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Analysis of elimination half-lives in MamTKDB 1.0 related to bioaccumulation: Requirement of repeated administration and blood plasma values underrepresent tissues

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ABSTRACT

When building the novel public mammalian toxicokinetic database (MamTKDB) we collected and included 3927 elimination half-lives ($^{elim}t_{1/2}$) for 1407 xenobiotics in various species (rat, human, mouse, dog, monkey, rabbit, cattle, pig, sheep, guinea pig, hamster, horse and goat) with specification of compartment (whole body, organ/tissue, cell type, medium) studied. Here we describe and analyse the collected data in MamTKDB 1.0. Most $^{elim}t_{1/2}$ are for humans and rats and their data differ in some ways: whereas the rat data are mainly for pesticides, the human data are mainly for pharmaceuticals and environmental contaminants. There are also differences in types of compartments studied and in metabolites followed: human $^{elim}t_{1/2}$ are mainly whole body based (i.e. based on blood plasma or excretion), animal data are additionally for various organs/tissues, cells or media. Contrary to human studies, animal studies regularly administrate radiolabeled (e.g. ^{14}C) substances and distribution of both parent and eventual metabolites are followed, measuring the radioactivity. In rats, substances had been given through single, preconditioning or repeated administration. Single administration studies dominated, but repeated studies generally had longer $^{elim}t_{1/2}$ than single or preconditioning studies for which $^{elim}t_{1/2}$ were similar. Repeated administration studies should better ascertain steady state conditions throughout the body, a process involving time-dependent tissue loading, and the data show that for most substances, repeated studies are required to address bioaccumulation potential. About 65% of the substances in MamTKDB 1.0 fulfilled the octanol–water and octanol–air partitioning-based screening criteria (log $K_{ow} > 2$ and log $K_{oa} > 5$) for further bioaccumulation assessment and/or testing, and most of the substances with long $^{elim}t_{1/2}$ in both humans and rats fulfill these criteria. Of note, however, there are also many chemicals with log $K_{ow} > 2$ with intermediate or short $^{elim}t_{1/2}$. Per- and polyfluoroalkyl substances (PFAS) stand out in that they often have log $K_{oa} < 5$. Rats are poor toxicokinetic test models for perfluoroalkyl acids (PFAAs) for which pigs (and possibly mice) $^{elim}t_{1/2}$ data resemble those of humans better. Perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs) of similar molecular weight had similar $^{elim}t_{1/2}$ in the species tested. For polychlorinated biphenyls (PCBs), $^{elim}t_{1/2}$ increases with the degree of chlorination in humans. In relation to other compartments, blood plasma/serum had among the shortest $^{elim}t_{1/2}$ in rats and often underrepresent $^{elim}t_{1/2}$ in tissues. Rat data were divided into 38 compartment (tissue or media) types out of which 20 had sufficient data for correlational tests. In general, there was a strong degree of correlation of rat $^{elim}t_{1/2}$ in-between most compartments, but there were also exceptions. Surprisingly, the correlation between brain and white fat was relatively weak. Interestingly, several substances or their metabolites bound to haemoglobin in red blood cells. MamTKDB 1.0 allows

Abbreviations: AUC, area under curve; BCF, bioconcentration factor; 'B', substances identified as bioaccumulating having a PBT, vPvB (REACH, Biocides) or POP label (Stockholm Convention); CL, clearance; DAR, Draft Assessment Report; $^{elim}t_{1/2}$, elimination half-life; i.v., intravenous; log $D_{7.4}$, logarithm of the octanol-water partition coefficient at pH 7.4; log K_a(HAS), logarithm of the human serum albumin affinity constant; log K_{ow}, logarithm of the octanol-water partition coefficient; log K_{oa}, logarithm of the octanol-air partition coefficient; MamTKDB, mammalian toxicokinetic database; MW, molecular weight; 'not B', substances currently not identified as PBT, vPvB or POP; PCB, polychlorinated biphenyl; PBT, persistent, bioaccumulative, and toxic; PFAA, perfluoroalkyl acid; PFAS, per- and polyfluoroalkyl substances; PFCA, perfluorinated carboxylic acid; PFSA, perfluorinated sulfonic acid; pK_a, negative log of the acid dissociation constant; PCDD, polychlorinated dibenzo-p-dioxin; PCDF, polychlorinated dibenzofuran; POP, persistent organic pollutant; PPP, plant protection product; QSAR, quantitative structure-activity relationship; SMILES, simplified molecular-input line-entry system; V_d, volume of distribution; vPvB, very persistent and very bioaccumulative.

