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Hazard/Risk Assessment

Development of Per and Polyfluoroalkyl Substances Ecological Risk-Based Screening Levels

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Abstract: Evaluation of chemical risks to threatened and endangered species is a requirement for Superfund ecological risk assessments; however, screening levels to evaluate the potential for toxicity associated with ecological receptor exposure to per- and polyfluoroalkyl substances (PFAS) are lacking. Therefore, PFAS risk-based screening levels (RBSLs) were developed. Wildlife RBSLs were developed using surrogate receptors selected to be representative of threatened and endangered species with different habitat types, feeding guilds, and trophic levels. Published uptake and toxicity data were combined with receptor exposure factors to derive RBSLs for terrestrial and aquatic wildlife for several PFAS, including perfluorononanoic acid, perfluorooctanesulfonic acid, perfluorooctanoic acid, perfluorohexanoic acid, perfluorobutanesulfonic acid, and pentafluorobenzoic acid. Uptake information for surrogate PFAS were considered to calculate RBSLs for PFAS with toxicity data and insufficient bioaccumulation data to develop an RBSL. Both no-observed–adverse effect level (NOAEL)– and lowest-observed–adverse effect level–based wildlife RBSLs were calculated to allow for a range of risk estimates appropriate to individual threatened and endangered species and populations of nonlisted wildlife receptors, respectively. Recommended water quality RBSLs protective of aquatic life were developed for 23 PFAS based on published literature reviews and peer-reviewed aquatic toxicity studies and Great Lakes Initiative methodology. For wildlife receptors, NOAEL RBSLs ranged from to 0.013 to 340 mg/kg for soil, 0.0014 to 370 mg/kg for sediment, and 0.000075 to 1600 mg/L for surface water. For aquatic life, chronic RBSLs ranged from 0.00022 to 3.4 mg/L. For terrestrial plants and soil invertebrates, the no-observed-effect concentration screening levels range from 0.084 to 642 mg/kg and from 1 to 50 mg/kg, respectively. *Environ Toxicol Chem* 2021;40:921–936. © 2020 SETAC

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INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are a large class of compounds with >4700 chemicals previously or currently on the market with wide-ranging uses in industrial and commercial products and processes (Interstate Technology and Regulatory Council 2020a). Use of aqueous film-forming foams represents an important known and/or potential source of PFAS contamination at many sites that frequently use these foams during maintenance activities and/or fire-training drills (Interstate Technology and Regulatory Council 2020a). Also, PFAS are used in a variety of applications (e.g., during chrome-plating) that represent additional potential sources of environmental release (Interstate Technology Regulatory Council 2020a). Because ecological screening levels are currently lacking for many PFAS and environmental media, ecological risk-based screening levels (RBSLs) were developed in the present study to assess potential PFAS risk for a variety of species and habitat types.

It is known that PFAS are extremely persistent in the environment. They are not mineralized or degraded to nonfluorinated compounds in the environment (although some PFAS will biotransform to other terminal PFAS end products under certain conditions; Interstate Technology and Regulatory Council 2020b) and have been measured in abiotic media as

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well as aquatic and terrestrial wildlife (Kannan et al. 2005; D'Hollander et al. 2014). Available toxicological data for ecological receptors (i.e., aquatic life, including fish and aquatic plants and invertebrates; terrestrial plants and invertebrates; and wildlife) are limited. For wildlife, most toxicity data are for mammals exposed to perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and indicate the potential for adverse effects, primarily associated with hepatic, immune, and developmental endpoints (Agency for Toxic Substances and Disease Registry 2018). These studies focus on a few laboratory species, primarily mice, rats, and monkeys (US Environmental Protection Agency 2016a, 2016b), in support of human health assessments. Avian toxicity data are limited to studies for PFOS and perfluorobutanesulfonic acid (PFBS) in mallard and quail (e.g., Gallagher et al. 2003a, 2003b, 2004a, 2004b). Aquatic ecotoxicity data for pentafluorobenzoic acid (PFBA), PFBS, and fluorotelomers including 8:2 fluorotelomer carboxylic acid (FTCA) and 8:2 fluorotelomer unsaturated carboxylic acid (FTuCA) are available for only a few aquatic species commonly used in standardized toxicity tests (Interstate Technology and Regulatory Council 2020c). Toxicity data for PFAS in benthic invertebrates, an important dietary resource for aquatic wildlife, are not currently available. Given the large number of PFAS chemicals, performing toxicity studies for each individual chemical in multiple species is not feasible. Therefore, approaches to assessing groups of PFAS (e.g., by functional group or structure) are being considered.

Because of their ubiquitous nature, persistence, and limited toxicity data, potential toxicity of PFAS to ecological receptors is a concern. The RBSLs in the present study were developed using standard US Environmental Protection Agency (USEPA) approaches (US Environmental Protection Agency 1997, 2005, 2012) and designed for use to evaluate both federal and state listed threatened and endangered species and other nonlisted wildlife potentially found at PFAS-impacted sites. Although the RBSLs were developed specifically for use at Department of Defense sites and rely primarily on approaches and data presented in USEPA documents, they are available and appropriate for use at most sites to rapidly assess potential for risk to ecological receptors that may be exposed to PFAS-contaminated soils, sediments, water, and prey.

METHODS

The methods used to develop RBSLs are summarized on Table 1.

Literature review

A comprehensive literature search was conducted to review and compile available PFAS toxicity data for aquatic and terrestrial biota. The literature review included a literature search on multiple search engines using keywords, a search on the USEPA ECOTOX database, and reviews of key secondary papers and US guidance documents. Review of each source paper focused on compiling toxicity data with endpoints, effects, and durations potentially relevant to RBSLs calculations based on US Environmental Protection Agency (1997, 2005, 2012) methods. Details of the literature review, including the search process, criteria for inclusion/exclusion of data, extracted data, and a reference list for reviewed materials, are presented in Supplemental Data I.

For aquatic life RBSLs, published studies were reviewed for toxicity data with endpoints and exposure durations relevant to calculating recommended water quality (RWQ) RBSLs following

TABLE 1: Approach to level screening development for each receptor and habitat

Receptor	Receptor group	Methodology	Exposure pathway evaluated	Screening level developed		
				Soil (mg/kg)	Sediment (mg/kg)	Surface water (mg/L)
Terrestrial receptor	Plant	EcoSSL	Media	Soil RBSL	NA	NA
	Invertebrate	EcoSSL	Media	Soil RBSL	NA	NA
	Bird	EcoSSL	Foodweb	Wildlife RBSL	NA	Wildlife RBSL
	Mammal	EcoSSL	Foodweb	Wildlife RBSL	NA	Wildlife RBSL
	Reptile	NE	NE	NE	NA	NE
Aquatic receptor	Plant	GLI	Media	NA	NA	Tier I/II RWQ RBSL
	Aquatic invertebrate[a]	GLI	Media	NA	NE	Tier I/II RWQ RBSL
	Benthic invertebrate[a]	EcoSSL	Media	NA	NE	NA
	Fish[b]	GLI/EcoSSL	Media/foodweb	NA	NE	Tier I/II RWQ RBSL
	Amphibian	GLI	Media	NA	NA	Tier I/II RWQ RBSL
	Bird	EcoSSL	Foodweb	NA	Wildlife RBSL	Wildlife RBSL
	Mammal	EcoSSL	Foodweb	NA	Wildlife RBSL	Wildlife RBSL
	Reptile	NE	NE	NA	NE	NE

[a]Insufficient data are available to evaluate benthic invertebrate toxicity from exposure to sediments using Ecological Soil Screening Level (EcoSSL) methodology (US Environmental Protection Agency 2007). The aquatic data set used to calculate Great Lakes Initiative (US Environmental Protection Agency 2011) Tier I/II recommended water quality risk-based screening levels based on water exposure includes benthic invertebrates exposed via water.
[b]Insufficient data are available to evaluate fish toxicity from exposure to sediments using dietary exposure studies and EcoSSL risk-based screening level (RBSL) methodology. The aquatic data set used to calculate Great Lakes Initiative Tier I/II recommended water quality RBSLs based on water exposure includes fish exposed via water.
BAF = bioaccumulation factor; BSAF = biota-sediment accumulation factor; NA = exposure pathway not applicable to methodology; NE = not evaluated due to insufficient data; RBSL = risk-based screening level; RWQ = recommended water quality.

Great Lakes Initiative (GLI) methodology (US Environmental Protection Agency 2012). Limited data availability for most PFAS supported using a data-inclusive approach when compiling toxicity values to include as much information as possible in the evaluations. Therefore, the RWQ RBSL calculations in the present study did not consider some factors included in standard GLI criterion calculations, including age of the organism, if it was fed or unfed during the experiment, or the static or flow-through conditions during the experiment. Censored 10% effect concentration/lethal concentration (EC/LC10) through 100% effect concentration/lethal concentration (EC/LC100) data were excluded in an effort to reduce uncertainty and avoid results skewed by censored values among a limited number of toxicity values. Applicable toxicity data as reported by the primary papers were included regardless of whether they were based on nominal or measured concentrations and regardless of methodology or software used to calculate the EC/LC50. Studies using mixtures of PFAS were generally excluded.

Toxicity data relevant for calculation of acute RWQ RBSLs included EC/LC50s typically based on exposure durations of 48 h for cladocerans (e.g., *Daphnia magna*) and 96 h for all other aquatic species. If the only EC/LC50 values available for a species were less than the preferred exposure duration for the tested species, they were included in the GLI evaluation.

