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REVIEW ARTICLE

Review of historical aquatic toxicity and bioconcentration data for the brominated flame retardant tetrabromobisphenol A (TBBPA): effects to fish, invertebrates, algae, and microbial communities

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Abstract

This paper summarizes the historical and recent research on the aquatic toxicology and bioconcentration potential of tetrabromobisphenol A (TBBPA), a major flame retardant in electronics. Historical studies on TBBPA are presented in detail, and are compared with more recent research. The historical studies have not been published to date, though they were pivotal in regulatory assessments by the European Union, Canada, and the USA. These assessments have enabled the use of TBBPA as a flame retardant in electronic applications, to the present. The studies were conducted under a Test Rule by the US Environmental Protection Agency in 1987, and were sponsored by member companies of the North American Flame Retardants Alliance (NAFRA) through the American Chemistry Council. The studies were conducted under Good Laboratory Practice procedures, and include 6 acute toxicity tests of TBBPA with fish, invertebrates, algae, and microbes, eight chronic tests, and three bioconcentration studies with fish and invertebrates. Methods and empirical data for each study are detailed in an electronic supplement. Results of the NAFRA studies are compared with recent findings on TBBPA toxicity. Molluscan shell growth may be uniquely sensitive to TBBPA, more sensitive than chronic fish or crustacean toxicity endpoints. Several of the NAFRA studies and several independent studies have reported toxicities exceeding the empirical water solubility limits of TBBPA (in the range of 2.0 mg/L depending on pH). The validity of these results is discussed.

Keywords Tetrabromobisphenol A (TBBPA) · Ecotoxicity · Bioconcentration · Sediment toxicity · Aquatic toxicity

Introduction

Tetrabromobisphenol A (TBBPA; CAS RN 79-94-7) (Fig. 1) is a widely used flame retardant in printed circuit boards and electronic enclosures, with a historical use of over 30 years (see e-supplement). It is among the most common flame retardant for electronic applications in the USA, Canada, and Europe. In 2011, US production was 120 million pounds (U.S. EPA 2015). Global production is estimated as 200,000 metric tons, the highest production volume among brominated flame retardants on the market (Howard and Muir 2010).

TBBPA (Fig. 1) is a highly effective flame retardant whose primary use is in printed circuit boards where it is chemically reacted into the epoxy resin backbone. A secondary use is as an additive flame retardant in electronic enclosures. Flame retardancy in these applications is needed given the inherently flammable nature of the substrates coupled with electric current. Manufacture and industrial use prior to incorporation into polymers represent points of potential release.

Summaries of the physical/chemical properties, commercial uses, environmental releases, and risk potential of TBBPA

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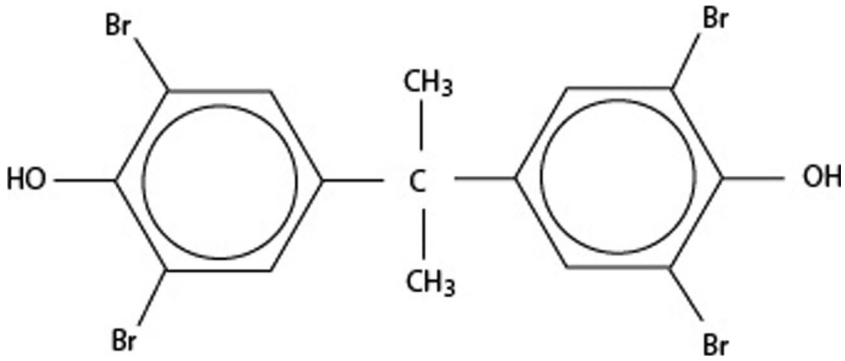

Fig. 1 Chemical structure of tetrabromobisphenol A (TBBPA)

have been published by Environment Canada (2013) and by the European Union (2008). TBBPA is a solid at room temperature, with the following properties that significantly influence the toxicity data presented here: vapor pressure < 1.19×10^{-5} Pa at 20 °C (US EPA 1987b; EC 2013); log Kow 5.903 at 25 °C (NAFRA 2001; EC 2013); and pKa 6.79 (1st) and 7.06 (2nd) (EU 2008; EC 2013). The water solubility of TBBPA increases with pH, e.g., 0.148 mg/L at pH 5; 1.26 mg/L at pH 7; and 2.34 mg/L at pH 9 (McAvoy et al. 2016).

TBBPA's toxicological database relating to human health and environmental effects dates to the late 1960s (U.S. EPA 1987a; EC 2013). A substantial body of information has been historically produced to satisfy product labeling, handling, and disposal, as well as chemical control regulations in various international jurisdictions including the European Union, Canada, and the USA. In the 1980s, an aquatic fate and effects testing program of TBBPA was initiated by manufacturers and member companies of the North American Flame Retardants Alliance (NAFRA) formerly titled the Brominated Flame Retardant Industry Panel (BFRIP). In 1987, the U.S. Environmental Protection Agency (EPA) issued an Aquatic and Environmental Fate Test Rule on TBBPA, recommending a broad series of biodegradation, aquatic toxicology, and bioconcentration tests (U.S. EPA 1987a, b). Prior to the Test Rule, a number of toxicity studies had already been completed or had commenced.

