HEPA

PFAS NEMP Supporting Document

Derivation of ecological guidelines for indirect exposure to perfluorooctanoic acid (PFOA)

National Chemicals Working Group of the Heads of EPAs Australia and New Zealand

September 2022

Cataloguing data

This publication (and any material sourced from it) should be attributed as: HEPA 2022, *Draft PFAS National Environmental Management Plan: PFAS NEMP Supporting Document Derivation of ecological guidelines for indirect exposure to perfluorooctanoic acid (PFOA)*, CC BY 4.0. Heads of EPA Australia and New Zealand

ISBN XXX-X-XXXXX-XXX-X

This publication is available at dcceew.gov.au/publications.
Department of Climate Change, Energy, the Environment and Water
GPO Box 3090 Canberra ACT 2601
Telephone 1800 900 090
Web dcceew.gov.au

Acknowledgements

The Heads of EPA Australia and New Zealand (HEPA) acknowledge the contributions of Commonwealth, State and Territory agencies and thank everyone who provided input and feedback to inform the development of this plan.

Acknowledgement of Country

We acknowledge the Traditional Custodians of Australia and their continuing connection to land and sea, waters, environment and community. We pay our respects to the Traditional Custodians of the lands we live and work on, their culture, and their Elders past and present.

Contents

Tables

Figures

Introduction

Background and purpose

This supporting document presents the background information on the derivation of the ecological criteria for PFOA presented in Section 8.6 of the PFAS National Environmental Management Plan (NEMP) (NEMP 3.0).

At the request of Environment Ministers around Australia, the Heads of EPAs Australia and New Zealand (HEPA) and the Australian Government Department of Climate Change, Energy, Environment and Water (DCCEEW) collaborated to develop and publish the PFAS National Environmental Management Plan (NEMP) (NEMP 1.0 in February 2018 and NEMP 2.0 in January 2020). The NEMP provides a nationally consistent approach to environmental management of PFAS, including environmental guideline values (GVs) intended to be protective of ecological and human health for a range of exposures.

To protect potential ecological exposures to PFOA, the PFAS NEMP 2.0 only includes a soil guideline value for direct exposure. This value is interim and was adopted from the human health soil investigation level (HIL) for public open space. In comparison, perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) soil ecological guideline values are included in the NEMP for direct exposure, indirect exposure, wildlife diet (mammalian and avian) and exposure of birds to protect their eggs.

As PFOA is bioaccumulative in terrestrial ecosystems and air breathing aquatic fauna (ECHA 2013), there is a need for ecological guideline values that are protective of these exposures. The tendency for PFOA to bioaccumulate in air breathing animals is attributed to the combination of efficient dietary assimilation, strong partitioning into protein rich tissues and fluids, high resistance to metabolism and low volatility. These factors translate into high gastrointestinal uptake and slow elimination rates (Kelly et al. 2009).

The current PFOS plus PFHxS indirect exposure via soil and wildlife diet GVs in the NEMP were adopted from the Canadian Federal Environment Quality Guidelines (FEQGs) developed by Environment and Climate Change Canada (ECCC 2018), with PFHxS included using the read across principle to PFOS.

For consistency, the same approach has been used in this paper to derive GVs for PFOA but taking into account Australian ecology and environmental objectives where appropriate with available data. Any deviation from EEEC (2018) is not intended as criticism of the Canadian methods, but rather is intended to adapt the approach where relevant considering the Australian context.

No specific recommendations to the NCWG are made in this report, as it is an information paper to support NCWG and HEPA decision making. For details on the Canadian GVs and methodologies the reader is referred to the Canadian Council of Ministers of the Environment (CCME) webpage. Drafts of this document have been peer-reviewed by members of the National Chemicals Working Group.

Scope

This supporting document is restricted to developing potential guidance to be included in the forthcoming NEMP 3.0 for indirect exposures for ecological receptors via consuming food or prey, which has accumulated PFOA from soil and, when assessing food items directly, from multiple potential sources such as water and sediments. Revision of the interim direct toxicity GV for PFOA is out of scope.

While the literature review aimed to include terrestrial and avian species, there was very limited research found on PFOA toxicity to birds. This included studies in which PFOA is injected into developing eggs (Nordén et al. 2016; Mattsson et al. 2019), a short-term acute toxicity study with Japanese quail (*Coturnix japonica*) (Simcik and Bursian 2021) and studies of elimination kinetics, biochemistry and tissue changes in chickens (*Gallus gallus*) using subcutaneous implantation (Yoo et al. 2009).

Due to the relative paucity of PFOA toxicity studies and data related to avian fauna, the draft GV derivations focus on mammals, in particular:

- Ecological criteria for wildlife diet for mammals (Section 2)
- Ecological criteria for indirect exposure via soil for mammals (Section 3).

1 Ecological guidance for wildlife diet

The ECCC Federal wildlife dietary guidelines calculate a dietary reference concentration for wildlife species, based on species-specific tolerable daily intakes (TDI), food ingestion rates and body weight (ECCC 2018). The species with the highest Food Ingestion: Body Weight (FI:BW) ratio will result in the lowest reference concentration. The lowest reference concentration from a broad range of species considered is chosen as the ecological criteria for wildlife diet under the ECCC guidelines (ECCC 2018). The equation for calculating reference concentration for wildlife diet is (CCME, 1998):

Equation 1

$$RCn = TDI \div (FI \div bw)$$

Where:

RCn = reference concentration (mg/kg), where n refers to one of several wildlife species for which an RC may be calculated

TDI = tolerable daily intake (mg/kg body weight per day)

FI = food ingestion (kg/day wet weight)

bw = body weight (kg)

Reference concentrations are calculated for key indicator wildlife species (e.g. piscivores) using information on body weight and daily food ingestion for these wildlife species, as well as the TDI derived from toxicity studies. Only the mammalian TDI is used to extrapolate to mammalian wildlife species.

Based on the above equation, the key inputs for guideline derivation are a TDI for the contaminant in question, and a relevant species with a sufficiently high FI:BW such that is protective of a large proportion of consumers. The wildlife diet guidelines are intended to protect mammalian species that consume aquatic biota.

The guidance value relates to the concentration of PFOA in the aquatic biota food item, expressed as whole body on a wet weight basis that could be eaten by terrestrial, aquatic or semi-aquatic mammalian wildlife. It also covers wildlife foods that derive from aquatic ecosystems, for example, emergent aquatic insects.

1.1 Tolerable daily intake (TDI)

The NCWG reviewed information on PFOA toxicity (Danish Ministry of the Environment 2015) and searches of more recent mammalian toxicity studies to determine the lowest observed adverse effects dose of PFOA found in mammalian toxicity data. Data on the lowest observed adverse effect level (LOAEL) from a consumptive exposure, expressed or able to be expressed as a concentration on a per unit of body weight basis, were sought.

The majority of studies found related to rodents, particularly rats and mice. A list of studies considered with ecologically relevant end points at lower doses are summarised in Appendix A.

General observations show that rats are less sensitive than mice, for the more sensitive end points, particularly developmental toxicity. These studies determined that rats are not ideal species to investigate PFOA-induced developmental effects due to the characteristic of the female rat to rapidly eliminate PFOA, with a half-life of only several hours (Lau et al. 2007). Due to the rapid elimination in the rat, steady state is not reached with daily dosing. This also results in episodic exposure of the foetus, rather than continuous exposure (Lau et al. 2007).

Mice do not have this characteristic and hence mice studies are preferred.

The chosen critical toxicity value related to developmental toxicity with an exposure concentration of 0.1 mg/kg bw per day and was based on abnormalities in mammary gland development. The study by Macon et al. (2011) involved a range of quantitative and qualitative measurements of mammary gland morphology to derive overall developmental mammary gland scores.

The lowest daily dose at which mammary gland developmental abnormalities were visible and statistically different was 0.01 mg PFOA/kg bw. At the next highest daily dose 0.1 mg PFOA/kg bw, abnormalities were more pronounced.

At postnatal day (PND) 14, the longitudinal epithelial growth of the mammary glands from the 0.1 and 1.0 mg/kg groups was significantly reduced compared with controls by 14.4% and 37.3% respectively, and the change in longitudinal growth from PNDs 1 to 14 was reduced by 27.4% and 56.5%, respectively. This form of aberrant gland development was observed to persist into adulthood.