Toxicity data relevant for calculation of chronic RWQ RBSLs included no-observed effect concentrations (NOECs), lowest-observed effect concentrations (LOECs), and EC/LC10s with growth, reproduction/development, and survival/mortality endpoints derived in laboratory tests on single species of aquatic animals (e.g., fish, aquatic invertebrates). The NOECs, LOECs, or EC/LC10s were based on early life cycle, partial life cycle, or life cycle exposure durations. Multigeneration studies were considered as well. Growth endpoints included changes in length or weight; malformations or delays in growth were not included as growth endpoints. Reproductive and developmental endpoints with a clear effect on fecundity, such as reduced total hatch, reduced live young, and reduced number of young per female, were included in the evaluation. Endpoints such as reductions in sperm or oocyte quality, changes in fertilization or hatching rates, and sublethal developmental malformations were not considered sufficiently related to a direct effect on fecundity.

For plant and soil invertebrate RBSLs, data selection was focused on relevant exposure and endpoints. For terrestrial plants, measured endpoints or media irrelevant to soil exposures, such as hydroponic and agar culture studies, were excluded. Chlorophyll absorbance and fluorescence values were excluded because these endpoints were not sufficiently correlated with reductions in growth or survival. Increased biomass and increased length were observed at low concentrations in multiple studies but were not considered adverse effects and therefore were excluded. Toxicity values considered for terrestrial and benthic invertebrates included adverse effects on growth, reproduction, and survival endpoints, such as decreased body weight, reduced number of offspring, and increased mortality. Measured endpoints or media irrelevant to the present study, such as drinking water, dietary exposure, or aqueous topical testing, were excluded from further evaluation. If the time points or treatment groups with significant effects did not follow a dose–response relationship, the values were excluded.

For wildlife RBSLs, primary and secondary papers were reviewed for acute, subchronic, and chronic toxicity data that would support development of a toxicity reference value (TRV) based on growth, reproduction, and survival endpoints, consistent with USEPA ecological risk assessment guidance (US Environmental Protection Agency 1997) and methods (US Environmental Protection Agency 2005). Relevant growth endpoints included decreased body weight or body length in juvenile animals; malformations or delays in development were not included as growth endpoints. Reproductive endpoints included in the present study were consistent with endpoints included in the ecological soil screening level (EcoSSL) TRV derivation (US Environmental Protection Agency 2007a). Only studies evaluating relevant exposure pathways for wildlife, such as ingestion of food or water or via oral gavage, were included in the TRV data sets.

Calculation of acute and chronic RWQ RBSLs

The methodology to develop the RWQ RBSLs is generally consistent with the GLI approach (US Environmental Protection Agency 2012). In the present study, the principles of GLI methodology are applied to calculate acute and chronic RWQ RBSLs for application to water bodies where aquatic life (i.e., fish, aquatic invertebrates, and aquatic plants and algae) may be exposed over acute or chronic durations. Acute surface water screening levels were calculated based on methods used for calculation of the criterion maximum concentration or secondary maximum concentration, and chronic surface water screening levels were calculated based on the continuous maximum criterion or secondary continuous concentration. Ideally, the acute and chronic values used to calculate acute-to-chronic ratios are reported from the same study. In the present study, acute and chronic values were occasionally used from the same author or laboratory and assumed similar test conditions between studies. The RWQ RBSL calculations and supporting data sets are available in Supplemental Data II.

Calculation of terrestrial plant and invertebrate soil RBSLs

Terrestrial plant and terrestrial invertebrate soil RBSLs were calculated as the geometric mean of available NOEC/EC10s or LOEC/EC20s. The NOEC RBSL was calculated as the geometric mean of all bounded NOEC/EC10s with reproduction or growth as the endpoint. The LOEC screening level was calculated as the geometric mean of all bounded or unbounded LOEC/EC20s with growth, reproduction, or survival as the endpoint. If the LOEC-based RBSL was lower than the NOEC-based RBSL, the LOEC-based RBSL was divided by an

uncertainty factor of 10 to calculate the final NOEC-based RBSL. Terrestrial plant RBSL calculations and supporting data sets are available in Supplemental Data II.

If multiple NOECs and LOECs with an appropriate endpoint were reported in a study, only the lowest of each was incorporated in the RBSL calculations. However, if the paper reported values from multiple related tests, such as a pilot and a main study, or tested multiple species, the lowest NOECs and LOECs of each study or species were incorporated.

Calculation of wildlife RBSLs

Wildlife RBSLs represent the PFAS concentration in soil (or sediment or water) that results in the receptor having an exposure equal to a threshold exposure dose (i.e., TRV). Levels were calculated for a suite of wildlife receptors representing various feeding guilds and habitat types (Table 2) using a food web model and EcoSSL equations (US Environmental Protection Agency 2005). The soil RBSL equation is presented as an example:

$$\text{Soil RBSL} = \text{TRV}/(\{[(\text{SIR} \times \text{FIR}) + (\text{FIR} \times \text{BAF})] \times \text{SUF}\}/\text{BW})$$

To calculate RBSLs, exposure assumption inputs are required for each receptor, including bioaccumulation factors (BAFs) for dietary items, ingestion rates (soil ingestion rate [SIR] and food ingestion rate [FIR]), body weight (BW), and site use factor (SUF) along with toxicity assumptions, represented by an appropriate TRV. When possible, both no-observed–adverse effect level (NOAEL)– and lowest-observed–adverse effect level (LOAEL)–based wildlife RBSLs were calculated, to allow for a range of risk estimates appropriate to individual threatened and endangered species and populations of nonlisted wildlife receptors, respectively. The inputs are presented in this section.

Selection of receptors and exposure assumptions. Selection of wildlife receptors focused on threatened and endangered species potentially present on multiple Department of Defense facilities. Representative threatened and endangered and surrogate species were selected for each feeding guild based on 1) expected high degree of exposure and/or sensitivity (e.g., small receptors with high dietary ingestion rates chosen over larger receptors with lower food ingestion rates); 2) applicability to other threatened and endangered species within the guild (e.g., a species of shrew chosen if several shrews listed); 3) confirmed or potential presence on Department of Defense installations; and 4) the amount of money spent by the Department of Defense to protect a threatened and endangered species. Toxicity, uptake, and exposure information is limited or unavailable for threatened and endangered species; therefore, a representative surrogate species with available toxicity and uptake data was identified for each threatened and endangered receptor. The representative surrogate species was selected for each threatened and endangered representative species based on similarity to the threatened and endangered species, common use as a surrogate species in ecological risk assessment (ERA), and availability of applicable exposure data. Exposure factors for the selected wildlife receptors are presented in Supplemental Data II.

Bioaccumulation factors. Factors were derived from published literature and data for a variety of tissue types and environmental media, as summarized in Table 3. Details of the literature review, including the search process, criteria for inclusion/exclusion of data, extracted data, and a reference list for reviewed materials, are presented in Supplemental Data I.

Briefly, aquatic surface water-to-biota BAFs and biota–sediment accumulation factors (BSAFs) were identified from values published in Giesy et al. (2010), McCarthy et al. (2017), and Valsecchi et al. (2017) and primary papers cited in these

TABLE 2: Selected threatened and endangered representative and surrogate species

Feeding guilds		Representative species	Representative surrogate species
Mammals			
Aquatic	Piscivore[a]	Guadalupe fur seal	Harbor seal, river otter, mink[b]
	Insectivore	Gray bat	Little brown bat
	Herbivore	Point arena mountain beaver	Muskrat
Terrestrial	Carnivore	Black-footed ferret	Long-tailed weasel
	Insectivore	Northern long-eared bat	Little brown bat
	Invertivore	Ornate shrew	Short-tailed shrew
	Herbivore	Amargosa vole	Meadow vole
Birds			
Aquatic	Piscivore	California least tern	Brown pelican
	Invertivore	Snowy plover	Tree swallow
	Omnivore	Yellow-shouldered blackbird	Red-winged blackbird
Terrestrial	Carnivore	Mexican spotted owl	Red-tailed hawk
	Insectivore	Red-cockaded woodpecker	House wren
	Herbivore	Palila (honeycreeper)	American goldfinch

[a]Inclusive of receptors with diets of crustaceans and cephalopods such as the blue whale.
[b]Harbor seal, river otter, and mink were selected as representative surrogate species. The harbor seal was considered a representative surrogate species for Guadalupe fur seal. River otters and mink were selected to model exposure for an inland aquatic piscivore or opportunistic carnivore receptor.