The testing program under the 1987 Rule was conducted and sponsored by NAFRA, including the major manufacturers: Chemtura, Albemarle, and ICL-IP. In 2001, NAFRA finalized testing and submitted a Data Summary and Test Plan to the U.S. EPA (updated in 2003 and 2005) (NAFRA 2001). In 2008, the European Union issued their Risk Assessment Report (EU 2008). In 2013, Environment Canada published their Screening Assessment Report (EC 2013). In these reports, both the EU and Environment Canada reviewed extensive data and concluded that TBBPA could continue to be used as a flame retardant for electronics applications. TBBPA is in commercial use internationally. Although there are no restrictions on the production of TBBPA, it was included in the OSPAR list of chemicals for priority action under the Hazardous Substances Strategy (OSPAR 2007). (OSPAR is a commission of the United Nations that works for the protection and preservation of the marine environment.) Despite TBBPA's historical use and close regulatory scrutiny, the empirical results of the NAFRA studies have not been published to date. The ecological risk assessments by EC (2013) and the EU (2008) cite extensive data, including the NAFRA study results. Neither EC nor the EU could publish the full contract laboratory study reports, including methods and empirical data. The American Chemistry Council commissioned the authors to summarize the study reports for publication, to enable access by the science community at-large. Given the active research on TBBPA today, and its relevance to bisphenol A (a controversial breakdown product), the data provided in this paper can help researchers find new research paths.

In this paper and two companion papers, NAFRA study reports were condensed from the original contract lab study reports, and supplemented with detailed electronic attachments. This paper summarizes the early data on TBBPA's aquatic effects and bioconcentration trends, and compares the results with extant studies conducted to the present. This publication enables synthesis and comparison of the results from the entire suite of TBBPA studies conducted across an 18-year time frame, and provides these data contrasted with the current exposure and hazard data available. Biodegradation studies of TBBPA in anaerobic digester sludge, surface waters, soils, and sediments conducted under the same initiative have been separately published (McAvoy et al. 2016). A third paper is being prepared to this journal to summarize key terrestrial toxicity studies, entitled: Review and summary of historical terrestrial toxicity data for the brominated flame retardant tetrabromobisphenol-A (TBBPA): Effects to soil microorganisms, earthworms, and seedling emergence. Publication of each of these papers has been sponsored by three companies (Albemarle, Chemtura, and ICL-IP), which collaborated in the testing program and regulatory submissions with coordination through the American Chemistry Council. Together, the three papers summarize the bulk of early fate and ecological effects research on TBBPA. They also compare the results with more recent independent research on TBBPA's ecotoxicity.

Materials and methods for the NAFRA TBBPA studies

Due to space considerations, the individual methods employed in each study are detailed in the e-supplement. However, specific details are summarized here.

Test chemical

The Industry Panel (BFRIP) coordinated consistent preparation and distribution of TBBPA test substances for toxicity testing. Manufacturers of the test substances were: Great Lakes Chemical Corp., Ethyl Corp., Albemarle Corp., and Bromine Compounds Ltd. Several specialty chemical manufacturers prepared the ^{14}C-TBBPA test substances used in bioconcentration testing. Trace concentrations of carrier solvents (acetone, dimethyl formamide, and dimethyl sulfoxide) were employed, as was customary at the time, to facilitate TBBPA's dissolution in toxicity testing. The potential impacts of these solvents on toxicity results are discussed in the Summary (Section 5).

Historical test protocols

The test protocols used in these studies reflect the historical standards and guidelines available in the early 1980s and 1990s, when standardized ecological toxicity and fate testing protocols were still undergoing development. Leading standard-setting organizations [i.e., the Organization for Economic Cooperation and Development (OECD), the U.S. EPA; the American Society for Testing and Materials (ASTM Committee D47)] were closely involved in the development of test protocols. While the TBBPA studies were considered state-of-the-art at the time, some of the protocols have since been updated. They were conducted by reputable contract testing laboratories, and followed existing Good Laboratory Practices (GLP) protocols. For certain older (pre-1990) studies, in-house test protocols of the contract laboratories followed government and/or standard-setting institutional guidelines (e.g., ASTM). Where GLP study standards had not been developed, the laboratories applied quality guidelines available in-house. This includes standard analytics of nominal and mean measured concentrations of TBBPA in test media, either by High Performance Liquid Chromatography (HPLC) or using ^{14}C-TBBPA in radiometric analyses. Additional details of the specific methods for each study can be found in the e-supplement.