Similar findings of dose dependent impaired mammary gland development were observed in a similar study of two mice strains conducted by Tucker et al. (2015), with persistence also noted into young adulthood. As the study noted a lower exposure of 0.01 mg/kg bw per day also caused statistically significant effects, a no–observed adverse effect level (NOAEL) could not be determined (Macon et al. 2011).

The exposure concentration of 0.1 mg/kg bw per day was chosen as the critical toxicity value based on the larger degree of gland impairment observed to strengthen the imputation of ecological relevance. Other adverse effects on mice reported at this dose include a reduction of pups per litter and an increased relative liver weight (Abbott et al. 2007).

The Macon et al. (2011) study was considered acceptable using the assessment system described in SERDP (2020), scoring 7 out of a possible 10. Three marks were lost for gavage dosing rather than feeding spiked food, dose made from analytical grade PFOA each day rather than measured each day and, despite a large range of exposure concentrations covering 3 orders of magnitude, the lowest dose which was 100 times lower than the highest dose still elicited an adverse effect, although of smaller magnitude (refer Appendix B for details).

The mammary gland is a unique organ in that most development occurs postnatally and thus is particularly sensitive to PFOA, which can act as an endocrine disruptor (Gore et al. 2015; Benninghoff et al. 2011). The observed adverse effects are attributed to *in utero* exposure, exacerbated by additional exposure due to lactation (Tucker et al. 2015).

Impairments to development with potential adverse impacts on nutritional support of young and delays in maternal ability to provide this are considered ecologically relevant. Prenatal exposure to atrazine is another substance found to delay mammary gland development in rodents, with delays in development most severe in the animals exposed prenatally and post-partum due to nursing from atrazine exposed dams (Rayner et al. 2005).

Young animals with delayed mammary gland development at breeding (to generate the F2 offspring) have been observed to raise pups that were significantly (from 12% to 25%) smaller than controls. This suggests that the exposed F1 dams were not able to produce the quality and/or quantity of milk necessary to sustain the body weight of their offspring (Rayner et al. 2005).

The processes of formation and secretion of casein and milk fat are the same in the glands of all three subclasses of the Mammalia. Ultra-structurally all mammary glands, prototherian, metatherian, and eutherian are identical, the alveoli consisting of a secretory epithelium invested by myoepithelium. (Griffiths et al 1973).

Mere delay in effective sexual maturity can be ecologically significant for Australian fauna. One circumstance is where mammal species have a rigid and highly synchronized mating period. Many dasyurids have a life history in which both sexes achieve sexual maturity at the same age and mate during a short (two to three week) and highly synchronised period each year (Morton et al. 1989). The mating is triggered by rate of change of day length, with delays observed with both increasing altitude and decreasing latitude (McAllan and Dickman 1986).

Another reason a mere delay in reproductive maturity could be ecologically disadvantageous is where reproduction must be timed with unpredictable availability of resources. In Australia's arid environments, the population dynamics of small mammals are strongly influenced by rainfall-driven pulses of primary productivity (Dickman et al. 1999; Southgate et al. 1996), inferring a competitive advantage for individuals able to rapidly reproduce and exploit favourable conditions.

Other sensitive adverse effects of PFOA at relatively lower dose rates include liver toxicity (Nakamura et al. 2009) and reproductive success (Abbott et al. 2007).

A TDI of **1 µg/kg bw per day** was derived by dividing the critical toxicity value (0.1 mg/kg bw per day) by an uncertainty factor (UF) of 100. ECCC (2018) apply a UF of 100 to account for extrapolation from laboratory to field conditions, and for extrapolation from observed effects to a no-effect level. As there are numerous small Australian mammal species that are threatened, vulnerable or endangered such as the water mouse *Xeromys myoides*, this degree of conservatism in uncertainty factor selection is considered appropriate.

Other potential uncertainty factors considered in developing ecological guidance would include a factor for intra and interspecies variability. These additional factors are not included in the ECCC (2018) for PFOS and not included here for reasons of consistency.

To some extent, intraspecies variability is addressed by using toxicity data from a mouse strain identified as more sensitive to PFOA for the chosen adverse effect. Interspecific variability is also indirectly considered by using a representative species with a large proportionate food intake in the GV calculation.

1.2 Food Intake rate

The second element necessary for the derivation is FI:BW ratio for a suitable and relevant species. ECCC (2018) derive Canadian wildlife diet guidance for PFOS using the maximum FI:BW from a range of relevant north American aquatic and semi aquatic species for which data are available (CCME 1998), with a FI:BW of 0.24 kg food (wet weight)/kg body weight for the American mink.

A review was undertaken of available food intake data for Australian species to confirm if a value of FI:BW of 0.24 would be sufficiently protective. In relation to Australian mammalian fauna, the platypus *Ornithorynchus anatinus* (Figure 1) is an Australian example of a small semiaquatic mammal that eats primarily aquatic prey for which food consumption data are available.

Holland and Jackson (2002) measured typical food consumption of platypus (when not breeding) at 20% to 30% of body weight per night. Food intake during late lactation reached 90% to 100% of body weight, indicating the large energy requirements during late lactation. Lactation has been observed for 3 to 4 months in the wild (Grant and Griffiths 1992) and also in captivity for 5 months (Fleay 1944).

Another study measuring platypus food consumption by lactating females found it reached a maximum of 36.4% of body weight during the final month of lactation, attributing the higher rate of the earlier study to measurement of uneaten as well as eaten food (Thomas et al. 2018).

Given that the critical toxicity value relates to developmental toxicity and that the adverse effect (deficiency in mammary gland development) is exacerbated by lactational exposure, consideration of food intake while providing nutrition to young is considered relevant. The FI:BW value of 0.36 as calculated by Thomas et al. (2018) is adopted for deriving a wildlife dietary guideline value using local species.

Figure 1: The platypus *Ornithorynchus anatinus*, an Australian example of a small semiaquatic mammal that eats primarily aquatic prey

Source: Queensland Government – Department of Environment and Science

1.3 Wildlife diet guideline derivation

Considering the TDI in Section 2.1 and the food intake rate in Section 2.2, the NCWG has calculated a guidance value for wildlife diet (reference concentration in food from Equation 1) of 1 µg/kg bw/day / 0.36 kg/kg bw/day = **2.8 µg/kg wet weight in food**.

If the FI:BW rate for a North American mink was used in preference to local data, the wildlife diet guidance value would be is 1 µg/kg bw/day / 0.24 kg/kg bw/day = **4.2 µg/kg wet weight in food**. This would likely not be protective of platypus or any other Australian mammal fauna with a higher food intake ratio than the mink.

2 Ecological guidance for indirect exposure via soil

The ECCC (2018) approach for soil guideline derivation is based on calculating PFAS transfer from soil into food chains on a dry weight mass transfer basis, following the general Canadian methodology for deriving soil guidance (CCME 2006). This contrasts with derivation of wildlife diet, which is based on wet weight concentrations in consumer food and prey sources.

The approach is based on daily intake models similar to derivation of maximum human daily uptake models. The modelling takes into account direct soil ingestion and bioaccumulation through the food chain. Canadian soil quality guidelines (SQG) are calculated using the general form of the following equation:

Equation 2

$$SQG_{2C} = \frac{0.75 \cdot DTED_{2C} \cdot BW_{2C}}{(SIR_{2C} \cdot BF) + (FIR_{2C} \cdot BAF_{2})}$$

Where:

SQG_{2C} refers to the soil quality guideline for soil and food ingestion for the secondary consumer (mg/kg dry weight soil).

0.75 is the proportion of the daily threshold effects dose allocated to this exposure pathway, namely 75%.

$DTED_{2C}$ is the daily threshold effects dose for the secondary consumer (mg/kg body weight-day).

BW_{2C} is the body weight of the representative secondary consumer (kg).

SIR_{2C} is the soil ingestion rate of the representative secondary consumer (kg dry weight soil/day).

BF is the bioavailability factor (unitless), assumed to be equal to one in all cases.

FIR_{2C} is the food ingestion rate for the species used in the $DTED_{2C}$ (kg dw food/day).

BAF_2 is the bioaccumulation factor (unitless).

The transfer or biomagnification factors needed to estimate mass transfer in modelling are:

- Transfer factor – Soil to plant
- Biomagnification factor – Soil to invertebrate
- Trophic biomagnification factor – Invertebrate to primary consumer
- Trophic biomagnification factor – Primary consumer to secondary consumer

Using these factors, mass transfer of a contaminant from the soil up the food chain into representative ecological receptor species can be calculated when combined with knowledge of the species' food intake as a proportion of body weight and the food types which it consumes. The contaminant mass transfer also includes incidental soil ingestion.