TABLE 3: Bioaccumulation factors for wildlife receptors

Media	Constituent	PFNA	PFOS	PFOA	PFHxA	PFBS	PFBA
Soil	Terrestrial plant BAF (unitless)	1.1	0.66	0.11	2.2	3.6	8.0
Soil	Terrestrial invertebrate BAF[a] (unitless)	4.6	19	2.0	1.9	31	7.1
Soil	Mammal, bird, reptile[b] BAF (unitless)	**11**	11	**11**	**11**	**11**	**11**
Sediment	Aquatic plant BSAF (unitless)	3.1	61	3.5	4.7	1.8	6.2
Sediment	Aquatic invertebrate BSAF[a,c] (unitless)	463	106	281	**263**	**263**	**263**
Sediment	Aquatic crustacean[c] BSAF (unitless)	65	25	77	**154**	**154**	**154**
Sediment	Fish[c,d] BSAF (unitless)	92	72	17	**86**	**86**	**86**
Surface water	Aquatic plant BAF (L/kg)	5188	1305	228	191	8	19
Surface water	Aquatic invertebrate[a,e] BAF (L/kg)	983	1549	379	2238	298	**298**
Surface water	Aquatic crustacean[e] BAF (L/kg)	3511	5608	703	777	388	**388**
Surface water	Fish BAF (L/kg)	3344	13 229	894	317	916	3200
Surface water	Amphibian[f] BAF (L/kg)	49	3322	0.8	2.9	**1198**	**2.9**

[a]The terrestrial invertebrate soil bioaccumulation factor (BAF) was used as a surrogate for the aerial insect soil BAF, the aquatic invertebrate sediment biota–sediment accumulation factor (BSAF) was used as a surrogate for aerial insect sediment BSAF, and the aquatic invertebrate surface water BAF was used as a surrogate for the aerial insect surface water BAF.
[b]The perfluorooctanesulfonic acid mammal bioaccumulation factor (BAF) was used as a surrogate for the remaining per- and polyfluoroalkyl substance mammal BAFs and bird and reptile BAFs.
[c]A perfluorohexane sulfonic acid bioaccumulation factor was used as a surrogate for perfluorohexanoic acid, perfluorobutanesulfonic acid, and pentafluorobenzoic acid for the aquatic invertebrate sediment biota–sediment accumulation factor (BSAF), aquatic crustacean sediment BSAF, and fish sediment BSAF.
[d]The fish biota–sediment accumulation factors (BSAFs) were used as a surrogate for the amphibian BSAF.
[e]A perfluorobutanesulfonic acid bioaccumulation factor (BAF) was used as a surrogate for the aquatic invertebrate surface water pentafluorobenzoic acid (PFBA) BAF and for the aquatic crustacean surface water PFBA BAF.
[f]A perfluorohexane sulfonic acid bioaccumulation factor (BAF) was used as a surrogate for perfluorobutanesulfonic acid, and a perfluorohexanoic acid BAF was used as a surrogate for pentafluorobenzoic acid amphibian surface water BAFs.
Bold indicates a surrogate bioaccumulation factor was used. The full suite of BAFs is presented in Supplemental Data III.
BAF = bioaccumulation factor; BSAF = biota–sediment accumulation factor; PFBA = pentafluorobenzoic acid; PFBS = perfluorobutanesulfonic acid; PFHxA = perfluorohexanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid.

sources. Whole-organism BAFs and BSAFs were calculated for aquatic plants, a variety of invertebrates, and fish based on field data. Field data were preferred because they provide a more representative measure of uptake from all exposure routes (whereas laboratory studies typically focus on a single exposure medium), and concentrations of PFAS in biota in the field would be expected to have reached equilibrium with concentrations in the environment. A representative BAF was calculated for each available medium and dietary item based on the geometric mean of the relevant BAF data. Tissue and sediment concentrations or BAFs presented as wet weight were converted to dry weight using moisture contents provided in the individual studies, if available, or in US Environmental Protection Agency (1993).

For soil-to-biota BAFs, a targeted literature search was conducted to identify BAFs or available terrestrial uptake data for PFAS, as described in Supplemental Data I. Soil or tissue concentrations presented as less than the limit of quantification or nondetects were excluded from BAF calculations. Final representative BAFs are based on the geometric mean BAF for each dietary item, and BAFs presented as wet weight were converted to dry weight using moisture contents provided in the individual studies, if available, or as reported in US Environmental Protection Agency (1993). Published BAFs calculated on a soil organic carbon basis were converted to a bulk soil basis based on the percentage organic carbon presented in the applicable studies. For PFOS, BAFs for linear and branched PFOS were reported. The linear BAFs were selectively incorporated in the RBSL calculations because BAFs for linear PFOS are higher than their branched counterparts and result in conservative uptake estimates (see Supplemental Data III).

Only studies providing measurements of PFAS uptake and accumulation in plants from soil or paired data for plants and soil were used to compile and calculate soil-to-plant BAFs. Studies that provide whole-plant measurements as well as individual plant compartment measurements were included in the review (see Supplemental Data I). For studies that provided only plant compartment concentrations (e.g., root and shoot), the arithmetic mean of the compartment concentrations was calculated for each plant to estimate a whole-plant concentration. Details on organism type, study type, and sources are presented in Supplemental Data I.

Similarly, for soil-to-invertebrate BAFs, only studies providing measurements of PFAS uptake and accumulation in invertebrates from soil or paired data for invertebrates and soil were used to compile and calculate BAFs for invertebrates.

For the 6 PFAS included in the wildlife RBSL calculations, a sensitivity analysis (presented in Supplemental Data III) was conducted to determine the difference between soil-to-plant and soil-to-invertebrate BAFs calculated using published data from 1) field studies, 2) field and laboratory/greenhouse studies excluding spiked soils, and 3) all field and laboratory/greenhouse studies. For plant uptake, BAFs for 5 of 6 PFAS differed by less than a factor of 2 regardless of the data set used. For PFOA, the BAF calculated using all laboratory/greenhouse studies and field data (0.26) is as much as 3.5 times greater than BAFs calculated using only field studies (0.074) or field and laboratory/greenhouse studies excluding spiked soils (0.11). Soil-to-invertebrate BAFs differed by up to 3-fold regardless of

the data set used, except for PFOS. The PFOS BAFs ranged from 19 (based on laboratory/greenhouse data excluding spiked soils) to 134 for field data only. Field, laboratory, and greenhouse studies based on field-contaminated soil were preferred because they provide a more representative measure of uptake in nature, and concentrations of PFAS in biota in the field would be expected to have reached equilibrium with concentrations in the environment. Therefore, both field and laboratory/greenhouse BAFs were included in the final data set used to calculate a representative BAF based on the geometric mean of the relevant BAF data. Laboratory/greenhouse studies utilizing soils directly spiked with PFAS were excluded.

A soil-to-small mammal PFAS BAF was not located in the published literature. However, a data set collected for the Fiskville Environmental Audit (AECOM 2014) included rabbit muscle and colocated soil PFAS concentrations allowing for calculation of a PFOS soil-to rabbit muscle BAF. Similarly, D'Hollander et al. (2014) report paired small mammal organ-specific and soil PFOS concentrations.

Ideally, soil-to-small mammal BAFs are calculated using whole-body concentrations. Papers describing partitioning of PFAS within small mammals were reviewed and used to convert organ- or tissue-specific BAFs to whole-body BAFs. A study by Bogdanska et al. (2011) that reports the tissue distribution of PFOS in mice was used as the basis for converting organ-specific BAFs based on data from Fiskville (AECOM 2014) and D'Hollander et al. (2014) to whole-body BAFs, as described in Supplemental Data I. Tissue and soil concentrations presented as wet weight were converted to dry weight using moisture contents provided in US Environmental Protection Agency (1993), Appendix C of US Environmental Protection Agency (1999), and Pla et al. (2010) to derive soil-to-mammal BAFs.

Toxicity reference values. The TRVs for birds and mammals were calculated based on the EcoSSL methodology (US Environmental Protection Agency 2007b). The NOAELs and LOAELs selected for inclusion in the TRV data sets were based on studies with exposure durations of 3 d or greater and survival, growth, or reproduction endpoints. Generally, only reproduction and growth NOAELs are considered in the development of the NOAEL TRV, whereas all 3 endpoints are included in the LOAEL TRV data set. If fewer than 3 reproduction and growth NOAELs were available, NOAELs based on mortality were considered following EcoSSL guidance. The minimum data requirements to derive a NOAEL and/or a LOAEL TRV were 1) at least 3 toxicity values (either NOAEL or LOAEL) with growth, reproduction, or mortality as the endpoint, and 2) at least 2 species tested. Although not used in the EcoSSL approach, uncertainty factors were applied in specific cases, such as use of a 10-fold uncertainty factor to estimate a chronic effect value from subchronic toxicity data, to ensure that the final NOAEL and LOAEL TRVs are appropriately conservative for use with threatened and endangered species. Use of the uncertainty factors, identification of subchronic and chronic exposure durations based on the life span of the test species, and additional details related to TRV calculations are described in Supplemental Data I and II. The NOAEL and LOAEL TRVs were calculated for 9 PFAS for mammals and 2 PFAS for birds (Table 4).

TABLE 4: Toxicity reference values for wildlife receptors

Constituent	NOAEL count[a]	LOAEL count[a]	NOAEL TRV (mg/kg body wt/d)	LOAEL TRV (mg/kg body wt/d)
Mammal TRVs				
PFNA	2	4	0.83	1.1
PFOS	27	30	0.1	0.166
PFOA	17	27	0.3	0.6
PFHxA	6	6	84	175
PFBS	6	3	50	200
PFBA	4	1	73	175
6:2 FTOH	3	4	43	100
GenX	14	8	0.5	5
N-EtFOSE	1	2	0.1	1
Bird TRVs				
PFOS	5	4	0.079	0.79
PFBS	3	2	92	153

[a]The no-observed–adverse effect level (NOAEL) count is based on the number of NOAELs with reproduction and growth as endpoints, and the lowest-observed–adverse effect level (LOAEL) count is based on the number of LOAELs with reproduction, growth, or survival as endpoints.
FTOH = fluorotelomer alcohol; GenX = hexafluoropropylene oxide dimer acid; LOAEL = lowest-observed–adverse effect level; N-EtFOSE = *N*-ethyl perfluorooctane sulfonamidoethanol; NOAEL = no-observed–adverse effect level; PFBA = pentafluorobenzoic acid; PFBS = perfluorobutanesulfonic acid; PFHxA = perfluorohexanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; TRV = toxicity reference value.

RESULTS

Literature review

A total of 256 toxicity tests including growth, reproduction, and lethality endpoints were included in the present evaluation. Of these, 126 tests were conducted with freshwater or marine aquatic organisms, 6 tests with terrestrial plants, 3 tests with terrestrial invertebrates, 11 tests with birds, and approximately 110 tests with mammals.