Test metrics and reporting conventions

The NAFRA studies also report toxicity test metrics in use at the time. Some of the metrics have since evolved. This is particularly true for certain chronic effect endpoints [e.g., no observed effect concentration (NOEC), lowest observed effect concentration (LOEC), maximum acceptable toxicant concentration (MATC), and effective concentration for 10% of the population (EC_{10})]. Here, we have elected to report the data and metrics in their original forms, without alteration or amendment. The study summaries in the e-supplement often cite *verbatim* the text used in the original study reports. Figures, tables, calculations, and statistics from each study are also reproduced in their original form, to the extent possible.

TBBPA aquatic toxicity testing results

Key results for the acute and chronic toxicity and bioconcentration tests conducted through NAFRA are presented below and in the e-supplement, and are compared with extant studies retrieved from a recent literature survey. The NAFRA studies presented in Tables 1, 2 and 3 are each cross-referenced to respective reports in the e-supplement. For each study, the supplement contains: 1. A condensed summary of the original laboratory study report, with testing and analytical methods; 2. The empirical data upon which the test metrics were estimated; and 3. Analyses of the relevance of the NAFRA studies in comparison with more recent data cited here.

Acute toxicity

Results of the six acute studies of TBBPA with fish, invertebrates, algae, and a microbial community conducted through NAFRA are reported in Table 1; the detailed methods and key empirical data are contained in the e-supplement. TBBPA's 96-h LC_{50} values were similar for the fathead minnow *Pimephales promelas* (Springborn Life Sciences 1988b) and rainbow trout *Oncorhynchus mykiss* (Wildlife International 2003b), with respective 96-h LC_{50} values of 0.54 and 1.1 mg/L. These estimates are consistent with results of independent fish and invertebrate studies. Godfrey et al. (2017) reported a 96-h LC_{50} for TBBPA of 1.3 mg/L (1.1–1.6 mg/L) to zebrafish embryos. This study utilized standardized methods for the parameters they describe: pH = 7–7.5; temperature = 28 °C; and photoperiod = 14 L:10D. For the copepod *Acartia tonsa,* Wollenberger et al. (2005) reported a 48-h LC_{50} of 0.40 mg/L (0.37–0.43 mg/L). This study was conducted under standard ISO international texting guidelines (Wollenberger et al. 2005). The 48-h *Daphnia magna* EC_{50} > 1.8 mg/L reported here is close to the solubility limit for TBBPA, as discussed below (Wildlife International 2003a). A summary of the NAFRA studies and the effect levels can be found in Table 1; data from the additional publications can be found in the scientific literature.

NAFRA reported the 96-h EC_{50} of TBBPA for the alga *Selenastrum capricornutum* as > 5.6 mg/L (Table 1)

Table 1 TBBPA acute toxicity studies with aqueous exposure. See e-supplement for detailed study protocol citations, and summaries of methods, empirical data, and discussions

Test organism and study duration	Contract laboratory	Toxicity endpoint(s)	Reported LC_{50} / EC_{50} (95% C.L.)	Remarks
48-h cladoceran (*Daphnia magna*)	Wildlife International (2003a)	Mortality; immobility	48-h LC_{50} > 1.8 mg/L NOEC = 1.8 mg/L	No mortality at either of two concentrations tested, 1.2 and 1.8 mg/L.
96-h eastern oyster (*Crassostrea virginica*)	Springborn Life Sciences (1989a)	Mortality; shell growth	96-h LC_{50} > 0.15 mg/L 96-h EC_{50} = 0.098 mg/L (0.020–0.210) 96-h NOEC < 0.018 mg/L	Mean measured concentrations in seawater were 0.018, 0.032, 0.051, 0.087, and 0.150 mg/L. No mortality at any test concentration. Toxicity estimates based on shell growth.
96-h green alga (*Selenastrum capricornutum*)	Springborn Life Sciences (1988a)	Algal cell density	96-h EC_{50} > 5.6 mg/L	Mean-measured concentrations in algal growth medium were: 0, 0.34, 0.76, 1.5, 3.0, and 5.6 mg/L. Stimulation in cell densities was observed relative to controls. Precipitate observed; likely exceeded solubility limit.
96-h rainbow trout (*Oncorhynchus mykiss*)	Wildlife International (2003b)	Mortality; lethargy	96-h LC_{50}/EC_{50} = 1.1 mg/L 96-h NOEC < 1.1 mg/L	Two mean-measured concentrations were tested: 0, 1.1, and 1.7 mg/L. NOEC based on mortality and lethargy.
96-h and 144-h fathead minnow (*Pimephales promelas*)	Springborn Life Sciences (1988b)	Mortality	96-h LC_{50} = 0.54 mg/L (0.45–0.63) 144-h LC_{50} = 0.49 (0.45–0.63) 144 h NOEC = 0.26 mg/L	Mean-measured ^{14}C TBBPA concentrations were: 0.63, 0.45, 0.32, 0.26, and 0.19 mg/L. Assay was extended from 96- to 144-h to assess toxicity trend.
3-h activated sludge (microbial community)	Wildlife International (2002a)	Respiration rate inhibition (mg O2/L/h)	3-h NOEC > 15 mg/L	Limit test at one TBBPA concentration (exceeded solubility limit). No significant inhibition in respiration rate.