For example, a primary consumer or herbivore, will daily ingest a certain amount of PFAS from soil plus additional PFAS that has accumulated into the plants is eats, as estimated by the relevant soil to plant transfer factor. This mass, when divided by the animal's body weight, can be compared to the relevant tolerable daily intake for the contaminant.

2.1 Soil to plant transfer factor

The NCWG collated PFOA soil to plant transfer factors (TFs) for above ground plant tissues from literature or calculated these from soil and plant concentrations in 12 publications using the following equation. The transfer factors and corresponding studies are summarised in Table 1.

Equation 3

$$TF = \frac{C_{plant}}{C_{soil}}$$

Where:

C_{plant} = concentration of PFOA in dry weight of plant

C_{soil} = concentration of PFOA in dry weight of soil

The transfer factors (TF) used for GV derivation are based on the maximum dry weight transfer factors across all the studies in Table 1 taken from each plant type (in bold). This approach was preferred so that each plant type was equally represented rather than an alternative where plant types with more data points would be overrepresented.

The measures of central tendency considered were the arithmetic mean, median and geometric mean. As the Canadian approach uses a geometric mean, this statistic was preferred for GV derivation.

The following TFs were adopted:

- 8.8 for above ground vegetation based on the geometric mean of the maximum TFs for each of the fourteen plant types
- 0.13 for grain based on the geometric mean of the maximum TF for each of the three grain types.

As grains are only present on a plant for part of the year, do not constitute the whole plant mass and not all the plants in the studies are cereals, a composite transfer factor was calculated using the transfer factors for vegetative compartments and grains weighted 90% and 10% respectively.

Equation 4

Composite PFOA TF = (Upper vegetative TF X 0.9) + (Grain TF X 0.1)

= (8.8 X 0.9) + (0.13 X 0.1)

= **7.9**

Table 1 PFOA soil to plant transfer factors, for plant portions relevant for ecological exposure, calculated from data in the listed data sources

	Plant	TF (mg/kg$_{plant}$)/(mg/kg$_{soil}$)	Calculation basis plant DW or WW	Data source
Vegetative parts DW	Alfalfa	3.2	DW	Wen et al. 2016
	Carrot	1.1 – 3.1	DW	Bizkarguenaga et al. 2016
	Cucumber	0.2 – 0.4	DW	Moshfeghi 2015
	Maize (corn)	0.1 – 0.3	DW	Stahl et al. 2009
	Maize (corn)	0.2	DW	Wen et al. 2016
	Mung bean	**8.4**	DW	Wen et al. 2016
	Oats	0.2 – **4.3**	DW	Stahl et al. 2009
	Radish	5.3	DW	Wen et al. 2016
	Ryegrass	**1.3**	DW	Wen et al. 2016
	Soybean	0.3	DW	Wen et al. 2016
	Wheat	1.9 – **6.8**	DW	Stahl et al. 2009
	Wheat	0.7 – 1.5	DW	Wen et al. 2014
	Wheat	0.09 – 0.3	DW	Zhao et al. 2014
	Wheat leaf	6.4	DW	Liu et al. 2019[a]
	Corn Leaf	**9.9**	DW	Liu et al. 2019[a]
	Celery leaf	**13**	DW	Liu et al. 2019[a]
	Onion leaf	**4.6**	DW	Liu et al. 2019[a]
	Chives leaf	**5.9**	DW	Liu et al. 2019[a]
	Radish	6.2	DW	Liu et al. 2019[a]
	Alfalfa	**10**	DW	Lasee et al. 2019
	Radish	**47**	DW	Lasee et al. 2019
	Carrot	**54**	DW	Lasee et al. 2019
	Red chicory leaf	**4.2**	DW	Gredelj et al. 2020
	Wheat	1.2 – 1.6	DW	Lan et al. 2018
Vegetative parts WW	Carrot	0.5	WW [= 4.2 DW][b]	Lechner and Knapp 2011
	Cucumber	0.8 – 1.0	WW [= 20 – **25 DW**][b]	Lechner and Knapp 2011
	Potato	0.4	WW [= **5.7 DW**][b]	Lechner and Knapp 2011
	Wheat grass	0.3 – 0.6	WW [= 1.6 – 3.2 DW][c]	Bräunig et al. 2019
Grains	Oats	0.03 – **0.1**	DW	Stahl et al. 2009
	Maize	0.003 – 0.009	DW	Stahl et al. 2009
	Wheat	0.009 – 0.1	DW	Stahl et al. 2009
	Wheat	0.1 – **0.2**	DW	Wen et al. 2014

	Plant	TF (mg/kg$_{plant}$)/(mg/kg$_{soil}$)	Calculation basis plant DW or WW	Data source
	Wheat	0.1	DW	Liu et al. 2019 [a]
	Corn	**0.1**	DW	Liu et al. 2019

Note

DW = dry weight, WW = weight weight.

[a] Data from Liu et al. 2019 from cropping field 10 km distant from fluoropolymer plant.

[b] Converted to dry weight using moisture content from Gebhardt and Thomas (2002), with potato plant leaves based on raw turnip leaves at 97% moisture.

[c] Wet weight values for values for cut wheat grass converted to a dry weight basis using the reported 21% average dry matter content of fresh wheat pasture (CCOF 2015).

2.2 Trophic bioaccumulation factors

There are limited studies on trophic transfer of PFAS in terrestrial food webs. ECCC (2018) used a food web study by Müller et al. 2011 comprising vegetation (plants and lichens), barren-ground caribou (*Rangifer tarandus groenlandicus*) and wolves (*Canis lupus*) for their PFOS soil GV derivation. The caribou in this food web are secondary consumers and the wolves are tertiary consumers. This study also reported trophic transfer values for PFOA. In the absence of Australian-specific terrestrial food web data, the NCWG has used the PFOA data from Muller et al. (2011) for GV derivation.

Use of trophic magnification factors from this research is considered appropriate as confounding factors are minimised as:

- The food web is relatively simple, as caribou feed mostly on lichen (in summer the diet also consists of willow, sedges and grasses) and wolves living near barren-ground caribou herds almost exclusively feed on them.
- It is therefore potentially easier to assess diet-consumer relationships than for more complex aquatic food webs
- As the environment is remote, the PFAS input is solely via the atmosphere as local sources are absent.

Muller et al. (2011) measured trophic biomagnification factors (TBMF) from two separate areas in Canada (Porcupine and Bathurst).

The PFOA values are:

- caribou (whole)/lichen 1.4 ± 0.4, and 2.6 ± 0.5
- caribou (whole)/vegetation 1.8 ± 0.7, and 0.3 ± 0.1
- wolf (whole)/caribou (whole) 2.4 ± 0.6, and 2.1 ± 0.5

Because the diet consists mostly of lichen during winter and other vegetation only forms a component over summer, the critical toxicity study manifests over a period much shorter than a season. Therefore, the mean of the lichen to caribou PFOA TBMF from the two environments was used to estimate trophic transfer from plants to primary consumers.

PFOA TBMF plants to primary consumers = (1.4 + 2.6)/2

= **2**

Similarly, the primary consumer to predator PFOA TBMF was the arithmetic mean of the caribou to wolf TBMFs from the two environments.

PFOA TBMF primary consumer to predator = (2.4 + 2.1)/2

= **2.25**

Combining the soil to plant TF and primary consumer to predator TBMF provides a composite bioaccumulation factor (BAF) for soil to herbivore, as calculated below.

BAF soil to herbivore = $TF_{\text{(soil to plant)}}$ x $TBMF_{\text{(plant to herbivore)}}$

= 7.9 x 2

= 15.8

2.3 Soil to invertebrate bioaccumulation factor

Octanol-water partition coefficients (Kow) are typically used as a proxy for tissue uptake of organic chemicals, but in the case of PFAS, the Kow relationship is unreliable for predictive modelling of PFAS partitioning into biota. Hence data from 12 studies that investigate transfer of PFAS from soil into biota are preferred.

Soil to biota uptake equations are based on simple ratios of the chemical concentrations in soil and tissues, but may be expressed in terms of wet or dry weight concentrations and in the case of soil, normalised to organic carbon content or not normalised (Stubberud 2006; Zhao et al. 2014; Zhao et al. 2013; Bräunig et al. 2019).