Surface water RWQ RBSLs

The RWQ RBSLs for protection of aquatic life were derived for 23 individual PFAS (Table 5). Chronic RWQ RBSLs range from 0.00022 mg/L for 10:2 FTCA to 3.4 mg/L for PFBS. Among the perfluoroalkyl compounds, perfluoroundecanoic acid (PFUnA) had the lowest acute RWQ RBSL, and PFOS had the lowest chronic RWQ RBSL. Chronic RWQ RBSLs for the fluorotelomer acids were generally lower than RWQ RBSLs for the perfluoroalkyl compounds.

The data sets for PFOS and PFOA are relatively robust, whereas the data available for other PFAS are quite limited. The RWQ RBSLs for perfluorododecanoic acid (PFDoA), PFUnA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoropentanoic acid, 7:3 Acid, 6:2 FTuCA, perfluorohexanoic acid (PFHxA), PFBA, perfluoropropionic acid, and 5H 4:1 fluorotelomer alcohol (FTOH) are all based on data from single studies (see Supplemental Data II).

TABLE 5: Acute and chronic surface water screening level summary

Constituent	GLI species groups[a]	Tier I or tier II methodology	Acute RWQ RBSL (mg/L)	Chronic RWQ RBSL (mg/L)
PFDoA	1	Tier II	6.4E-01	7.2E-02
PFUnA	1	Tier II	4.4E-01	4.9E-02
PFDA	2	Tier II	1.2E+00	1.4E-01
PFNA	2	Tier II	1.1E+00	1.2E-01
PFOS	8	Tier I	6.1E-01	3.9E-02
PFOA	8	Tier I	5.4E+01	3.0E+00
PFHpA[b]	1	Tier II	7.8E+00	8.7E-01
PFHxA	3	Tier II	8.8E+00	2.2E+00
PFPeA[c]	2	Tier II	1.2E+00	1.4E-01
PFBS	4	Tier II	1.7E+01	3.4E+00
PFBA	2	Tier II	4.2E+00	4.7E-01
PFPrA[b]	1	Tier II	1.8E+00	2.0E-01
10:2 FTCA	1	Tier II	6.8E-04	2.2E-04
10:2 FTuCA	1	Tier II	6.4E-03	1.3E-03
8:2 FTCA	1	Tier II	6.0E-02	6.7E-03
8:2 FTuCA	2	Tier II	1.4E-01	1.5E-02
7:3 Acid	2	Tier II	3.7E-02	4.1E-03
6:2 Cl-PFESA[b]	1	Tier II	3.3E-01	6.9E-02
6:2 FTCA[c]	1	Tier II	5.7E-01	6.4E-02
6:2 FTuCA	1	Tier II	6.8E-01	7.5E-02
6:2 FTAB[b]	1	Tier II	1.5E+00	1.6E-01
5H 4:1 FTOH	1	Tier II	4.5E+00	5.0E-01
FC807[b]	1	Tier II	4.8E+00	5.4E-01

[a]The Great Lakes Initiative species group count is the number of species groups (out of 8 possible groups) fulfilled by the data set and is used to determine the secondary acute factor.
[b]The Great Lakes Initiative tier II methodology recommends deriving a value only when a *Daphnia* sp. toxicity value is available, and a *Daphnia* value was not identified for this per- and polyfluoroalkyl substance.
[c]The Great Lakes Initiative tier II methodology recommends deriving a value only when a *Daphnia* sp. toxicity value is available, and all *Daphnia* values identified were greater or less than.
Cl-PFESA = chlorinated polyfluoroalkyl ether sulfonic acid; GLI = Great Lakes Initiative; FC807 = di-perfluoroalkyl phosphate; FTAB = fluorotelomer sulfonamide alkylbetaine; FTCA = fluorotelomer carboxylic acid; FTuCA = fluorotelomer unsaturated carboxylic acid; FTOH = fluorotelomer alcohol; PFBA = pentafluorobenzoic acid; PFBS = perfluorobutanesulfonic acid; PFDA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; PFPeA = perfluoropentanoic acid; PFPrA = perfluoropropionic acid; PFUnA = perfluoroundecanoic acid; RWQ RBSL = recommended water quality risk-based screening level.

Terrestrial plant and invertebrate RBSLs for soil

The plant NOEC-based soil RBSLs for 6 PFAS range from 0.084 to 642 mg/kg (Table 6). Consistent with the aquatic evaluation, the terrestrial plant PFOS and PFOA data sets include substantially more studies than are available for the remaining PFAS.

The soil invertebrate toxicity data were based on tests with earthworms. The NOEC-based soil RBSLs ranged from 1 to 50 mg/kg, and the LOEC-based soil RBSLs ranged from 100 to 141 mg/kg (Table 7). The RBSLs calculated for soil invertebrates are based on a single LOEC or NOEC for most PFAS and are, therefore, considered to have a higher degree of uncertainty in their ability to predict risk to these organisms than for other more data-rich RBSLs calculated in the present study.

Bird and mammal RBSLs

Both NOAEL- and LOAEL-based wildlife RBSLs were calculated to allow for a range of risk estimates appropriate to individual threatened and endangered species and populations of nonlisted wildlife receptors, respectively. The NOAEL-based TRVs and NOAEL-based RBSLs are based on toxicity values where no adverse effect was observed. Therefore, these RBSLs are assumed to be conservative screening benchmarks where no adverse effect to either an individual receptor or the receptor population would be expected. The LOAEL-based RBSLs are derived using TRVs based on the lowest dose at which an adverse effect relevant to wildlife populations was observed in tests with individual organisms; LOAEL RBSLs are conservatively assumed to be protective of wildlife populations.

TABLE 6: Plant screening-level summary

Constituent	NOEC count	LOEC count	NOEC soil RBSL (mg/kg soil)	LOEC soil RBSL (mg/kg soil)
PFDA	1	0	51	NA
PFNA	1	0	46	NA
PFOS[a]	6	7	11	33
PFOA[b]	8	14	0.084	0.84
PFBA	1	0	642	NA
5H 4:1 FTOH	1	0	23	NA

[a]The no-observed-effect concentration (NOEC) count for perfluorooctanesulfonic acid includes only bounded NOECs.
[b]The no-observed-effect concentration (NOEC) count for perfluorooctanoic acid includes only bounded NOECs and 10% effect concentration values.
FTOH = fluorotelomer alcohol; LOEC = lowest-observed-effect concentration; NA = not applicable; NOEC = no-observed-effect concentration; PFBA = pentafluorobenzoic acid; PFDA = perfluorodecanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; RBSL = risk-based screening level.

NOAEL-based RBSLs. The receptor- and media-specific NOAEL-based RBSLs for wildlife are presented in Table 8 for

TABLE 7: Soil invertebrate screening-level summary

Constituent	NOEC count	LOEC count	NOEC soil RBSL (mg/kg soil)	LOEC soil RBSL (mg/kg soil)
PFNA[a]	1	1	1	100
PFOS[a,b]	1	1	7.7	141
PFOA[a,b]	1	0	50	NA
PFHpA[a]	1	1	1	100
PFHxS[a]	1	1	1	100
PFBS[a,b]	1	0	10	NA

[a]The perfluorononanoic acid (PFNA), perfluorooctanesulfonic acid, perfluoroheptanoic acid (PFHpA), and perfluorohexane sulfonic acid (PFHxS) soil risk-based screening levels (RBSLs) are based on a single paired no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC). The perfluorooctanoic acid and perfluorobutanesulfonic acid soil RBSLs are based on a single unbounded NOEC. Although all are based on survival, an uncertainty factor was not applied to the PFNA, PFHpA, and PFHxS NOEC RBSLs because the NOEC is already 2 orders of magnitude lower than the LOEC.
[b]An uncertainty factor of 10 was applied to the perfluorooctanesulfonic acid no-observed-effect concentration (NOEC) of 77 mg/kg because it was less than an order of magnitude lower than the lowest-observed-effect concentration of 141 mg/kg. The perfluorooctanoic acid (PFOA) and perfluorobutanesulfonic acid (PFBS) NOEC soil risk-based screening levels (RBSLs) are based on an unbounded NOEC with a survival endpoint. Based on this uncertainty, an uncertainty factor of 10 was applied to calculate the final PFOA and PFBS RBSLs.
NA = not applicable; PFBS = perfluorobutanesulfonic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; RBSL = risk-based screening level.

aquatic receptors and Table 9 for terrestrial receptors. The minimum RBSLs for each environmental medium and the associated receptor are also presented. The soil NOAEL-based RBSLs range from 0.013 mg/kg (PFOS RBSL for house wren) to 340 mg/kg (PFHxA RBSL for short-tailed shrew). Sediment NOAEL-based RBSLs range from 0.0014 mg/kg (PFOS RBSL for tree swallow) to 370 mg/kg (PFBS RBSL for muskrat). Surface water NOAEL-based RBSLs had the greatest range: 0.000075 mg/L (PFOS RBSL for brown pelican) to 76 mg/L (PFBS RBSL for muskrat) among aquatic receptors and 0.3 mg/L (PFOS RBSL for house wren) to 1600 mg/L (PFBS RBSL for red-tailed hawk) for terrestrial receptors.

The majority of minimum aquatic NOAEL-based RBSLs for each PFAS are based on invertivore or insectivore receptors, such as the tree swallow and little brown bat. There is greater variability in the terrestrial receptors associated with the minimum soil NOAEL-based RBSLs. For PFNA and PFBS the minimum is associated with the insectivorous little brown bat, and the PFOS minimum is associated with the house wren. The long-tailed weasel, a carnivore, is associated with the minimum soil NOAEL-based RBSL for PFOA. The meadow vole, an herbivore, is associated with the minimum NOAEL soil RBSL for PFHxA and PFBA. Terrestrial receptors tended to have higher RBSLs than aquatic receptors, as might be expected because of generally lower potential for uptake from dietary sources.

