Guidance on interpreting biota tissue concentrations for bioaccumulation assessment

Chief Scientist's Group report

July 2022

We are the Environment Agency. We protect and improve the environment.

We help people and wildlife adapt to climate change and reduce its impacts, including flooding, drought, sea level rise and coastal erosion.

We improve the quality of our water, land and air by tackling pollution. We work with businesses to help them comply with environmental regulations. A healthy and diverse environment enhances people's lives and contributes to economic growth.

We can't do this alone. We work as part of the Defra group (Department for Environment, Food & Rural Affairs), with the rest of government, local councils, businesses, civil society groups and local communities to create a better place for people and wildlife.

Published by:

Environment Agency
Horizon House, Deanery Road,
Bristol BS1 5AH

www.gov.uk/environment-agency

Further copies of this report are available from our publications catalogue: www.gov.uk/government/publications or our National Customer Contact Centre: 03708 506 506

Email: research@environment-agency.gov.uk

Authors:
Paul, K.B., Handy, R.D., Barnett, C.L., Beresford, N.A., Bowyer M.P.C., Carson, D.S., Walker, L.

Keywords:
Total body burden, whole body concentration, tissue concentration, extrapolation, vertebrate, organic pollutants

Research contractors:
UK Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster, LA14AP. Tel.: 01524 595800
Subcontractors:
Blue Frog Scientific Limited, Edinburgh, 23 Old Fishmarket Close, EH1 1AE. Tel.: 0131 3747422
Richard Handy (Consulting), 9 Maple Close, Tavistock, Devon, PL19 9LL

Environment Agency's Project Manager:
Pippa Curtis-Jackson

Environment Agency's Project Lead:
Morné van der Mescht

Citation:
Environment Agency (2022) Guidance on interpreting biota tissue concentrations for bioaccumulation assessment. Environment Agency, Bristol.

Research at the Environment Agency

Scientific research and analysis underpins everything the Environment Agency does. It helps us to understand and manage the environment effectively. Our own experts work with leading scientific organisations, universities and other parts of the Defra group to bring the best knowledge to bear on the environmental problems that we face now and in the future. Our scientific work is published as summaries and reports, freely available to all.

This report is the result of research commissioned by the Environment Agency's Chief Scientist's Group.

You can find out more about our current science programmes at https://www.gov.uk/government/organisations/environment-agency/about/research

If you have any comments or questions about this report or the Environment Agency's other scientific work, please contact research@environment-agency.gov.uk.

Dr Jo Nettleton
Chief Scientist

Contents

1. Introduction

There is a need to make better use of existing scientific literature/monitoring data in regulatory frameworks. This is to reduce both animal testing and effort required to achieve regulatory compliance. One key data requirement under several UK regulatory frameworks[1] (e.g. UK REACH[2] /REACH Regulation (EC) 1907/2006 and GB BPR[3]/ Regulation (EU) No. 528/2012) is bioaccumulation, most commonly body burdens are converted into a bioconcentration factor (BCF). Bioaccumulation assessments are integral to chemical safety assessment strategies (i.e. is there a need to consider exposure to predators via the oral route or human exposure via the environment) and are also used in the identification of substances that fulfil the hazard criteria of persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulate (vPvB). In a regulatory context a substance with a BCF > 2000 is considered (bioaccumulative) B and one with a BCF > 5000 is considered very bioaccumulative (vB; e.g. UK REACH Annex XIII). Substances that exceed these thresholds can potentially accumulate through food chains and webs. The biological accumulation may lead to significant concentrations that could result in possible adverse effects in organisms. The assessment aids in the establishment of chemicals that may be considered for risk management measures.

Many academic papers report organic substance concentrations in specific wildlife tissues or organs (e.g. muscle, liver, etc.). Wildlife monitoring schemes also tend to focus on specific organs and tissues (see Tables 1 and 2). Hence, there are two data rich repositories for the indication of potential bioaccumulation that could be used for chemical risk management. These data are usually reported as either wet weight or lipid normalised values. Studies that specifically focus on food chain bioaccumulation compare such values between species or with concentrations in potential food items/environmental compartments (e.g. soil or water) to calculate bioaccumulation factors (BAF) or biomagnification factors (BMF). This contrasts with standard laboratory measures of bioaccumulation potential which rely on whole body concentrations (e.g. OECD TG 305 (OECD, 2012)) that are used to calculate BCFs or BMFs.

---

[1] Currently, UK and EU regulations are highly comparative as the legislative frameworks were largely adopted directly into UK law.

[2] UK REACH: UK regulation concerning the registration, evaluation, authorisation and restriction of chemicals. REACH etc. (Amendment) Regulations 2021, UK Statutory Instrument 2021 No. 904.

[3] GB BPR: GB regulation concerning the making available on the market and use of biocidal products (Biocidal Product Regulation). GB regulatory framework for Biocidal Products Regulations and CLP; Statutory Instrument 2019, No 720.

However, currently there is limited to no guidance in the literature of how to make use of tissue/organ concentrations in a regulatory context and/or how they can be converted to whole body concentration values and subsequently BCFs.

There is guidance for extrapolation of radionuclides and metals tissue/organ concentrations to whole body concentrations (e.g. Yankovich *et al*., 2010), but how reliable such a method is for regulatory use or how broadly applicable it would be to other chemicals has not been fully explored. For those chemicals where there are no laboratory derived bioaccumulation data a bioaccumulation conclusion drawn from field data are often not possible or comparable with the regulatory required endpoint derived from laboratory studies. Moreover, there is currently only limited detail in existing regulatory guidance documents on how monitoring, field and/or literature data can be used in a regulatory context (e.g. sections R.11.4.1.2.6 of the R.11 PBT assessment guidance (ECHA, 2017a) and R.7.10.3.3 of the R.7c Endpoint Specific Guidance (ECHA, 2017b)).

The following review will discuss the current state-of-science in the area of bioaccumulation and try to establish ways to extrapolate tissue concentrations to whole body concentrations. The review will also assess if and how these can be converted into information useful for regulatory use, what knowledge gaps exist, and the current uncertainty associated with such extrapolations.

2. UK wildlife monitoring schemes

Current contaminant biomonitoring in the UK uses both individual organ/tissue and whole body samples; whole body samples often being pooled. For monitoring used as part of the '*Exposure and adverse effects of chemicals on wildlife in the environment: 'H4 indicator*' (Defra 2019; EA 2021) contaminant concentrations in whole fish and mussels are measured; https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/992555/Exposure_and_adverse_effects_of_chemicals_on_wildlife_in_the_environment_interim_H4_indicator.pdf . Monitoring schemes utilising larger vertebrates, including the Cardiff University Otter Project (CUOP), Predatory Bird Monitoring Scheme (PBMS), red fox monitoring (operated by FERA Science Ltd (FERA))[4] and the Centre for Environment, Fisheries, and Aquaculture Science (CEFAS) and Cetacean Strandings Investigation Programme (UK CSIP) predominantly monitor contaminant concentrations in liver sub-samples. However, in addition to the liver many of these schemes retain other tissue samples (see Table 1). The contaminants currently monitored as part of the H4 indicator monitoring varies between species (see Table 2 for summary).

The wildlife monitoring schemes represent a potential source of samples that could undergo further analyses to determine tissue/organ specific and/or whole organism concentrations in the same animals if required.

[4]Previously operated through the Wildlife Incident Investigation Scheme (WIIS).

Table 1. Summary of UK wildlife monitoring schemes showing species collected and organs/tissues sampled (see also https://www.wildcoms.org.uk/); UKCEH – United Kingdom Centre of Ecology & Hydrology, IoZ Institute of Zooloogy, APHA – Animal and Plant Health Agency, DRAHS - Disease Risk Analysis and Health Surveillance, WIIS - Wildlife Incident Investigation Scheme (other organisations/schemes are defined in text).

	UKCEH	UKCEH	IoZ	IoZ	IoZ / CEFAS	APHA	FERA	FERA	Cardiff University[4]
Scheme	Predatory Bird Monitoring Scheme	National Fish Tissue Archive	Garden Wildlife Heath	DRAHS[3]	CSIP	Diseases of Wildlife Scheme	WIIS	H4 Fox sampling	Cardiff University Otter Project
Species (tissues/organs sampled listed below)	All UK resident diurnal and nocturnal raptors	Roach (*Rutilus rutilus*)	A range of vertebrate species found in peri-urban environment	Red kite (*Milvus milvus*); Hen harrier (*Circus cyaneus*);Eurasian Beaver (*Castor fiber);* Reptiles[1]; and Amphibians[2]	Cetaceans; pinnipeds; marine turtles and basking shark	Vertebrates	Vertebrates	Fox (*Vulpes vulpes*)	Eurasian otter (*Lutra lutra*)
Whole body		X						X	
Liver	X		X	X	X	X	X		X
Kidney	X		X	X			X		X
Muscle	X		X	X			X		X

	UKCEH	UKCEH	IoZ	IoZ	IoZ / CEFAS	APHA	FERA	FERA	Cardiff University[4]
Brain	X		X	X					X
Feather	X		X	(X)[5]					
Bone	X		X	X					X
Blood						X			X
Fat	(X)		(X)	(X)	X				
Stomach contents			X	X			X		
Intestines						X			

[1]Smooth snake (*Coronella austriaca*), Adder (*Vipera berus*), Sand lizard (*Lacerta agilis*); [2]Pool frog (*Pelophylax lessonae*), Natterjack toad (*Epidalea calamita*); [3]Additional species collected by DRAHS - Red squirrel (*Sciurus vulgaris*), Common dormouse (*Muscardinus avellanarius*), Corncrake (*Crex crex*), Marsh harrier (*Circus aeruginosus),* Cirl bunting *(Emberiza cirlus)*; [4]Additional samples retained by the Cardiff University Otter Project include adrenals, fur, heart, spleen, ectoparasites, vibrissae, baculum, lung, kidney stones, gall bladder, testes, scent, skull, faeces, thymus, thyroid, ear and tongue; [5](X) denotes - tissue sampled when available.

Table 2. Summary of species monitored as part of current monitoring efforts under H4 monitoring, and the contaminants measured.

Scheme	Species	Tissue/Organ	Contaminants[1]
PBMS	Sparrowhawk (*Accipiter nisus*)	Liver	Total mercury, lead, cadmium, zinc, copper and nickel
PBMS	Red kite (*Milvus milvus*)	Liver	SGARs
WIIS / Fera Ltd	Red fox (*Vulpes vulpes*)	Liver	SGARs
CUOP	Eurasian Otter (*Lutra lutra*)	Liver	Total mercury, cadmium, PBDEs and PFOS
CSIP	Harbour porpoise (*Phocoena phocoena*)	Liver	PBDEs, PCBS, PFOS
MSFD[2]	Common dab (*Limanda limanda*)	Pooled individuals	Total mercury, lead, cadmium, zinc, copper, nickel, PBDEs, PCBS and PFOS
CSEMP[3]	Blue mussel (*Mytilus edulis*)	Pooled individuals	Mercury, lead, cadmium, zinc, copper, nickel PBDEs and PCBS
EA[4] freshwater fish monitoring	Common roach (*Rutilus rutilus*), Chub (*Squalius cephalus*) and Brown trout (*Salmo trutta*)	Pooled individuals	Mercury, lead, cadmium, zinc, copper, nickel PBDEs, PCBS and PFOS

[1]PBDEs - polybrominated diphenyl ethers, PCBs - polychlorinated biphenyls; PFOS – perfluorooctanesulfonic acid, SGARs – second generation anticoagulant rodenticides; [2]Marine Strategy Framework Directive (OSPAR); [3]Clean Seas Environment Monitoring Programme (Scottish marine waters); [4]Environment Agency.

3. Literature review

The literature review followed methods outlined in the guidance on Quick Scoping Reviews as presented by Collins *et al*. (2015) and ECHA Chapter R.4 (2011, v1.1). A full description of the review can be found in Appendix 1.

3.1 Literature review - results

Table 3 presents an overview of the literature searches and critical review; all stages of the evaluation are recorded in the Excel® worksheets. Whilst approximately 600 manuscripts were identified by the literature searches approximately two-thirds of these were dismissed as not being relevant during the rapid relevance review. Relatively few manuscripts were found to contain data relevant to estimating total body burden (TBB) from tissue/organ specific measurements. Data from those critically reviewed manuscripts meeting our criteria have been used in the next section to investigate the potential to extrapolate from tissue burdens to whole body concentrations. The data used in these extrapolations can be found in Appendix 2; data considered were for accumulating organs/tissues and those commonly sampled and analysed in wildlife monitoring schemes.

Table 3. Overview of the literature review and critical evaluation.

Organism Group	Number Records Identified Web of Science	Number Records Identified Pubmed	Number Duplicates Removed	Number Records Added From Extra Searches	Total Number Unique Records Obtained	Number Identified As Potentially Relevant From Title/Abstract	Number Containing Relevant Data
Amphibians	11	9	4	2	18	11	1
Birds	17	38	10	2	47	26	2
Fish	132	151	60	5	228	33	6
Mammals	218	126	48	6	302	92	5
Reptiles	4	3	1	0	6	4	0

4. Extrapolating from tissue burdens to whole body concentrations

The use of single organs/tissues instead of whole body concentrations (also called total body burden or TBB) for the assessment of bioaccumulation potential relies heavily on the concept of target organ pathology (see, Turton and Hooson (1998) on chemicals generally, and Handy and Al-Bairuty (2019) on nanomaterials). The concept of 'target organs' is a long-established theory, where chemicals are taken up, and are deposited in and/or have effects on specific internal organs in the animal. Consequently, these 'target organs' are of interest in understanding the mechanisms of toxicity, the likely effects on specific body systems (e.g., cardiovascular system, nervous system) as well as where the substance is accumulating inside the body. The latter is of prime interest here and in order to use an individual type of organ for bioaccumulation assessments, the organ would need to be able to accumulate the pollutant to a degree where detection was possible. This makes the target organ a prime candidate for extrapolation of organ/tissue to whole body concentrations.

The relationship between chemical concentrations in a single internal organ and the whole body concentration depends on the absorption, distribution, metabolism and excretion (ADME) in the animal of interest. The route of absorption (e.g., exposure via the skin, respiratory system, or gut) can influence the subsequent pattern of accumulation in the organs. Arguably, more is known about these processes for metals in aquatic species and some terrestrial wildlife due to their historic impact ecologically (e.g. mining and associated industries etc.) and partly because the methods for the digestion of biological samples and subsequent determination of total metal are relatively straightforward and often less expensive, compared to the analysis of organic chemicals in tissues. Nonetheless, in fish the accumulation of total metal in the organs varies between aqueous and dietary exposures (Handy *et al*., 1992a,b), and this also seems to be case for organic chemicals (Qiao *et al*., 2000; Kwong *et al*., 2008). Notably, it was recognised early on that the water solubility of organic chemicals greatly influenced their uptake rates from aqueous exposure relative to dietary sources in fish, with uptake via the gut tending to become more important as lipophilicity increased (Bruggeman *et al.,* 1981). In animals with closed circulatory systems, the distribution of the substance to the internal organs is dependent on several factors. These include: (i) how the substance is carried in the blood, (ii) the proportion of the blood flow to each organ, and (iii) the lipid content of the organ or tissue concerned. So, for example, lipophilic compounds tend not to be freely dissolved in blood plasma, but are carried by lipoproteins, as lipid emulsion in the blood, or on the blood cells (e.g., Jandacek and Tso, 2001). In contrast, substances that are very water soluble, such as dissolved metals, may be taken up into the blood plasma as ions, although they also attach to proteins such as albumins in the blood. New insights on different types of perfluoroalkyl substances (PFAS) show that binding to albumins depends on the hydrophobicity of the substance, chain length and the number of fluoride atoms present on the carbon backbone; with the shorter and more water soluble PFAS only weakly binding to albumin (Alesio *et al*., 2022). Broadly for the target organ concept, lipophilic substances tend to accumulate in fatty tissues (e.g., endocrine organs, subcutaneous fat, mesenteric fat around the intestine), while metals and other hydrophilic substances tend to accumulate in lean tissues with high blood flow (e.g., liver, kidney, lung/gill). The brain of vertebrate animals is an area of special circulation

because of the blood-brain barrier and traditional thinking is that only lipid soluble chemicals that can diffuse through the lipid of the blood-brain barrier can be taken up by facilitated diffusion into the brain (e.g., Hagenbuch *et al*., 2002). This includes substances like methyl-mercury where the brain is a main target organ (Ostertag *et al*., 2013). However, it is also now clear that metals have a role in brain function, and these can also be taken up via carrier-mediated mechanisms to cross the blood-brain barrier (e.g., aluminium, Yokel *et al*., 1999).