Table 2 Chronic studies of TBBPA in aqueous and dosed-sediment exposures to fish and invertebrates. See e-supplement for detailed study protocol citations, and summaries of methods, empirical data, and discussions

Test organism and study duration	Contract laboratory	TBBPA dosing regimen	Reported EC_{50}/LC_{50} (95% C.L.)	NOEC	LOEC	Remarks
Aqueous exposures to TBBPA						
14-day, 2nd instar midge larvae (*Chironomus tentans*)	Springborn Laboratories (1989)	Mean measured: 0. 0.07, 0.12, 0.20, 0.41, and 0.85 mg/L	LC_{50} = 0.13 mg/L (0.11–0.15) based on survival	< 0.07 mg/L based on growth 0.12 mg/L based on survival	0.07 mg/L based on growth 0.2 mg/L based on survival	Larval growth reduced at all doses tested. Survival reduced at 0.20 mg/L and higher doses. Precipitate observed at highest concentration (0.85 mg/L).
21-day cladoceran (*Daphnia magna*)	Springborn Life Sciences (1989b)	Mean measured: 0, 0.056, 0.10, 0.19, 0.30, and 0.98 mg/L	Not reported	> 0.30 mg/L based on reproduction	≥ 0.98 mg/L based on survival, growth, and reproduction	Decrease in number of offspring at highest concentration. No effect on daphnid survival or growth.
35-day fathead minnow embryos and larvae (*Pimephales promelas*)	Springborn Life Sciences (1989c)	Mean measured: 0, 0.024, 0.040, 0.084, 0.16, and 0.31 mg/L	Not reported	0.16 mg/L based on embryo and larval survival	0.31 mg/L based on embryo and larval survival	Reduced day 5 embryo survival and day 30 larval survival at 0.31 mg/L. No effects on growth.
70-day blue mussel (*Mytilus edulis*)	AstraZeneca (2005a, b)	Mean measured: 0, 0.017, 0.032, 0.062, 0.126, and 0.226 mg/L	Not reported	0.017 mg/L based on shell length and dry tissue weight 0.062 mg/L based on wet tissue weight	0.032 mg/L based on shell length and dry tissue weight 0.126 mg/L based on wet tissue weight	No effects on mussel survival at any concentration.
Dosed sediment exposures to TBBPA						
28-day midge larvae (*Chironomus riparius*)	Wildlife International (2005)	Nominal: 0, 63, 125, 250, 500, and 1000 mg/kg dw	EC_{50} = 235 mg/kg (207–268) based on percent emergence	125 mg/kg based on emergence ratio, development rate, and development time	250 mg/kg based on emergence ratio, development rate, and development time	Significant differences in emergence ratio, development rate, and development time at 250 mg/kg and higher.
28-day amphipods (*Hyalella azteca*)	Wildlife International (2006)	Nominal: 63, 125, 250, 500, and 1000 mg/kg dw	LC_{50} = > 1000 mg/kg based on survival	250 mg/kg based on survival	500 mg/kg based on survival	Decreased survival at 500 and 1000 mg/L after 28 days. No effects on growth.
28-day oligochaete (*Lumbriculus variegatus*): Two studies at 2.5% and 5.9% sediment organic carbon (SOC)	Wildlife International (2002c) 2.5% OC; (2002d) 5.9% OC	Mean measured: 0, 90, 151, 254, 426, 715, and 1200 mg/kg dw	2.5% OC EC_{50}/LC_{50} = 294 (140–391) mg/kg based on survival and reproduction 5.9% OC EC_{50}/LC_{50} = 405 (314–869) mg/kg	2.5% OC: 90 mg/kg based on survival/ reproduction 5.9% OC: 254 mg/kg based on survival, reproduction, and growth	2.5% OC: 151 mg/kg based on survival/reproduction 5.9% OC: 426 mg/kg based on survival, reproduction, and growth	Increased NOECs and LOECs at higher sediment OC, similar with *C. tentans* results (Table 3).

Table 3 Bioconcentration (BCF) studies of TBBPA with fish and invertebrates

Test organism and study duration	Contract laboratory	Mean measured ^{14}C-TBBPA concentration in overlying water	^{14}C-Whole-body tissue residue concentrations at steady-state	BCFs estimated assuming no TBBPA metabolism	Percentage of parent TBBPA remaining in whole body tissues at steady-state	BCFs of parent TBBPA estimated by the EU [5]
30-day fathead minnow (*Pimephales promelas)*	Springborn Life Sciences (1989d)	4.7 μg/L	5800 ± 1300 μg/kg	1200 (900–1700)	13%	156
34-day Eastern oyster (*Crassostrea virginica*)	Springborn Life Sciences (1989e)	1 μg/L	720 ± 160 μg/kg	720 (±160)	20.60%	148
14-day midge (*Chironomus tentans*)	Springborn Laboratories (1989)	BCF estimates based on pore water concentrations	Not reported	240–510: High organic carbon 490–1100: Middle organic carbon 650–3200: Low organic carbon	No data. Did not distinguish parent TBBPA from metabolites	Not estimated