Generally, PFHxA and PFBA mammal RBSLs and bird or mammal PFBS RBSLs are higher than the other RBSLs, in part because of their higher TRVs relative to TRVs for the other 3 PFAS.

LOAEL-based RBSLs. The receptor- and media-specific LOAEL-based RBSLs for wildlife are presented in Table 8 for aquatic receptors and Table 9 for terrestrial receptors. The minimum RBSLs are also presented along with the associated receptor. Soil, sediment, and surface water LOAEL-based RBSLs for each PFAS are higher than their NOAEL-based counterparts by 1.3- to 10-fold. Little brown bat is the receptor most frequently associated with the lowest RBSLs.

Comparison of the mammalian and bird PFOS TRVs indicates that mammals and birds are similar in sensitivity to PFOS. However, data from only 6 studies were available to derive the bird PFOS TRV, whereas toxicity data from 33 studies were included in the mammalian PFOS TRV data set. Comparison of the bird and mammal PFBS TRVs does not allow for a clear interpretation of respective sensitivities because their TRV NOAEL to LOAEL ranges overlap. Both bird and mammal PFBS TRVs are at least 10 times higher than the PFOS bird and mammal TRVs.

DISCUSSION

A widely recognized uncertainty associated with evaluating ecological PFAS exposures is limited availability of toxicity data. For surface water, the majority of toxicity data compiled to derive RWQ RBSLs were for PFOS and PFOA. Perfluorohexane sulfonic acid (PFHxS) had no identified aquatic toxicity literature applicable to deriving RWQ RBSLs. Compounding the uncertainties associated with limited toxicity data, only a few common test species have been tested with PFAS other than PFOS and PFOA. Overall, the amount of data and therefore the range of test species are limited, introducing uncertainty as to whether species that are more sensitive to PFAS have been tested.

Surface water RWQ RBSLs

The US Environmental Protection Agency (2012) advises using caution when intraspecies acute toxicity data have considerable variability, such as more than a factor of 10. The species mean acute value for *Daphnia magna* exposed to PFBA and *Dugesia japonica* exposed to PFOA both include data that vary by >10-fold. Because of the limited data available for these chemical–species pairs, it was not possible to determine which values were unacceptably variable from the norm of the data set; inclusion of all data in the calculations was generally considered more robust than excluding some of these data. One available PFOS acute-to-chronic ratio, based on fatmucket clam (Hazelton et al. 2012), was excluded from the final calculated acute-to-chronic ratio used to calculate the chronic RWQ RBSL because it was approximately 50-fold greater than 6 other available PFOS acute-to-chronic ratios and >10-fold greater than the seventh PFOS acute-to-chronic ratio.

One method of bounding uncertainty is to compare the RWQ RBSLs derived in the present study to other published water quality benchmarks for PFOS and PFOA (Figure 1). For PFOS, chronic values were located and range from 0.13 µg/L (Australian Government 2016) to 250 µg/L (Yang et al. 2014), with the lower end of the range based primarily on lower percentile species-sensitivity distribution (SSD) approaches and the upper end of the range based on other methods (e.g., tier I/II GLI). When selecting screening values for a PFAS site, consideration should be given to the types of data included (e.g., relevant effects, use of effect and/or no-effect values, test methods and durations, and specific species) and methodology (e.g., based on acute toxicity data with an acute-to-chronic adjustment factor, as in the GLI approach; based on lowest available chronic toxicity data with uncertainty factors, as in the Water Framework Directive approach [European Commission 2011a]; or an SSD approach using chronic data) because these factors affect the final RWQ RBSL. For example, the Environment and Climate Change Canada (2018) federal water quality guideline (FWQG) of 6.8 µg/L is based on the 5th percentile of an SSD that includes chronic freshwater NOEC and LOEC data for 2 amphibian, 5 fish, 5 invertebrate, and 8 plant species. Three of the 5 lowest values are reported as chronic NOECs. Using US Environmental Protection Agency approaches (1985, 2012), chronic NOECs are not used alone; species-specific chronic values based on the geometric mean of the NOEC and LOEC are calculated for use in an SSD or for calculation of an acute-to-chronic ratio. Thus, when selecting screening benchmarks for a particular site, the basis of the benchmarks should be considered. If benchmarks are based in large degree on protection of sensitive species not relevant to a specific site or for which the selected effects (e.g., behavioral

TABLE 8: Aquatic risk-based screening level summary for wildlife

		Aquatic RBSL							
		Herbivore	Insectivore	Invertivore	Carnivore	Carnivore	Omnivore	Invertivore	Piscivore
Receptor		Muskrat	Little brown bat	River otter	Harbor seal	Mink	Red-winged blackbird	Tree swallow	Brown pelican
Constituent	Units								
NOAEL-based RBSLs									
Sediment									
PFNA	mg/kg	3.6E+00	**1.0E-02**	2.4E-01	2.0E-01	2.5E-01	NA	NA	NA
PFOS	mg/kg	2.3E-02	5.3E-03	4.7E-02	4.6E-02	3.8E-02	7.0E-03	**1.4E-03**	1.4E-02
PFOA	mg/kg	1.2E+00	**6.0E-03**	2.8E-01	1.9E-01	4.0E-01	NA	NA	NA
PFHxA	mg/kg	2.4E+02	**1.8E+00**	2.9E+01	2.6E+01	2.5E+01	NA	NA	NA
PFBS	mg/kg	3.7E+02	1.1E+00	1.8E+01	1.6E+01	1.5E+01	2.4E+01	**7.3E-01**	1.3E+01
PFBA	mg/kg	1.6E+02	**1.6E+00**	2.6E+01	2.3E+01	2.2E+01	NA	NA	NA
Surface water									
PFNA	mg/L	**2.2E-03**	4.7E-03	9.2E-03	9.7E-03	6.8E-03	NA	NA	NA
PFOS	mg/L	1.1E-03	3.6E-04	2.8E-04	3.1E-04	2.1E-04	3.4E-04	9.1E-05	**7.5E-05**
PFOA	mg/L	1.8E-02	**4.4E-03**	1.2E-02	1.3E-02	9.4E-03	NA	NA	NA
PFHxA	mg/L	6.1E+00	**2.1E-01**	6.4E+00	5.0E+00	6.9E+00	NA	NA	NA
PFBS	mg/L	7.6E+01	9.4E-01	2.0E+00	2.1E+00	1.5E+00	1.7E+01	**6.4E-01**	1.3E+00
PFBA	mg/L	4.9E+01	1.4E+00	8.6E-01	9.3E-01	**6.6E-01**	NA	NA	NA
LOAEL-based RBSLs									
Sediment									
PFNA	mg/kg	4.7E+00	**1.3E-02**	3.2E-01	2.7E-01	3.3E-01	NA	NA	NA
PFOS	mg/kg	3.8E-02	**8.8E-03**	7.7E-02	7.7E-02	6.3E-02	7.0E-02	1.4E-02	1.4E-01
PFOA	mg/kg	2.3E+00	**1.2E-02**	5.7E-01	3.9E-01	8.0E-01	NA	NA	NA
PFHxA	mg/kg	5.1E+02	**3.8E+00**	6.1E+01	5.5E+01	5.3E+01	NA	NA	NA
PFBS	mg/kg	1.5E+03	4.3E+00	7.0E+01	6.3E+01	6.0E+01	4.0E+01	**1.2E+00**	2.2E+01
PFBA	mg/kg	3.8E+02	**3.8E+00**	6.1E+01	5.5E+01	5.3E+01	NA	NA	NA
Surface water									
PFNA	mg/L	**2.9E-03**	6.3E-03	1.2E-02	1.3E-02	9.0E-03	NA	NA	NA
PFOS	mg/L	1.8E-03	6.0E-04	4.7E-04	5.1E-04	**3.6E-04**	3.4E-03	9.1E-04	7.5E-04
PFOA	mg/L	3.6E-02	**8.9E-03**	2.4E-02	2.6E-02	1.9E-02	NA	NA	NA
PFHxA	mg/L	1.3E+01	**4.4E-01**	1.3E+01	1.0E+01	1.4E+01	NA	NA	NA
PFBS	mg/L	3.0E+02	3.8E+00	8.0E+00	8.5E+00	6.0E+00	2.9E+01	**1.1E+00**	2.1E+00
PFBA	mg/L	1.2E+02	3.3E+00	2.1E+00	**2.2E+00**	1.6E+00	NA	NA	NA

Bold indicates the minimum risk-based screening level for each per- and polyfluoroalkyl substance and media.
LOAEL = lowest-observed–adverse effect level; NA = not available; NOAEL = no-observed–adverse effect level; PFBA = pentafluorobenzoic acid; PFBS = perfluorobutanesulfonic acid; PFHxA = perfluorohexanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; RBSL = risk-based screening level.

effects) do not require protection at that site, the benchmarks may overestimate the potential for adverse effects at the site in question. For example, Environment and Climate Change Canada (2018) notes that the FWQG value of 6.8 μg/L based on the 5th percentile SSD represents the concentration at which one would expect either no, or only a very low, likelihood of adverse effects on aquatic life. In addition to this guideline, 2 additional concentration ranges are provided for use in risk management. At concentrations between greater than the FWQG and the 50th percentile of the SSD (i.e. >6.8 to 1100 μg/L) there is a moderate likelihood of adverse effects to aquatic life. Concentrations greater than the 50th percentile (>1100 μg/L) have a higher likelihood of adverse effects. The "moderate" and "higher" benchmarks may be used in setting less protective interim targets for waters that are already degraded or where there may be socio-economic considerations that preclude the ability to meet the FWQG.