Thus, when selecting an organ to analyse for bioaccumulation predictions it is necessary to consider the physico-chemical properties of the substance, including its molecular weight, water solubility and its lipid solubility (e.g., where *log* K_{ow} value can be used as a surrogate), the blood flow and anatomy of the organism and the route of exposure. There are a few exceptions and caveats to this thinking on physico-chemistry. For example, the bioaccumulation of perfluorinated alkyl acids (PFAAs) depends both on their lipid solubility within biological membranes and their complex interactions with plasma proteins with respect to chain length and charge of the PFAA molecule (Ng and Hungerbühler, 2014). Also, predicting the bioaccumulation potential for substances such as perfluorinated acids from the *log* K_{ow} may be problematic as those perfluorinated acids with shorter chain lengths do not follow the usual rules (i.e. *log* K_{ow} < 3 not B). However, this is only identified when assessing bioaccumulation in air breathing organisms whereby, the lower *log* K_{ow}, shorter chain perfluorinated acids are bioaccumulative even when expected to not be (Miranda *et al*., 2022). Thus, it is not just a matter of lipid solubility, perfluorinated acids with chain lengths of less than seven fluorinated carbons would not be considered bioaccumulative using the *log* K_{ow} value in a regulatory context (Conder *et al*., 2007), but this is only the case due often to the reliance of aquatic bioaccumulation studies.

Some organs may not be suitable, for example, selecting a very lean tissue like skeletal muscle for a very lipophilic substance such as benzene, or looking for dissolved metals in fatty tissue like the thymus, as in either example it is less likely the chemical will have quantifiable amounts in the noted tissues. There are, of course, exceptions, but for broad applicability of extrapolating tissue burdens to whole body concentrations the general thinking in the target organ approach when selecting organs for measurements of bioaccumulation should be applied. Fortunately, the liver is a well-known central target organ for both metals and organic chemicals as there are both ‘lean’ and ‘fatty’ parts to the soft tissue of the liver. The liver has a role in energy metabolism and storage, and when food is plentiful, animals deposit glycogen inside the hepatocytes (e.g., see Hampton *et al*., 1985 for liver histology). The glycogen, along with the cell membranes, represents a lipophilic compartment and when the hepatocytes typically occupy 80 % or more of the liver volume (e.g., trout, Hampton *et al.*, 1989) this inevitably represents an important location for the deposition of organic chemicals. The cytoplasm of the hepatocytes not occupied by glycogen storage, and the extracellular sinusoid space and blood vessels, offer a place for hydrophilic substances to accumulate. Thus, the organ would be a good choice for an initial exploration of predictions of organ concentrations versus whole body concentrations. The liver will often contain much higher concentrations of chemicals than the equivalent skeletal muscle sample in the same animal. Liver is also a tissue commonly sampled and measured by existing wildlife monitoring schemes (see Tables 1 and 2). However, it should be noted the liver is especially involved in the metabolism and/or excretion of organic chemicals, and

the concentrations of substances in the liver are likely to be dynamic, and with dynamics not necessarily the same as other internal organs. For example, given the importance of the liver to the metabolism of organic chemicals, there could be initial transient decreases in hepatic concentrations arising from first-pass metabolism (where the liver quickly metabolises the substance during the initial exposure), and even accumulation in the post-exposure phase as the organic substance is redistributed from other internal organs to the liver for excretion (Timbrell, 2002).

A further consideration in the choice of organ(s) is the relative mass of the organ or tissue type and its contribution to the whole body weight of the animal. For example, in adult trout, the skeletal muscle can make around 66% of the body weight and so a relatively low concentration of a substance in the muscle could make a significant contribution to the whole body burden (e.g., cadmium Handy *et al*., 1992a; PFOS, Vidal *et al*., 2019). For substances that bioaccumulate and are persistent in the internal organs, the body burden will also tend to increase with the age of the animals, and so adult animals can have higher body burdens than juveniles. However, the morphometrics of animals also change with growth, and so the proportions of organ mass contributing to body mass in juveniles may not be the same as adults if the body form is changing. For any calculations, it would therefore be essential to use morphometrics from animals at roughly the same size (size acting as a surrogate for age and developmental stage) as those collected from the contaminated field site, or to have data on total organ weights by dissection and direct measurement, prior to collecting tissue for subsequent chemical analysis.

Ideally, for studies of fish, the BCF is derived from the whole body burden at steady state with the surrounding water (Veith *et al*., 1979). The OECD TG 305 (2012) for determining the bioaccumulation potential in fish measures the whole body burden during ‘aqueous’ exposures (i.e., where the test substance added to the water column), which may then be used to calculate a BCF directly, with the option for correcting for the lipid content of the animal. It is also possible to calculate the BCF using a kinetic method based on the uptake and elimination rate constants from the aqueous exposure method in OECD TG 305. For organic chemicals, there is also a relationship between the BCF and the propensity of a chemical to partition toward into lipid rather than water phases. This partitioning is usually expressed as the *log* K_{ow} value (Veith *et al*., 1979), where *log* K_{ow} values >3 indicate the substance is lipophilic and more likely to accumulate. Thus, the bioaccumulation potential in fish can be predicted with a degree of accuracy from the *log* K_{ow} (Veith *et al*., 1979; Meylan *et al*., 1999). For substances that are ‘difficult to handle in water’, or where exposure via the food is a main concern in the ecosystem, there is the alternative of using a dietary exposure method in TG 305 where the biomagnification factor (BMF) is determined (see discussion, Handy *et al*., 2018), also with caveats about correcting for the lipid content of the animals. BMFs are calculated from the results of the dietary test method, usually from the exposure concentration in the food and the whole body burden. A BMF value >1 implies trophic transfer of the test substance has occurred in the test (i.e., uptake from the food to the internal organs of the fish).

Considering the above, there are several ways that individual organs could be used to determine the BCF:

1. Using direct measurements within studies to convert the tissue/organ concentration to a whole body concentration, which can then be used with the concentration in the test medium or in the food to calculate a BCF or BMF respectively. This direct approach requires data on organ and whole body concentrations from the same study to make calculations or comparisons. For example, by plotting organ concentration values against whole body values in order to fit a relationship to the data; the resultant equations for the fitted line could then be used to predict whole body concentrations relative to a particular organ. Collecting data from individual studies that have both organ and whole body concentrations was the focus of the literature review above. However, as noted above, few studies report all this information within the same experiment, so the scope for direct calculation may be limited at the present time. There are studies that report multiple organs, but not the carcass or whole body concentration, and vice versa. It is possible to calculate the total body burden from the organ concentrations, organ weights and body mass of the animals according to Handy *et al*. (1992a,b); but only if these factors are known or directly measured within the individual study.
2. Conduct a meta-analysis of data from the scientific literature to correlate organ concentrations with whole body concentrations ideally at steady-state to then derive a predictive equation to estimate the whole body concentration for groups of similar organic chemicals. The most reliable prediction equations may identify the 'best' organ for this approach.

If concentration values are known for single organs these could be plotted against the measured body burdens, and with data from sufficient studies in the meta-analysis, this could give a prediction equation that enables an organ value to be extrapolated to a whole body estimate for a chemical, or perhaps even similar groups of chemicals. It would require the organ concentrations and whole body values to be in the same units (e.g., µg/g dry weight) from all the studies included the meta-analysis. However, it is also worth considering that single organ values may not be the best predictors of the total body burden. The ratios of organ concentrations (e.g., liver:whole body) may be more informative, or that other variables, such as the *log* K_{ow}, are key factors in prediction. Multi-variate regressions may refine or extend the meta-analysis to derive a prediction equation for BCF from organ concentration including such factors. The focus in this report is on *in vivo* data only. It does not include *in vitro* data from organs, as this may depart significantly from the *in vivo* condition(s). However, once an organ approach is validated from *in vivo* data, it may then be worth exploring how *in vitro* data from cells or organ preparations could be used in the future.

Inevitably, much of the data available on the bioaccumulation of organic chemicals comes from fish species used in OECD TG 305 (2012) and similar standardised tests, using model organisms such as rainbow trout or carp. In comparison there is a relative lack of data on amphibians, and reptiles especially, as well as birds. For mammals, there are some data on model organisms such as the laboratory rats used in OECD toxicity tests, but less information about wildlife. The question therefore arises if the existing fish data has utility for: (a) cross-species extrapolation to other vertebrate animals, or (b) if a BMF in a fish might be predictive of bioaccumulation at other trophic levels in food webs. The bioaccumulation

data on amphibians, reptiles, birds and mammals in the scientific literature may find greater utility in a regulatory context for environmental protection, if this 'non-fish' data could be correlated in some way to whole body burdens or BCFs from TG 305 on fish.

Consider cross-species extrapolation within the vertebrate animals. From the perspective of anatomy and functional physiology, the gut is designed by feeding habit (i.e., carnivore, herbivore, omnivore), not by phylogeny (see review on gut anatomy, van der Zande *et al*., 2020). So, it may be best to compare carnivores with other carnivores. For example, the trout has acid digestion in the stomach, and could be compared with other carnivores with a similar digestive function. Environmental regulations and risk assessment tend to process data on water- and air-breathing animals separately, because they come from different parts of the ecosystem (aquatic versus terrestrial). Inevitably, a BCF is for bioconcentration from an aqueous media or air, but a BMF is from an oral exposure and fundamentally whether the organism is aquatic or terrestrial does not alter the BMF calculation. Notably, the gut barrier of vertebrate animals is broadly similar with the main layers of mucosa, submucosa, and muscularis. Even aspects of the gut lumen chemistry are surprisingly similar within vertebrates, such as the high ionic strength of the chyme (van der Zande *et al*. 2020). So, by extension there may be similar uptake mechanisms and anatomical barriers across species as well as similar toxicokinetics as a whole (i.e. ADME). Thus, there is a reasonable scientific foundation in comparative physiology for the notion of extrapolating the uptake of chemicals from one species of vertebrate animal to another.

One way forward, would be to gather existing BMFs for fish from the OECD TG 305 (2012) test as a 'benchmark' and to compare these against other species of vertebrate animal, with body temperature or mass-specific metabolic rate to correct for temperature differences between species (known as allometric scaling). Metabolic rate increases with body temperature; with typically a two- or three-fold increase in aerobic metabolic rate with a 10 ^{o}C rise in body temperature, depending on the anatomy of the animal. Cold-blooded animals such as trout simply follow the ambient temperature, while in mammals the body temperature has a set-point of 37 ^{o}C. Thus, even at summer temperatures in freshwater (e.g., 15 ^{o}C), a trout may have a body temperature that is some 20 ^{o}C lower than that of a mammal and a much lower metabolic rate. Consequently, the uptake and excretion rates of chemicals in fish would need to be corrected for the effects of body temperature in order to make a fair comparison with mammals, birds (body temperature, 42 ^{o}C), or reptiles that often use behavioural strategies to stay warm. It may also be necessary to include biotic factors known to influence gut transit time and therefore exposure duration in the gut, such as ration size the type of food eaten, and body size for each animal. The OECD guidance on calculating bioaccumulation factors does include some allometric-style equations to correct for the growth of fish when estimating uptake rate constants from uptake kinetics data obtained using TG 305 (OECD, 2017). There are also some BMF values for different chemicals and feeding rates in fish in the OECD literature (OECD, 2013). Data on aspects of allometry, gut transit time, typical ration size, etc., are available for other animals in the zoological literature (see van der Zande *et al*., 2020). A training data set of known BMF values (e.g., for birds and mammals) from the scientific literature to compare against those of fish from TG 305 would be needed to validate any predictive equations, but while careful data correction for cross-species effects is needed, this overall approach might derive some extrapolation factors from fish to other species of vertebrate animals.

Cross-species extrapolation by organ concentration alone, is more challenging with numerous biological differences in factors affecting ADME in organisms, including the blood volume, the percentage of blood flow to each organ, the location of the organ in the cardiovascular network, differences in lipid content between organs in different species, etc. Some of this basic zoological information may not be known, and extrapolation by BMF across species may be a more useful starting point. However, Du *et al*. (2020) attempted to calculate BMF values for the transfer of chlorinated paraffins from a frog (prey item) to a snake (predator) using only the chemical concentrations in the skeletal muscle. The skeletal muscle was chosen because both species had similar fat contents in that tissue, but any such BMF calculation by organ assumes the target organs and the distribution of the test substance to that organ is the same in both animals, and this was not validated by Du *et al*. (2020).

It is also important not to confuse BMFs with the concept of biomagnification at higher trophic levels in food webs. While some persistent organic chemicals that are hard to metabolise do show biomagnification to the apex predator in the food web (e.g., some types of flame retardants, Sørmo *et al*., 2006), it is not necessarily the case that all trophic levels will show biomagnification within a food web. In any event, the critical body burden that causes toxic effects in the organism might be achieved without biomagnification (e.g., where the trophic transfer is to a sensitive or susceptible animal). Similarly, any relationship between the organ concentration of a chemical substance and trophic level may not necessarily inform on the threat to animal species arising from the organ contamination, because the critical organ concentration for target organ dysfunction may vary between the types of animals. Currently, there seems to be insufficient data in the scientific literature on critical body burdens and the relationship to organ concentrations to make useful predictions of effects on organ function or organism survival. A more detailed analysis of literature could identify what is possible and the data gaps.

4.1 Attempts at meta-analysis to correlate whole body with organ concentrations using the existing literature.

The literature searches resulted in a limited number of robust manuscripts for each organism type that contained data on both whole body and organ concentrations for use in the evaluation of correlations between organ and whole body concentrations (Table 4; data from these papers are available in Appendix 1). Nearly half of these papers reported field studies (Table 4). Not all the papers with whole body and organ concentration data could be used in this evaluation, for example, one of the mammal papers presented data in units relative to tin concentration (ng Sn/g) that were not compatible with the other reported studies (see Appendix 1). The range of species was limited with no useful data on reptiles, one paper on amphibian (black-spotted frog), two on bird (glaucous gulls and red-throated divers), and two on mammals (laboratory rats and bottlenose dolphins). The fish data consisted of a wider range of species, but these were all freshwater species. From the manuscripts only a few organic chemicals were reported and often the values were total concentrations for a group of substances (e.g., ∑PFAS) rather than the individual substances. The data mainly consisted of liver or muscle (assumed to be skeletal muscle) chemical burdens. For other

manuscripts where only tissue burden data were available a further search on regulatory databases was conducted to see if the whole body concentrations could be gained from BCF (OECD 305) studies. However, none of the studied chemicals had such data. In many instances this is likely because these chemicals are intermediates which do not require large datasets for regulatory compliance. Therefore, it was not possible to expand on the *n*-number for current dataset.

Table 4. Summary of data used in attempts at meta-analysis (data are available in Appendix 2).

Type of Animal	Number of Papers	Type of Study	Number of species	Types of Chemicals
Freshwater Fish	6	5 laboratory, 1 field study	9	BDEs, ibuprofen, PFAS/PFOS
Amphibians	1	Field study	1	PFAS
Birds	2	Field studies	2	BDEs, PCBs, chlordanes, PFAS
Mammals	3	2 laboratory, 1 field study	2	Dioxins, PCBs, DDT, BDEs

The first iteration of the meta-analysis considered the data by type of vertebrate animal. Using the fish literature alone, the plots of whole body burden versus the liver, or muscle, showed no apparent trend or valid correlation (Figure 1). The limited data were mostly for ∑PFAS or ∑PFOS in the organs, with most of the studies not reporting individual isomers or substances, only 3 values on individual BDEs and one for ibuprofen were obtained. These data clustered near the intercept, but also with some very high values for 2,2',4,4'-tetrabromodiphenyl ether (BDE 47) in the liver of zebrafish and ibuprofen in the muscle of carp, giving rise to spurious Pearson correlation coefficients. The data on the amphibian (black-spotted frog) was entirely for per- and poly-fluorinated substances including PFSA, PFCA and PFESA; again the values were for sum totals of each type of substance, not individual compounds. The amphibian data offered a modest correlation coefficient for whole body concentrations with the liver (Pearson correlation, 0.67), but the linear fit of the data had an r^2 value of only 0.43 (Figure 2). However, with more data the Pearson Correlation and r^2 may be improved and Figure 2 at least shows some promise for the extrapolation of frog liver burdens to whole body burdens. The whole body versus muscle for amphibians (Figure 2) showed a poorer correlation (Pearson correlation, 0.53) and no useful linear fit could be made to the data (attempts gave r^2 values < 0.3). This could also be because the

best fit for the data is not linear but with the limited data it is not currently possible to determine what this fit might be. The liver is around 2-5% of the body mass of a frog (Smith, 1950) and it contained typically tenfold higher concentrations of each substance than the muscle (Figure 2); demonstrating the importance of the amphibian liver as a target organ for organic chemicals. Most of the muscle mass in frogs is in the large hind legs, which are consequently a good location for collecting a muscle sample. However, a large portion of the systemic blood flow will go to organs such as the liver, kidney and viscera before reaching the hind limb vasculature, and so the hind leg muscles may not provide a sample that is reflective of the body burden.