Table 4 Comparison of the effects of TBBPA exposure on shell deposition (growth) in mussels in 96-h and 70-day assays. Asterisks (*) indicate significant differences ($p = 0.05$) between the treatment and pooled control

70-day study in *Mytilus* (AstraZeneca 2005a, b)	Negative control	Solvent control			Mean measured TBBPA concentration (mg/L)		
			0.017	0.032	0.062	0.126	0.226
	0.642 ± 0.182	0.576 ± 0.159	0.532 ± 0.171	0.498 ± 0.137 *	0.432 ± 0.121 *	0.302 ± 0.138 *	0.234 ± 0.147 *
96-h study in *Crassostrea* (Springborn Life Sciences 1989a)	Negative control	Solvent control			Mean measured TBBPA Concentration (mg/L)		
			0.018	0.032	0.051	0.087	0.150
	1.6 ± 1.1	1.3 ± 0.8	1.0 ± 1.0*	1.0 ± 0.8*	0.8 ± 0.9*	0.8 ± 0.8*	0.6 ± 0.7*

(Springborn Life Sciences 1988a). This value exceeds TBBPA's water solubility estimates determined by Wildlife International (2002b): 1.26 mg/L at pH 7, and 2.34 mg/L at pH 9. In a different study, algal toxicity estimates by Walsh et al. (1987) were consistent with the solubility limits determined by NAFRA (See discussion for more details on solubility limits). The Walsh et al. (1987) study did not provide details on the standardized methods utilized in their study, including water quality parameters. However, the NAFRA study estimates of 72-h EC_{50} values varied from 0.09–0.89 mg/L for *Skeletonema costatum,* and from 0.13–1.0 mg/L for *Thalassiosira pseudonana*, which were below the TBBPA solubility limits.

Among the TBBPA acute tests, a particularly sensitive acute endpoint in an invertebrate species was for shell growth in the Eastern oyster (*Crassostrea virginica*) (Springborn Life Sciences 1989a). Significant effects on shell growth were observed after 96 h exposure to the lowest (mean measured) TBBPA concentration tested, 0.018 mg/L (Table 4). No effects on oyster mortality or siphoning behavior were observed, even at a nearly 10-fold higher dose of 0.15 mg/L. Effects on molluscan shell growth in a 96-h assay are somewhat unusual, as these types of effects typically take longer to manifest (i.e., chronic duration studies). It is worth noting that the 96-h study had high standard derivations relative to the mean, in some cases exceeding the mean. However, similar results were observed in an initial 96-h assay at higher doses, and the percent reduction in shell deposition increased with the concentration of TBBPA showing a clear dose-response for this effect. Data generated during both sets of exposures produced similar concentration response curves and similar effect levels. This lends further weight to the effect being a real effect even given the short exposure time frame. Finally, these effects were corroborated by the 70-day study with the mussel, *Mytilus edulis* (Table 4). In the *M. edulis* study, the NOEC and LOEC based on shell growth and dry tissue weight were 0.017 mg/L and 0.032 mg/L, respectively (AstraZeneca 2005a, b) (see e-supplement). From results of the *C. virginica* acute and *M. edulis* chronic studies, molluscan shell growth is a uniquely sensitive endpoint for TBBPA. As the NAFRA acute toxicity studies did not include embryo-larval survival or reproductive indices, extant studies reporting chronic endpoints are discussed below in Section 3.2.

Chronic toxicity

NAFRA sponsored eight chronic toxicity studies of TBBPA with seven test organisms, summarized in Table 2. Four of these studies used aqueous exposures of TBBPA to the midge *Chironomus tentans*, fathead minnow *P. promelas,* cladoceran *D. magna*, and the mussel *M. edulis*. Four of the additional NAFRA studies tested sediments dosed with TBBPA in two tests with the oligochaete *Lumbriculus variegatus*, and single tests with the amphipod *Hyalella Azteca* (Wildlife International 2006) and midge larvae *Chironomus riparius* (Wildlife International 2005).