Soil to earthworm bioaccumulation factors (BAF) have been extracted or calculated from the literature and summarised in Table 2 using the following equation:

Equation 5

$$BAF = \frac{C_{worm}}{C_{soil}}$$

Where:

C_{worm} = concentration of PFOA in dry weight of worm

C_{soil} = concentration of PFOA in dry weight of soil

The BAFs have been converted to the ratio dry weight of worm to dry weight of soil, without normalisation to organic carbon. This is due to the fact that organic carbon is not the only factor that influences the sorption of PFOA to soil (Li et al. 2018) nor bioavailability and uptake of PFAS into worms (Jager et al. 2003). Earthworms exposed to soil have two exposure routes, through ingestion of soil and gut adsorption as well as by passive diffusion from pore water through the skin (Sijm et al. 2000).

Two approaches are used to determine a soil to earthworm bioaccumulation factor to be used in the soil derivation. Firstly, use of the geometric mean value, **8.5,** calculated from all the maximum BAF concentrations from all the studies summarised in Table 2. Apart from one study (Zhao et al., 2014), these all relate to earthworms exposed to soil with no plants growing in the soil.

The second approach was to note that PFOA bioaccumulation into worms has been found to be enhanced when plants are also growing in the same soil (Zhao et al., 2014). This is considered especially relevant for an ecological soil guideline value as the majority of ecological exposures are likely to involve vegetated rather than bare soil.

Thus, the geometric mean value of **15.1**, calculated of the three BAF values from the combined earthworm plus vegetation treatments in Zhao et al. (2014) was also used to calculate a soil criterion. Although using a wider range of soil types is preferred, the GV is also calculated using the higher BAF value (15.1) as a sensitivity assessment.

Table 2 PFOA soil to earthworm bioaccumulation factors (BAF)

Group	Exposure	BAF	Calculation basis	BAF dry, not OC normalised [a]	Data source
		$(ng/g_{worm\ dry})/(ng/g_{soil\ dry})$	OC or Non-OC	$(ng/g_{worm\ dry})/(ng/g_{soil\ dry})$	
Dry worm BAFs	30 days, spiked soil together with wheat OC 4.11%	0.57, 0.6, 0.7	OC	13.8, 14.7, 17.1	Zhao et al. 2014
	30 days, spiked soil with without wheat OC 4.11%	0.29 – 0.32	OC	6.8 – 7.7	Zhao et al. 2014
	28 days, 2 soils with biosolids and 2 soils with AFFF present OC 1.6 – 6.5%	2 – 8.3	Non-OC	2 – 8.3	Rich et al. 2015
Wet worm BAFs		$(ng/g_{worm\ wet})/(ng/g_{soil\ dry})$		$g/g_{worm\ dry})/(ng/g_{soil\ dry})$	
				Dry basis	
	30 days, spiked soil OC 4.88%	0.014 – 0.037	OC	1.8 – 4.7[b]	Zhao et al. 2013
	28 days, AFFF contaminated soil OC 0.5 – 2.9 %	0.7 – 1.1	Non-OC	4.2 – 6.9[b]	Bräunig et al. 2019
	30 days, spiked soil, treatment without heavy metals present OC 2.84%	0.557	Non-OC	19.6[b]	Zhao et al. 2018
	Artificial OECD soil, OECD test 222 methods	0.5 – 0.72	Non-OC	3.1 – 4.5[b]	Stubberud 2006[c]

Notes

OC = soil normailsed to organic carbon. Non-OC = soil not normalised to organic carbon

[a] BAFs expressed on an organic carbon (OC) normalised basis were converted to non-OC normalised BAF by dividing the OC normalised BAF by the respective fraction of organic carbon started for the soil. Zhao et al. (2014) soil characterisation data is assumed expressed in organic carbon basis, not organic matter.

[b] BAFs expressed using wet weight of worms were converted to BAF worm dry weight using the worm moisture content of 84% as advised in Table 4.1 page 555 in US EPA (1993). This accords with the approximate 85% moisture content indicted in Rich et al. (2015), who measured and reported both wet and dry worm BAFs.

[c] Summary in English, report in Norwegian, note dry soil concentrations labelled t.v. (tørrvekt in Norwegian).

2.4 Daily effects threshold dose

The selection of a critical toxicity value for PFOA is outlined in Section 2 (LOAEL = 0.1 mg/kg-bw/day). The question then arises as to what uncertainty factor (UF) to apply to derive a tolerable daily intake, referred to in Canadian guidance as a daily threshold effects dose (DTED). ECCC (2018) applied a UF of 2 for the PFOS indirect soil GV derivation based on a 2-year chronic effect study of liver toxicity in rats (Thomford 2002). This UF selection also took account of the availability of toxicity data for other taxa, particularly birds.

The CCME (2006) derivation protocol used by ECCC (2018) is quite rigid in that it allows a maximum UF of 5 for soil guidance based on the assumption that the GV is developed for the most threatened species and applies on a contaminated site, rather than the wider ambient environment protected by the federal environmental quality guidelines such as for wildlife diet. This Canadian UF also accounts for the assessment considering risks using established dietary requirements for a large range of sensitive Canadian mammalian and avian species.

Internationally, there is a range of UF used for establishing ecological oral dietary toxicity thresholds. The European Union allows higher UFs, within the range of 30 to 300 for mammals depending upon test duration but based on an acceptable no observed effect level (NOEL) rather than a LOAEL (ECHA 2008).

The National Environment Protection (Assessment of Site Contamination) Measure 1999 (NEPC 2013) schedule B5b provides advice on UF for deriving soil guideline values for direct toxicity. The NEPC (2013) UF are also greater than the CCME (2006) UF, with a minimum of 10 recommended for extrapolation from field to laboratory settings for a chronic NOEL and additional multiplicative factors of between 10 and 100 applied, depending on the nature of the underlying toxicity data (acute or chronic) and range of taxonomic groups considered in the data.

The critical toxicity value chosen for PFOA is a short-term exposure causing developmental toxicity. This shorter-term exposure to elicit an adverse effect is suggestive of a more sensitive exposure than the 2-year chronic toxicity underpinning the corresponding PFOS GV. A greater UF is warranted for shorter exposure duration, and uncertainty arising from the comparative lack of dietary information on Australian fauna and lack of avian and reptilian data.

However, a minimum UF of 10 could generally be recommended in the Australian context, acknowledging that this GV is designed to protect fauna on single contaminated sites rather than the broader environment as in the case of the wildlife diet. The minimum UF of 10 accounts for extrapolation from field to the laboratory setting. A UF of 10 would yield a DTED of

0.01 mg/kg bw per day, being the LOAEL of 0.1 mg/kg bw per day divided by an uncertainty factor of 10.

As the intention is to follow the Canadian approach as used in the corresponding PFOS secondary soil derivation, the NCWG in this case suggests an uncertainty factor of 5 is adopted. This results in a DTED of **0.02 mg/kg bw per day**, being the LOAEL of 0.1 mg/kg bw per day divided by an uncertainty factor of 5. If instead the UF of 10 was preferred, a corresponding soil GV could be found by dividing the final soil GV derived from using the DTED of 0.02 mg/kg bw per day by a factor of 2, that is halving the GV.

2.5 Representative species and food intake rates

The mammalian species and their respective dry-weight food intake rates used by ECCC (2018) in developing their PFOS Federal Soil Quality Guideline for indirect exposure based on dietary pathways appears appropriate to be used in the first instance for PFOA. The input values for representative species, soil ingestion and diet together with the bioaccumulation factors detailed above are summarised in Table 3. Respective values for Australian species with large food intake to body weight requirements could also be utilised if representative data are available.

2.6 Indirect toxicity soil guideline derivation

This section details the input values used for deriving soil guidance using the (CCME 2006) daily intake equation (Equation 2).

The EEEC (2018) values for the variables of body weight, soil ingestion rate, and food ingestion rate for Equation 2 for representative species are reproduced in Table 3. These data, together with bioavailability factor of 1, the soil to plant transfer factor (6.2), soil to invertebrate bioaccumulation factor (8.5, 15.1 sensitivity) and soil to herbivore bioaccumulation factor (12.4) have been substituted into the Canadian (CCME 2006) equation and soil guidelines calculated.