The data for whole body versus liver or blood for birds gave excellent to fair correlations (Pearson correlation coefficients, 0.98 and 0.63 respectively), but again nearly all the values were for sum totals of types of substances (e.g., ∑PCBs, ∑PBDE) not individual compounds. The liver gave the best linear fit with the whole body burden, with an r^2 value of 0.99 compared to only 0.40 in the blood (Figure 3). In the data on birds there was one high value for the whole body concentration for the sum of chlorodanes (701 µg/g). Removal of this data point did not appreciably change the fitted equations and without the data point the equation for the liver was: $y = 2.3967x-3.4209$, $r^2 = 0.89$. The values for blood are not whole blood, but the plasma after centrifugation to remove the blood cells (Verreault *et al*., 2005; Verreault *et al*., 2007) and the values were not corrected for the lipid concentration in the plasma. The cause for the scatter of the data is unclear, and without the full haematology of the animals it is not possible to correct the data for factors such as how ‘dilute’ or ‘concentrated’ the plasma may be; this is an important aspect of the physiology of gulls that drink seawater. In addition to plasma lipids and lipoproteins, the blood cells may adsorb organic substances, so understanding the composition of the blood is important. Plasma lipids and other blood parameters are often not reported in the literature in a way that allows correction of the organic chemical concentration in the blood and no correction could be made here. Little is known about first-pass metabolism and how the activity of the liver dynamically alters plasma concentrations of chemical substances in birds. The good correlation here between body burden and liver, and to the plasma, are noteworthy because they are mainly on one species of gull from one study and therefore not influenced by variance introduced across multiple studies. Data from multiple studies and species of animal could add more variance to the data, but such effects could be normalised in some manner (i.e., to account for body size, anatomical differences, etc.).

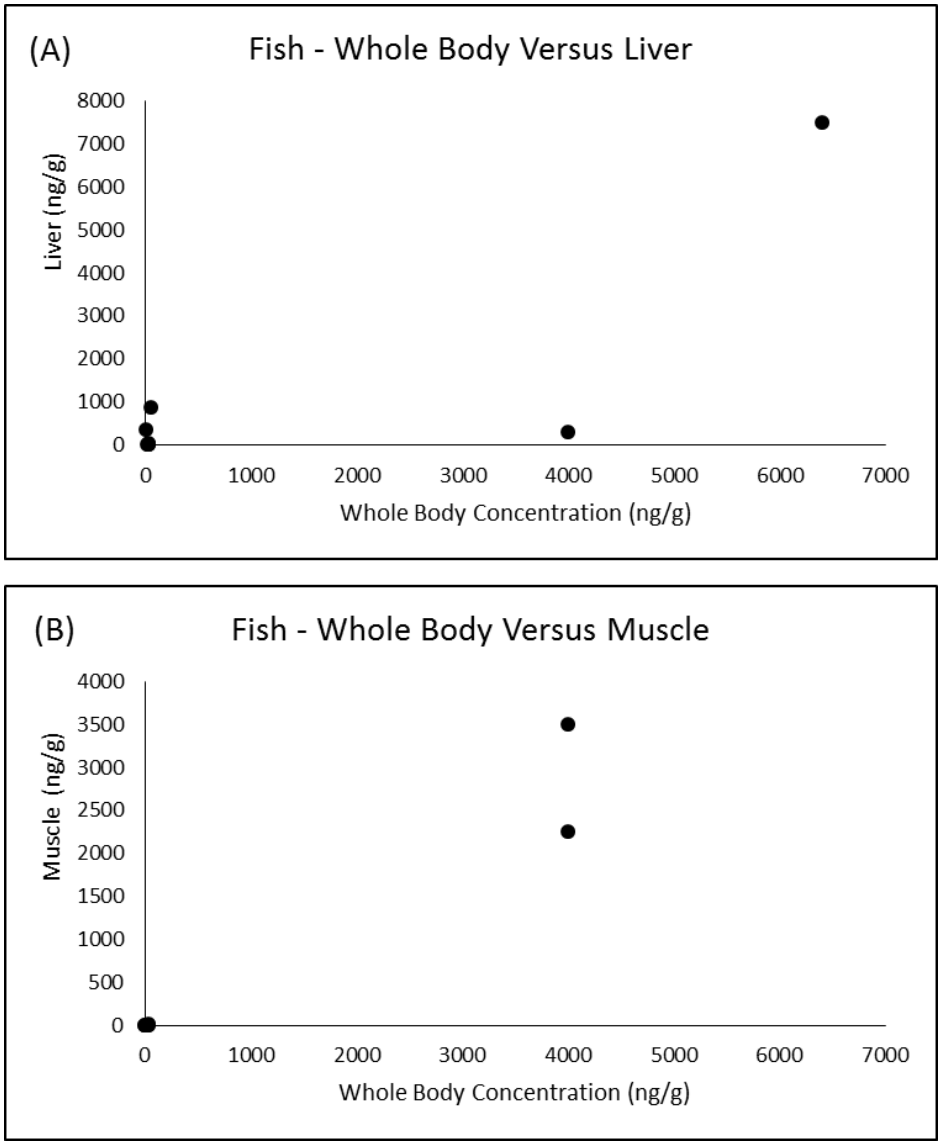

Figure 1. Scatter plots of whole body values for freshwater fish versus (A) liver (*n* = 6) and (B) muscle (*n* = 10). Note the data clusters on top of each other for some substances. No useful correlations could be derived.

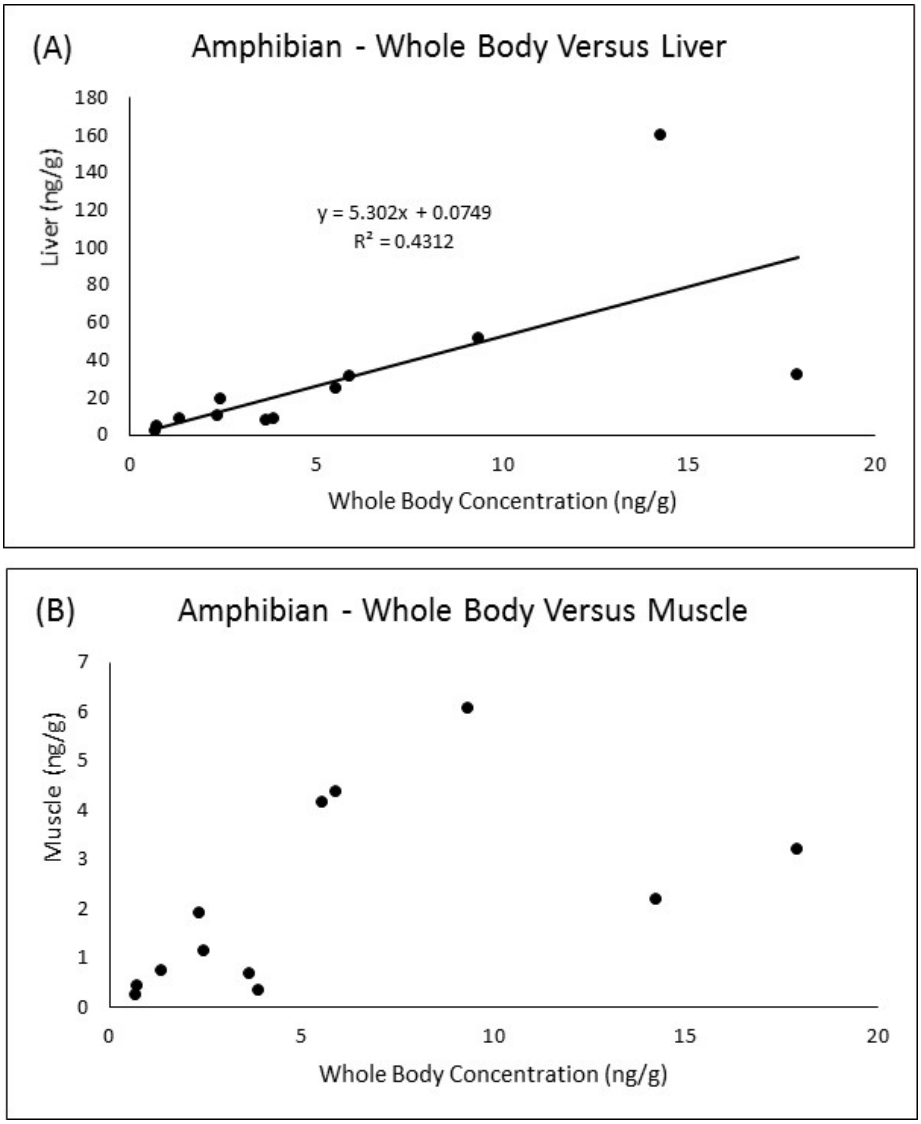

Figure 2. Scatter plots of whole body values for amphibians versus (A) liver (*n* = 12) and (B) muscle (*n* = 12). Note the data clusters on top of each other for some substances and the data are derived from one species; the black-spotted frog (*Pelophylax nigromaculatus*). No useful correlations could be derived for the muscle.

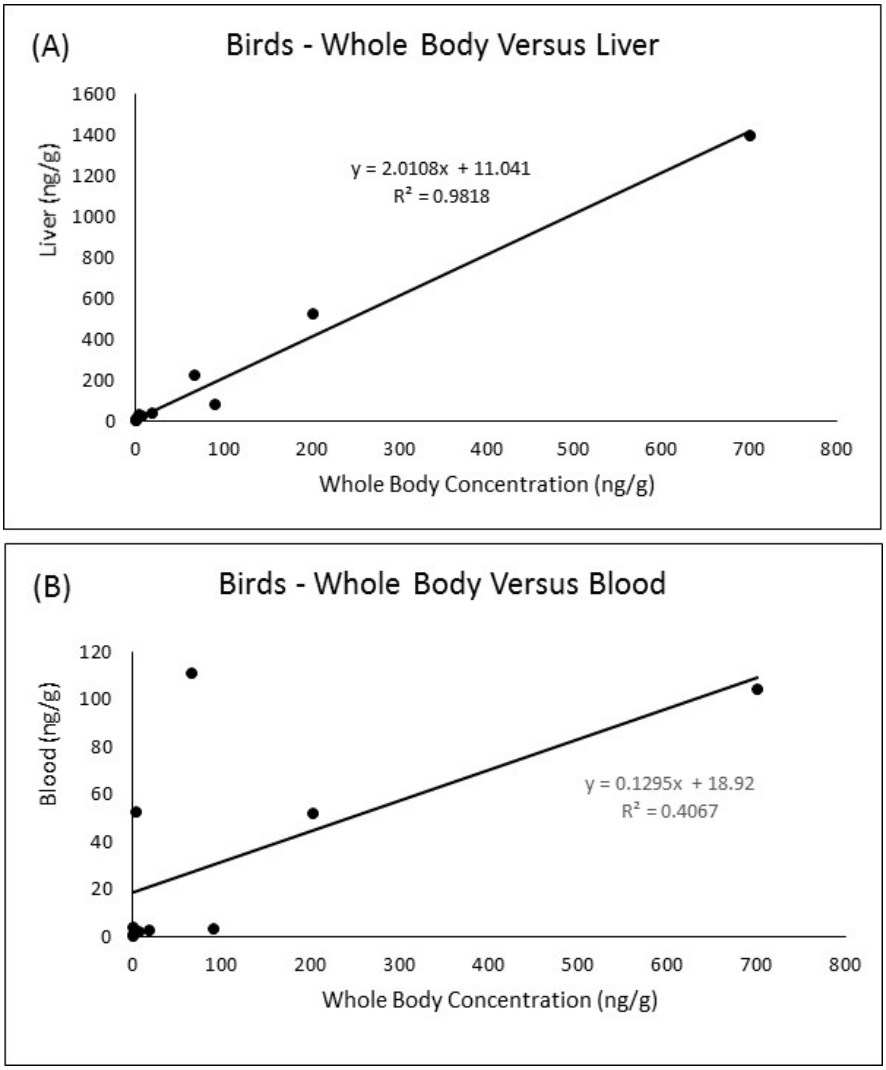

Figure 3. Scatter plots of whole body values for birds versus (A) liver (*n* = 10) and (B) blood plasma (*n* = 10). Note the data clusters on top of each other for some substances and the data are derived from only two species; mainly the glaucous gull (*Larus hyperboreus*) and one data point from the red-throated diver (*Gavia stellata*).

For the mammals, the data were for two species, the laboratory rat and the bottlenose dolphin. Data are shown for the whole body versus the liver or muscle (Figure 4). There were insufficient data on blood or blubber to plot those parameters against whole body burden values. Both liver and muscle correlated well with the whole body burden (Pearson correlation coefficients were, 0.88 and 0.95 respectively). The linear plots of whole body versus liver and muscle also gave good r^2 values (0.78 and 0.90 respectively). However, the slope on the expression for liver is much lower than that derived for birds and amphibians. This is because for dolphins the body burden value was driven by high concentrations in blubber. The data for rats (individual compounds of PBDEs) were more like the birds and amphibians, where the liver generally had a higher concentration that the whole body for the relevant chemical (Figure 6. presents the relationship for liver and whole body for rats only).

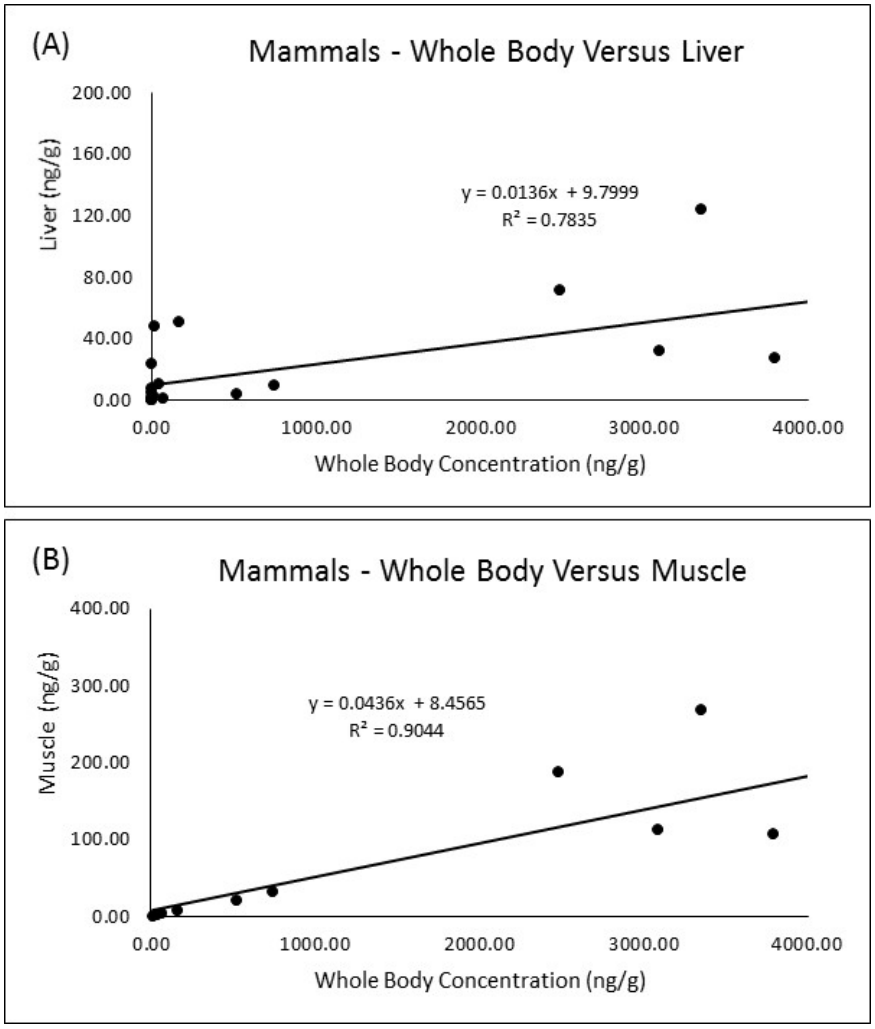

Figure 4. Scatter plots of whole body values for mammals versus (A) liver and (B) muscle. Note the data clusters on top of each other for some substances and the data are derived from only two species; laboratory rats and the bottlenose dolphin (*Tursiops truncatus*). The data did not cluster by species, with a wide range of values in both animals.

Taken together, this initial meta-analysis suggests that the liver is worth exploring further, especially in the birds and mammals, and possibly amphibians. More data is needed to explore the relationship between fish liver and whole body values. The skeletal muscle appears promising as a tool in mammals with good correlations, but it is important to plot separate relationships for marine mammals that have a high proportion of fat in the body burden, or correct data for the fat content. In contrast, the muscle was a poor descriptor of body burden in amphibians, but more data on different species is needed. Blood plasma is generally more problematic because it should be corrected for the osmotic status of the animal (haemodilution, haematocrits, etc.,) and any lipid in the blood plasma that might bind organic chemicals. The composition of the blood, especially nutrients like lipids, may vary with season/nutritional state in animals, as well as with age, sex and body size – as it does in humans. Using whole blood has the advantage of minimal sample but the haematology would still need to be measured to understand how much blood cell volume relative to that

of plasma is present, osmotic status in terms of any potential dilution or concentration of the substance in the plasma, etc., in order to correctly interpret the data.

