 (1.33–5.24)
	48h-EC50	5.92 (4.04–7.80)	16.66 (4.42–28.91)	3.48 (3.03–3.92)	1.24 (0.87–1.62)	3.51 (0.93–6.09)
P. pilsbryi	7d- LC10	10.49 (7.04–13.95)	7.31 (3.84–10.77)	7.61 (4.68–10.54)	7.74 (5.42–10.06)	1.62 (1.08–2.16)
	7d- LC25	13.92 (9.21–18.64)	10.68 (8.05–13.31)	11.39 (8.48–14.31)	10.26 (6.90–13.62)	2.39 (1.87–2.91)
	7d- LC50	17.84 (9.56–26.11)	14.89 (8.35–21.42)	16.22 (11.86–20.57)	13.13 (7.06–19.20)	3.36 (2.57–4.15)
P. promelas	96h-EC10	1.61 (0.85–2.37)	5.05 (3.14–6.95)	6.64 (5.54–7.72)	1.18 (0.62–1.74)	1.06 (0.66–1.74)
	96h-EC25	2.94 (2.08–3.80)	8.50 (6.68–10.32)	7.61 (6.78–8.44)	2.16 (1.53–2.79)	1.78 (1.40–2.16)
	96h-EC50	4.99 (4.07–5.92)	13.41 (11.93–14.90)	8.57 (8.02–9.12)	3.67 (2.99–4.35)	2.81 (2.50–3.12)

Table 3
Change in the growth rate (expressed as day^{-1}) of *Chlamydomonas reinhardtii* after 96 h of exposure to *n*-ABS, BABS, and APBS.

	***n*-ABS**		**BABS**		**APBS**[#]
Nominal concentration (mg/L)	**Measured concentration (mg/L)**	**Growth rate (day^{-1})**	**Measured concentration (mg/L)**	**Growth rate (day^{-1})**	**Growth rate (day^{-1})**
0	0	0.56 ± 0.07	0	0.73 ± 0.02	0.67 ± 0.03
0.001	0.001	0.55 ± 0.02	0	0.70 ± 0.02	0.65 ± 0.03
0.01	0.01	0.59 ± 0.03	0.002	0.67 ± 0.02	0.65 ± 0.10
0.1	0.07	0.61 ± 0.01	0.02	0.62 ± 0.01	0.66 ± 0.01
1	0.74	0.45 ± 0.03	0.21	0.52 ± 0.08	0.60 ± 0.09
10	7.36	0.29 ± 0.02	2.09	0.54 ± 0.08	0.55 ± 0.02
100	73.56	−0.31 ± 0.01	20.91	−0.49 ± 0.01	0.56 ± 0.03

Data are expressed as mean growth rate ± standard deviation.
[#]: only nominal concentrations are available for this compound.
Significant differences from controls:* $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