In common with the ECCC (2018) PFOS derivation, the most sensitive species is the secondary consumer, the common shrew, due to its small size and comparatively large proportionate food intake. The fact that PFOA bioaccumulates from soil into soil organisms which constitute the bulk of its diet also contributes to this outcome.

A PFOA indirect soil guideline value of **5 µg/kg** is indicated. If the more conservative assumptions are adopted, this falls to **3 µg/kg**. The more conservative value is driven by a larger bioaccumulation into earthworms based on research findings showing that bioaccumulation into earthworms is greater in the presence of plants compared to bare soil, which is a realistic expectation. As mentioned above, if the use of a higher uncertainty factor of 10 was preferred, the soil GV concentrations would be half these respective concentrations.

3 Discussion and conclusions

Draft mammalian protective guideline values for PFOA have been calculated for wildlife diet and indirect toxicity for soil generally following the Canadian guidelines (CCME 2006 and ECCC 2018) respectively. These are based on scientifically justifiable risk-based approaches and have been previously used in deriving the equivalent PFOS + PFHxS ecological guidance included in the for the Australian guidance (HEPA 2020). The following draft guideline values for PFOA are proposed in this report:

Ecological criteria for wildlife diet for mammals –

- **2.8 µg/kg** wet weight in food (based on platypus base); [preferred]
- **4.2 µg/kg** wet weight in food (based on American mink) [alternate].

Ecological criteria for indirect exposure via soil for mammals

- **3 µg/kg** (using transfer factor for earthworm with plant); [sensitivity, alternate]
- **5 µg/kg** (using transfer factor for earthworms chiefly in bare soil) [preferred].

There are some dietary differences between Canadian and Australian species that regulatory authorities may wish to consider when setting GVs for Australia. The platypus *Ornithorhynchus anatinus* is an aquatic mammalian predator endemic to creeks and rivers of eastern Australia and with presence also in South Australia. It is considered near threatened on the IUCN Red List of Threatened Species (Woinarski and Burbidge 2016) and is experiencing significant population decline (Hawke et al. 2019).

It has a higher food intake rate than that adopted by ECCC (2018) for the American mink, which would lead to a lower wildlife dietary GV than if North American species are used. This raises the issue of whether it is desirable to utilise this information in setting the GV or use food intake information derived from Canadian species in adherence to the ECCC 2018 approach.

Using Australian data for the PFOA GV would be inconsistent with the current PFOS + PFHxS wildlife diet GV for mammals as it does not consider Australian species. Ideally, the PFOS + PFHxS and PFOA GV should be consistent. Options are retaining derivations based solely on the north American species, with a note that they may not be protective of Australian fauna or use of data from Australian species for both the PFOA and PFOS+PFHxS GV.

Apart from a reduction in relevance to local factors, an additional concern in using the mink FI:BW used for the PFOS GV is whether that intake encompasses any increased food intake by mink during lactation, as this is the period most relevant to the PFOA GV. Ideally, Australian guidance should, wherever practicable, be based on data for Australian species. It is for this reason that the PFOA wildlife diet GV based on platypus is preferred.

Table 3: Summary of representative species, diet and input values for calculation of PFOA soil guideline for indirect toxicity

	Feeding guild **Representative species**	**Species & diet[a]**	**Body weight (kg)**	**Soil ingestion rate** **(kg dw/day)**	**Food ingestion rate** **(kg dw/day)**	**Biocentric concentration factor(s)** **(unitless)** **Soil to plant** **Soil to invertebrate** **Soil to animal**	**Value to protect the receptor** **(mg PFOA/kg dry soil)[b]**
Primary consumer (1C)	Herbivorous mammal Meadow Vole	100% plants	0.035	0.000041	0.00173	7.9	0.038
Secondary consumer (2C)	Omnivorous mammal Deer Mouse	50% plants 50% invertebrates	0.2	0.000018	0.0009	7.9 8.5 (15.1)	0.041 (0.029)
Secondary consumer (2C)	Insectivorous mammal Common Shrew	2.5% plants 95% invertebrates 2.5% small mammals	0.004	0.000032	0.0013	7.9 8.5 (15.1) 15.8	0.005 (0.003)
Tertiary consumer (3C)	Carnivorous mammal Wolf	100% mammals	80	0.0118	0.042	15.8	1.777
Tertiary consumer (3C)	Omnivorous mammal Red Fox	60% mammals and birds 25% invertebrates 15% plants	3.8	0/0015	0.05	7.9 8.5 15.8	0.089 (0.079)

[a] Animal body weight, ingestion rates and diet information provided in ECCC (2018).

[b] Values in brackets are concentrations determined for sensitivity analysis using soil: invertebrate BAF of 15.1 from combined earthworm with plant study, where invertebrates are part of diet.

Considering the PFOS+PFHxS TDI of 1.1 µg/kg bw/day (ECCC 2018) and the platypus food intake rate in Section 2.2, the NCWG has calculated a revised PFOS+PFHxS guidance value for wildlife diet (reference concentration in food from Equation 1) of 1.1 µg/kg bw/day / 0.36 kg/kg bw/day = **3.1 µg/kg wet weight in food**.

In terms of PFOS ecological guidance, there is a growing body of information that immunotoxicity is a more sensitive end point for PFOS than the liver toxicity that underpins the current PFAS NEMP guidance (EFSA 2020, ASTDR 2012, Guruge et al. 2009). The NCWG may consider whether it is desirable to also review the PFOS ecological guidance in the light of more recent toxicological information, as well as intake data on Australian fauna while considering ecological guidance for PFOA to address consistency and more recent science.

Using the most sensitive species, the common shrew, the resulting PFOA indirect soil guideline value from the calculations is **5 µg/kg**. If the more conservative assumptions are adopted, this falls to **3 µg/kg**. The more conservative value is driven by a larger bioaccumulation into earthworms based on research findings that show bioaccumulation into earthworms is greater in the presence of plants compared to bare soil, which is a realistic expectation in an Australian setting. However, given the large range of BAF values over different soils, the **5 µg/kg** value is preferred.

These guideline values are based on the secondary consumer, the common shrew, which is the most sensitive due to its small size and comparatively large proportionate food intake. The fact that PFOA bioaccumulates from soil into soil organisms which constitute the bulk of its diet also contributes to this outcome.

In the Australian context, recalculations for dietary exposure to birds and reptiles could be useful, rather than relying on calculations for mammals only. However, this will require alternate toxicity information for PFOA, as the mode of action in this case is not applicable to these animal groups and they do not feed their young via lactation.

An interim direct soil exposure guideline value to protect reptiles has also been developed using an application factor approach and is discussed in a separate submission. Given that reptiles are important elements of the majority of Australian ecosystems, reptilian guidance would be valuable, even if it is of an interim nature.

The draft guideline values are submitted to the National Chemicals Working Group for consideration for consideration by HEPA and inclusion in the next version of the PFAS NEMP, version 3.0.

Potential draft alternate amended text (highlighted) for PFAS NEMP 3.0 is attached in Appendix C and Appendix D. Appendix C provides draft text for section 8.6.2 Biota guideline values and an updated Table A1 including PFOA and PFOS+PFHxS wildlife diet criteria based on platypus as the alternative representative species. Appendix D provides a draft amended PFAS NEMP Table 3 incorporating an indirect soil GV for PFOA.

References

Abbott, BD, Wolf, CJ, Schmid, JE, Das, KP, Zehr, RD, Helfant, L, Nakayama, S, Lindstrom, AB, Strynar, MJ & Lau, C 2007, 'Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha', *Toxicological Sciences.* vol. 98, no. 2, pp. 571-81.

ASTDR 2021, *Toxicological Profile for Perfluoroalkyls. U.S. Department of Health and Human Services,* The Agency for Toxic Substances and Disease Registry, Atlanta.

Bräunig, J, Baduel, C, Barnes, CM, Mueller, JF 2019, 'Leaching and bioavailability of selected perfluoroalkyl acids (PFAAs) from soil contaminated by firefighting activities', *Science of The Total Environment*, vol. 646, pp. 471-479.

Benninghoff, AD, Bisson, WH, Koch, DC, Ehresman, DJ, Kolluri, SK & Williams, DE 2011, 'Estrogen-like activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors in vitro', *Toxicological Sciences*, vol. 120, pp. 42–58.