Unsuccessful attempts were made to combine all the species together in one meta-analysis of whole body versus liver or muscle. However, it should be possible to focus on models for individual groups of animals (i.e., a model for birds, another for fish, and so on), provided that each model has multiple parameter inputs to correct for species differences with the group for morphometrics, organ weights, blood volume, etc. In the fish data, where individual compounds were identified, attempts were made to improve the correlations by correcting the data with the *log* K_{ow} value of the substances – the logic being that BCFs in fish may be related to *log* K_{ow} for specific substances (Veith *et al*., 1979). This did not help and made the correlations worse, suggesting that it is not the lipid solubility of the substance alone driving the spread in the data in this example with respect to organ concentration. This is perhaps not surprising given the comments on biological factors above. It could also be that the attribution of a single *log* K_{ow} value for a group of chemicals (e.g. ∑PFAS) will not allow appropriate correction as within this group there will be wide variance of this parameter with chain length etc. and situations where *log* K_{ow} may not be reliable for short-chain length molecules (Alesio *et al*. 2022).

Finally, an alternative approach to plotting whole body concentrations against individual organs for regression analysis is to determine if there is a constant or factor that would enable a whole body value to be calculated from an individual organ to whole body ratio of concentrations. For an organic chemical that diffuses into the whole body with relatively similar concentrations in all tissues/organs, perhaps the organ will reflect that body burden according to the mass of the organ as a proportion of the total body mass. For example, in the case of trout and other salmonid fish the muscle represents about 66 % of the body mass, so it is possible to hypothesise that the muscle may contain about two thirds of the body burden. However, this is not reflected in the ratios of concentrations of muscle:whole body, or the ratios for liver:whole body (Figure 5). The values range enormously for the liver:whole body ratio from 0.08 to 70and vary considerably within the same chemical types. This likely reflects the fact that the liver is a central target organ involved in the metabolism of organic chemicals, and inevitably the concentration in the fish liver will change relatively quickly over time and not be the same as muscle that makes the bulk of the whole body mass. Metabolism in the liver and excretion via the bile, may decrease the concentration of the chemical substance available to the rest of the body, and organs such as the muscle. However, the muscle:whole body ratio might be a more promising tool, with the ratios for PFOS being around 0.4 regardless of the species of fish (note: a 1:1 relationship is unlikely due to distribution, metabolism and excretion). It should be noted though that the ratio will also be substance specific and chemicals like ibuprofen have different organ ratios to PFOS (Figure 5) due to the differences in ADME. Attempts to further sort the organ:whole body ratios by the *log* K_{ow} value were not successful, suggesting that lipid solubility is not the only driving factor effecting the ratio in the livers or muscle compared to whole body in fish. A study by O'Neil *et al*. (2013) also found that concentrations of PCBs in the muscle relative to the whole body did not partition according to the lipid content of the respective compartments. Bevelhimer *et al*. (1997) attempted to correlate PCBs in fillets of freshwater bass (*Micropterus* spp.) or catfish (*Ictalurus* spp.) with whole body burdens, but the data was too scattered for freshwater bass to give predictions that could be used for regulatory

decision making, although total PCBs in fillets of catfish correlated well with the whole body values.

Across the amphibians, birds and mammals, the liver:whole body ratios were extremely varied, suggesting any analysis should be done within each group of animals, but even within the birds alone, the data was scattered for the same reasons relating to the liver as a target organ involved in metabolism and excretion; where the liver concentration may change rapidly, but the whole body concentration may not. The muscle:whole body ratio could be explored further in amphibians and mammals, if more data becomes available. In the birds, muscle data were not available for most compounds for the two references considered. Some caution will also be needed with marine mammals that have a high proportion of body mass as blubber. The muscle:whole body ratios for marine mammals were low (< 0.08 or less), suggesting the whole body is not reflecting the muscle tissue. There was insufficient data on blubber and whole body values to explore that as an alternative for marine mammals. Yordy *et al*. (2010) argues that the organic chemical concentrations in the blubber could be used as a predictor of the total body burden, if the organ weights and morphometrics of the marine mammal were understood.

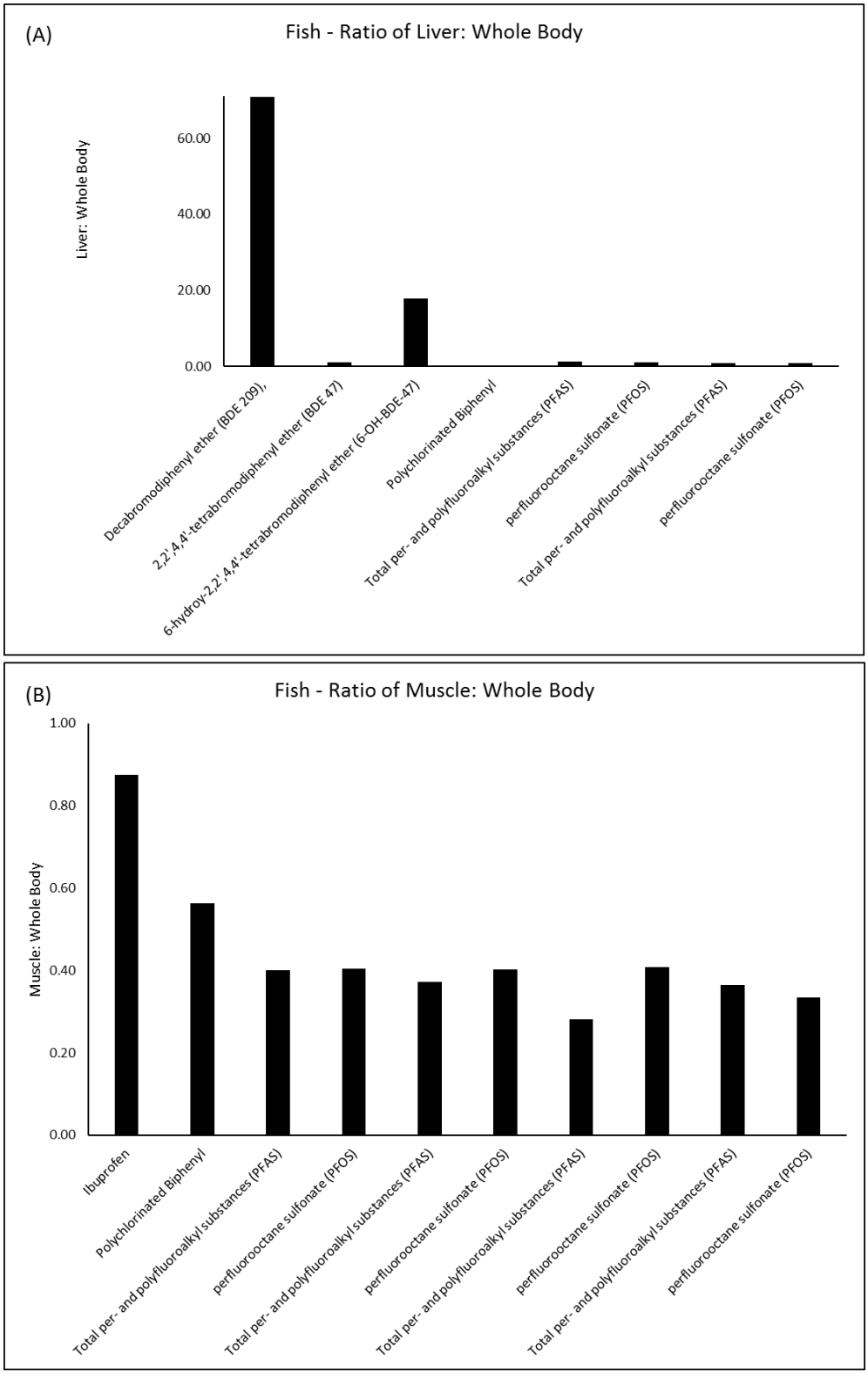

Figure 5. Ratios of organ concentrations:whole body concentration in fish using (A) liver and (B) muscle. Note the values are for different chemicals and also different species of fish; there was not enough data to make average values per substance for one species of fish. The ratios are calculated from single means, so do not have error bars.

4.2 More complex computational tools that relate whole body burden with organs concentrations

A more sophisticated approach would be to develop pharmacokinetic models that describe the relationship between the whole body burden and organ concentrations. Some models are available for humans and laboratory rats (e.g., Dong *et al*., 2020), but there has been less effort on wildlife. The approach relies on detailed knowledge of the morphometrics of the animal and the proportions of regional blood flow going to each organ in the body as well as aspects of the membrane biology of the chemical substance. For the latter this may include the permeability across the gills and other organs involved in uptake, solubility in blood compared to water, fat content of organs and so on, such that “slow” and “fast” tissues can be identified with respect to uptake and/or clearance. There are very few reports that do this with the aim of predicting the organ concentrations. However, Parhizgari and Li (2014) developed such a model for the distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) in two fish species - fathead minnow and medaka. The model was validated by plotting measured whole body burdens against the model predictions, then the model was used to predict the organ concentrations. As expected, the kinetics were dominated by uptake and excretion via the liver and the body fat was identified as a tissue for the bioaccumulation of dioxin (limited excretion). Importantly, the study showed that once a model was constructed, it could predict the organ concentrations, not just the whole body burden. Pharmacokinetic models that predict the concentrations in individual organs are certainly worth considering, although each model will initially be species and chemical substance-specific and require considerable data to develop. However, once the inner workings of such models are understood it may be possible to change the input parameters to make them less species-specific and to simultaneously give data predictions on several organs compared to the whole body concentrations, or indeed the exposure (or uptake rate) that caused it.

5. Regulatory relevance & recommendations

In the modern regulatory landscape, there is an increased emphasis on the reduction of animal testing. For example, under Article 25(1) of the UKREACH states:

"In order to avoid animal testing, testing on vertebrate animals for the purposes of this Regulation shall be undertaken only as a last resort. It is also necessary to take measures limiting duplication of other tests."

Therefore, regulators and regulatory scientists are continually looking at alternative methods to fulfil data requirements. For example, in such programmes as the NC3Rs programme (www.nc3rs.org.uk). It has also been concluded in many instances during bioaccumulation testing (OECD TG 305) that one concentration should be sufficient rather than needing two, dramatically reducing animal requirements (https://www.nc3rs.org.uk/our-portfolio/applying-one-concentration-approach-fish-bioaccumulation-studies). Of course, this also aligns with the 3 Rs of toxicology (Replacement, Reduction and Refinement) which have been at the core of biological sciences since the 1960s (Russel and Burch, 1959). In a regulatory context this can be done by following an integrated testing strategy, whereby certain endpoints trigger the need for higher-tier endpoints, or not. For example, in accordance with Annex XIII (Criteria for the identification of persistent, bioaccumulative and toxic substances, and very persistent and very bioaccumulative substances) under (UK)REACH, for bioaccumulation there are screening criteria (lower tier endpoints) that can be assessed prior to triggering the need for any vertebrate testing (higher tier testing). Typically, organic chemicals which accumulate *via* lipid binding mechanisms can be screened by using the *log* K_{ow}, whereby a *log* K_{ow} > 4.5 indicates the potential of a substance to be B/vB. If the substance is also persistent or very persistent (P/vP) more data would need to be generated/gathered to understand the bioaccumulative nature of the substance (ECHA, 2017a v3.0; Figure R.11-4, pg. 68). It should be noted that bioaccumulation studies are required at Annex IX and above when a substance's *log* K_{ow} is > 3. Beyond the UK REACH legislation new human pharmaceutical (UK amendments for the Human Medicines Regulations, 2012; Directive 2001/83/EC) and biocide (GB BPR) active substances with *log* K_{ow} > 3 require performance of an OECD TG 305 (OECD, 2012) study to investigate the potential of the substance to cause secondary poisoning.

If no relevant/usable information exists to fulfil the legislative data requirements, then testing will be required. It is noted in the ECHA guidance, that *"in normal cases where experimental information on bioaccumulation is needed, a flow-through bioaccumulation test with fish according to OECD TG 305-I or OECD TG 305-II (2012) is preferred due to the best possibilities of reliably comparing the results from such test with the B/vB criteria* Viable alternatives will have the most impact at this higher-tier level, where data demands and vertebrate testing are highest. This may be done by direct replacement of the vertebrate study, maximising use of existing data and/or expanding the screening level studies which may be used to negate the need for higher-tier studies. However, for these alternatives to be a viable strategy they must first be proven to be scientifically robust.

Such strategies always have limitations due to the complexity of different chemistries and biology such as morphometrics, varying anatomies and subsequently differing toxicokinetics. Again, *log* K_{ow} provides a useful indicator of bioaccumulation but can be both over- and under-conservative. For example, a PBT assessment under Annex XIII of (UK)REACH for fentin hydroxide (EC No.: 200-990-6; CAS No.: 76-87-9) would be under conservative. The *log* K_{ow} of the substance is around 3.53 this is only suggestive of a minor possibility of bioaccumulation and as such under the PBT assessment guidance testing for bioaccumulation would not be triggered and the substance would be concluded as not being B/vB. However, the BCF for this substance is > 5000 i.e. it is a vB substance (http://www.safe.nite.go.jp/english/db.html). Whilst this is a useful illustrative example, it is worth noting that the occurrence of these outliers is infrequent and the above example is an exception as it is an organometallic which are largely not representative of organic substances. This shows the importance of molecular make up, here the presence of tin (Sn) may increase uptake over and above that expected when purely looking at *log* K_{ow} alone. Regardless, if the tool is accurate in most instances and its limitations are known it can still be appropriately used for regulatory purpose as long as uncertainty analysis is robust and conducted by an appropriate expert. For example, one alternative to animal testing is the use of (quantitative) structure analysis models ((Q)SARs). In accordance with ECHAs 'Practical Guide – How to use and report (Q)SARs' (v3.1, July 2016), an r^2 value <0.7 is considered to serve warning that the model may have a potentially low performance. But such models should always be used with caution and fully justified with supporting documents. In this sense, the regulation appreciates the limitations (not 100 % accurate) but places a practical limit as well as further procedures to increase the robustness of the data (reduce uncertainty). In the case of building an extrapolative model for tissue burden to whole body burden many of the principles outlined for the validity of a (Q)SAR apply, it is first and foremost about building a statistical model. These will be outlined in the context of this report later.

The data above already applies one principle within the ECHA guidance in that the use of other taxonomic groups other than fish (e.g. mussel bioconcentration test, ASTM, 2003) can be used to create valid BCFs but the concept goes further and takes this to tissue specific levels of chemicals not simply whole body burdens. The exercise looks to reduce animal testing beyond that of some of the *in vitro* and *in chemico* testing and in many ways can be a more reliable real-world indication of a substance's B properties. Detailing these *in vitro* alternatives is currently beyond the scope of this review but they are detailed to some degree in ECHA guidance document R.7c: Endpoint specific requirements v3.0 (ECHA, 2017b).

5.1 Current applicability of tissue burden extrapolative models to whole body burdens

As reported in Section 4, scientific literature and monitoring investigations containing both tissue and whole body burden data are sparse. Therefore, the models have been developed

with a limited number of data. This leads to high levels of uncertainty in any interpretation regardless apparent correlations.

As mentioned, such an extrapolative model that has been attempted here can be checked for reliability, relevance and adequacy, amongst other things, using the regulatory guidance from ECHAs *Practical Guide – How to use and report (Q)SARs* (v3.1) (ECHA (2016). There are four main criteria that it should borne in mind, these are:

1. A defined endpoint
2. An unambiguous algorithm
3. A defined domain of applicability
4. Appropriate measures of goodness of fit, robustness and predictivity

Regarding point 1, the model must predict the same endpoint that would be measured to fulfil the requirement for the relevant regulation. Taking (UK)REACH as an example this would be data requirements defined in Annexes VII to X, XIII. For bioaccumulation, the most relevant endpoint is the BCF, whereby this value can be used in the chemical safety assessment as well as the PBT/vPvB assessment. Current attempts here only seek to develop a relationship between tissue burdens and whole body burdens. The step from an extrapolated whole body burden to BCF has not yet been attempted, as the data are not currently robust enough to make this a meaningful exercise. However, it is expected that any extrapolation in principle should be the same as for the extrapolation from tissue concentrations to whole body concentrations. To estimate BCF from the extrapolation, chemical analysis of the environmental substrate would also be required and this is often not performed. Moreover, the current endpoint (tissue burden) which best correlates with whole body burdens has not been substantiated, though promise for liver and muscle has been noted. To add further complexity to the interpretation is that in field results the main contributions or the proportionality of uptake between diet or respiration are often not known (Handy et al., 1992a,b). Therefore, it is not known which value may be most appropriately calculated e.g., whether it should be the bioconcentration factor (BCF-aqueous/air exposure), biota-sediment accumulation factor (BSAF – sediment dwellers via pore water), biomagnification factor (BMF, dietary exposure) or bioaccumulation factor (BAF, where the whole-body concentration is compared to a dissolved water concentration, even though there is no defined route of exposure (i.e. there is a contribution from both the surrounding medium and the diet)). In most instances it can be assumed a BAF can be derived for field data. However, deriving a BAF would only ever be useful in a weight-of-evidence argument for PBT/vPvB assessment, as there are no defined thresholds for this parameter to conclude on whether the substance is B/vB. Further, if the extrapolation is to work across different species correction for lipid content must also be conducted in the original data. Though information on lipid content tends to be available for mammal bioaccumulation studies, lipid correction during fish or reptile studies is sporadic. The latter point is again highlighted by this review as correcting for lipid while deriving tissue concentration:whole body concentration relationships was not possible due to it not being done in the original studies or data on lipid contents not being presented.