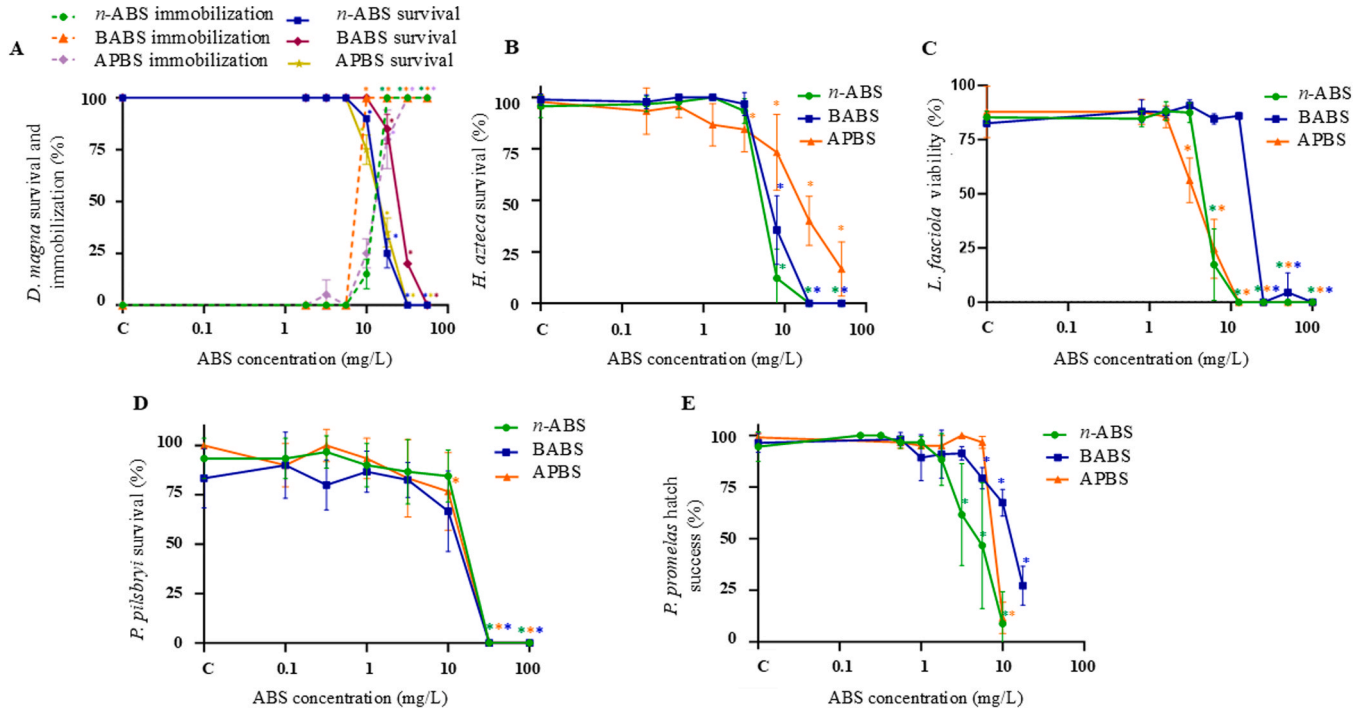


Fig. 2. Effect of *n*-ABS and BABS on the survival and immobilisation (%) of *D magna,* survival (%) of *H. azteca* (B), viability (%) of *L. fasciola* glochidia (C), survival (%) of *P. pilsbryi* (D), and on hatching success (%) of *P. promelas* (E). The letter 'C' on the x-axis represents control data points. Data are expressed as mean ± SD and the asterisks represent a significant difference from the controls.

most to least toxic, was APBS > *n*-ABS > BABS (Table 2).

3.2.5. *Planorbella pilsbryi mortality*

Fig. 2D summarizes the toxicity of the three ABS in snails after 7 d of exposure. All tested compounds caused 100 % mortality in snails at concentrations of 32 and 100 mg/L ($p < 0.001$). In addition, exposure to 10 mg/L of APBS resulted in a significant decrease in snail survival ($p < 0.05$), while exposure to concentrations of the three ABS below 10 mg/L did not cause any significant mortality (Fig. 2D). The toxicity ranking based on the 7-d LC50s in order of most to least toxic was BABS > APBS > *n*-ABS (Table 2).

3.2.6. *Pimephales promelas multiple endpoints*

The toxicity of the three ABS to fathead minnow embryos was assessed using the endpoints of time-to-hatch, hatching success (%), deformities (%), and length. Results indicated that time-to-hatch and heart rate were not affected by exposure to the different ABS (data not shown). In contrast, exposure to concentrations ≥ 3.16 mg/L of *n*-ABS led to a significantly decreased hatching success (reaching 91 % at 10 mg/L; $p < 0.001$) compared to the control groups, while exposure to concentrations ≥ 5.62 mg/L of BABS decreased embryo hatching success by 18 % ($p < 0.05$), 29 % ($p < 0.05$), and 69 % ($p < 0.05$), respectively (Fig. 2E). Only 10 mg/L of APBS led to significantly decreased hatching success by about 88 % ($p < 0.001$), an effect similar to that recorded with *n*-ABS at the same concentration (Fig. 2E). In addition, results showed a significant increase in the rates of deformities in newly hatched fry after exposure to concentrations ≥ 3.16 mg/L of *n*-ABS (33–71 %, $p < 0.05$) as well as 10 mg/L of APBS (14 %, $p < 0.05$) and BABS (25 %, $p < 0.05$) compared to the control group. Total lengths of fish were globally unaffected by APBS exposure. However, there were observations of slightly smaller lengths of newly hatched fathead minnows in the presence of *n*-ABS at 5.62 and 10 mg/L (5.36 and 5.06 mm, $p < 0.05$) and BABS at 10 and 17.78 mg/L (5.56 and 5.70 mm), in comparison with the control group (Table S6). In summary, hatch success was the most sensitive endpoint in 6-d embryo-larval testing and the relative toxicity of the three tested ABS (from most to least toxic) was *n*-ABS > APBS > BABS (Table 2).