Though the test organisms varied, comparisons between aqueous and dosed-sediment exposures to TBBPA collectively suggest that aqueous exposure is significantly more toxic than sediment exposure, suggestive of TBBPA sorption to sediment and sequestration. TBBPA NOECs for the three benthic species ranged from 90 to 250 mg/kg dry weight sediment. In aqueous exposure studies, NOEC estimates were each less than 1 mg/L TBBPA. In the *C. tentans* study (Springborn Laboratories 1989), the reported 14-day NOEC was < 0.07 mg/L, based on growth endpoints. For the congeneric midge species tested (*C. tentans* and *C. riparius*) under both aqueous and sediment exposures, the presence of sediment appeared to attenuate TBBPA toxicity at least on the basis of comparative EC_{50} values. For *C. tentans* in aqueous exposure, the EC_{50} was 0.13 mg/L (0.11–0.15 mg/L), based on survival. For *C. riparius* in dosed sediments, the EC_{50} was 235 mg/kg (207–268 mg/L) based on percent emergence (Wildlife International 2005). The mitigation of TBBPA toxicity by sorption to sediment is consistent with TBBPA's high sorption coefficient (log Koc 4.52–5.43), and results of Level III EQC (EQuilibrium Criterion) fugacity modeling (EC 2013; EQC 2003). Among the three sediment dosing studies with the midge *C. riparius*, oligochaete *L. variegatus,* and amphipod *H. azteca, L. variegatus* was most sensitive. In tests comparing 2.5% (Wildlife International 2002c) and 5.9% (Wildlife International 2002d) sediment organic carbon (SOC) levels, toxicity was reduced at the higher SOC level. The NOEC increased from 90 mg/kg at 2.5% SOC, to 254 mg/kg at 5.9% SOC. The sorption of TBBPA in sediments, and effects on toxicity mitigation, was directly related to SOC.

In the 35-day TBBPA exposure studies, similar sensitivities were observed in the embryo/larval survival of the fathead minnow, and in reproduction in *D. magna* (Springborn Life Sciences 1989c). Respective NOEC/LOEC values were 0.16/0.31 mg/L for the minnow, and 0.30/0.98 mg/L for *Daphnia*. In independent research, Carlsson and Norrgren (2014) reported a LOEC of 1000 μg/L (1 mg/L) TBBPA in short-term (24 and 48 h) exposures to embryos of two frog and one fish species: *Xenopus (Silurana) tropicalis*, *Rana arvalis*, and *Danio rerio*. Sub-lethal effects included edema, lack of spontaneous movement, and decline in embryo heart rate; no mortality was observed. The Carlsson and Norrgren (2014) studies were published as a short communication, and therefore little data on test parameters were presented. However, they cite standard guidelines in their methods suggesting their studies conformed to standard toxicity testing guidelines. In a partial life-cycle exposure of TBBPA to zebrafish adults and their embryos, Kuiper et al. (2007) reported effects on egg

production, juvenile survival, and gender development of offspring at TBBPA-body burdens around 5–7 mg/g lipid.

As noted above, a more sensitive endpoint was the mussel *M. edulis* shell growth response to TBBPA after 70 days. NOEC and LOEC values (0.017 and 0.032 mg/L, respectively) were an order of magnitude lower than in *D. magna* (AstraZeneca 2005a, b). No effects on mussel survival were observed at any TBBPA concentration up to 0.226 mg/L. In a study of TBBPA effects on zebrafish egg production, similarly low toxicity values (30-day NOEC 0.013 mg/L) have been reported (Kuiper et al. 2007; EC 2013). Hence, particularly sensitive species and endpoints have been reported in TBBPA studies with both fish and mollusks.

Bioconcentration results

Three bioconcentration studies are summarized with the fathead minnow, Eastern oyster, and midge *C. tentans* (Table 3). The 30-day study with the fathead minnow (Springborn Life Sciences 1989d) and the 34-day study with the Eastern oyster (Springborn Life Sciences 1989e) included sufficient analytical detail to enable the European Union (2008) to calculate credible and consistent estimates of bioconcentration factors (BCFs) for the parent TBBPA molecule. Polar (unidentified) metabolites accounted for 87% of total ^{14}C-activity in the minnows after 30 days, and 79% in oysters after 34 days. The midge study did not distinguish between parent TBBPA and metabolites; consequently, the midge BCF estimate is deemed a less reliable estimate of BCF.

The original contract lab study reports reported BCFs of 1200 for the fathead minnow, and 720 for the oyster. Neither estimate accounted for the presence of metabolites. The respective BCFs were subsequently re-calculated by the EU (2008) and were proportionally lower, consistent with the metabolites found in the minnow and oyster tissue residues. BCFs calculated by the EU (assuming 87 and 79% metabolite contributions to ^{14}C tissue residues) were 156 for the fathead minnow, and 148 in oysters. In both studies, TBBPA reached steady-state tissue concentrations within 4–5 days, and relatively short depuration half-lives were calculated (< 1 day for fish, 3–5 days for oysters). The fathead minnow BCF estimate was independently corroborated in a 28-day study with the bluegill sunfish, *Lepomis macrochirus* (Stoner Laboratories, Inc. 1978). BCFs in sunfish were estimated to be 20 in edible tissue and 170 in visceral tissue. Concentrations in both tissues decreased rapidly through a 14-day depuration period.

The 14-day *C. tentans* midge study (Springborn Life Sciences 1989b) did not distinguish TBBPA metabolites, as was done for the fathead minnow and the oyster. Therefore, the reported BCF of 240 to 3200 mg/L (based on pore water concentrations) likely overestimates the BCF. This is consistent with the EU (2008) conclusion that estimates derived from ^{14}C-measurements alone may overestimate TBBPA BCF. The midge study also compared accumulation trends in three sediments with varying organic carbon content. The respective BCFs for each sediment (though uncorrected for metabolism) decreased with increasing sediment organic carbon content. Like the *Lumbriculus* chronic study (Table 2), sediment organic carbon was shown to significantly sequester TBBPA from *C. tentans*.