Although the reliability of the various calculation approaches depends on the robustness of the underlying data set, the RWQ RBSLs derived by the GLI approach in the present study use a relatively prescriptive set of requirements for inclusion of acute toxicity data and calculation of acute-to-chronic ratios using paired acute and chronic data for each species. As a result, some data that may be included using different benchmark calculation approaches may have been excluded from the present study. Notably, effects data not included in the RWQ RBSLs per the exposure duration specifications in the GLI approach include 10-d and life-cycle PFOS toxicity data for *Chironomus tentans* (MacDonald et al. 2004), 14-d adult and multigenerational PFOS toxicity data for Japanese medaka (*Oryzias latipes*; Ji et al. 2008), and 180-d multigenerational PFOS toxicity data for zebrafish (Keiter et al. 2012), which are among the lowest reported effect concentrations used in derivation of several current PFOS standards (e.g., Annual Average - Environmental Quality Standard for ecological receptors [AA-EQSeco] [European Commission 2011b], FWQG [Environment and Climate Change Canada 2018]). For *C. tentans*, a total emergence NOEC of <2.3 μg/L was reported in a life cycle test by MacDonald et al. (2004); however, EC10s were higher (89.3 μg/L for total emergence, 49 μg/L for 10-d growth, 88.2 μg/L for 20-d growth, and 107 μg/L for 10-d survival). Ji et al. (2008) reported an LOEC of 10 μg/L based on reduced

TABLE 9: Terrestrial risk-based screening level summary for wildlife

		Terrestrial RBSL						
Receptor		Herbivore Meadow vole	Invertivore Short-tailed shrew	Insectivore Little brown bat	Carnivore Long-tailed weasel	Herbivore American goldfinch	Insectivore House wren	Carnivore Red-tailed hawk
Constituent	Units							
NOAEL-based RBSLs								
Soil								
PFNA	mg/kg	2.3E+00	1.5E+00	**1.0E+00**	1.5E+00	NA	NA	NA
PFOS	mg/kg	3.1E-01	4.8E-02	3.0E-02	1.7E-01	3.8E-01	**1.3E-02**	8.7E-02
PFOA	mg/kg	5.8E+00	1.3E+00	8.4E-01	**5.7E-01**	NA	NA	NA
PFHxA	mg/kg	**1.2E+02**	3.4E+02	2.5E+02	1.6E+02	NA	NA	NA
PFBS	mg/kg	3.8E+01	1.4E+01	**9.1E+00**	7.8E+01	8.9E+01	9.3E+00	1.0E+02
PFBA	mg/kg	**2.9E+01**	7.8E+01	5.8E+01	1.3E+02	NA	NA	NA
Surface water								
PFNA	mg/L	6.0E+00	5.6E+00	**5.2E+00**	7.2E+00	NA	NA	NA
PFOS	mg/L	7.3E-01	6.7E-01	6.3E-01	8.6E-01	3.2E-01	**3.0E-01**	1.4E+00
PFOA	mg/L	2.2E+00	2.0E+00	**1.9E+00**	2.6E+00	NA	NA	NA
PFHxA	mg/L	6.1E+02	5.6E+02	**5.3E+02**	7.2E+02	NA	NA	NA
PFBS	mg/L	3.6E+02	3.4E+02	**3.2E+02**	4.3E+02	3.7E+02	3.5E+02	1.6E+03
PFBA	mg/L	5.3E+02	4.9E+02	**4.6E+02**	6.3E+02	NA	NA	NA
LOAEL-based RBSLs								
Soil								
PFNA	mg/kg	3.0E+00	2.0E+00	**1.3E+00**	2.0E+00	NA	NA	NA
PFOS	mg/kg	5.1E-01	7.9E-02	**5.0E-02**	2.8E-01	3.8E+00	1.3E-01	8.7E-01
PFOA	mg/kg	1.2E+01	2.6E+00	1.7E+00	**1.1E+00**	NA	NA	NA
PFHxA	mg/kg	**2.6E+02**	7.0E+02	5.3E+02	3.3E+02	NA	NA	NA
PFBS	mg/kg	1.5E+02	5.7E+01	3.6E+01	3.1E+02	1.5E+02	**1.5E+01**	1.7E+02
PFBA	mg/kg	**7.0E+01**	1.9E+02	1.4E+02	3.2E+02	NA	NA	NA
Surface water								
PFNA	mg/L	8.0E+00	7.4E+00	**6.9E+00**	9.5E+00	NA	NA	NA
PFOS	mg/L	1.2E+00	1.1E+00	**1.0E+00**	1.4E+00	3.2E+00	3.0E+00	1.4E+01
PFOA	mg/L	4.4E+00	4.0E+00	**3.8E+00**	5.2E+00	NA	NA	NA
PFHxA	mg/L	1.3E+03	1.2E+03	**1.1E+03**	1.5E+03	NA	NA	NA
PFBS	mg/L	1.5E+03	1.3E+03	1.3E+03	1.7E+03	6.2E+02	**5.8E+02**	2.7E+03
PFBA	mg/L	1.3E+03	1.2E+03	**1.1E+03**	1.5E+03	NA	NA	NA

Bold indicates the minimum RBSL for each per- and polyfluoroalkyl substance and media.
LOAEL = lowest-observed–adverse effect level; NA = not available; NOAEL = no-observed–adverse effect level; PFBA = pentafluorobenzoic acid; PFBS = perfluorobutanesulfonic acid; PFHxA = perfluorohexanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; RBSL = risk-based screening level.

larval survival and growth when the F0 generation was exposed for 14 d and followed by exposure in the F1 generation. Keiter et al. (2012) report statistically reduced growth in female fish continuously exposed to 0.6 µg/L PFOS in water for 180 d, although the EC10 appears to be higher for all growth endpoints measured. These studies are conducted over some of the longest durations typically reported in the literature. Although it is unclear if small reductions in growth (e.g., <10%) would be ecologically significant at the individual or population level, these data suggest that the acute-to-chronic ratios calculated from the available paired data may not be sufficiently high for chemicals that elicit effects at lower concentrations following continuous multigenerational exposures or for sensitive life stages.

Physical properties of specific PFAS may also contribute uncertainty to test results and their applicability to environmental conditions. For example, PFBA has been demonstrated to reduce the pH of test water, affecting toxicity (Hagenaars et al. 2011; Ding et al. 2012b). A study presenting both pH adjusted and unadjusted results indicated that toxicity data from unadjusted conditions are notably lower (Ding et al. 2012b). In the present study, pH unadjusted data were preferentially chosen when both were provided by the primary paper. In addition, PFOA is commonly available as an ammonium or potassium salt, and PFAS and their salts were included in the present study. The dissociated ammonia from the ammonium salt of PFAS may contribute to the toxicity observed in aquatic tests, as has been observed for PFOA (ammonium perfluorooctanoate) and hexafluoropropylene oxide dimer acid (Colombo et al. 2008; Hoke et al. 2016).

Terrestrial plant and invertebrate RBSLs

Plant NOECs for several PFAS reported in Ding et al. (2012a) were notably higher than the rest of the data set comprised of 6 published studies; LOECs were not reported by Ding et al (2012a). Because their study was the sole basis for the PFDA, PFNA, PFBA, and 5H 4:1 FTOH NOEC RBSLs, there is uncertainty around these RBSLs.

The PFOA NOEC RBSL was revised to be based on a LOEC RBSL divided by a uncertainty factor of 10 because the initially calculated NOEC RBSL was higher than the LOEC RBSL. There