The extrapolation of a BAF to a BCF has recently been explored but requires detailed information to be accurate including the *log* K_{ow}, lipid and non-lipid content (e.g. protein content), chemical concentration in water and diet, the ratio of the rate constants for dietary uptake and respiration, the multiple which may be required where uptake exceeds expected steady state due to food uptake, depuration, food preference and growth (Mackay *et al*., 2013). All these data are rarely available, especially within a single study, making robust conversion impractical. Therefore, the more practical approach may be to develop thresholds directly for BAF though this is problematic. One immediate issue is the fact that some contribution to the BAF is due to the dietary uptake (i.e. relates to the BMF) and that uncertainties in deriving such a threshold for BMF is problematic in itself i.e. there seems to be no widely applicable correlation between BMF or BCF which would make such a threshold possible. For example, though a BMF > 1 may be indicative of bioaccumulation up the food chain it has been noted that a BMF of 0.1 may be indicative of both a BCF over or under 2000 dependent on the chemical studied (ECHA R.11, 2017a v3.0). A benchmarking study has previously been conducted with carp, based on a regression between BCF and BMF for nine compounds tested, it was shown that a BCF value of 5000 L/kg, normalised to a lipid content of 5%, corresponded to a lipid normalised BMF of 0.3 kg food/kg fish, and a BCF of 2000 L/kg corresponds to a BMF of 0.1 kg food/kg fish. (Inoue, Hashizume et al., 2012, ECHA R.11, 2017a). This is much lower than the threshold of a BMF > 1 for indicating trophic transfer. For perflourinated chemicals, a BCF value of 5000 L/kg corresponded to a BMF from the dietary test of 0.5 kg food/kg fish, and a BCF of 2000 L/kg corresponded to a BMF of 0.4 kg food/kg fish. Three substances had a BCF > 2000 but had BMF values both greater than and less than of 1.0 (, ECHA R.11, 2017a v3.0). This shows again that a simple extrapolation from one measure of biological persistence to another is not yet possible and to make a robust conversion of BMF to BCF the same data would be required as outlined for the BAF to BCF conversion. The fact the same BMF value can show different chemicals to be bioaccumulative, or not, emphasises the importance of defining an applicability domain based around chemical characteristics for such extrapolations. Therefore, it would be logical to define if particular chemical groups have specific thresholds, as shown above, rather than find a value that is widely applicable. However, building a database where such conversions have been attempted and building large datasets based on reliable data may begin to provide a reasonable indication as to where such thresholds might be and perhaps for which chemical types they apply. The current state of research means much more data are required. The current status of the attempts made here, is an endpoint has been partially defined i.e. whole body burden. However, it is not yet clear which parameters are required as inputs for the endpoint derivation e.g. which tissue should be used, should the tissue burden be corrected for e.g. lipid content. The answer to either is likely to be dependent on a substances physical-chemistry, which ultimately dictates the substances toxicokinetics. Once the inputs are established it is still unclear how to then best estimate whole body burden from tissues/organs and subsequently how to use an extrapolated whole body burden in a regulatory framework such as UK REACH and GB BPR in a practicable manner.

Concerning point 2 from the list above, although the algorithms suggested are unambiguous, they are not currently well supported by the limited amount of data.

Defining an applicability domain (point 3) for the extrapolations will be of paramount importance, especially considering the limited data in the open literature. At the moment this is not possible due to the limited data. However, the data indicate that without using morphometrics and other input data for higher tier modelling (e.g. PBPK models), the applicability domain would likely need to be organism specific to give any confidence in such an interpretation. It will be organ/tissue specific and this may be driven by the chemical characteristics as well as the organism type. For instance, a substance with a high lipid solubility measured in marine mammals may indicate that the best tissue for study would be blubber. However, for those chemicals with a D_{max} > 1.7 nm (Note: D_{max} is the diameter of the molecule which is dependent on molecular weight and density) regardless of *log* K_{ow}, the liver may be more appropriate as translocation and systemic distribution are more unlikely, thus the only organs which may have a measurable and relatable burden will be those involved in first-pass and detoxification. Moreover, it may be more appropriate to look at chemical groups rather than attempt to create a model that has wider applicability across chemical groups. For example, it has been noted that chlorinated paraffins with higher molecular weights/D_{max} values can be translocated and do bioaccumulate, this does not follow traditional rules of toxicokinetics. In conclusion defining the boundaries of an applicability domain for the extrapolation of tissue burdens to whole body burdens is currently not possible.

Obtaining appropriate measures of goodness-of-fit, robustness and predictivity (point 4) in itself is a relatively straight forward exercise. However, what must be borne in mind are the data behind these measures. For example, a linear fit between two data points will yield a correlation coefficient and r^2 of 1 (i.e. 100 % fit/correlation). This does not mean there is a true relationship just that there is a limited dataset that seems to show a good fit. The model is, therefore, less robust and the algorithm can change wildly with the inclusion of single additional datapoints. Using the model fits that are presented above there is some promise that relationships may prove to be robust for the extrapolation of tissue to whole body burden, but this is likely chemical and organism dependent. Again, referring to the ECHA guidance (ECHA, 2008), an r^2 value < 0.7 may reflect poor performance, so r^2 values above this for specific relationships are of course desirable. For example, when comparing liver to whole body burdens for birds the r^2 was 0.99, easily good enough to give confidence in the model, though the chemical types were limited as were the number of data points so the applicability domain currently is restricted to contain only specific chemical type studied and the specific birds. An extrapolation with such restrictions is not desirable as its applicability and therefore use is limited, for instance, ideally the extrapolation should work on several bird species otherwise the implementation becomes impracticable. The number of data are modest but workable (n = 10) but, the dataset is made up of chemical burdens from groups (“sums of”) chemicals, which may skew the relationship to be either over- or under-precautionary. This is because the possible relationships between tissue burdens and a specific single chemical will be missed as each chemical within a group can have a highly

diverse range of bioaccumulation potential. In most instances when taking the sum of a substance from a single tissue the data will likely be skewed by the most bioaccumulative substance in the chemical group. For example, PFAS cover a wide range of chemicals which covers fluorinated alkyl chains of varying lengths and can in itself cover other subclasses. In Table 5 different PFAS, their chain length, *log* K_{ow} and measured BCF can be seen, these were derived from the NITE database (http://www.safe.nite.go.jp/english/db.html).

Table 5. PFAS taken from NITE database and the corresponding chain lengths, *log K_{ow}* and fishBCF values (red text shows lower BCF than expected based on *log K_{ow}*).

Chemical name	Chain Length	*Log K_{ow}*	BCF
1-Pentanol, 2,2,3,3,4,4,5,5-octafluoro- CAS No. 355-80-6	5	1.97	<29
1-Heptanol, 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoro- CAS No. 335-99-9	7	3.31	22
Heptane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-hexadecafluoro- CAS No. 335-57-9	7	5.5	8740
Octane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-octadecafluoro- CAS No. 307-34-6	8	6.17	13600
3,3,4,4,5,5,6,6,6-nonafluorohexan-1-yl acrylate CAS No. 52591-27-2	8	4.43	<53
1,1,1,2,2,3,3,4,4,5,5,6,6-Tridecafluorooctane CAS No. 80793-17-5	8	5.3	2600
1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- CAS No. 678-39-7	9	5.58	310
3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctan-1-yl methacrylate CAS No. 2144-53-8	10	6.32	12
1-Undecanol, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11-eicosafluoro- CAS No. 307-70-0	11	5.99	2600
henicosafluoroundecanoic acid CAS No. 2058-94-8	11	6.82	5300
Dodecanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-tricosafluoro- CAS No. 68015-87-2	12	7.49	25000
Tetradecanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,14-heptacosafluoro- CAS No. 376-06-7	14	8.83	25000
Hexadecanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,15,15,16,16,16-hentriacontafluoro- CAS No. 67905-19-5	16	10.17	5900
Octadecanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-pentatriacontafluoro- CAS No. 16517-11-6	18	11.51	460

Based on data collected on PFAS from the NITE database (Table 5), BCFs can range from < 3 for low molecular weight (MW) PFAS (MW = 232; 2,2,3,3,4,4,5,5-octafluoro-1-pentanol, EC No. 206-593-4, CAS No. 355-80-6) up to 25,000 for higher molecular weight longer chain derivatives. At the higher ends of molecular weight the relationship begins to reverse, likely around where the D_{max} is closer to 1.7 nm (MW = 914 (D_{max} 1.23 nm)); perfluorooctadecanoic acid, EC No.: 240-582-5, CAS No.: 16517-11-6) and *log* K_{ow} > 10 (ECHA R.11, 2017a v3.0). The long chain PFAS (carbon chain length exceeding seven) can be considered traditionally bioaccumulative *via* a lipid driven mechanism despite being ionogenic substances and this can be detected in the traditional fish bioaccumulation tests. However, the BCF values can vary by orders of magnitude but barring the two PFAS highlighted in red (Table 5), the relationship of *log* K_{ow} with bioaccumulation holds true. Those highlighted in red appear to have much lower BCFs than would be expected.

Due to short chain PFAS often gaseous nature a measure of BCF in aquatic species is likely of little value and air-breathing animals will be of far more importance when deriving any relationship. These shorter chain length PFAS (carbon chain lengths < 7) appear to not follow the rules based around lipophilicity and bioaccumulation in organisms other than fish. Therefore, PFAS of carbon chain length ≤ 7, bioaccumulation estimated from fish studies or using *log* K_{ow} as a surrogate may underestimate bioaccumulation in air breathing organisms (Miranda *et al*., 2022). However, these numeric cut-offs for vB and B should often never be used in isolation and require expert input, considering such things as the impact of the substances persistence and physico-chemical properties and the influence these may have on the substances ADME (see Section 4 for further discussion). Such a weight-of-evidence has been used in hazard assessments for this substance type, specifically Perfluorohexane-1-sulphonic acid and its salts (EC No. 206-587-1; https://echa.europa.eu/documents/10162/a5cb08e3-7909-c28d-8fbc-5f1e4f9cc377). Note, in the NITE dataset the majority of PFAS have a chain length of ≥ 7, therefore, as discussed it is expected that the bioaccumulation is to extent lipid driven and can be identified in fish studies, which has been shown in the BCF results (Table 5).

A relationship between *log* K_{ow} and carbon chain length is well documented for non-ionic organic chemicals. Therefore, an algorithm which considers data from the sum of a chemical group is maybe inaccurate even if it allows proof of concept. It is also important to note that particular functional groups, as shown for PFAS above, may impact uptake and not accounting for this or being able to include correction factors for these differences again leads to uncertainty in the algorithm. Such corrections are well documented for models that predict BCFs or biological response (e.g. the BCFBAF (Q)SAR: https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface). The concentrations will likely be dominated (skewed) by the highest accumulating chemical of the group. Though analysis of the sums of chemicals in tissues (e.g. sum of PFAS) are useful for monitoring and establishing how particular policies are impacting removal of such chemicals from the market or remediation schemes, they are unfortunately less useful for developing such algorithms.

Regardless of the any established relationships between tissue/organ and whole body concentrations the other criteria (Points 1-3) needed to make a robust calculation also require further definition before relying on such a model for regulatory purpose.

The mammal data provides an example of how an interpolative/extrapolative equation can be built with more confidence when the parameters are more defined, for example one species and one chemical category (i.e. a more defined applicability domain). It also shows how a chemical category will contain a broad spectrum of more and less bioaccumulative substances. In the dataset there are data on several substances from the chemical class polybrominated diphenyl ethers (PBDEs) and the whole body and liver burdens are available for each. In Figure 6, a linear relationship can be seen for PBDEs and the high r^2 number (r^2 = 0.998) when using only one species (Sprague-Dawley rat) and one chemical type (PBDEs). Even with this though, the data are limited so it is difficult to understand where the boundaries of such an extrapolation might lie, i.e. where would the extrapolation begin to fail. For example the extrapolation may only work with specific chemistries, molecular weight ranges, chain lengths or at within specific boundaries of other physicochemical boundaries. Another point to note is that the single point with the highest values, relatively far from the other data may drive the relationship with some skew introduced, this again highlights the need for larger datasets to create confidence in any model.

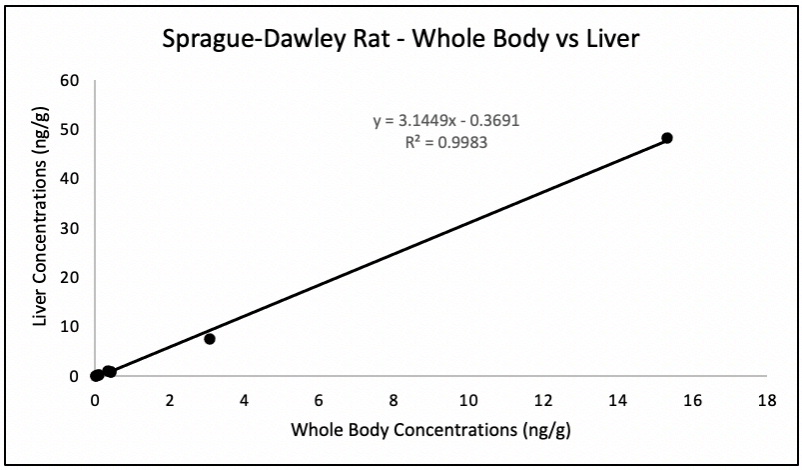

Figure 6. Scatter plots of whole body versus liver concentrations for Sprague-Dawley Rats (*n* = 9).

5.2 Use in a regulatory framework

This review has outlined ways that it may be possible to maximise the use of tissue concentration data for regulatory use, namely how to derive a whole body concentration from tissue burdens, but ultimately how this might contribute to an assessment of bioaccumulation. At this time it is not advisable to use such models due to lack of understanding and lack of data, with the most promising option perhaps being to refine such models by broad organism type and chemical class as discussed in Section 5.1.

Conceptually it is of value to understand how such an extrapolation (i.e. tissue to whole body burden to whole body concentration) might fit within any regulatory scheme, either as part of tiered testing approach, or a weight-of-evidence. This will depend on the certainty (i.e. robustness, reliability and adequacy) and conservatism of the method. For example, for substances registered under (UK)REACH at a supply level of 100-1000 tonnes per year (Annex IX) a *log* K_{ow} > 3 triggers the need for a bioaccumulation study and during PBT assessment (at Annex VIII) a *log* K_{ow} of > 4.5 triggers concern that the substance may be bioaccumulative. Rather than move immediately to vertebrate testing for Annex IX such methodologies (tissue burden extrapolation to BCF), though not yet possible, might be seen as robust enough in future to act as an intermediary screening level assessment whereby any uncertainty from such a method is considered acceptable for those substances with a *log* K_{ow} > 3 but < 4.5. This would lead to a significant reduction in the number of animal and amount of resources as many substances being registered are within this *log* K_{ow} range. To add a further layer of conservatism, should a method for conversion ever be available, it could be stipulated that any extrapolation from a tissue burden that leads to a BCF ≥ 1000 should then be tested using the standard bioaccumulation test (OECD TG 305, 2012). See Figure 7 for a possible schematic.

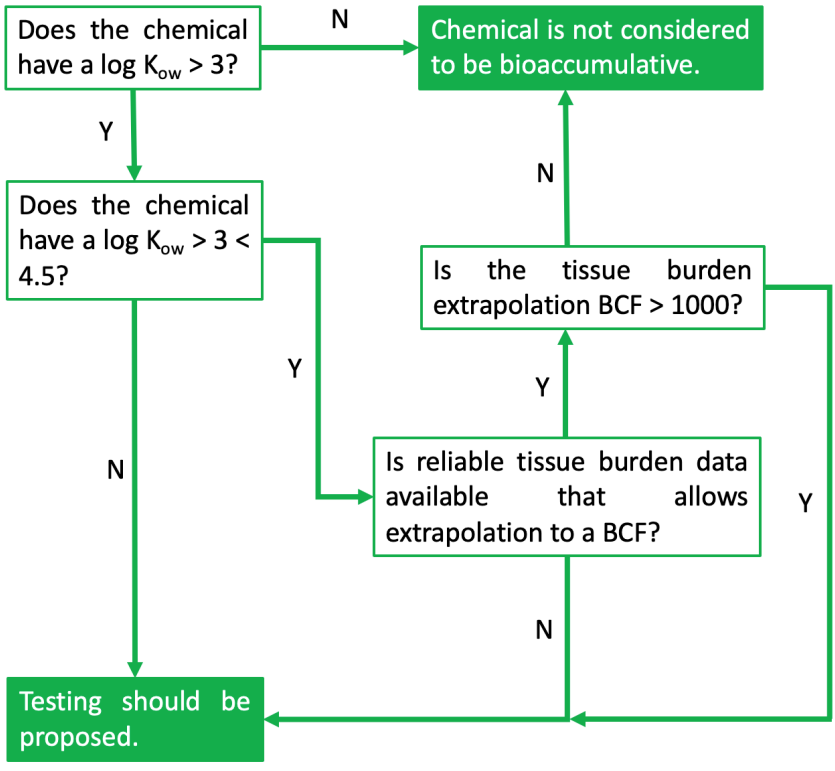

Figure 7. A possible schematic of how to use tissue burden extrapolations to BCF values in an integrated testing strategy approach for regulatory purpose.