4. Discussion

Considerable efforts have been invested in evaluating the adverse effects of anionic surfactants on the environment and understanding the relationship between the toxicity and the molecular structures of these surfactants (Cserháti et al., 2002, and references therein). However, the summarized ecotoxicological data are typically focused on the effects of LAS (HERA, 2013; see Table S7 for a review on ABS toxicity in aquatic species most relevant to the current study) and very little/no information has been published on BABS or APBS, which is of particular interest because APBS has been recently detected in municipal wastewater influent and effluent from various locations in Canada (Van Stempvoort et al., 2020). In this context, the goal of the current study was to assess and compare the potential effects of three ABS of interest on a comprehensive suite of six aquatic species, thus providing important toxicological information for these data-poor compounds in support of environmental risk assessment.

Comparisons among the ABS tested in the present study were based on nominal concentrations, as APBS could not be quantified. The results clearly indicated that the three tested ABS exhibited different acute toxicity to the six freshwater species studied, although each species differed in its sensitivity to these chemicals. This confirms observations from other studies indicating that the sensitivity of aquatic organisms to surfactants, including LAS, may vary among species and may be influenced by physiological and anatomical characteristics within systemic groups (Swedmark et al., 1971).

When comparing the toxicity outcomes among the studied species, algae exhibited greater variability in their sensitivity to ABS exposures; the 96-h EC50 values for growth rate varied more than 11-fold (8.69 mg/L to more than 100 mg/L) among compounds. These results are in agreement with those reported by Kimerle (1989) indicating that 90 % of algae species display a wide range of sensitivities to LAS, with EC50 values ranging from 0.1 to 100 mg/L. The lowest EC50 value in the current study was derived for *n*-ABS and that value was towards the low end of the range of toxicity reported for similar species, compounds, and exposure durations (0.9–116 mg/L; Table S7 and references therein). The observed decrease in cell growth after exposure to the three tested ABS was probably due to several adverse effects (such as cell wall damage, alterations of nutrient uptake, DNA synthesis, and protein turnover) induced by the surfactants as previously reported in other studies performed with LAS (Chawla et al., 1986, 1987). However, as only the growth rate of algae was evaluated in this study, further studies at structural, biochemical (such as biomarkers of oxidative stress, protein folding and turnover, membrane cell integrity and DNA damage) and molecular levels would be required to elucidate the mechanisms involved in ABS toxicity.

Daphnia magna was previously reported to be more sensitive to surfactants than several aquatic invertebrates (flatworm, roundworm, oligochaete, amphipod, isopod, midge) (Lewis and Suprenant, 1983). However, in the present study, *D. magna* was less sensitive to *n*-ABS than amphipods and mussels. This finding corroborates the ecotoxicological tests completed with alkyl ethoxy sulphates (AES) showing that *Daphnia* was more tolerant to this anionic surfactant ($4.2 < LC50 < 72$ mg/L) (BKH, 1994) than organisms from other trophic levels such as *Tubifex tubifex* and *Gammarus pulex* (LC50s ranged from 26.69 to 39.24 mg/L) (Bhattacharya et al., 2021; Singh et al., 2002). In comparison to the literature, the 48-h EC50 for *D. magna* immobilization after exposure to *n*-ABS (10.97 mg/L) calculated in the present study fell within the range of sensitivity reported for *D. magna* and other cladocerans (LC/EC50s = 3.0–26.94 mg/L; Table S7 and references therein). However, the 48-h LC50 (15.38 mg/L) generated in this study for *D. magna* exposed to *n*-ABS was 2–6 times greater than those previously determined for *D. magna* after exposure to C11.8 LAS (1.8–5.6 mg/L; Lewis and Suprenant, 1983) and to pure C11 and C12 LAS homologues (5.7 and 3.5 mg/L, respectively) (Kimerle and Swisher, 1977). As the toxicity of LAS has been reported to increase with chain length, notably in cladocerans (Table S7; Kimerle and Swisher, 1977; Verge et al., 2001; Belanger et al., 2016), differences could also be due in part to the chemical composition of *n*-ABS, as it contained various mixtures of C9–14 homologues with C11 as the most abundant congener, followed by C12.