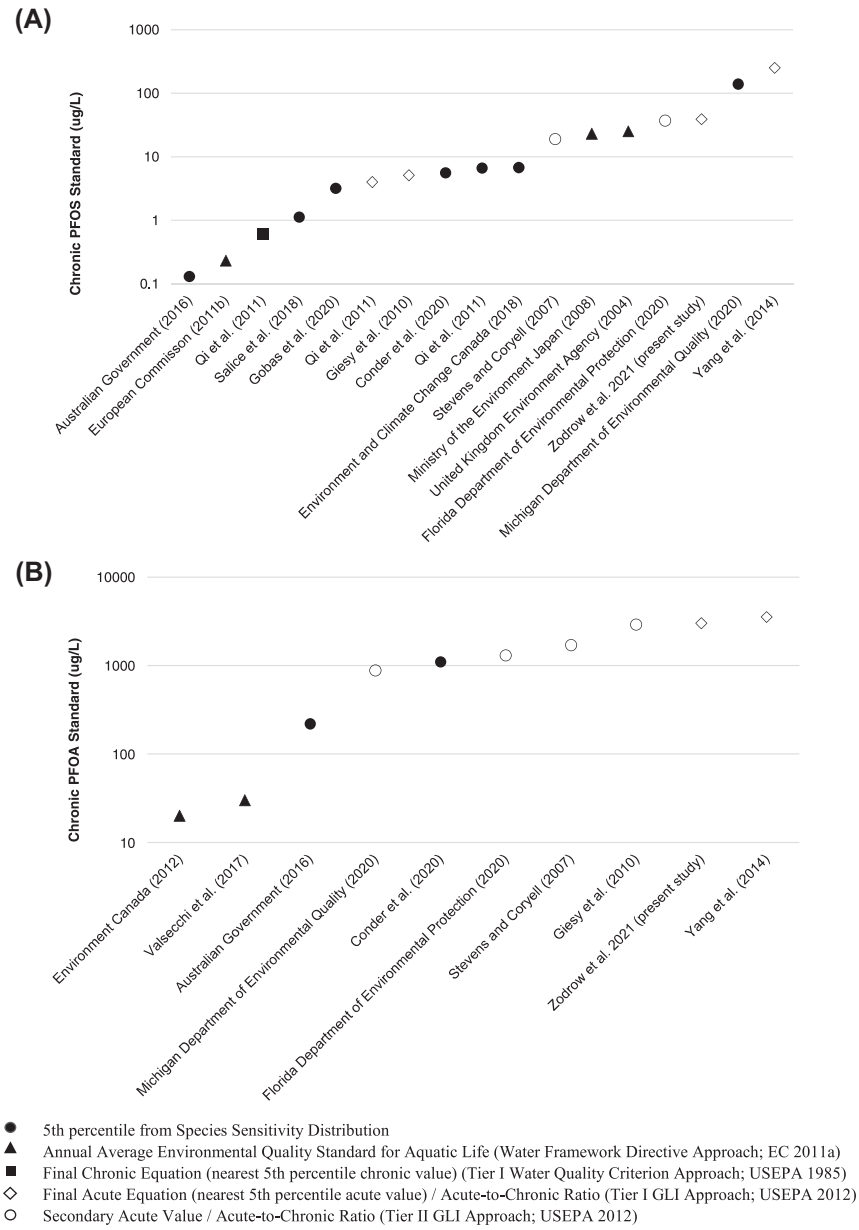

FIGURE 1: Comparison of chronic water quality values protective of aquatic life. Values published for perfluorooctanesulfonic acid are shown in (**A**); values for perfluorooctanoic acid are shown in (**B**). Symbols indicate the approach used to calculate the water quality values: ● = 5th percentile from species sensitivity distribution; ▲ = annual average environmental quality standard for aquatic life (Water Framework Directive approach; European Commission 2011a); ■ = final chronic equation (nearest 5th percentile chronic value; tier I water quality criterion approach; US Environmental Protection Agency 1985); ◇ = final acute equation (nearest 5th percentile acute value)/acute-to-chronic ratio (tier I Greak Lakes Initiative [GLI] approach; US Environmental Protection Agency 2012); ○ = secondary acute value/acute-to-chronic ratio (tier II GLI approach; US Environmental Protection Agency 2012). PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid.

is additional uncertainty around this value because it is based on application of a uncertainty factor to a LOEC rather than experimentally measured NOECs.

Invertebrate soil RBSLs

The terrestrial invertebrate NOEC RBSL calculation methods, like other NOEC RBSLs developed in the present study, are preferentially based on reproduction or growth endpoints. However, reproduction and growth toxicity data were unavailable. Therefore, all terrestrial invertebrate NOECs were based on survival and may underestimate the potential for adverse reproductive and growth effects. A uncertainty factor of 10 was applied to calculate the final RBSL in certain cases (see Table 7) to account for this uncertainty. Invertebrate RBSLs are likely to evolve in future evaluations as additional toxicity data become available.

Wildlife TRVs

The mammalian PFOS NOAEL and LOAEL TRVs (Table 4) are similar to the PFOS NOAEL- and LOAEL-based TRVs estimated by McCarthy et al. (2017) of 0.1 and 0.4 mg/kg body

weight/d, respectively. McCarthy et al. (2017) estimated PFOA NOAEL and LOAEL TRVs of 1 and 3 mg/kg body weight/d, which are higher than the PFOA TRVs calculated in the present study but differ by less than a factor of 10. McCarthy et al. (2017) selected PFOS TRVs as the lowest NOAEL and its paired LOAEL and PFOA TRVs as the lowest LOAEL above the highest NOAEL. Although McCarthy et al. (2017) generally followed only an abbreviated approach to the EcoSSL methodology, the agreement between TRVs derived by them and in the present study suggests a low relative degree of uncertainty in the mammalian TRVs, at least for PFOS and PFOA.

For all PFAS with mammalian TRVs derived in the present study, the data sets contain at least 3 toxicity studies, the minimum required number of toxicity studies to calculate a TRV according to EcoSSL methods. The toxicity studies were reviewed and selected for inclusion in the data sets using data quality evaluations similar to but somewhat less stringent than the EcoSSL approach. Therefore, the level of uncertainty in the mammalian TRVs is similar to that for other compounds for which EcoSSLs have been derived based on data sets of the same size.

Bird TRVs were calculated for only PFOS and PFBS (Table 4) because applicable toxicity data were unavailable for the remaining PFAS with mammalian TRVs. The PFOS NOAEL-based TRV is >1000 times lower than its PFBS counterpart. Both avian TRVs derived in the present study are based on a limited number of studies and the same 2 test species: northern bobwhite quail and mallard duck. As a result, sensitivities of other bird species or exposure environments may not be captured in the TRV data set.

Similar to the mammalian TRVs, bird TRVs calculated in the present study were compared to other published bird TRVs. In following EcoSSL methodology to derive the TRVs, both the PFOS and PFBS TRVs in the present study are higher than previously selected values (Newsted et al. 2005; Beach et al. 2006; Giesy et al. 2010; McCarthy et al. 2017; Gobas et al. 2020). For PFOS, Newsted et al. (2005) and Beach et al. (2006) calculated NOAEL TRVs of approximately 0.021 mg/kg body weight/d, and Giesy et al. (2010) calculated a NOAEL TRV of 0.032 mg/kg body weight/d; these TRVs are based on a LOAEL of 0.77 mg/kg body weight/d but were calculated using different uncertainty factors (36, 36, and 24, respectively). Gobas et al. (2020) calculated a similar TRV (0.025 mg/kg body wt/d) based on an SSD and uncertainty factor of 36. For PFBS, Giesy et al. (2010) calculated a bird PFBS TRV of 7.3 mg/kg body weight/d after applying a uncertainty factor of 12 to a NOAEL of 87.7 mg/kg body weight/d. McCarthy et al. (2017) suggests the same PFOS LOAEL of 0.77 mg/kg body weight/d as the basis of a bird TRV. Although the LOAEL was unbounded, this value was selected as the LOAEL TRV in the present study; however, a calculated measured concentration of 0.79 mg/kg body weight/d was used instead of the nominal concentration of 0.77 mg/kg body weight/d. The NOAEL TRV was the LOAEL TRV divided by a uncertainty factor of 10. Therefore, the LOAEL TRVs between the 3 evaluations are effectively the same, and differences in NOAEL TRVs are a result of different uncertainty factors applied. Similarly, Giesy et al.'s (2010) selected avian NOAEL for PFBS is included in the present evaluation's data set. However, it was not the highest bounded NOAEL below the lowest bounded LOAEL and, therefore, was not carried through as the final NOAEL-based TRV in the present study.

Wildlife RBSLs

The wildlife NOAEL RBSLs calculated in the present study span a wide range of concentrations (Tables 8 and 9). The lowest water PFOS RBSL is 0.075 μg/L, and the lowest water PFOA RBSL is 4.4 μg/L. Values for protection of wildlife calculated using European Commission (2011a) methods are lower by approximately an order of magnitude for both PFOS (0.002 μg/L; European Commission 2011b) and PFOA (0.1 μg/L; Valsecchi et al. 2017).

Bioaccumulation factors

A key uncertainty in the selection of BAFs was the use of surrogates. Either BAFs, BSAFs, or data used to derive BAFs and BSAFs were more available for certain dietary components such as terrestrial plants and fish than for other dietary components such as reptiles and birds. When no data were available to estimate a BAF for a dietary component, surrogate BAFs from a dietary component with similar predicted exposure were used to develop RBSLs. Terrestrial invertebrate BAFs were used as surrogates for terrestrial aerial insects. The terrestrial soil-to-small mammal BAF was used to represent PFAS bioaccumulation by terrestrial birds, terrestrial reptiles, and aquatic mammals when developing RBSLs. The aquatic invertebrate BAFs and BSAFs were used as surrogates for aquatic aerial insect BSAFs. Fish BSAFs were used as surrogates for amphibian BSAFs. If BAFs were available within a dietary component but not for all 6 PFAS identified for RBSL development, surrogate BAFs from another PFAS were selected based on similarity in carbon chain length (e.g., PFBS was commonly used as a surrogate for PFBA). No RBSLs were developed for PFHxS in the present study; however, BAFs and BSAFs were identified for PFHxS as part of the BAF review. The BAFs and BSAFs for PFHxS were used as surrogate BAFs and BSAFs for PFHxA, PFBS, or PFBA. In most cases, the structure of PFAS used as surrogates was relatively similar to the PFAS for which the RBSL was being derived, varying by no more than 3 carbons in the fluorotelomer chain. In some cases, the functional groups differed as well (e.g., sulfonate instead of carboxylate). As a surrogate for PFBA, PFHxS had the largest difference in chain length and differed in functional groups. One exception is the PFOS soil-to-small mammal BAF used for all PFAS. This BAF was used for terrestrial bird and reptile dietary groups as well as the aquatic bird and mammal BSAFs. Sufficient data are not currently available to quantitatively evaluate the magnitude and direction of uncertainty related to the use of surrogate BAFs for each dietary component. The relative importance of the BAF uncertainties in each calculated RBSL depends on both the magnitude of the uncertainty and the fraction of the diet represented by a specific dietary