In this way the extrapolation could become an integral part of tiered testing strategies while caution is exercised in its interpretation and conservatism is introduced specifically into the

regulatory testing framework (i.e. a BCF cut-off of > 1000 needing further testing), rather than any direct interpretation of B/vB for such borderline substances.

It could also be possible that such extrapolation could replace the vertebrate study if there was a significant body of scientific evidence to prove its validity, or that it could be used in a weight-of-evidence with other approaches e.g. *in vitro*. However, without a robust extrapolation method for tissue to whole body burdens it does not provide such an opportunity.

Also, in this review, the extrapolations of BMFs to BCFs is discussed. This is particularly important for chemicals such as nanomaterials or those that are highly adsorptive or poorly water soluble whereby testing via the water phase becomes impractical if not impossible. However, in terms of risk assessment or PBT/vPvB assessment the relevance of a BMF is not immediate, though a BMF > 1 is often indicative of a BCF > 5000, it does not rule out that BMF values < 1 are not ≥ 2000 (i.e. still B; see Section 5.1 for further discussion). As such no definitive threshold has been assigned for BMF values which would indicate a substance as B or vB. Although there are varying equations for the conversion of a BMF to BCF (see: https://www.oecd.org/chemicalsafety/testing/section-3-environmental-fate-behaviour-software-tg-305.htm) each is limited to specific situations and none are widely applicable and should be selected with care using expert judgement. Therefore, concluding a B assessment using these data already has inherent uncertainty and adding to this uncertainty by extrapolation cross-species or otherwise may currently be unwise considering the current knowledge gaps. Also discussed is how data-intensive extrapolating from each bioaccumulation factor (BCF, BAF, BSAF and BMF) can be.

The current state-of-science presents unacceptable uncertainty for extrapolations as discussed here to be used in a regulatory context and more data are required as well as a better understanding of chemical interactions, morphometrics, ADME and species differences in order to allow the robust modelling of such an extrapolation. It is likely a multi-factor analysis is needed and other information may need to be gained beyond that of simply the tissue burden in order to make the extrapolation reliable. This could come from specific non-animal testing or basic knowledge of animal anatomy, physiology and behaviour (e.g. feeding patterns) as well as understanding of the physical-chemical characteristics of the molecule of interest. However, exactly what these multiple factors should be and which are essential or superfluous is yet to be established.

5.3 Tissue to whole body concentration extrapolation for purposes other than estimating BCF

To assist in the monitoring of progress against contaminants in the UK Governments 25-Year Environment Plan (UK Government 2018) the H4 indicator ‘Exposure and adverse effects of chemicals on wildlife in the environment’ was established (Defra 2019; EA 2021). To support this various wildlife monitoring schemes are sampling a range of species and

analysing tissues/organs for a number of chemicals (Table 2). However, these schemes tend to only analyse specific tissues/organs, most commonly liver.

Concurrently ‘thresholds’ are being developed for different organism-chemical combinations, where the threshold is the concentration in a receptor indicative of potential harm (Shore & Walker 2020). Such threshold values may be for whole organism concentrations. Therefore, to exploit the results of the wildlife monitoring schemes it would be necessary to convert an organ/tissue concentration (most likely liver) to a whole body concentration to compare to the threshold value.

Similarly, as part of the risk assessment process, models may be used to predict whole organism concentrations though its use under most UK chemical legislations is limited, simply due to uncertainty surrounding these models. For compliance purposes the wildlife monitoring scheme results could be compared to such model predictions and both in conjunction could be used in a weight-of-evidence approach. However, again tissue concentrations would need to be converted to whole organism concentrations to allow this comparison.

6. Future Focus

The most rudimentary and unescapable finding from this report is that there is a lack of data to stablish robust tissue to whole body concentration ratio relationships, especially for regulatory use. It also further highlights the large amount of data in the literature that is unusable outside of the target of the manuscript and the review demonstrates that many, if not most, authors do not make their underlying data available. Although only a small number of usable papers were identified, some of the other studies that were rejected likely generated data which would have been useful if made available. Data harmonisation is now a key focus of some major EU projects as there is a drive to increase the cross disciplinary usability of data (e.g. NanoCommons https://www.nanocommons.eu). Data harmonisation can include reporting templates or specific minimum reporting requirements for certain types of studies in order to make the results robust and transferable. Moreover, and data availability is a requirement of some funders and publishers though this unfortunately is not rigorously policed.

In order to establish relationships between tissue/organ and whole body concentrations and drive the number of available data upwards it may be prudent in future when commissioning work specifically looking a tissue burdens that certain other data must also be collected to fully understand the implications of the measure as well as move toward the building of models. Another aspect would be to ensure principal investigators were aware of the regulatory frameworks that there project work could directly impact and be mindful of this throughout the process. This, combined with more rigour during experimental design and planning stages would start to see a large upshift in usable data for regulatory purposes. For example, it might stipulate that when tissue burden data are collected that whole body burden should also be measured as well as background concentrations of the chemical of interest. It could also be stipulated that other basic parameters should be derived where relevant such as derivation of the chemicals basic physico-chemical properties. In the context of (UK)REACH if the substance is registered this data may be available due to the regulations data requirements. Of course, it must be cautioned that such stipulations only lend themselves to situations where they are practicable and that they do not inhibit the protection of humans and the environment. For example, biomonitoring programmes for chemical classes or chemicals of concern should not be subject to such constraints that would make them time and financially consuming to the point they would not be viable and as such could not inform policy.

It may also be important to further explore the concept per species/organism and chemical substance class and build each class before a model which includes all classes can be made. In this way as each model becomes available it becomes immediately usable but also it allows for problem solving on a smaller scale and should the extrapolation not work only the efforts on this particular class are lost. Equally, models could be explored for several chemicals of known properties on one species (e.g., trout) then subsequently see if these models work with some modifications to similar animals in the same group (e.g., carp). The

process could continue to other vertebrates (e.g. birds) to see if the relationships continue to hold true or if correction is required or possible.

Once this research has moved further forward it will also be important to define any other key parameters that must be accounted for in relationships, be it biological or physical-chemical. It will also be possible to understand if a simple model or more complex model might be required (e.g. PBPK) and if specific *in vitro* tests might be required to gain the required inputs. Another option would be to move beyond the more simplistic mathematical models and start using advanced computing techniques (artificial intelligence) which allows the data to derive its own associations and networks (Serra et al., 2019). Of course, the challenge would still be regulatory acceptance of such a model.

An interesting next step might also be to find data on the tissue burden of a substance or substance class for a species highlighted here where the r^2 tissue to whole body burden extrapolation is > 0.7 and there is regulatory data available on the BCF/whole body burden to see if the whole body burden extrapolation model derives a reasonable result. Such an exercise was outside the current scope and in most instances would be easier to test for fish due to the regulatory focus on BCF and the fish BCF study (OECD, 2012). Though, it should be noted currently no good relationships were found in this review between fish tissue/organs and whole body concentrations, this may change with more data.

As noted above there are many on-going wildlife monitoring schemes which tend to only analyse a narrow range of tissues/organs. However, the schemes collect and retain other sample types and hence offer the opportunity to estimate whole organism concentrations. These schemes therefore offer the opportunity to gain more field data on comparative organ/tissue and whole organism concentrations. There are of course challenges during field sample collection and ideally to stop any change post-mortem samples should be snap frozen on site with liquid nitrogen, or at the very least only kept on ice for a few hours (Handy et al., 2002). Moreover, it needs to be acknowledged that there will be some uncertainty in using field data as the organism exposure history will be unknown. Such a caveat is also relevant to the application of any organ/tissue - whole organism relationships if applied to field collected data to estimate whole organism concentrations from collected organs/tissues.

7. Conclusion

Already in Annex XIII of UKREACH all available information/evidence on bioaccumulation must be considered in a weight-of-evidence approach. This includes bioaccumulation experiments, monitoring data from the field and toxicokinetic information from mammalian studies as well as other testing and non-animal testing indicators. The above review represents an attempt to increase the value of biomonitoring and literature data which often focus on tissue burden data only and how this may feed into such a weight-of-evidence for the specific purpose of B/vB assessment. Moreover, the importance of such a review and finding useful outputs from such data will also aid policy makers' and regulators' decision making. The premise of this review was to establish the potential of adding to the "alternative" data toolset.

The report set out to determine if it was possible to extrapolate from tissue burdens to whole body burdens. The developed linear relationships on a small set of acceptable literature showed that both the r^2 and correlation coefficient can vary dependent on the measured tissue and the organism type with r^2 ranging from < 0.30 to 0.99 and the correlation coefficient ranging from <0.40 to 0.98. The data showed that the extrapolation in many instances is likely possible but was tissue and organism specific as to its goodness-of-fit. The difference in goodness-in-fit could not be fully explained based on the dataset here but it is postulated that several factors would lead to a poor fit from poor reporting to differences in ADME for different chemicals which was not addressed by the model and simply a lack of data. The lack of data and understanding leads to inherent uncertainty in such an extrapolation making it difficult to justify its use in a regulatory context.

The report does suggest some promise for the use of both liver and muscle tissue concentrations for extrapolation to whole body burdens, but limitations in the dataset inhibit full interpretation, such as relying on sums of chemical classes rather than defining each individual substance. It should be stressed that, though there may be scope to extrapolate tissue to whole body concentrations, no one sampled tissue should be taken to represent whole body concentrations (i.e., it is not possible to assume muscle or liver concentrations are equal whole organism concentration). If suitable models to extrapolate between tissue/organ and whole body concentrations can be established, how this would then lead to a useful value for regulatory context remains unaddressed. The determination of a BCF/BAF/BMF/BSAF is only possible when sampling of the environment from which the biological sample was taken was conducted. Further, when a BCF is not derived but instead e.g. a BAF, the extrapolation from these factors (e.g. BAF/BSAF/BMF) to BCF is in itself data-intensive, making the concept more impractical.

The evaluation presented here did highlight that the concept is worth further exploration and that if data was presented more uniformly and a drive was given toward collecting whole body and tissue burden data, the models may yet prove successful. As such it was also worth exploring there use in a regulatory context. It is easy to see how such data could be

used to limit vertebrate testing and aid in the use of the plethora of information from monitoring studies and the literature.

It was not possible to understand if such a concept would work for chemicals where bioaccumulation is not dictated by *log* K_{ow} due to the limited data set (e.g. halogenated chemicals often do not fit the classic paradigm for bioaccumulation with some halogenated chemicals above a molecular weight of 1000 Da able to penetrate cell membranes and be found systemically when it is expected they would be largely excreted; De Silva *et al.* 2021). Nor was it possible to identify if the model worked well for one uptake route over another. It was only possible to postulate on the possible toolbox of data that may be required as inputs to such an extrapolation in order to make it more robust and reliable.

The report highlights where future work could be focused as well as the current data gaps. At this time the use of extrapolated whole body burdens from tissue burdens is not possible in a regulatory context. Moreover, it is also currently not practicable to convert this whole body burden to BCF when in many instances the BCF will need to be derived from a BAF/BMF and so forth (see Section 5.2). There may currently be better alternatives to reduce vertebrate testing such as using invertebrate models (e.g. Schlechtriem *et al*., 2019; Handy *et al*., 2021), which are closer to regulatory acceptance, or *in vitro* fish models (Handy *et al*., 2022). However, the power of a tissue burden to whole body concentration extrapolation that can be used in a regulatory context is the eventual use of non-invasive sampling on animals *in situ* such as analysis of excretions, nail clippings, or hedgehog spines, which will be a far more accurate reflection of chemical behaviour than a laboratory study and not cost animal life.

Further work in the area of tissue concentration to whole body concentration is required and holds promise due to the wide impact it may have on data utilisation and animal reduction.

References

AHRENS. L., NORSTRÖM, K., VIKTOR, T., COUSINS, A.P., JOSEFSSON, S., 2015. Stockholm Arlanda Airport as a source of per- and polyfluoroalkyl substances to water, sediment and fish. Chemosphere, 129, 33-38.

ALESIO, J.L., SLITT, A. AND BOTHUN, G.D., 2022. Critical new insights into the binding of poly-and perfluoroalkyl substances (PFAS) to albumin protein. Chemosphere, 287, 131979.

American Society for Testing and Materials, ASTM E1022 - 94 Standard Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks, 2013.

BEVELHIMER, M.S., BEAUCHAMP, J.J., SAMPLE, B.E., SOUTHWORTH, G.R., 1997. Estimation of Whole-Fish Containment Concentrations from Fish Fillet Data. Report No. ES/ER/TM-202. Tennessee, USA: Oak Ridge National Laboratory (ORNL).

BRUGGEMAN, W.A., MARTRON, L.B.J.M., KOOIMAN, D. AND HUTZINGER, O., 1981. Accumulation and elimination kinetics of di-, tri-and tetra chlorobiphenyls by goldfish after dietary and aqueous exposure. Chemosphere, 10, 811-832.

CHEN, Q., YIN, D., HU, X., WANG, R., ZHANG, C., 2014. The effect of nC60 on tissue distribution of ibuprofen in *Cyprinus carpio*. Science of The Total Environment, 496, 453-460.

COLLINS, A., COUGHLIN, D., MILLER, J., KIRK, S., 2015. The Production of Quick Scoping Reviews and Rapid Evidence Assessments: A How to Guide.

CONDER, J.M., HOKE, R.A., WOLF, W.D., RUSSELL, M.H. AND BUCK, R.C., 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environmental Science & Technology, 42, 995-1003.

CUI, Q., PAN, Y., ZHANG, H., SHENG, N., WANG, J., GUO, Y., DAI, J., 2018. Occurrence and tissue distribution of novel perfluoroether carboxylic and sulfonic acids and legacy per/polyfluoroalkyl substances in black-spotted frog (*Pelophylax nigromaculatus*). Environmental Science and Technology, 52 (3), 982-990.

DE SILVA, A.O., ARMITAGE, J.M., BRUTON, T.A., Dassuncao, C., Heiger-Bernays, W., Hu, X.C., Kärrman, A., Kelly, B., Ng, C., Robuck, A., Sun, M., Webster, T.F., Sunderland, E.M., 2021. PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding. Environmental Toxicology and Chemistry, 40(3):631-657.

DEPARTMENT FOR ENVIRONMENT FOOD AND RURAL AFFAIRS (Defra), 2019. Measuring Environmental Change: Outcome Indicator Framework for the 25 Year Environment Plan. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/925779/25-yep-indicators-2019.pdf [Accessed February 2022]

DIRECTIVE 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community Code Relating to Medicinal Products for Human Use.

DONG, Z., LI, T., WAN, Y., SUN, Y. AND HU, J., 2019. Physiologically based pharmacokinetic modeling for chlorinated paraffins in rats and humans: importance of biliary excretion. Environmental Science & Technology, 54, 938-946.

DU, X., YUAN, B., ZHOU, Y., DE WIT, C.A., ZHENG, Z., YIN, G., 2020. Chlorinated paraffins in two snake species from the Yangtze River Delta: tissue distribution and biomagnification. Environmental Science & Technology, 54 (5), 2753-2762.

ENVIRONMENT AGENCY (EA), 2021. Exposure and Adverse Effects of Chemicals on Wildlife in the Environment: Interim H4 Indicator: Supporting Information and Data, Version 1. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/992555/Exposure_and_adverse_effects_of_chemicals_on_wildlife_in_the_environment_interim_H4_indicator.pdf [Accessed 11 February 2022]

EUROPEAN CHEMICALS AGENCY (ECHA), 2008. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.6: QSARs and grouping of chemicals, Guidance for the Implementation of REACH. Helsinki, Finland: ECHA.

EUROPEAN CHEMICALS AGENCY (ECHA), 2011. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.4: Evaluation of Available Information, Version 1.1. Guidance for the Implementation of REACH. Helsinki, Finland: ECHA.

EUROPEAN CHEMICALS AGENCY (ECHA), 2016. How to use and report (Q)SARs. Practical Guide 5, Version 3.1. Helsinki, Finland: ECHA.

EUROPEAN CHEMICALS AGENCY (ECHA), 2017a. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/Vpvb Assessment, Version 3.0. Helsinki, Finland: ECHA.