Bizkarguenaga, E, Zabaleta, I, Mijangos, L, Iparraguirre, A, Fernández, LA, Prieto, A & Zuloaga, O 2016, 'Uptake of perfluorooctanoic acid, perfluorooctane sulfonate and perfluorooctane sulfonamide by carrot and lettuce from compost amended soil', *Science of the Total Environment*, vol. 571, pp. 444–451.

CCME 1998, *Protocol for the Derivation of Canadian Tissue Residue Guidelines for the Protection of Wildlife that Consume Aquatic Biota*, Canadian Council of Ministers of the Environment, Winnipeg.

CCME 2006, *A Protocol for the Derivation of Environmental and Human Health Soil Quality Guidelines*. Canadian Council of Ministers of the Environment, Winnipeg.

CCME 2018, *Scientific Criteria for the Development of the Canadian Soil and Groundwater Quality Guidelines for Perfluorooctane sulfonate (PFOS)*, Canadian Council of Ministers of the Environment, Winnipeg.

CCOF 2015, *Average Dry Matter Percentages for Various Livestock Feeds*, California Certified Organic Farmers Information Guide, pp. 1-10p https://www.ccof.org/sites/default/files/Feed%20Type%20DMI%20Table%20Final.pdf.

Danish Ministry of Environment 2015, *Perfluoroalkylated substances: PFOA, PFOS and PFOSA Evaluation of health hazards and proposal of a health-based quality criterion for drinking water, soil and ground water*, Danish Environmental Protection Agency, Environmental Project No. 1665, pp 1-89, Copenhagen.

Dickman, CR, Mahon, PS, Masters, P & Gibson, DF 1999, 'Long-term dynamics of rodent populations in arid Australia: the influence of rainfall', *Wildlife Research,* vol. 26, pp. 389–403.

ECCC 2018, *Canadian Environmental Protection Act, 1999, Federal Environmental Quality Guidelines Perfluorooctane Sulfonate (PFOS)*, Environment and Climate Change Canada, Toronto.

ECHA (European Chemicals Agency), 2008. 'Guidance on information requirements and chemical safety assessment, Chapter R.10: Characterisation of dose [concentration]-response for environment', Guidance for the implementation of REACH, May 2008, pp. 1-65.

ECHA 2013, *Substance of Very High Concern Support Document - Pentadecafluorooctanoic Acid (PFOA)*, European Chemicals Agency, Helsinki, Accessed 27 September 2021.

European Food Safety Authority 2020, 'Risk to human health related to the presence of perfluoroalkyl substances in food', *EFSA Journal 2020*, vol. 18, no. 9, pp. 6223.

Fleay, D 1944, *We breed the platypus*, Robertson & Mullens, Melbourne.

Gebhardt, SE & Thomas, RG 2002, Nutritive values of foods, United States Department of Agriculture, Agricultural Research Service, Home and Garden Bulletin, no.72.

Gore, AC, Chappell, VA, Fenton, SE, Flaws, JA, Nadal, A, Prins, GS, Toppari, J & Zoeller, RT 2015, 'EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals', *Endocrine Reviews*, vol. 36, no. 6, pp. E1–E150.

Grant, TR & Griffiths, M 1992, *Aspects of lactation and determination of sex ratios and longevity in a free-ranging population of platypuses, Ornithorhynchus anatinus, in the Shoalhaven River, NSW*, in Augee, ML (Ed.), *Platypus and echidnas,* pp. 80-89, Royal Zoological Society of New South Wales, Sydney.

Gredelj, A, Nicoletto, C, Valsecchi, S, Ferrario, C, Polesello, S, Lava, R, Zanon, F, Barausse, A, Palmeri, L, Guidolin, L and Bonato, M 2020, 'Uptake and translocation of perfluoroalkyl acids (PFAA) in red chicory (*Cichorium intybus L.*) under various treatments with pre-contaminated soil and irrigation water', *Science of the Total Environment*, vol. 708, pp. 134766.

Griffiths, M, Elliott, MA, Leckie, RMC & Schoefl, GI 1973, 'Observations of the comparative anatomy and ultrastructure of mammary glands and on the fatty acids of the triglycerides in platypus and echidna milk fats', *Journal of Zoology*, vol 169, pp. 255-279.

Guruge, KS, Hikono, H, Shimada, N, Murakami, K, Hasegawa, J, Yeung, LW, Yamanaka, N & Yamashita, N 2009. 'Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F1 mice', *Journal of Toxilogical Sciences*, vol. 34, no. 6, pp. 687-691.

Hawke, T, Bino, G & Kingsford, RT 2019, 'A silent demise: Historical insights into population changes of the iconic platypus (Ornithorhynchus anatinus)', *Global Ecology and Conservation*, vol. 20, pp. e00720.

HEPA 2018, PFAS National Environmental Management Plan (NEMP). January 2018, The Heads of EPAs Australia and New Zealand (HEPA).

HEPA 2020, PFAS National Environmental Management Plan (NEMP) Version 2.0. January 2020, National Chemicals Working Group of the Heads of EPAs Australia and New Zealand (HEPA).

Holland, N & Jackson, SM 2002, Reproductive behaviour and food consumption associated with the captive breeding of platypus (Ornithorhynchus anatinus), *Journal of Zoology,* vol. 256, issue 3, pp. 279–288.

Jager, T, Fleuren, RHLJ, Hogendoorn, EA & de Korte, G 2003, 'Elucidating the routes of exposure for organic chemicals in the earthworm, *Eisenia andrei* (Oligochaeta)', *Environmental Science and Technology*, vol. 37, no. 15, pp. 3399-3404.

Kelly, B, Ikonomou, M, Blair, J, Surridge, B, Hoover, D, Grace, R, & Gobas, F 2009. 'Perfluoroalkyl contaminants in an Arctic marine food web: trophic magnification and wildlife exposure', *Environmental Science and Technology,* vol. 43, no. 11, pp. 4037-43.

Lan, Z, Zhou, M, Yao, Y & Sun, H, 2018. 'Plant uptake and translocation of perfluoroalkyl acids in a wheat–soil system', *Environmental Science and Pollution Research International*, vol. 25, pp. 30907–30916.

Lasee, S, Subbiah, S, Thompson, WA, Karnjanapiboonwong, A, Jordan, J, Payton, P & Anderson, TA. 2019, 'Plant Uptake of Per-and Polyfluoroalkyl Acids under a Maximum Bioavailability Scenario', *Environmental Toxicology and Chemistry*, vol. 38 no. 11, pp. 2497-2502.

Lau, C, Anitole, K, Hodes, C, Lai, DY, Pfahles-Hutchens, A, & Seed, J 2007, 'Perfluoroalkyl acids: a review of monitoring and toxicological findings', *Toxicological Sciences,* vol. 99, pp. 366–394.

Lechner, M & Knapp, H, 2011, 'Carryover of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plant and Distribution to the Different Plant Compartments Studied in Cultures of Carrots (*Daucus carota ssp. Sativus*), Potatoes (*Solanum tuberosum*), and Cucumbers (Cucumis Sativus)', *Journal of Agricultural and Food Chemistry*, vol. 59, pp. 11011–11018.

Li, Y, Oliver, DP & Kookana, RS 2018, 'A critical analysis of published data to discern the role of soil and sediment properties in determining sorption of per and polyfluoroalkyl substances (PFASs)', *Science of the Total Environment*, vol. 628-629, pp. 110-120.

Liu, Z, Lu, Y, Song, ., Jones, K, Sweetman, AJ, Johnson, AC, Zhang, M, Lu, X & Su, C. 2019, 'Multiple crop bioaccumulation and human exposure of perfluoroalkyl substances around a mega fluorochemical industrial park, China: Implication for planting optimization and food safety', *Environment International*, vol. 127, pp. 671-684.

Macon, MB, Villanueva, LR, Tatum-Gibbs, K, Zehr, RD, Strynar, MJ, Stanko, JP, White, SS, Helfant, L, & Fenton, SE 2011, 'Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry', *Toxicological Sciences*, vol. 122, no. 1, pp. 134–145.

Mattsson, A, Sjöberg, S, Kärrman, A and Björn Brunström, B 2019, 'Developmental exposure to a mixture of perfluoroalkyl acids (PFAAs) affects the thyroid hormone system and the bursa of Fabricius in the chicken', *Scientific Reports*, vol. 9, pp. 19808.