Although *H. azteca* and mussel glochidia exhibited similar acute toxicity to *n*-ABS (LC/EC50s = 5.63 and 5.92 mg/L, respectively) in this study, mussels could be considered the most sensitive species to this surfactant as their EC50 was obtained after 48 h of exposure compared to the 7-d LC50 derived for *H. azteca*. In comparison to the literature, the calculated 7-d LC50 for *H. azteca* was in the same range as that reported for the same or similar species, exposure types and duration, and compounds (0.92–3.3 mg/L; Table S7). Although the effects of ABS on the early life stages of unionid mussels remain largely unknown, previous studies with marine mussels reported that LAS concentrations as low as 0.05 mg/L affected *M. edulis* fertility (Granmo, 1972), which is more than 100-fold lower than the EC50 for *n*-ABS reported in this study. Previously reported effects on adult mussels range from 4.4 to > 100 mg/L (Table S7). Mussels at later life stages tend to be less sensitive than at early life stages, as they can close their valves during exposure to toxicants, including LAS (Swedmark et al., 1971).

In contrast with mussels, snails were the least sensitive organism to *n*-ABS, with a difference of approximately four-fold between EC/LC50s for the two species. This suggests that *P. pilsbryi* is relatively more tolerant to *n*-ABS compared to the other species tested, possibly due to differences in mode of respiration (*P. pilsbryi* is a pulmonate snail that breathes at the surface, and therefore does not pass large volumes of water through their gills), since surfactants may have an influence on the respiration rate (Swedmark et al., 1971) and thereby on toxicity. The 7-d LC50 value for *n*-ABS (17.84 mg/L) generated in the current study for juvenile snails was in the same range as that reported for the pulmonate snail *Physa acuta* exposed for 24 h to LAS (16.65 mg/L) (Liwarska-Bizukojc et al., 2005), but was 13-fold higher than the mean 10-d LC50 value (1.39 mg/L) reported for the marine mud snail, *Hydrobiae ulvae* (Mauffret et al., 2010), a prosobranch snail (i.e., which breathes through gills).

Fathead minnows demonstrated a similar sensitivity to *n*-ABS as amphipods and mussels, with LC50s of 4.99, 5.63, and 5.92 mg/L, respectively. The LC50 obtained in our study agreed well with the range reported in the literature for fathead minnows (0.63–16.0 mg/L) and zebrafish (2.10–19.5 mg/L) when considering similar exposure durations and test compounds (Table S7 and references therein). Information regarding adverse effects of surfactants in early-life stages of freshwater fish is often reported and several studies have established that LAS is an embryotoxic agent. For example, embryos of zebrafish exposed at early developmental stages to concentrations up to 5 mg/L of C11.2 LAS group for 96 h had anomalies such as extra numerary eyes, yolk enlargement or distension, and smaller brain area (Dewese, 1974). This agrees with our findings showing that *n*-ABS induced deformities in newly hatched fry of fathead minnow at concentrations ≥ 3.16 mg/L. It has also been reported that acute exposure to LAS (0.3–6 mg/L) caused significant inhibitory effect on the fertilization success of seabream *Sparus aurata* sperm (Rosety et al., 2001). However, Hampel et al. (2002) reported that seabream larvae were three times more sensitive to C_{12}-LAS exposure than eggs, with 24h-LC50 values of 0.6 and 0.2 mg/L for eggs and larvae, respectively.

Comparisons of ABS toxicity across published data are challenging due to the lack of studies reporting LAS composition (i.e., measured or nominal, ambiguity in analysis of full homologous series, and influence of sodium ion), which may vary greatly (Kusk and Petersen, 1997). Moreover, it has been suggested that the toxicity of surfactants may differ depending on the molecular structures (linear versus branched), but there are discrepancies about the most toxic molecular form (Jackson et al., 2016). In fact, previous studies on freshwater and marine species (fishes, crustaceans, and algae) reported that acute toxicity of linear ABS was two to four times greater than branched ABS (Maggi and Cossa, 1973; Gard-Terech and Palla, 1986), and several acute studies

with fathead minnow reported that the toxicity of linear ABS was greater than branched ABS when young/adult fish and fish eggs were exposed to various commercial LAS (Hirsch, 1963; Pickering, 1966; Gledhill, 1974). Similarly, our results showed that the toxicity of *n*-ABS was greater than BABs for *D. magna*, *H. azteca* and *P. promelas* (LC50s were about 1.6, 1.3, and 2.7 times lower, respectively). However, this trend was not confirmed for mussels, snails, and algae, as the estimated EC/LC50s for both *n*-ABS and BABS were in similar ranges (overlapping confidence intervals). Due to the differences in experimental conditions (time of exposure, tested ABS, and exposure conditions), it is difficult to make comparisons among previous studies and assess which of linear or branched ABS were more toxic to the tested species.