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investigation on how certain chemical characteristics influence $^{elim}t_{1/2}$ and is a promising database for assessment of bioaccumulation potential.

1. Introduction

Guaranteeing that a foreign chemical (xenobiotic) does not magnify within a food chain or accumulate in any of the many organs/tissues, cell types and structures in our bodies, is an important task since bioconcentration and biomagnification (both covered under the concept of bioaccumulation) increase the risk of toxicity. Most xenobiotics enter humans through dietary intake, although some (e.g. those in air pollution) are inhaled or dermally absorbed (e.g. cosmetics). Hazard and human risk assessment of chemicals is mainly based on rodent data since testing potentially bioaccumulating and/or toxic substances in higher species than the rat (which is the preferred species in regulatory guidelines) can be ethically questionable.

Bioaccumulation is assessed under the EU Regulation Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) in the context of the identification of substances as Persistent, Bioaccumulative and Toxic (PBT), or very Persistent and very Bioaccumulative (vPvB). Current regulatory criteria for assessment of bioaccumulation are mainly based on data from aquatic species, particularly on laboratory derived bioconcentration factors (BCF) in fish. Since lipophilic chemicals tend to bioaccumulate more than hydrophilic ones, substances are first screened based on their octanol-water partition coefficients (K_{ow}). Fish and other gill-breathers are rather efficient in clearing themselves via ventilated water. In contrast, air-breathers cannot clear themselves effectively from chemicals via physicochemical based partitioning into exhaled air or through urine and faeces excretion/egestion because the respective sorption capacities of these media are small, and their excreted volumes are insufficient for clearance of hydrophobic chemicals (Goss et al., 2018). Hence, it has been concluded that chemicals with a log $K_{ow} > 2$ and a log K_{oa} (octanol–air partitioning) > 5 would typically exceed the biomagnification factor (BMF) > 1 threshold in mammals if no metabolism occurs (ECHA Guidance R.11) (ECHA, 2017). Due to species differences, chemicals with similar partitioning properties may have different bioaccumulation potential due to biotransformation (McLachlan et al., 2011) and excretion/egestion differences. Highly halogenated chemicals such as polychlorinated biphenyls (PCBs, lipophilic) and per- and polyfluoroalkyl substances (PFAS, often dual hydrophobic and hydrophilic surfactant characteristics) have the capacity to remain chemically stable against biodegradation or other processes such as oxidation or hydrolysis (persistence; see also (ECHA-term)), and bioaccumulate in humans.

Testing a substance's bioaccumulation potential in terrestrial air-breathers can be done as part of absorption, distribution, metabolism (biotransformation) and elimination (ADME) studies. The OECD Test Guideline (TG) 417 *Toxicokinetics* is currently the prime choice for ADME studies. The 2010 edition mainly for rats (OECD, 2010) replaced the 1984 edition which was for testing in an 'appropriate animal species' (OECD, 1984). Use of elimination half-life ($^{elim}t_{1/2}$) as a bioaccumulation metric has been suggested for both water- and air-breathing organisms (Goss et al., 2013; Gottardo et al., 2014). To meet a BMF < 1 criteria, a whole body depuration based $^{elim}t_{1/2}$ cut-off of 17 days for rats and 70 days for humans has been suggested (the threshold is directly related to the feeding rate) (Goss et al., 2013; Goss et al., 2018). For a comprehensive ADME profile also other parameters such as bioavailability (F; how much reaches the circulatory system and tissues) or % absorption (which in addition includes uptake into the gut wall and portal venous system from the gastrointestinal (GI) tract to the liver), half-life of absorption, volume of distribution (V_d) and total body (or blood plasma) clearance (CL) are often investigated. The absorption of a substance is often rapid in comparison to its elimination. After reaching a pseudo-equilibrium, elimination of a substance from blood plasma is largely dependent on V_d and CL; substances with a high V_d and/or low blood plasma CL can be expected to have a long $^{elim}t_{1/2}$ ($t_{1/2} = 0.693 x V_d/CL$) in blood plasma (Toutain and Bousquet-Melou, 2004). Among drugs, acids tend to have low V_d, neutrals medium, and bases ($pK_a > 7$) the highest V_d due to base (e.g. R_3N^+) ion-pair interactions with acidic membrane phospholipids ($R\text{-}PO_4^-$). Lack of biotransformation has been pointed out as one important factor for bioaccumulation (Arnot et al., 2014).