EUROPEAN CHEMICALS AGENCY (ECHA), 2017b. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7c: Endpoint Specific Guidance. Version 3.0. Helsinki, Finland: ECHA.

GB regulation concerning the making available on the market and use of biocidal products (Biocidal Product Regulation; GB BPR). GB regulatory framework for Biocidal Products Regulations and CLP; Statutory Instrument 2019, No 720.

HAGENBUCH, B., GAO, B., MEIER, P.J., 2002. Transport of xenobiotics across the blood-brain barrier. Physiology, 17, 231-234.

HAMPTON, J.A., MCCUSKEY, P.A., MCCUSKEY, R.S. AND HINTON, D.E., 1985. Functional units in rainbow trout (*Salmo gairdneri*) liver: I. Arrangement and histochemical properties of hepatocytes. The Anatomical Record, 213, 166-175.

HAMPTON, J.A., LANTZ, R.C. AND HINTON, D.E., 1989. Functional units in rainbow trout (*Salmo gairdneri*, Richardson) liver: III. Morphometric analysis of parenchyma, stroma, and component cell types. American Journal of Anatomy, 185, 58-73.

HANDY, R.D., JHA, A.N, DEPLEDGE, M.H., 2002. Biomarker Approaches for Ecotoxicological Biomonitoring at Different Levels of Biological Organisation. In: Handbook of Environmental Monitoring (BURDEN, F., MCKELVIE, I., FÖRSTNER, U., GUENTHER, A., (Eds.). Chapter 9, 9.1-9.32. New York: McGraw Hill.

HANDY, R.D., 1992a. The assessment of episodic metal pollution. I. Uses and limitations of tissue contaminant analysis in rainbow trout (*Oncorhynchus mykiss*) after short waterborne exposure to cadmium or copper. Archives of Environmental Contamination and Toxicology, 22, 74-81.

HANDY, R.D., 1992b. The assessment of episodic metal pollution. II. The effects of cadmium and copper enriched diets on tissue contaminant analysis in rainbow trout (*Oncorhynchus mykiss*). Archives of Environmental Contamination and Toxicology, 22, 82-87.

HANDY, R.D., AHTIAINEN, J., NAVAS, J.M., GOSS, G., BLEEKER, E.A., VON DER KAMMER, F., 2018. Proposal for a tiered dietary bioaccumulation testing strategy for engineered nanomaterials using fish. Environmental Science: Nano, 5, 2030-2046.

HANDY, R.D. AND AL-BAIRUTY, G., 2019. Effects of Nanomaterials on the Body Systems of Fishes. In: Ecotoxicology of Nanoparticles in Aquatic Systems. Boca Raton, Florida: CRC Press. pp. 156-168.

HANDY, R.D., CLARK, N.J., VASSALLO J., GREEN, C., NASSER, F., TATSI, K., HUTCHINSON, T.H., BOYLE, D., BACCARO, M., VAN DEN BRINK, N., SVENDSEN, C., 2021. The bioaccumulation testing strategy for manufactured nanomaterials: physico-chemical triggers and read across from earthworms in a meta-analysis. Environmental Science: Nano, 8, 3167-3185.

HANDY, R.D., CLARK, N.J., BOYLE, D., VASSALLO J., GREEN, C., NASSER, F., BOTHA T.L., WEPENER V., VAN DEN BRINK, N.W., SVENDSEN, C., 2022. The bioaccumulation testing strategy for nanomaterials: correlations with particle properties and a meta-analysis of in vitro fish alternatives to in vivo fish tests. Environmental Science: Nano, 9, 684-701.

HUWE, J.K., SMITH, D.J., 2007. Accumulation, whole-body depletion, and debromination of decabromodiphenyl ether in male sprague-dawley rats following dietary exposure. Environmental Science and Technology, 41 (7), 2371-2377. Erratum in: Environmental Science and Technology, 2007 41, 4486.

INOUE Y., HASHIZUME N., YOSHIDA T., MURAKAMI H., SUZUKI Y., KOGA Y., TAKESHIGE R., KIKUSHIMA E., YAKATA N. and OTSUKA M., 2012. Comparison of Bioconcentration and Biomagnification Factors for Poorly Water-Soluble Chemicals Using Common Carp (*Cyprinus carpio* L.). Archives of Environmental Contamination and Toxicology, 63, 241–248.

JANDACEK, R.J. AND TSO, P., 2001. Factors affecting the storage and excretion of toxic lipophilic xenobiotics. *Lipids*, 36 (12), 1289-1305.

KLIMISCH, H.J., ANDREAE, M., TILLMANN, U., 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology,, 1-5.

KWONG, R.W., YU, P.K., LAM, P.K. AND WANG, W.X., 2008. Uptake, elimination, and biotransformation of aqueous and dietary DDT in marine fish. Environmental Toxicology and Chemistry, 27, 2053-2063.

MACKAY, D., ARNOT, J.A., GOBAS, F.A.P.C., POWELL D.E., 2013. Mathematical relationships between metrics of chemical bioaccumulation in fish. Environmental Toxicology and Chemistry, 32, 1459-1466.

MARTIN J.W., MABURY S.A., SOLOMON K.R. AND MUIR D.C.G., 2003a. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology Chemistry*, 22,189-95.

MARTIN J.W., MABURY S.A., SOLOMON K.R. AND MUIR D.C.G., 2003b. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology Chemistry* 22:196-204.

MEYLAN, W.M., HOWARD, P.H., BOETHLING, R.S., ARONSON, D., PRINTUP, H., GOUCHIE, S., 1999. Improved method for estimating bioconcentration/bioaccumulation factor from octanol/water partition coefficient. Environmental Toxicology and Chemistry, 18, 664-672.

MIRANDA, D. de A., PEASLEE, G.F., ZACHRITZ A.M. AND LAMBERTI G.A., 2021. A worldwide evaluation of trophic magnification of per- and polyfluoroalkyl substances in aquatic ecosystems. Integrated Environmental Assessment and Management 2022;00:1-13.

O'NEIL, J.A., JOHNSON, T.B., DROUILLARD, K.G., 2013. Validation of rapid assimilation of PCBs following IP dosing in the Round Goby (*Neogobius melanostomus*). Bulletin of Environmental Contamination and Toxicology, 91, 135-140.

ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT (OECD), 2008. Test No. 315: Bioaccumulation in Sediment-Dwelling Benthic Oligochaetes. OECD Guidelines for the Testing of Chemicals, Section 3. Paris: OECD Publishing.

ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT (OECD), 2012. Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure. OECD Guidelines for the Testing of Chemicals, Section 3. Paris: OECD Publishing.

ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT (OECD), 2013. Validation Report of a Ring Test for the OECD 305 Dietary Exposure Bioaccumulation Fish Test. Additional Report Including Results Using a Lower Feeding Rate. Series on Testing and Assessment No. 191, ENV/JM/MONO(2013)15. Paris: OECD Publishing.

ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT (OECD), 2017. Guidance Document on Aspects of OECD TG 305 on Fish Bioaccumulation. Series on Testing & Assessment No. 264, ENV/JM/MONO(2017)16, Paris: OECD Publishing.

OSTERTAG, S.K., STERN, G.A., WANG, F., LEMES, M. AND CHAN, H.M., 2013. Mercury distribution and speciation in different brain regions of beluga whales (*Delphinapterus leucas*). Science of the Total Environment, 456, 278-286.

REGULATION (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

REGULATION (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products (BPR). Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:167:0001:0123:EN:PDF [Accessed February 2022]

NG, C.A. AND HUNGERBÜHLER, K., 2014. Bioaccumulation of perfluorinated alkyl acids: observations and models. Environmental Science & Technology, 48, 4637-4648.

PARHIZGARI, Z. AND LI, J., 2014. A physiologically-based pharmacokinetic model for disposition of 2, 3, 7, 8-TCDD in fathead minnow and medaka. Environmental Toxicology and Chemistry, 33, 1064-1071.

QIAO, P., GOBAS, F.A.P.C. AND FARRELL, A.P., 2000. Relative contributions of aqueous and dietary uptake of hydrophobic chemicals to the body burden in juvenile rainbow trout. Archives of Environmental Contamination and Toxicology, 39, 369-377.

RUBARTH, J., DREYER, A., GUSE, N., EINAX, J.W., EBINGHAUS, R., 2011. Perfluorinated compounds in red-throated divers from the German Baltic Sea: new findings from their distribution in 10 different tissues. Environmental Chemistry, 8, 419-428.

RUSSELL, W.M.S. AND BURCH, R.L., 1959. The Principles of Humane Experimental Technique. London: Methuen & Co. Limited.

SCHLECHTRIEM, C., KAMPE, S., BRUCKERT, H.-J., BISCHOF I., EBERSBACH, I., KOSFELD, V., KOTTHOFF, M., SCHÄFERS C., 2019. Bioconcentration studies with the freshwater amphipod *Hyalella azteca*: are the results predictive of bioconcentration in fish? Environmental Science and Pollution Research, 26, 1628-1641.

SERRA, A., LETUNIC, I., FORTINO, V., HANDY R.D., FADEEL, B., TAGLIAFERRI, R. GRECO, D., 2019. INSIdE NANO: a systems biology framework to contextualize the mechanism-of-action of engineered nanomaterials. Scientific Reports, 9, 179.

SHORE, R.F. AND WALKER, L.A., 2020. Issues to consider for the use of a dashboard for the H4 chemicals indicator. Report to Defra. Lancaster, UK: UKCEH.

SMITH, C.L., 1950. Seasonal changes in blood sugar, fat body, liver glycogen, and gonads in the common frog, *Rana temporaria*. Journal of Experimental Biology, 26, 412-429.

SØRMO, E.G., SALMER, M.P., JENSSEN, B.M., HOP, H., BÆK, K., KOVACS, K.M., LYDERSEN, C., FALK-PETERSEN, S., GABRIELSEN, G.W., LIE, E., SKAARE, J.U., 2006. Biomagnification of polybrominated diphenyl ether and hexabromocyclododecane flame retardants in the polar bear food chain in Svalbard, Norway. Environmental Toxicology and Chemistry: An International Journal, 25, 2502-2511.

STAPLETON, H.M., BRAZIL, B., HOLBROOK, R.D., MITCHELMORE, C.L., BENEDICT, R., KONSTANTINOV, A., POTTER, D., 2006. In vivo and in vitro debromination of decabromodiphenyl ether (BDE 209) by juvenile rainbow trout and common carp. Environmental Science and Technology, 1, 40, 4653-4658.

TIMBRELL, J., 2002. Introduction to Toxicology. 3rd Edition, Boca Raton, USA: CRC Press.

TURTON, J. AND HOOSON, J., 2008. Target Organ Pathology. London: Taylor and Frances Ltd.

UK GOVERNMENT, 2018. A Green Future: Our 25 Year Plan to Improve the Environment. London: HM Government. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/693158/25-year-environment-plan.pdf [Accessed February 2022]

UNITED STATES ENVIRONMENT PROTECTION AGENCY (EPA), 2009. KABAM Version 1.0 User's Guide and Technical Documentation. Available from: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/kabam-version-10-users-guide-and-technical#Section1_1 [Accessed February 2022]

UK amendments for the Human Medicines Regulations, 2012: https://www.legislation.gov.uk/uksi/2012/1916/contents/made.

UK regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (UK REACH). REACH etc. (Amendment) Regulations 2021, UK Statutory Instrument 2021 No. 904

VAN BIRGELEN, A.P.J.M., AND VAN DEN BERG, M., 2000. Toxicokinetics, Food Additives & Contaminants, 17, 267-273.

VALSECCHI, S., BABUT, M., MAZZONI, M., PASCARIELLO, S., FERRARIO, C.D.E., FELICE, B., BETTINETTI, R., VEYRAND, B., MARCHAND, P., POLESELLO, S., 2021. Per- and polyfluoroalkyl substances (PFAS) in fish from European lakes: Current contamination status, sources, and perspectives for monitoring. Environmental Toxicology and Chemistry, 40, 658-676.

VAN DER ZANDE, M., KOKALJ, A. J., SPURGEON, D., LOUREIRO, S., SILVA, P., KHODAPARAST, Z., DROBNE, D., CLARK, N. J., VAN DEN BRINK, N., BACCARO, M., VAN GESTEL, K., BOUWMEESTER, H., HANDY, R.D., 2020. The gut barrier and the fate of engineered nanomaterials: A view from comparative physiology. Environmental Science: Nano, 7, 1874-1898.

VEITH, G.D., DEFOE, D.L., BERGSTEDT, B.V., 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. Journal of the Fisheries Board of Canada, 36, 1040-1048.

VERREAULT, J., LETCHER, R.J., MUIR, D.C., CHU, S., GEBBINK, W.A., GABRIELSEN, G.W., 2005. New organochlorine contaminants and metabolites in plasma and eggs of glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic. Environmental Toxicology and Chemistry, 24, 2486-2499.

VERREAULT, J., SHAHMIRI, S., GABRIELSEN, G.W., LETCHER, R.J., 2007. Organohalogen and metabolically-derived contaminants and associations with whole body constituents in Norwegian Arctic glaucous gulls. Environment International, 33, 823-830.

VIDAL, A., LAFAY, F., DANIELE, G., VULLIET, E., ROCHARD, E., GARRIC, J. AND BABUT, M., 2019. Does water temperature influence the distribution and elimination of perfluorinated substances in rainbow trout (*Oncorhynchus mykiss*)?. Environmental Science and Pollution Research, 26, 16355-16365.

WEN, Q., LIU, H.L., ZHU, Y.T., ZHENG, X.M., SU, G.Y., ZHANG, X.W., YU, H.X., GIESY, J.P., LAM, M.H., 2015. Maternal transfer, distribution, and metabolism of BDE-47 and its related hydroxylated, methoxylated analogs in zebrafish (*Danio rerio*). Chemosphere, 120, 31-36.

WHITE, S.L., DEMARIO, D.A., IWANOWICZ, L.R., BLAZER, V.S., WAGNER, T., 2020. Tissue distribution and immunomodulation in channel catfish (*Ictalurus punctatus*) following dietary exposure to polychlorinated biphenyl aroclors and food deprivation. International Journal of Environmental Research and Public Health, 14, 17, 1228.

YANG, J., HARINO, H., MIYAZAKI N., 2007. Transplacental transfer of phenyltins from a pregnant Dall's porpoise (*Phocoenoides dalli*) to her fetus. Chemosphere, 67, 244-249.

YANKOVICH, T.L., BERESFORD, N.A., WOOD, M., AONO, T., ANDERSSON, P., BARNETT, C.L., BENNETT, P., BROWN, J., FESENKO, S., HOSSEINI, A., HOWARD, B.J., JOHANSEN, M., PHANEUF, M., TAGAMI, K., TAKATA, H., TWINING, J., UCHIDA, S., 2010. Whole-body to tissue concentration ratios for use in biota dose assessments for animals. Radiation and Environmental Biophysics, 49, 549-565.

YOKEL, R.A., ALLEN, D.D., ACKLEY, D.C., 1999. The distribution of aluminium into and out of the brain. Journal of Inorganic Biochemistry, 76, 127-132.

YORDY, J.E., PABST, D.A., MCLELLAN, W.A., WELLS, R.S., ROWLES, T.K., KUCKLICK, J.R., 2010. Tissue-specific distribution and whole-body burden estimates of persistent organic pollutants in the bottlenose dolphin (*Tursiops truncatus*). Environmental Toxicology and Chemistry, 29, 1263-1273.

Appendix 1. Details of literature review

The literature review followed methods outlined in the guidance on Quick Scoping Reviews as presented by Collins *et al*. (2015) and ECHA Chapter R.4 (2011, v1.1).

The targeted literature review addressed the question 'how to estimate/extrapolate total body burden (TBB) from tissue/organ specific measurements'. Specific Boolean search terms including a list of chemicals were chosen to ensure the relevance of the search. Exclusion terms were included to avoid identifying literature which was not appropriate for this work (e.g. on metals or radionuclides). Searches were conducted using *Web of Science* (https://www.webofscience.com/) and repeated using *Pubmed* (https://pubmed.ncbi.nlm.nih.gov/) these were executed using the same query format for all organism groups (amphibians, birds, fish, mammals and reptiles). The searches were conducted in November 2021 with no date constraint on the search; *Web of Science* contains information on literature dating back to 1950 and *Pubmed* to 1966.