In addition, it is known that comparing the sensitivity of species using measured concentrations is more reliable and accurate than nominal results (Jackson et al., 2016). Therefore, in the current study, based on the measured concentrations, it seems that *n*-ABS and BABS displayed similar toxicity for algae, mussels, and fish, while BABS was 2–4 times more toxic than *n*-ABS for the other species (Table 2). This difference in the sensitivity to branched and linear ABS could be due to differences in the behaviour of the two chemicals in the media. It has been reported that aquatic toxicity of linear (Kimerle and Swisher, 1977) and branched ABS (Gard-Terech and Palla, 1986) decreases during primary biodegradation, and therefore effects measured in the *n*-ABS and BABS experiments may be due to the parent compound as well as transformation products.

In general, biotransformation of ABS is affected by molecular architecture. In fact, linear alkyl substituents are less persistent and can undergo biotransformation more readily than branched alkyl substituents. Earlier research has shown that linear ABS undergo metabolism through ω-oxidation, which converts the terminal carbon to a carboxylic acid (i.e. sulfophenylcarboxylic acids). The sulfophenylcarboxylic acids are further biotransformed via α- and β-oxidation, which proceeds by shortening the alkyl chain. All of these processes transform the parent linear ABS into smaller and more water-soluble chemicals which are more easily excreted by the organism (Álvarez-Muñoz et al., 2007). Thus, it is possible that linear ABS may present lower toxicity due to faster biotransformation and excretion of metabolites. However, it is probable that the diversity of structures within the branched ABS umbrella may not allow generalization of toxicity trends for linear versus branched ABS. For example, Hall et al. (1989) did not find significant differences in toxicity between linear and branched forms of nonyl phenol ethoxylate surfactants to *Mysidopsis bahia*. Martinez et al. (1989) reported higher IC50 (concentration that inhibits an endpoint 50 % of exposed organisms) for a branched ABS mixture compared to linear ABS mixtures in acute studies with *Daphnia,* but the effect of chain length was not controlled and the specific branched composition was not determined. It is therefore not completely understood how the molecular composition and biodegradation pathways of ABS formulations influence their toxicity. In this context, and in order to contribute to structure activity models, future studies should focus on testing toxicity using individual purified standards that are well characterized for position of branching, position of substitution on the phenyl ring, and chain length**.**

From the current study, we observed that it is challenging to compare the toxicity of APBS with *n*-ABS and BABS, as there is little published toxicological information, the technical mixtures are chemically complex, and detailed chemical analysis is often not reported. The extent of LAS toxicity to aquatic organisms was reported to be closely related to the length of the carbon chain (Fendinger et al., 1994) (which is proportional to the molecular weight of the LAS used) and the phenyl position on the alkyl chain (Martinez et al., 1989). It has been suggested that the alkyl chains in APBS are linear, but this has not been confirmed (IMAP Group Assessment Report, 2016). In addition to the uncertainty about the diversity in chemical identity in APBS, there are no peer-reviewed studies examining APBS toxicity in aquatic species to date. However, data from the current study allow an initial ranking of ABS in terms of toxicity. According to the Globally Harmonized System of Classification and Labeling of Chemicals scheme (UNEP, 2011), a chemical is considered highly toxic if LC50/EC50 $\leq$ 1 mg/L, toxic if 1 < LC50 / EC50 $\leq$ 10 mg/L, harmful if 10 < LC50/EC50 $\leq$ 100 mg/L, and nontoxic if LC50/EC50 > 100 mg/L. Based on that classification system, *n*-ABS was generally the most toxic (toxic to 4 species, harmful to 2 species), BABS was less toxic (toxic to one species, harmful to 5), and the toxicity of APBS was variable depending on the species (Table 4). However, it is worth noting that ABS toxicity in this study is expressed in milligrams per liter and does not take into consideration the molecular weight of the chemicals and the proportion of active ingredient, so the relative toxicity of ABS may be different than assessed here. Therefore, further studies are required to confirm if and how the observed toxicity of BABS and APBS is related to active ingredients, which represent only 18 % of the tested formulation of BABS and are unknown for APBS.