Use of radiolabeled chemicals have facilitated toxicokinetic studies, e.g. the half-life of water in the human body (when half the water taken in has left the body) was determined to be 9 ± 1 days in 1934 by use of isotopically labeled heavy water (D_2O) (Hevesy and Hofer, 1934). For TG 417 studies, oral gavage administration of a ^{14}C-labeled (in the core portion) xenobiotic in rats is standard, after which blood, plasma, tissues, blood cells and excrete (urine, faeces, air) are measured for radioactivity after homogenization commonly using liquid scintillation counting (LSC).

Due to a present lack of toxicokinetic databases with access to substance specific toxicokinetic parameters, we recently established the novel mammalian toxicokinetic database (MamTKDB 1.0; 1st version in Excel table format) (Hofer et al., 2021). In MamTKDB 1.0, the focus was on collecting late, slow phase, $^{elim}t_{1/2}$ from toxicokinetic studies in several animal species and humans. The goal with MamTKDB 1.0 was to provide easy public access to $^{elim}t_{1/2}$ of various substances and allow investigation on how certain chemical characteristics (functional groups, physiochemical properties such as lipophilicity, acidity, etc.) influence $^{elim}t_{1/2}$, which can be relevant for bioaccumulation assessment of chemicals.

From foods, humans are daily exposed to low concentrations of chemicals from plant protection products (pesticides) often used to prevent harm by insects, which therefore was one focus in MamTKDB 1.0. Some previously used toxic pesticides were found to biomagnify in the food chain, e.g. Dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenyldichloroethylene (DDE) built up in birds and other predatory animals (Carson, 1962). For future read-across purposes and to check if certain structural or physiochemical properties increase bioaccumulation, also other types of xenobiotics (e.g. biocides, environmental pollutants and veterinary medicines) were included in order to obtain a wide chemical diversity/space. Another aim was to check some known bioaccumulating PFAS and PCBs for $^{elim}t_{1/2}$ trends. We also wanted to investigate how the commonly used oral administration types (single, preconditioning or repeated) in rats affects $^{elim}t_{1/2}$. Preconditioning involves dosing with an un-labelled substance, often repeatedly, prior to dosing with a radiolabeled substance. We hypothesize that there would be a strong correlation between $^{elim}t_{1/2}$ in rats for fatty tissues such as white fat (also called visceral adipose tissue) and brain, possibly also to skin since it is the largest organ of the body (~2 m^2 and 7 kg in adults), containing a considerable amount of dermal adipose tissue (Chen et al., 2019). We also ask if substances having long $^{elim}t_{1/2}$ in certain tissues (e.g. brain) also have long $^{elim}t_{1/2}$ in more easily measured compartments such as blood (plasma) or in excrete (urine and faeces) that reflect the whole body.

2. Materials and methods

2.1. MamTKDB 1.0 – A freely accessible toxicokinetic database

Data analysed in this paper are from the novel toxicokinetic database MamTKDB 1.0 which is freely accessible to everyone through the EU Open Data Portal (Hofer et al., 2021). MamTKDB 1.0 is hosted by ECHA.