The queries used to search both these databases for publications related to fish, as an example, were:

Web of Science query

(((((("body burden" OR "whole body concentration") AND ("Organ concentration" OR "tissue residue" OR tissue* OR liver OR muscle* OR blood OR lipid OR protein* OR kidney* OR fat OR blubber OR organ*)) AND ("Organ concentration" OR "tissue residue" OR tissue* OR liver OR muscle* OR blood OR lipid OR protein* OR kidney* OR fat OR blubber OR organ*)) AND (herbicide* OR pesticide* OR halogen* OR PFAS OR POPS OR "flame retardant*" OR polymer* OR monomer* OR fluorinated OR PBT OR PvB OR "Chlorinated paraffin*" OR surfactant* OR fungicide* OR "fire suppressant*" OR hydrocarbon* OR fragrance* OR phenol* OR detergent* OR ketone* OR alcohol* OR aldehyde* OR "organic acid*")) AND (**fish* OR teleost OR "blue gill" OR medaka OR trout OR "zebra fish"**)) NOT (metal* OR mercury OR silver OR copper OR tin OR zinc or "Pb" OR cadmium OR uranium OR arsenic OR strontium OR caesium OR cesium OR actinide* OR irradiation)

Pubmed query

"body burden" OR "whole body concentration" (Topic) and "Organ concentration" OR "tissue residue" OR tissue* OR liver OR muscle* OR blood OR lipid OR protein* OR kidney* OR fat OR blubber OR organ* (Topic) and herbicide* OR pesticide* OR halogen* OR PFAS OR POPS OR "flame retardant*" OR polymer* OR monomer* OR fluorinated OR PBT OR PvB OR "Chlorinated paraffin*" OR surfactant* OR fungicide* OR "fire suppressant*" OR hydrocarbon* OR fragrance* OR phenol* OR detergent* OR ketone* OR alcohol* OR aldehyde* OR "organic acid*" (Topic) AND **fish* OR teleost OR "blue gill" OR medaka OR trout OR "zebra fish"** (Topic) NOT metal* OR mercury OR silver OR copper OR tin OR zinc OR "Pb" OR cadmium OR uranium OR arsenic OR strontium OR caesium OR cesium OR actinide* OR irradiation (All Fields)

To conduct searches for other organism groups (the text highlighted in bold above) was replaced with:

- amphibian* OR frog* OR tadpole* OR toad*
- bird* OR quail OR bobwhite* OR partridge* OR pigeon* OR fowl OR poultry OR avian OR raptor*
- mammal* OR mouse OR mice OR rabbit* OR rat* OR "sprague-dawley" OR wistar OR cetacean* OR mustelid* OR mink*;
- reptile* OR crocodile* OR snake* OR turtle* OR lizard*

Note * is a wildcard in the above searches generally allowing plurals to be identified (e.g. fish* will search for fish and fishes etc.).

Search results for each organism type were collated into separate Excel® worksheets and any duplicate references were removed. A limited number of additional references were identified whilst reviewing the initially identified manuscripts and these have been included in the overall review.

The data then underwent a rapid relevance assessment based on the title and the abstract. Any manuscripts that were not relevant were marked red and removed from further assessment. Potentially relevant manuscripts were highlighted in green for further review.

The potentially relevant papers then underwent critical evaluation including if they contained information/data useful for estimating total body burden from tissue specific measurements. Critical evaluation was conducted using a predetermined set of scientific quality criteria to ensure a thorough and consistent review process among reviewers and remove bias when establishing robustness and reliability. Where the manuscripts contained the required information, they were scored using an adaptation of the Klimisch rating system (Klimisch *et al*. 1997). All stages of the evaluation are recorded in the Excel® worksheets. Table 3 presents an overview of the literature searches and critical review.

The quality criteria used were:

1. Well-defined primary test material, representative substance or transformation product including purity/content for active ingredient or formulation details for product.

2. For laboratory studies, dosing strategy acceptable (e.g. exposure period, number of doses sufficient, nominal exposure concentration)

3. Exposure concentration is measured in the vehicle or medium unless otherwise justified (e.g. based on physical chemical properties)

4. Sufficient information on test organisms (e.g. age category)

5. Results are directly related to a useful and quantifiable endpoint (e.g. whole organism and tissue concentration) of the test material in question

6. The methodology used is clearly and transparently presented as well as robust

7. For laboratory studies test organisms have not been previously exposed to the test material or other contaminants or their history is well-known.

8. Where individual data are not available evidence that any statistical method used to derive the endpoint is acceptable and appropriate.

9. The methods used for measurements and analytical techniques are robust, reliable and have been validated and verified.

The adapted Klimisch rating used was:

Klimisch 1 Reliable without restriction: This includes studies or data from the literature or reports which meet the quality criteria defined above.

Klimisch 2 Reliable with restriction: This includes studies or data from the literature, reports, which do not totally comply with the quality criteria (e.g. low sample numbers), but which are nevertheless well documented and scientifically acceptable.

Klimisch 3 Not reliable: This includes studies or data from the literature/reports which do not meet most of the quality criteria.

Klimisch 4 Not assignable: This includes studies or data from the literature, which do not give sufficient experimental details (e.g. a paper citing data from another source) or which are only listed in short abstracts or secondary literature (books, reviews, etc.).

Appendix 2. Data used in meta-analysis

Table 1. Fresh mass concentration (FM) of various compounds in total whole body and tissues.

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Fish										
Oncorhynchus mykiss (Rainbow trout)	Decabromodiphenyl ether (BDE 209) CAS number: 1163-19-5 EC number: 214-604-9	5.3	375		32			ng/g FM	1	Stapleton *et al*. 2006
Danio rerio Zebrafish	2,2',4,4'-tetrabromodiphenyl ether (BDE 47) CAS: 5436-43-1 EC number: 690-137-8	6400	7500					ng/g FM	1	Wen *et al*. 2015

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Danio rerio Zebrafish	6-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (6-OH-BDE-47) CAS Number: 79755-43-4 EC number: None	49	870					ng/g FM	1	Wen *et al*. 2015
Perca fluviatilis (European perch)	ΣPer- and polyfluoroalkyl substances (PFASs) CAS number: N/A EC number: N/A	334	77	64	25	7		µg absolute (content not concentration)	1	Ahrens *et al*. 2015

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Cyprinus carpio (Carp)	Ibuprofen CAS number: 15687-27-1 EC number: 239-784-6	4		3.5		4		µg/g FM	1	Chen *et al*. 2014
Ictalurus punctatus (Channel Catfish)	ΣPolychlorinated Biphenyls CAS number: N/A EC number: N/A	4	0.3	2.25				µg/g	2	White *et al*. 2020
Lota lota (Burbot)	ΣPer- and polyfluoroalkyl substances (PFAS)	31.0	36.9	12.4				ng/g FM	1	Valsecchi *et al*. 2021

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
	CAS number: N/A EC number: N/A									
Lota lota (Burbot)	Perfluorooctane sulfonate (PFOS) CAS number: 1763-23-1 EC number: 217-179-8	24.3	28.5	9.8				ng/g FM	1	Valsecchi *et al*. 2021
Rutilus rutilus (Roach)	ΣPer- and polyfluoroalkyl substances (PFAS) CAS number: N/A EC number: N/A	31.5	27.8	11.7				ng/g FM	1	Valsecchi *et al*. 2021

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Rutilus rutilus (Roach)	Perfluorooctane sulfonate (PFOS) CAS number: 1763-23-1 EC number: 217-179-8	22.6	20.2	9.1				ng/g FM	1	Valsecchi *et al*. 2021
Alosa agone Mediterranean Shad; freshwater	ΣPer- and polyfluoroalkyl substances (PFAS) CAS number: N/A EC number: N/A	6.4		1.8				ng/g FM	1	Valsecchi *et al*. 2021
Alosa agone Mediterranean	Perfluorooctane sulfonate (PFOS)	2.7		1.1				ng/g FM	1	Valsecchi *et al*. 2021

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Shad; freshwater	CAS number: 1763-23-1 EC number: 217-179-8									
Salmo trutta Brown trout	Σper- and polyfluoroalkyl substances (PFAS) CAS number: N/A EC number: N/A	5.2		1.9				ng/g FM	1	Valsecchi *et al*. 2021
Salmo trutta Brown trout	Perfluorooctane sulfonate (PFOS) CAS number: 1763-23-1	2.7		0.9				ng/g FM	1	Valsecchi *et al*. 2021

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
	EC number: 217-179-8									
Amphibians										
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFCAs CAS number: N/A EC number: N/A	5.54	24.63	4.16				ng/g FM	2	Cui *et al*. 2018
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFCAs CAS number: N/A EC number: N/A	14.24	160	2.18				ng/g FM	2	Cui *et al*. 2018
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFSAs CAS number: N/A	1.35	8.32	0.760				ng/g FM	2	Cui *et al*. 2018

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
	EC number: N/A									
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFSAs CAS number: N/A EC number: N/A	3.64	7.71	0.670				ng/g FM	2	Cui *et al*. 2018
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFESAs CAS number: N/A EC number: N/A	2.45	18.96	1.16				ng/g FM	2	Cui *et al*. 2018
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFESAs CAS number: N/A EC number: N/A	3.86	8.86	0.350				ng/g FM	2	Cui *et al*. 2018

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFASs CAS number: N/A EC number: N/A	9.34	51.91	6.08				ng/g FM	2	Cui *et al*. 2018
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFASs CAS number: N/A EC number: N/A	17.92	32.57	3.2				ng/g FM	2	Cui *et al*. 2018
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFCAs CAS number: N/A EC number: N/A	2.36	10.7	1.92				ng/g FM	2	Cui *et al*. 2018
Pelophylax nigromaculatus	ΣPFSAs	0.692	2.57	0.250				ng/g FM	2	Cui *et al*. 2018

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
(Black-spotted frog)	CAS number: N/A EC number: N/A									
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFESAs CAS number: N/A EC number: N/A	0.722	4.57	0.450				ng/g FM	2	Cui *et al*. 2018
Pelophylax nigromaculatus (Black-spotted frog)	ΣPFASs CAS number: N/A EC number: N/A	5.89	31.2	4.38				ng/g FM	2	Cui *et al*. 2018
Birds										
Gavia stellata (red-throated divers)	ΣPFC	67	222	42	111			µg/kg FM	2	Rubarth *et al*. 2011

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
	CAS number: N/A EC number: N/A									
Larus hyperboreus (glaucous gull)	Σchlorodanes	701	1399		104			ng/g FM	1	Verreault *et al*. 2007
Larus hyperboreus (glaucous gull)	ΣPCB CAS number: N/A EC number: N/A	8319	20114		1853			ng/g FM	1	Verreault *et al*. 2007
Larus hyperboreus (glaucous gull)	ΣOH-PCB CAS number: N/A EC number: N/A	4.84	28.5		52.5			ng/g FM	1	Verreault *et al*. 2007

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Larus hyperboreus (glaucous gull)	ΣMeSO2-PCB CAS number: N/A EC number: N/A	6.97	24.7		2.02			ng/g FM	1	Verreault *et al*. 2007
Larus hyperboreus (glaucous gull)	3-MeSO2-p,p′-DDE	0.34	1.28		0.36			ng/g FM	1	Verreault *et al*. 2007
Larus hyperboreus (glaucous gull)	Total-(α)-HBCD CAS number: N/A EC number: N/A	91	75.6		3.29			ng/g FM	1	Verreault *et al*. 2007
Larus hyperboreus (glaucous gull)	BB101 CAS Number.: 67888-96-4	1.5	2.04		0.28			ng/g FM	1	Verreault *et al*. 2007

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
	EC number: None									
Larus hyperboreus (glaucous gull)	ΣPBDE CAS number: N/A EC number: N/A	202	522		51.5			ng/g FM	1	Verreault *et al*. 2007
Larus hyperboreus (glaucous gull)	ΣMeO-PBDE CAS number: N/A EC number: N/A	19.4	32.2		2.78			ng/g FM	1	Verreault *et al*. 2007
Larus hyperboreus (glaucous gull)	ΣOH-PBDE CAS number: N/A EC number: N/A	0.27	3.57		3.54			ng/g FM	1	Verreault *et al*. 2007

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Mammals										
Rat	TCDD CAS Number.: 1746-01-6 EC number: 217-122-7	0.06	0.54					ng/g FM	3	van Birgelen & van den Berg 2000
Rat	TCDD CAS Number.: 1746-01-6 EC number: 217-122-7	0.294	5.1					ng/g FM	3	van Birgelen & van den Berg 2000
Rat	TCDD CAS Number.: 1746-01-6	1.436	24					ng/g FM	3	van Birgelen & van den Berg 2000

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
	EC number: 217-122-7									
Tursiops truncatus (Bottlenose dolphin)	ΣPCBs CAS number: N/A EC number: N/A	0.167	0.051	0.008			0.763	µg/g FM	2	Yordy *et al*. 2010
Tursiops truncatus (Bottlenose dolphin)	ΣPBDEs CAS number: N/A EC number: N/A	0.010	0.003	0.001			0.048	µg/g FM	2	Yordy *et al*. 2010
Tursiops truncatus (Bottlenose dolphin)	ΣDDTs CAS number: N/A EC number: N/A	0.040	0.011	0.002			0.183	µg/g FM	2	Yordy *et al*. 2010

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Tursiops truncatus (Bottlenose dolphin)	ΣPCBs CAS number: N/A EC number: N/A	12.519	0.191	0.613			28.9	µg/g FM	2	Yordy *et al*. 2010
Tursiops truncatus (Bottlenose dolphin)	ΣPBDEs CAS number: N/A EC number: N/A	0.746	0.01	0.033			1.73	µg/g FM	2	Yordy *et al*. 2010
Tursiops truncatus (Bottlenose dolphin)	ΣDDTs CAS number: N/A EC number: N/A	3.091	0.032	0.112			7.19	µg/g FM	2	Yordy *et al*. 2010
Tursiops truncatus	ΣPCBs	9.687	0.117	0.335			30.1	µg/g FM	2	Yordy *et al*. 2010

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
(Bottlenose dolphin)	CAS number: N/A EC number: N/A									
Tursiops truncatus (Bottlenose dolphin)	ΣPBDEs CAS number: N/A EC number: N/A	0.520	0.004	0.021			1.61	µg/g FM	2	Yordy *et al*. 2010
Tursiops truncatus (Bottlenose dolphin)	ΣDDTs CAS number: N/A EC number: N/A	3.797	0.027	0.107			11.9	µg/g FM	2	Yordy *et al*. 2010
Tursiops truncatus (Bottlenose dolphin)	ΣPCBs CAS number: N/A	3.350	0.124	0.269			13.9	µg/g FM	2	Yordy *et al*. 2010

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
	EC number: N/A									
Tursiops truncatus (Bottlenose dolphin)	ΣPBDEs CAS number: N/A EC number: N/A	0.068	0.001	0.004			0.289	µg/g FM	2	Yordy *et al*. 2010
Tursiops truncatus (Bottlenose dolphin)	ΣDDTs CAS number: N/A EC number: N/A	2.484	0.072	0.187			10.3	µg/g FM	2	Yordy *et al*. 2010
Sprague-Dawley rats	PBDE (BDE209) CAS number: 1163-19-5 EC number: 214-604-9	15.34	48.2		3.6			ng/g FM	2	Huwe & Smith 2007

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Sprague-Dawley rats	PBDE (BDE206) CAS number: 63387-28-0 EC number: 264-56-7	0.42	0.9		0.04			ng/g FM	2	Huwe & Smith 2007
Sprague-Dawley rats	PBDE (BDE207) CAS number: 437701-79-6 EC number: None	3.07	7.6		0.5			ng/g FM	2	Huwe & Smith 2007
Sprague-Dawley rats	PBDE (BDE208) CAS number: 437701-78-5 EC number: None	0.33	1		0.05			ng/g FM	2	Huwe & Smith 2007

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Sprague-Dawley rats	PBDE (BDE196) CAS number: 446255-39-6 EC number: Not found	0.10	0.2		0.004			ng/g FM	2	Huwe & Smith 2007
Sprague-Dawley rats	PBDE (BDE203) CAS number: 337513-72-1 EC number: None	0.020	0.03		0.002			ng/g FM	2	Huwe & Smith 2007
Sprague-Dawley rats	PBDE (BDE197) CAS number: 117964-21-3 EC number:	0.42	0.8		0.02			ng/g FM	2	Huwe & Smith 2007

Species	Compound	Total body	Liver	Muscle	Blood	Gill	Blubber	Units	Review Klimisch score	Reference
Sprague-Dawley rats	PBDE (BDE201) CAS number: 446255-50-1 EC number: None	0.083	0.2		0.004			ng/g FM	2	Huwe & Smith 2007
Sprague-Dawley rats	PBDE (BDE183) CAS number: 207122-16-5 EC number: None	0.030	0.02		0.002			ng/g FM	2	Huwe & Smith 2007
Phocoenoides dalli (Dall's porpoise)	ΣPhenyltins CAS number: N/A EC number: N/A	1.6	7.26	2.15	0.3		1.36	ng Sn g^{-1}	2	Yang *et al*. 2007

Would you like to find out more about us or your environment?

Then call us on

03708 506 506 (Monday to Friday, 8am to 6pm)

Email: enquiries@environment-agency.gov.uk

Or visit our website

www.gov.uk/environment-agency

incident hotline

0800 807060 **(24 hours)**

floodline

0345 988 1188 **(24 hours)**

Find out about call charges ()

Environment first

Are you viewing this onscreen? Please consider the environment and only print if absolutely necessary. If you are reading a paper copy, please don’t forget to reuse and recycle.