McAllan, BM & Dickman, CR 1986, 'The Role of Photoperiod in the Timing of Reproduction in the Dasyurid Marsupial *Antechinus stuartii*', *Oecologia*, vol. 68, no. 2, pp. 259–264.

Morton, SR, Dickman, CR, & Fletcher, TP 1989, 'Dasyuridae. In 'Fauna of Australia'. Eds D. W. Walton and B. J. Richardson', *Australian Government Publishing Service: Canberra*, pp. 560–582.

Müller, CE, De Silva, AO, Small, J, Williamson, M, Wang, X, Morris, A, Katz, S, Gamberg, M & Muir, DC 2011, 'Biomagnification of Perfluorinated Compounds in a Remote Terrestrial Food Chain: Lichen-Caribou-Wolf', *Environmental Science and Technology*, vol 45, no. 20, pp. 8665 – 8673.

NEPC 2013, National Environment Protection (Assessment of Site Contamination) Measure 1999, Amended 2013. *Schedule B5b Guideline on Methodology to Derive Ecological Investigation Levels in Contaminated Soils*, The National Environment Protection Council. Canberra.

Nordén, M, Berger, U & Engwall, M 2016, 'Developmental toxicity of PFOS and PFOA in great cormorant (Phalacrocorax carbo sinensis), herring gull (*Larus argentatus*) and chicken (*Gallus gallus domesticus*)', *Environmental Science and Pollution Research*, vol. 23, no.11, pp. 10855-10862.

Rayner, JL, Enoch, RR & Fenton, SE 2005, 'Adverse effects of prenatal exposure to atrazine during a critical period of mammary gland growth', *Toxilogical Sciences*, vol. 87, no. 1, pp. 255-66.

Rich, CD, Blaine, AC, Hundal, L, & Higgins, CP 2015, 'Bioaccumulation of perfluoroalkyl acids by earthworms (*Eisenia fetida*) exposed to contaminated soils,' *Environmental Science and Technology*, vol. 49, no. 2, pp. 881-888.

SERDP 2020, *Guidance for Assessing the Ecological Risks of PFASs to Threatened and Endangered Species at Aqueous Film Forming Foam-Impacted Sites*, SERDP Guidance Document, Project ER18-1614, pp. 1-181, Strategic Environmental research and Development Program, Alexandria.

Simcik, MF & Busian, SJ 2021 *Development of Toxicity Reference Values (TRVs) for Birds Exposed to PFOS, PFOA and Associated Mixtures of Fluorinated Compounds*, SERDP Project ER-2624. SERDP Final report, pp. 1-128, Alexandria.

Sijm, D, Kraaij, R & Belfroid, A 2000 'Bioavailability in soil or sediment: exposure of different organisms and approaches to study it', *Environmental Pollution*, vol. 108, no. 1, pp. 113-119.

Song, P, Li, D, Wang, X & Zhong, X 2018 'Effects of perfluorooctanoic acid exposure during pregnancy on the reproduction and development of male offspring mice', *Andrologia*, vol. 50, pp. e13059.

Southgate, R & Masters, P 1996, 'Fluctuations of rodent populations in response to rainfall and fire in a central Australian hummock grassland dominated by *Plectrachne schinzii*,' *Wildlife Research*, vol. 23, pp. 289–303.

Stahl, H, Heyn, J, Thiele, H, Hüther, J, Failing, K, Georgii, S & Brunn, H 2009, 'Carryover of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plants', *Archives of Environmental Contamamination and Toxicology*, vol. 57, pp. 289–298.

Stubberud, H 2006, 'Ecotoxicological effects of PFOS, PFOA and 6:2 FTS on earthworms (*Eisenia fetida*) (TA-2212/2006)', pp. 31, Norwegian Pollution Control Authority (SFT) Oslo, Norway.

Thomas, JL, Handasyde, KA, Temple-Smith, P & Parrott, ML 2018, 'Seasonal changes in food selection and nutrition of captive platypuses (*Ornithorhynchus anatinus*)', *Australian Journal of Zoology*, vol. 65, pp. 319–327.

Thomford, PJ 2002, *Final report: 104-week dietary chronic toxicity and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in rats*, Covance Laboratories Inc, Madison.

Moshfeghi, MM 2015, 'The bioavailability of perfluoroalkyl substances (PFASs) and polycyclic aromatic hydrocarbons (PAHs) in soil to *Eisenia fetida* and *Cucurbita pepo'*, Swedish University of Agricultural Sciences, Faculty of Natural Resources and Agricultural Sciences. Uppsala, 2015.

Nakamura, T, Ito, Y, Yanagiba, Y, Ramdhan, DH, Kono, Y, Naito, H, Hayashi, Y, Li, Y, Aoyama, T, Gonzalez, FJ & Nakajima, T 2009, 'Microgram-order ammonium perfluorooctanoate may activate mouse peroxisome proliferator-activated receptor α, but not human PPARα', *Toxicology*, vol. 265, no. 1–2, pp. 27-33.

Tucker, DK, Macon, MB, Strynar, MJ, Dagnino, S, Andersen, E & Fenton, SE 2015, 'The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure', *Reproductive Toxicology*, vol. 54, pp. 26-36.

U.S. EPA. Wildlife Exposure Factors Handbook (Final, 1993). U.S. Environmental Protection Agency, Washington, D.C., EPA/600/R-93/187, 1993.

Wen, B, Zhang, H, Li, L, Hu, X, Lui, Y, Shan, X & Zhang, S 2015, 'Bioavailability of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in biosolids-amended soils to earthworms (*Eisenia fetida*),' *Chemosphere*, vol. 118, pp. 361–366.

Wen, B, Wu, Y, Zhang, H, Liu, Y, Hu, X, Huang, H & Zhang, S 2016, 'The roles of protein and lipid in the accumulation and distribution of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in plants grown in biosolids-amended soils', *Environmental Pollution*, vol. 216, pp. 682–688.

Wilson, BA, & Bradtke, E 1999, 'The diet of the New Holland mouse, *Pseudomys novaehollandiae* (Waterhouse) in Victoria', *Wildlife Research*, vol. 26, no. 4, pp. 439-451.

Woinarski, J & Burbidge, AA 2016, *Ornithorhynchus anatinus*. *The IUCN Red List of Threatened Species* 2016: e.T40488A21964009. https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T40488A21964009.en. Downloaded on 19 October 2021.

Yoo H, S, Guruge K, Yamanaka ,N, Sato, C, Mikami, O, Miyazaki, S, Yamashita, N & Giesy, JP 2009, 'Depuration kinetics and tissue disposition of PFOA and PFOS in white leghorn chickens (*Gallus gallus*) administered by subcutaneous implantation', *Ecotoxicology and Environmental Safety*, vol. 72, no. 1, pp. 26-36.

Zhao S., Zhu L., Liu L., Liu Z., Zhang Y. (2013) Bioaccumulation of perfluoroalkyl carboxylates (PFCAs) and perfluoroalkane sulfonates (PFSAs) by earthworms (*Eisenia fetida*) in soil *Environ. Pollut.*, 179, 45-52.

Zhao, S, Fang, S, Zhu, L, Liu, L, Liu, Z, & Zhang, Y 2014, 'Mutual impacts of wheat (*Triticum aestivum* L.) and earthworms (*Eisenia fetida*) on the bioavailability of perfluoroalkyl substances (PFASs) in soil', *Environmental Pollution*, vol. 184, pp. 495–501.

Zhao, S, Zhou, T, Wang, B, Zhu, L, Chen, M, Li, D & Yang, L 2018, 'Different biotransformation behaviors of perfluorooctane sulfonamide in wheat (*Triticum aestivum L.*) from earthworms (*Eisenia fetida*)', *Journal of Hazardous Materials*, vol. 346, pp. 191-198.