A preliminary assessment of the relative sensitivity of the six aquatic species tested to ABS can be drawn therefore from the present study. Daphnids and snails were less sensitive; amphipods, mussels, and fish were more sensitive, and algal sensitivity was variable depending on the compound (Table 4**)**. Environmental factors such as media composition could explain at least in part sensitivity differences between organisms. Indeed, the bioavailability of anionic surfactants is influenced by the electrostatic properties of the hydrophilic head group, which can form ion pairs with divalent cations (e.g., Ca^{2+} or Mg^{2+}) (Yan et al., 2010). Lewis and Perry (1981) indicated that increased water hardness from 25 to 150 mg/L (as $CaCO_3$) significantly decreased the toxicity of LAS in *D. magna*. In the present study, although hardness may have contributed to the decreased sensitivity of freshwater snails (water hardness 170 mg/L + addition of calcium carbonate to support shell building), it did not explain the lower sensitivity of *D. magna* (water hardness 81–89 mg/L) compared to that of *H. azteca* (water hardness 170 mg/L). Therefore, differences among traits in the aquatic species tested likely contributed to the observed variation in sensitivity to ABS. In fact, it has been reported that physiological (e.g. metabolic capacity, detoxification mechanisms, and antioxidant defences), morphological, and anatomical characteristics (e.g., surface area-to-body mass scaling relationship) as well as behaviour, are factors determining sensitivity of organisms to toxic substances by influencing their ability to detect, absorb, tolerate, and detoxify these chemicals (Edo et al., 2024; Spurgeon et al., 2020). The relationship between organism traits and sensitivity to xenobiotics is therefore complex, and additional studies will be needed to better understand the mechanisms of action underlying the sensitivity or tolerance of these species to ABS.

5. Conclusion

In the present study, we assessed differences in the sensitivity of six freshwater species to environmental concentrations of three ABS compounds, using representative species from algae, crustaceans, mollusks, and fish. The most sensitive species to *n*-ABS were fish, mussels, and amphipods, while amphipods and mussels were the most sensitive to BABS and APBS, respectively. When expressed in nominal

Table 4
ABS toxicity according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). GHS hazard statements include Toxic if 1 < LC50 / EC50 $\leq$ 10 mg/L, harmful if 10 < LC50 / EC50 $\leq$ 100 mg/L, and nontoxic if LC50 / EC50 > 100 mg/L (UNEP, 2011).

Species	n-ABS	BABS	APBS
C. reinhardtii	Toxic	Harmful	Nontoxic
D. magna	Harmful	Harmful	Harmful
H. azteca	Toxic	Toxic	Harmful
L. fasciola	Toxic	Harmful	Toxic
P. pilsbryi	Harmful	Harmful	Harmful
P. promelas	Toxic	Harmful	Toxic

concentrations, snails had similar sensitivity to all surfactants and were among the most tolerant species tested. Given the complexity of the substances assessed and in the absence of pure analytical standards for BABS and APBS, confirmation of the concentrations of each substance was intricate (BABS) or impossible (APBS). This study provides important information addressing the knowledge gap on the aquatic toxicity of ABS, and the results will support risk assessment activities for this complex group of compounds.

CRediT authorship contribution statement

Hanana H.: Methodology, Validation, Formal analysis, Writing - Original draft and Editing. **Gilroy È.:** Methodology, Validation, Formal analysis, Writing-Review and Editing. **Bartlett A.:** Methodology, Validation, Formal analysis, Writing-Review and Editing. **Bennett C.J.:** Methodology, Investigation. **Brinovcar C.J.:** Methodology, Validation, Formal analysis, Investigation. **Brown L.:** Methodology, Validation, Formal analysis, Investigation. **Clarence S.:** Methodology, Validation, Formal analysis, Investigation. **De Silva A.:** Methodology, Validation, Writing-Review and Editing, Resources, Data Curation, Supervision. **Gillis P.:** Methodology, Validation, Writing-Review and Editing. **Hedges A.:** Methodology, Validation, Formal analysis, Investigation. **Khan H.:** Methodology, Validation, Formal analysis, Investigation. **Lavalle C.:** Methodology, Validation, Formal analysis, Investigation. **Parrott J.:** Methodology, Validation, Formal analysis, Writing-Review and Editing. **Pham-Ho V.:** Methodology, Investigation. **Salerno J.:** Methodology, Validation, Formal analysis, Investigation. **Shires S.:** Methodology, Validation, Formal analysis, Investigation. **Houde M.:** Methodology, Validation, Resources, Supervision, Writing-Review and Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118127.

Data availability

Data are available on the Canada Open Data portal." Link: https://data-donnees.az.ec.gc.ca/data/substances/assess/acute-toxicity-of-three-alkylbenzene-sulfonates-in-six-freshwater-aquatic-species?lang=en.

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