Discussion

Sensitive taxa and endpoints

Molluscan shell growth was the most sensitive aquatic taxa and endpoint tested by NAFRA. Effects included reduced shell growth in a 96-h exposure to the Eastern oyster (*C. virginica*) and in a 70-day exposure to the mussel *M. edulis*. No effects on oyster mortality or siphoning behavior were observed in either study. In the *Mytilus* study, the NOEC and LOEC based on shell growth and dry tissue weight were 0.017 mg/L and 0.032 mg/L, respectively. In another study of TBBPA's effects on zebrafish egg production, similarly low toxicity values (30-day NOEC 0.013 mg/L) have been reported (Kuiper et al. 2007; EC 2013). Hence, particularly sensitive species and endpoints have been reported in TBBPA studies with both fish and mollusks.

Water solubility considerations in TBBPA aquatic toxicity

Solubility limits were tested by NAFRA under the EPA's Test Rule, and reported by Wildlife International (2002b). Water solubility of TBBPA is both pH- and temperature–dependent (Kuramochi et al. 2008). Water solubility in the NAFRA study increased from: 0.148 mg/L at pH 5; to 0.24 mg/L at pH 7; to 2.34 mg/L at pH 9. An early Velsicol Corporation report (as cited by Environment Canada 2013) reported solubility limits ranging from 0.72–4.16 mg/L at neutral pH. From these data, we conclude that TBBPA toxicity estimates greater than 4.16 mg/L are inaccurate and unrealistic. This review has identified a number of TBBPA toxicity estimates that exceeded empirical water solubility ranges at relevant pH levels [OECD recommends pH of water during acute toxicity testing should be in the range of 6.5–8.5, with variation throughout the test of ±0.5 pH units (OECD 2013)]. These include the *Selenastrum* and activated sludge toxicity studies reported here, and algal toxicity estimates reported by Debenest et al. (2010, 2011). The 96-h EC_{50} of TBBPA to the alga *S. capricornutum* was > 5.6 mg/L (Table 1) (Springborn

Table 5 Summary of TBPPA Ecological Risk Assessment Conclusions by International Regulatory Authorities

Environment Canada (EC 2013)	"Based on the information available, there is currently low risk of harm to organisms or the broader integrity of the environment from TBBPA... It is therefore concluded that TBBPA… [does] not meet the criteria set out in paragraphs 64(a) or 64(b) of CEPA 1999, as these substances are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends".
European Union (EU 2008)	Aquatic: "The risk characterization ratios indicate that risk to surface water is low from regional sources, and also from manufacturing and processing of epoxy and polycarbonate resins, where tetrabromobisphenol-A is used as a reactive flame retardant, and conversion sites where tetrabromobisphenol-A is used as an additive flame retardant in ABS. For the other areas, the risk characterization ratios indicate a risk. Sediment: "No risks to sediment are currently identified from the formation of bisphenol-A via degradation of tetrabromobisphenol-A for any use. However, further work is ongoing at present within the UK that could affect the aquatic and hence sediment PNEC. This conclusion should therefore be re-examined when the sediment PNEC for bisphenol-A has been finally agreed. This applies to the assessment for sediment from regional sources, and also from manufacturing and processing of epoxy and polycarbonate resins and conversion sites using tetrabromobisphenol-A as an additive flame retardant in ABS".
OSPAR (2011)	Marine: "The risk assessment for the marine environment indicates a potential risk to water and sediment from the compounding step for the additive uses of tetrabromobisphenol-A in ABS. The manufacture and processing of epoxy and polycarbonate resins, and the conversion step for ABS, do not appear to present a risk. It would be possible to revise the PECs for the other endpoints by collection of further exposure information. Industry has indicated that none of the major manufacturing sites in the EU using tetrabromobisphenol-A as a reactive flame retardant, or compounding sites using tetrabromobisphenol-A as an additive flame retardant, are situated close to coastal areas, and that the sole ABS plant in the EU where a risk was identified has since closed."

Life Sciences 1988a). The 3-h limit test with activated sludge microorganisms estimated a NOEC > 15 mg/L (Wildlife International 2002a). Debenest et al. (2010, 2011) reported 72-h algal EC_{50} values for *Pseudokirchneriella subcapitata* and *Nitzschia palea* ranging from 5 to 250 mg/L.