2.2. Creation of MamTKDB 1.0

Collection of toxicokinetic data, all from available public sources, took place from March 2016 until February 2020. Focus was on collecting the elimination half-life ($^{elim}t_{1/2}$); the time required for the concentration to fall by 50% during the terminal phase in the compartment studied (units are often reported in hours but were converted to days to be more comprehensible). The European Food Safety Authority (EFSA; www.efsa.europa.eu) assesses active substances (pesticides) used in plant protection products (PPPs) and publishes EU Draft Assessment Reports (DARs). Often the DARs contain summaries over toxicokinetic studies (in Annex B.6) provided by the registrant, and if so, these sometimes provide calculated $^{elim}t_{1/2}$ for blood (and/or its components, i.e. blood plasma, serum or cells), organs/tissues, or for whole body excretion into urine/faeces. Due to the high number (hundreds and growing) of available pesticide DARs, we limited MamTKDB 1.0 to just include screening DARs for PPPs listed on EFSA's homepage during years 2005–end of 2014 (no DARs had been listed before 2005). Likewise, we collected $^{elim}t_{1/2}$ from biocide dossier study summaries available via ECHA's website (www.echa.europa.eu). Furthermore, $^{elim}t_{1/2}$ data from the REACH Candidate List of substances identified as PBT, vPvB or persistent organic pollutants (POPs; e.g. environmental contaminants) under the Stockholm convention, as well as some PFAS (perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs) that are perfluoroalkyl acids (PFAAs)) from registration dossiers under the REACH Regulation, published at ECHA's website, were also collected. Many of the PBT/vPvB/POP substances are polyhalogenated, e.g. PCBs, polychlorinated dibenzofurans (PCDFs), polychlorinated dibenzo-p-dioxins (PCDDs) and PFAS. Veterinary medicine $^{elim}t_{1/2}$ data were included from European Public Maximum residue limit Assessment Reports (EPMARs) available early in year 2016 at European Medicines Agency's (EMA's) website (www.ema.europa.eu). The reports sometimes referred to studies in the open scientific literature that were then screened for $^{elim}t_{1/2}$.

No quality or reliability assessments of the collected $^{elim}t_{1/2}$ were made but the data appeared to be of high quality in general although with limited study details provided in some cases. No attempt was made to calculate $^{elim}t_{1/2}$ from presented toxicokinetic data, e.g. from tables. Full original study dossiers were not accessible. For a limited number of substances, elimination rates were converted to $^{elim}t_{1/2}$ based on well accepted formulas, e.g. $t_{1/2} = \ln(2)/k$ for first order kinetics, where k is the depuration (elimination) rate constant for a compartment during exponential decay. When several animal studies were available for the same substance, the study considered most representative was chosen (long or intermediate $^{elim}t_{1/2}$ were favoured over short). When this was difficult to determine, data from several studies were included for the same compound. Collection of $^{elim}t_{1/2}$ from repeated administration studies was of particular interest. When data were given as a range (lower $^{elim}t_{1/2}$ to upper $^{elim}t_{1/2}$ bound) this was noticed, and the arithmetic mean was used.