component. The individual BAFs used to calculate the final BAF are presented in Supplemental Data I, and the fraction of diet is presented in Supplemental Data II. Thus, for example, although uncertainty in the reptile and amphibian BAFs and BSAFs may be high (surrogate reptile BAFs are based on mammal BAFs using data converted from tissue to whole body; amphibian BSAFs are based on fish data as a surrogate), these food items typically make up a relatively small proportion of wildlife diets and were included in the RBSL calculations only for mink (3% of diet) and red-tailed hawk (13% of diet), reducing the overall effect of uncertainty from these BAFs on calculated RBSLs. In addition, a surrogate approach is common in screening and baseline ERAs when site-specific or tissue-specific bioaccumulation data are unavailable. For example, small mammals (i.e., rodents) are commonly used to estimate uptake for predators that may consume a variety of vertebrate prey. For neutral organic bioaccumulative compounds (e.g., chlorinated organics such as polychlorinated biphenyls), use of soil-to-earthworm BAFs as a surrogate for estimating uptake in all terrestrial invertebrates is considered to be conservative because of earthworm behavior and feeding strategy (living in and ingesting soil, as opposed to inhabiting aboveground habitats) and lack of physical structures (e.g., exoskeletons present in arthropods) that reduce uptake through the skin. However, data suggest that the standard lipid-based equilibrium partitional model that makes earthworms a conservative surrogate may not be appropriate for PFAS that have a complex chemistry due to the presence of multiple and diverse functional groups that can bind to proteins and/or cell membranes (Jones et al. 2003; Agency for Toxic Substances and Disease Registry 2018; Fitzgerald et al. 2018). Nevertheless, the terrestrial invertebrate BAFs calculated for use in the wildlife RBSLs were derived based on earthworm data because no relevant uptake data for other invertebrate species were located. Similarly, although differences in metabolism and clearance of PFAS may be more pronounced between species than for neutral organic bioaccumulative compounds, a soil-to-mammal BAF is commonly used in risk assessment to estimate uptake for avian wildlife. For aquatic invertebrates, data available for a variety of species (Supplemental Data I) were used, which may give a more robust estimate of PFAS uptake from water. As additional bioaccumulation data become available, the uncertainty in surrogates can be quantified and BAFs updated, as needed.

The process for developing BAFs generally favored values more representative of field conditions or more conservative values (i.e., predicting higher uptake); however, as discussed, some degree of uncertainty is introduced by the BAF data selection. Terrestrial BAFs incorporated both laboratory-based and field-based BAFs, whereas aquatic BAFs incorporated only field-based BAFs to account for additional variability in the aquatic environment.

It has been assumed that PFAS with shorter chain lengths, considered perfluoroalkyl carboxylic acids with 6 carbons or less, generally demonstrate lower bioaccumulation compared to the longer-chain PFAS (Brendel et al. 2018). Among the PFAS with RBSLs calculated in the present study, PFNA, PFOS, and PFOA are considered long-chain PFAS, whereas PFHxA, PFBS, and PFBA are short-chain PFAS. The data retrieved for the present study present no clear relationship between chain length and bioaccumulation for any of the terrestrial or aquatic groups, which may be due to factors including lack of a robust data set, species-specific uptake differences, and differences in the test conditions. As more data are collected for additional receptors and compounds, more consistent relationships may become more apparent.

Biomagnification uncertainty

While evaluating bioaccumulation of PFAS in aquatic and terrestrial biota, studies were identified that evaluated the potential for PFAS biomagnification and estimated biomagnification factors (BMFs) between trophic levels and trophic magnification factors (TMFs). Biomagnification of PFAS between trophic levels is not fully understood. Regional differences have been observed between TMFs, and the potential for biotransformation of PFAS into other, more stable PFAS compounds is a complicating factor in calculating BMFs (Houde et al. 2006b; Tomy et al. 2009; Müller et al. 2011).

There is a large variation in measures of biomagnification between constituents, geographic locations, and complexity of food webs. Tomy et al. (2009) reported estimated liver:liver BMFs for a western Canadian Artic aquatic food web ranging from 27.7 to 276 for PFOS and 1.2 to 12.9 for PFNA. Tomy et al. (2009) estimated whole-body adjusted TMFs of 4.8 for PFNA and 19.6 for PFOS. Loi et al. (2011) reported a whole-body homogenate PFOS TMF of 1.3 for an aquatic food web in China. Three additional TMFs, for PFDA, PFUnA, and PFDoA, were 1.50, 1.74, and 1.38, respectively (Loi et al. 2011). Müller et al. (2011) reported whole-body adjusted BMFs and TMFs for terrestrial food webs from 2 areas in northern Canada. The caribou:vegetation BMFs were estimated as 8.5 and 5.3 for PFNA, 9.1 and 7.9 for PFOS, and 1.8 and 0.3 for PFOA (Müller et al. 2011). The wolf:caribou BMFs were estimated as 3.8 and 5.4 for PFNA, 3.3 and 1.2 for PFOS, and 2.4 and 2.1 for PFOA (Müller et al. 2011). Wolf:caribou:vegetation TMFs were estimated as 2.0 and 1.9 for PFNA, 1.1 and 1.3 for PFOA, and 2.2 and 2.3 for PFOS (Müller et al. 2011). Estimations of biomagnification and trophic magnification suggest that PFAS with carbon chain lengths of 6 or greater have the potential to biomagnify through the food web and across trophic levels (Houde et al. 2006a, 2006b; Kelly et al. 2009; Tomy et al. 2009; Giesy et al. 2010; Loi et al. 2011; Müller et al. 2011).

In the present study, BMFs were not available for the threatened and endangered surrogate species considered. Therefore, biomagnification was not quantitatively included in the calculation of RBSLs. However, the BAFs and BSAFs included in the food web and used in the calculations of RBSLs do account for accumulation through the food web. For example, the field-based BAFs and BSAFs based on trout, seabass, snakehead, sole, and flounder include diets consisting of other fish and aquatic organisms. In the United States, threatened and endangered species that are higher in the food web

TABLE 10: Background and impacted soil, sediment, and surface water per- fluoroalkyl substance concentrations

Media	Constituent	Background concentration range	Reference	Impacted concentration range[a]	Chronic RWQ RBSL	Min RBSL[b]	Basis
Soil (mg/kg)	PFOS	5.8E-04–2.6E-03	[c]	1.7E+00–1.9E+00	—	1.3E-02	Wildlife RBSL
	PFOA	3.2E-04–3.2E-01	[d]	5.8E-02–1.4E-01	—	8.4E-02	Plant RBSL
Sediment (mg/kg)	PFOS	2.0E-04–3.0E-03	[e]	1.9E+02	—	1.4E-03	Wildlife RBSL
	PFOA	1.4E-04–3.9E-03	[d,e]	9.5E-01	—	6.0E-03	Wildlife RBSL
Surface water (mg/L)	PFOS	1.6E-06–7.6E-04	[f]	9.0E+01	3.9E-02	7.5E-05	Wildlife RBSL
	PFOA	6.5E-07–6.0E-04	[d,g]	2.1E-01	3.0E+00	4.4E-03	Wildlife RBSL

Table 10 presents a summary of select background and site impacted per- and polyfluoroalkyl substance (PFAS) concentrations; however, it does not represent a comprehensive literature search and may not be representative of the full range of background or impacted-site PFAS concentrations.
[a]Anderson et al. (2016).
[b]Minimum RBSL is the minimum terrestrial plant, terrestrial invertebrate, and aquatic and terrestrial wildlife RBSLs.
[c]Strynar et al. (2012).
[d]González-Naranjo and Boltes (2014).
[e]Higgins et al. (2005).
[f]Hansen et al. (2002), Boulanger et al. (2004, 2005), Sinclair et al. (2006).
[g]Hansen et al. (2002).
PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; RBSL = risk-based screening level; RWQ = recommended water quality.

and potentially subject to higher levels of biomagnification include the Canada lynx, Florida panther, gray wolf, jaguar, Mexican wolf, and red wolf.

CONCLUSIONS

The surface water RWQ RBSLs are lowest for fluorotelomer PFAS as well as PFDoA, PFUnA, and PFOS. The limited toxicity data available for terrestrial plants and soil invertebrates preclude comparisons of the relative toxicity of different PFAS at this time. For wildlife, the calculated RBSLs indicate that wildlife receptors may be most sensitive to PFOS. Aquatic and terrestrial insectivorous and invertivorous receptors appear most sensitive to PFAS in the environment based on the magnitude of the RBSLs. The little brown bat was most often the most sensitive surrogate species. Therefore, the northern long-eared bat, as well as the other threatened and endangered species within the insectivore and invertivore feeding guilds, may be expected to be the most sensitive to elevated environmental PFAS concentrations based on default exposure assumptions. Application of the RBSLs paired with a site-specific site use factor could increase the site-specific RBSLs and modify the most sensitive receptor because the site use factor incorporates receptor home ranges.

Although the shorter-chain PFAS wildlife RBSLs are generally higher (i.e., are less toxic) than those for longer-chain PFAS, shorter chains have been documented to be more mobile in the environment (Brendel et al. 2018). This may lead to larger PFAS-impacted areas as a result of PFAS migration in soil or water. Therefore, the lower risk associated with PFAS with higher RBSLs may be offset in an ERA by higher site use factors associated with a larger impacted area.

The minimum RBSLs are consistently within or below the range of PFAS concentrations measured at select impacted sites (Table 10), indicating that areas with historical or current releases may contain environmental media with PFAS above screening levels and warrant further evaluation. The minimum NOAEL-based RBSL is above the range of select background concentrations for PFOS in soil, and the minimum NOAEL-based RBSL is within the range of background concentrations for PFOS in sediment and surface water and PFOA in soil, sediment, and surface water. For aquatic life, the PFOS acute and chronic RWQ RBSLs are below the select impacted concentrations and above the background ranges presented in Table 10. The PFOA acute and chronic RWQ RBSLs are above the impacted concentration as well as the background concentration range. As demonstrated by PFOS and PFOA RBSLs falling within the background concentration range, the feasibility of some of the minimum NOAEL RBSLs as screening benchmarks may depend on additional refinement or site-specific background concentrations.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4975.

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