Appendix A – Toxicological studies considered in selection of the critical toxicity value for developing ecological guidance for mammals

Table A1 Toxicological studies considered in selection of the critical toxicity value for developing ecological guidance for mammals

Animal	Treatment	Adverse effects	LOAEL mg/kg BW	Study
Female mice	PFOA via gavage 17 gestational days	Increased liver weight	0.1	Abbott et al. 2007
		Decreased pup survival	0.6	
		Delayed eye opening	1	
		Decreased body weight	1	
		Decreased pups/litter	0.1	
Mice	PFOA via gavage 14 days gestational days	Increased liver weight	0.3	Nakamura et al. 2009
		Increased liver lipids	0.3	
		Increased PPARα activation	0.3	
Mice	PFOA via gavage 17 gestational days (GD) PFOA via gavage 10 late gestational days	Increased liver weight	0.3	Macon et al. 2011
		Abnormal/delayed mammary gland development	0.3 (17 GD study)	
			0.01 (10 GD study)	
			(Larger adverse effects at 0.1, 0.3)	
Mice	PFOA via gavage 17 gestational days (GD) PFOA via gavage 10 late gestational days	Delayed abnormal mammary gland development in 2 strains, Strain 1 more sensitive attributed, to slower PFOA excretion	Strain 1 (outbred) Dose dependant from 0.01 through 0.1, 0.3, 1 Strain 2 Dose dependant from 0.3 to 1	Tucker et al. 2015
Mice	PFOA via gavage 17 gestational days (GD 1, 2.5 or 5 mg/kg BW PFOA daily	Decreased pup survival	5	Song et al. 2018
		Damaged testis	1, 2.5, 5	
		Disrupted reproductive hormones	1	
			Some adverse effects seen	

Animal	Treatment	Adverse effects	LOAEL mg/kg BW	Study
			at lowest dose	

Appendix B – Scoring of Macon (et al. 2011) Study for wildlife toxicity evaluation

Table B1 Scoring of Macon (et al. 2011) Study for wildlife toxicity evaluation

Study attribute	Scoring value assignment		Points and comments
	1	2	
Data source	Primary source available publicly for review	Primary source not publicly available for review (e.g. only referenced)	1 research paper publicly available
Dose route	Dosed via spiked food	Dosed via gavage, capsule, liquid, injection, or other method	0 gavage dosing
Test substance	Concentrations doses measured or spiking of dose confirmed via measurement	Doses based on nominal values	0 doses prepared daily from analytical grade >98% pure PFOA into distilled water
Contaminant form	Dose comprised of analytical grade PFAS	Dose contains unverified mixture of PFAS (i.e., AFFF) and/or other chemicals	1 analytical grade PFOA
Dose quantification	Dose expressed by authors in mass chemical per body mass per unit time	Doses expressed on other basis	1 dose in mg/kg per kg body mass per day
Endpoint	Ecologically sensitive and ecologically relevant effects such as reproduction and growth	Other effects, such as lethality, physiology, behavioural, biochemical, and pathology	1 endpoint ecologically significant. Development impairment affecting nutrition of F2
Dose range	Studies with both no-effect and lowest-effect values	Studies with only no-effect or lowest-effect value	0 range of concentrations. Effects evident at lowest concentration 0.01 mg/kg/kg bw
Statistical power	Statistical significance of effects presented by study authors	Statistical significance of effects not presented or analysed by study authors	1 statistical significance stated, varies <0.05 to <0.001 at higher doses
Exposure duration	Chronic duration or multigenerational studies	Sub-chronic and acute studies	1 sub-chronic but relates to development window i.e. gestational period
Test conditions	Exposure conditions and effect measurement methods described	Exposure conditions not described or most information missing	1 conditions stated e.g. light, temperature, water, nil PFAS in feed and water

Source: SERDP (2020) Table 3. Overall comment Score 7 out of 10 considered acceptable. Three marks lost for gavage dosing rather than feeding spiked food, dose made from analytical grade PFOA each day and, despite a large range of exposure concentrations covering 3 orders of magnitude, the lowest dose which was 100 times lower than the highest dose still elicited an adverse effect, although of smaller magnitude.

Appendix C – Draft amendments (highlighted) of NEMP section 8.6.2 and Table C1 Platypus as the representative species for wildlife diet

8.6.2 Biota guideline values

The wildlife diet values for PFOS + PFHxS provided in the second version of the NEMP (NEMP 2.0) are considered to have been derived in a manner consistent with the Australian context. The wildlife diet value for PFOA has been derived following the same approach but using wildlife consumption data for a representative mammalian Australian species. This consumption data has been used to also update the PFOS + PFHxS mammalian guideline.

The bird tissue egg value adopted from the ECCC (2018) which was listed in NEMP 1.0 has been updated. The change is due to an additional uncertainty factor that reflects the paucity of toxicological data for birds, and therefore the additional uncertainty factor accounts for potential for intra and interspecies variability. The adjusted uncertainty factor is 100 while the original uncertainty factor was 10 (ECCC 2018).

The purpose of the tissue guideline for acceptable contaminant levels in bird egg is to assess potential risks to avian populations where these receptors may be relevant. When assessing sensitive avian receptors, it is important to note that some birds may be considered endangered species, and therefore sampling eggs may not be appropriate. In such instances, if bird eggs were to be sampled, this would need to rely on samples of other species which have similar relevant ecological niches.

Table C1 Biota guideline values

Exposure scenario	Sum of PFOS and PFHxS	PFOA	Description	Comments and source
Ecological direct exposure for wildlife diet	3.1 µg/kg	2.8 µg/kg	Mammalian diet – consumption of biota as wet weight food	PFOS and PFHxS – Canadian Federal Environment Quality Guidelines (ECCC 2018) using food intake for representative local species[a]. PFOA – Tolerable daily intake 1 µg/kg BW/day based on ECCC (2018) approach using same representative local species. This guideline value is to be used on sampled biota tissue for assessing risk to mammal and avian receptors based on their diet.
	8.2 µg/kg		Avian diet – consumption of biota as wet weight food	

Exposure scenario	Sum of PFOS and PFHxS	PFOA	Description	Comments and source
				The avian diet value may not be protective of migratory wading birds that have a high food intake due to the need to gain weight rapidly. PFOS and PFHxS diet values may also not be protective of reptiles and amphibians. PFOA diet values may not be protective of birds, reptiles or amphibians.
Ecological exposure protective of birds	0.2 µg/g		Whole bird egg as wet weight	Adapted from Canadian Federal Environment Quality Guidelines (ECCC 2018) using an additional uncertainty factor. This guideline value is to be used on sampled bird eggs to assess risk to sensitive avian ecological receptors.

Notes:

[a] As the PFOA mammalian toxicity derivation is based on adverse effects that occur during development and lactation, food intake rates are based on lactating females are preferred. The food intake rate of 0.36 kg/kg bw/day is based on consumption data for the platypus *Ornithorynchus anatinus* supporting lactation (Thomas et al. 2018) – Note: Add the following reference: Thomas, J. L., Handasyde, K.A., Temple-Smith, P. and Parrott M.L. (2018) Seasonal changes in food selection and nutrition of captive platypuses (*Ornithorhynchus anatinus*). *Australian Journal of Zoology* 65:319–327.

Where the guideline values refer to the sum of PFOS and PFHxS, this includes PFOS only, PFHxS only, and the sum of the two. The Canadian guidelines refer to the criterion for PFOS only; in the NEMP 2.0 the guideline values for ecological direct exposure for wildlife diet refer to the levels of PFOS and PFHxS in food consumed by mammals or birds. This has been adapted to allow for uncertainties and potential similar toxicities of PFHxS with PFOS.

The guideline value for ecological exposure protective of birds refers to the levels of PFOS and PFHxS in bird eggs.

Tolerable daily intakes (µg/kg BW/day): Mammalian – PFOA 1; sum PFOS and PFHxS 1.1; Avian – 7.7.

As the PFOA mammalian toxicity derivation is based on adverse effects that occur during development and lactation, food intake rates are based on lactating females.

Appendix D – Draft amendments (highlighted) of Table D1 of NEMP to include ecological indirect guidance for PFOA in soil

Table D1 Ecological guideline values for soil

Exposure scenario	PFOS	PFOA	Land use	Comments and source
Ecological direct exposure	1 mg/kg	10 mg/kg	All land uses	Future work may be undertaken to review available soil guideline values proposed by Australian research and industry organisations. For example, CRC CARE (2017). The human health screening value for public open space is used as an interim value (see Table 2).
Ecological indirect exposure	0.01 mg/kg	0.005 mg/kg	All land uses	The guideline value is based on dietary exposure of a secondary consumer as the most sensitive exposure pathway assessed. This value may not be protective of specific animals relevant to Australia, including predatory animals such as quolls, antechinus and reptiles. For intensively developed sites with no secondary consumers and minimal potential for indirect ecological exposure, a higher criterion of up to 0.14 mg/kg PFOS may be appropriate as outlined in the accompanying text in section 8.2.1.