MamTKDB 1.0 contains information on substance identity (substance name, CAS and EC numbers), $^{elim}t_{1/2}$ for the last/slowest (often β) phase (when several phases, other $^{elim}t_{1/2}$, e.g. for the initial (α) phase, were often noticed under 'Other study info' but is not used in data analysis below) as well as other study related information. For animal studies, details on strain, sex, age and/or weight, route and type of administration, chemical construct details with label position, dose, vehicle, test duration, time of excretion, excretion information (e.g. % into urine and/or faeces), chosen compartment model, and references to the original study were recorded. It is also mentioned if total radioactivity was measured (predominant) or if parent substance or specific metabolites were measured. On occasion also supplementary toxicokinetic parameters such as V_d, CL, area-under-curve (AUC; concentration × time) values were collected although it seemed unclear how they could be pragmatically used, and the study summaries varied in reporting them. In addition, various (predicted) substance-specific parameters and descriptors have been included if available: molecular weight (MW), predicted logarithm of the octanol–water partition coefficient (log Kow, log P), predicted logarithm of the octanol–water partition coefficient taking ionization at pH 7.4 into account (log $D_{7.4}$), acid dissociation constant (strongest pK_a (acid), strongest pK_a (base)), estimated logarithm of the octanol-air partition coefficient (log K_{oa}), human serum albumin affinity constant (log K_a(HAS)), absorption parameters (maximum passive absorption, absorption rate (ka), estimated permeability (Pe (jejunum), Pe (Caco-2)), substance group information, regulatory status in relation to the bioaccumulation potential and simplified molecular-input line-entry system (SMILES). Log K_{ow} and log K_{oa} were predicted with EPI Suite KowWin v1.68 and KoaWin v1.10, respectively, from the US Environmental Protection Agency. Log P, log $D_{7.4}$, pK_a, log K_a(HAS), maximum passive absorption, Ka and Pe were calculated with Percepta from ACD/Labs (Toronto, Ontario, Canada), release 2019.2.1. Disclaimer: physio-chemical data predictions were done without applicability domain check of the model and no reliability verification. Log D accounts for how a chemical's eventual ionization affects lipophilicity at specific pHs. A positive log D means that a chemical prefers a lipophilic environment, a negative that it prefers aqueous environment, and a log D around 0 that it has no preference. Extremely lipophilic chemicals may be less well absorbed. For Fenbutatin oxide, a large bulky PPP (MW 1052.7 g/mol), absorption in rats was just 4% and reported $^{elim}t_{1/2}$ (24–40 h) based on faecal excretion were not included in MamTKDB since gastrointestinal passage without absorption may have taken place.

2.2.1. Human data

A previously assembled collection of human (adults) $^{elim}t_{1/2}$ for 1105 xenobiotics composed of 80% pharmaceuticals and 20% environmental contaminants (Arnot et al., 2014) was included in MamTKDB 1.0. That compilation, also available through OECD QSAR Toolbox (www.qsartoolbox.org), contains few study details (e.g. no or little information regarding dose, administration type, compartment or phase studied, with no references to original publications) and provided $^{elim}t_{1/2}$ are stated to be whole body total $^{elim}t_{1/2}$. In that collection, most of the pharmaceutical data had been taken from a compilation of single administration intravenous (i.v.) pharmacokinetic parameters of 670 drugs (Obach et al., 2008) for which $^{elim}t_{1/2}$ can be expected to mainly be for blood plasma or serum, a compilation that also lacks references to original publications. However, a follow-up paper by the same group (Obach) does include references (Lombardo et al., 2018) in a supplementary document. Some pharmacokinetic data had also been taken (Arnot et al., 2014) from the on-line database www.drugbank.ca which also contains few details and no references. Environmental contaminant $^{elim}t_{1/2}$ data (Arnot et al., 2014) had been taken from TOXNET (http://toxnet.nlm.nih.gov/) as well as a limited number of publications including: a literature review with $^{elim}t_{1/2}$ measured (e.g. in blood or adipose tissue) or modelled for some dioxins, furans and dioxin-like PCBs (Milbrath et al., 2009), $^{elim}t_{1/2}$ estimates for some PCBs based on analyses in blood and adipose tissue (Ritter et al., 2011), and $^{elim}t_{1/2}$ for some PFAS based on decreasing blood serum levels in retired workers (Olsen et al., 2007). A large portion of the human environmental contaminant data (Arnot et al., 2014) are presumably from repeated exposures (unless a single dose was administrated which is rarely performed for environmental contaminants). In the collection (Arnot et al., 2014) we included, the median $^{elim}t_{1/2}$ half-life was 7.6 h for pharmaceuticals and environmental contaminants altogether. Going through mainly animal based toxicokinetic data for PPPs, biocides, etc. with a focus also on the environmental contaminants PCBs and PFAS, we came across and collected 158 single replicate human $^{elim}t_{1/2}$ for 58 substances (some not among the 1105 xenobiotics mentioned above) that were included into MamTKDB 1.0 with added references to original publications and study details when available. These $^{elim}t_{1/2}$ were often based on when somebody has left a factory where occupational exposure has taken place, micro-dosing in volunteers, suicide attempts, accidental

exposures, or other incidental exposures. With our additions, there are now 1133 substances having 1261 $^{elim}t_{1/2}$ in humans.