The effect of carrier solvents used in toxicity studies with TBBPA was one possible factor considered for those studies reporting TBBPA effect levels above solubility limits. Use of carrier solvents for poorly soluble chemicals such as TBBPA was not uncommon in early testing protocols, but its relevance has been questioned (Green and Wheeler 2013). Typically, the potential for solvent toxicity was addressed with a solvent-only control. In the fathead minnow and rainbow trout acute studies reported here, trace concentrations (typically 100 μL/L) of acetone and dimethyl formamide, respectively, were used in dosing to solubilize TBBPA. The independent zebrafish studies employed dimethyl sulfoxide, and acetone was used in the *A. tonsa* study. In the studies reported here and in the literature, we did not detect a pattern indicating that carrier solvents played a role in elevating toxicity values, i.e., by skewing analytical results. With the exceptions noted, the majority of acute and chronic toxicity estimates were less than 2 mg/L, consistent with TBBPA's solubility data.

TBPPA ecological risk assessments by international regulatory authorities

The toxicity data from the NAFRA studies and other sources were compiled by regulatory authorities in the European Union (2008), the U.S. EPA (1987a, b), and Environment Canada (2013) (summarized in Table 5). They each conducted ecological risk assessments of TBBPA using the NAFRA toxicity data here, and TBBPA monitoring data relevant to their jurisdictions. Monitoring data for air, water, soil, and sediment were independently compiled by the European Union (2008), Environment Canada (2013), and by Fraunhofer IME (2011). These are summarized in more detail in the e-supplement. Fraunhofer IME (2011) reported mean surface water and sediment concentrations from monitoring sites in Europe of 0.058 ng/L ($n = 48$) and 3.78 μg/kg dw ($n = 101$). From time series studies, they concluded that there was no historical evidence of increasing environmental levels of TBBPA.

The highest reported surface water concentration reported by Environment Canada was 0.05 μg/L from monitoring in Japan from 1977 to 1989. (It was detected in one of 240 samples; most samples were below detection limits.) In

freshwater sediments, the highest monitored concentrations reported in Fraunhofer IME (2011) were from sites in China in areas with suspected TBBPA emissions. Among 18 sites, the median sediment concentration was 22.2 μg/kg dw, and the mean was 82.3 ± 189.0 μg/kg dw. This was more than 1000 times lower than the chronic effect concentrations determined in the spiked sediment toxicity studies. A lone report of 330 mg/kg dw in sediments in the vicinity of US manufacturing site was identified, but the original report is not available (Zweidinger et al. 1979, as cited in EC 2013). By comparison in Europe, the mean sediment concentration was 3.78 ± 10.77 μg/kg dw (n = 101). In sewage sludge, Fraunhofer (2011) reported median and mean concentrations of 14.0 μg/kg dw and 133.8 ± 285.0 μg/kg (n = 41). Environment Canada (EC 2013) reported higher and more variable freshwater sediment concentrations of TBBPA globally. The majority of samples contained < 150 μg/kg dw, though one site in the UK was reported to have 9750 μg/kg [40]. Environmental concentrations of TBBPA in surface waters, soils, and sediments were generally orders of magnitude below the toxicity levels reported here.

TBBPA is a registered chemical under the EU's Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) regulation. Risk characterization ratios (RCRs) for TBBPA calculated by the EU (2008) indicated minimal risk to aquatic organisms from regional sources and from manufacturing and processing of epoxy and polycarbonate resins. RCRs were in the range of 0.001 for these sources. RCRs for benthic organisms in sediment were similarly low. RCRs calculated using worst-case assumptions exceeded one (4.3 to 7.1) only at compounding sites where TBBPA is used as an additive flame retardant in acrylonitrile-butadiene-styrene resins (EU 2008). Environment Canada (EC 2013) calculated low risk quotients for aquatic, benthic, and terrestrial compartments of 0.21, 0.054, and 0.00031, respectively. They concluded that environmental risks from the use of TBBPA in Canada are unlikely. Supported by health and environmental assessments of TBBPA by global regulatory authorities, TBBPA has been used internationally as an electronics flame retardant for over 35 years.

Summary

Collectively, this paper and two companion papers on TBBPA's fate and terrestrial toxicity (McAvoy et al. 2016; Rothenbacher and Pecquet, 2018) constitute a comprehensive database on the environmental fate and ecotoxicology of TBBPA. Results of the NAFRA studies were consistent with those of more recent TBBPA research for overt toxicological endpoints and effect levels. Particularly sensitive taxa and endpoints identified were molluscan shell growth in the NAFRA studies and zebrafish egg production reported by Kuiper et al. (2007).

The majority of toxicity estimates from the NAFRA studies and other research on TBBPA to aquatic organisms are less than 2 mg/L, consistent with TBBPA's empirical water solubility. Toxicity estimates that significantly exceed a solubility of 4.16 mg/L are deemed suspect.

Given the continuing use of TBBPA and related chemicals, broad access to the empirical TBBPA data in the NAFRA studies may help to better understand the fate and effects of TBBPA and related chemicals. The data may stimulate new insights in data analysis, modeling, and ecological risk assessment. Credit for the quality of the original studies is due to the technical expertise of the laboratory study directors, who in large part laid the groundwork for conventional ecotoxicity test methods today (see Acknowledgements).

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