2.2.2. Animal data

Animal ADME studies conducted for regulatory purposes (industry) often followed OECD TG 417 *Toxicokinetics* (OECD, 1984, 2010) and were often of good laboratory practice (GLP) quality. Even so, TG 417 offers flexibility to suit the chemical of interest. In brief, TG 417 (2010) suggests gavage administration of a radiolabeled substance (e.g. ^{14}C-labeled in the core portion of the molecule) of high purity (>95% radiopurity) at a minimum of two different single doses (often low and high, in both sexes if evidence for sex-related differences in toxicity), and occasionally also repeated (often low dose, one sex only unless gender specific toxicity) daily administration at a constant dose over 14 days. The preferred species is the rat. Repeated administration for 14 days may sometimes not be long enough for steady state conditions to establish and then the period can be extended, checking if the plasma concentration is still increasing can be done at intermediate time points (OECD, 2010). There may be an interest in ascertaining whether a steady state concentration has also been attained in target tissues. Pre-conditioning administration can be one way of checking eventual enzymatic induction or inhibition effects, typically involving daily dosing over 13 consecutive days with an un-labelled substance followed by a single administration with a radiolabelled substance at the same dose on day 14, but repeated dosing with radiolabelled substance is preferred to check bioaccumulation (OECD TG 417 (2010), §57) (OECD, 2010). A description of how animal toxicokinetic studies are commonly performed with derivation of $^{elim}t_{1/2}$ is available in Supplement A.

2.3. Statistical analyses

Statistical analyses were performed using JMP Pro 15 from SAS Institute Inc. (Cary, NC) and Prism 9 from GraphPad Software Inc. (San Diego, CA). Level of statistical significance was set to $p < .05$.

2.3.1. Overall data description

Data were checked for normality using the D'Agostino and Pearson normality test. Sample data are described by quantiles (25%, median, 75% percentile), their geometric and/or harmonic means with 95% confidence intervals (CI). Untransformed $^{elim}t_{1/2}$ data were often right-skewed and were commonly log_{10}-transformed to improve normal distribution before performing statistical tests. For data analysis, no differentiation was made regarding strain, sex, age, or route (oral gavage was dominant) of administration.

2.3.2. Calculation of mean $^{elim}t_{1/2}$ per substance, compartment and administration type

In order to avoid overrepresentation (bias) of data, arithmetic $^{elim}t_{1/2}$ means were calculated for each substance, compartment and administration type, thus giving one mean for single ($N = 1$), preconditioning ($N = 1$) and repeated ($N = 1$) administration each when data for several types of administration were available.

2.3.3. Influence of administration type on $^{elim}t_{1/2}$

For the substances having $^{elim}t_{1/2}$ from more than one administration type (single, preconditioning and repeated) in animals, the effect of administration type on $^{elim}t_{1/2}$ was analysed using paired *t*-tests. Because data were right-skewed, the data were first log_{10}-transformed to attain normal distribution.

2.3.4. Tests for correlations and associations between rat compartments

Rat data for the three administration types were analyzed together (single administration was dominant) using the calculated arithmetic mean $^{elim}t_{1/2}$ for each substance and compartment. Correlations between $^{elim}t_{1/2}$ data from two different compartments was performed using Spearman rank tests. Calculated Spearman correlation coefficients rho (ρ) are the same regardless if data is transformed or not as a rank test.

Linear regressions were performed to find associations. Since the data was not normally distributed and as the resulting slopes (β) were found to be strongly influenced by outliers, $^{elim}t_{1/2}$ data were first log_{10}-transformed. Slopes for log_{10}-transformed data are denoted β'.

3. Results

3.1. Overall description of MamTKDB 1.0

MamTKDB 1.0 contains 3927 single replicate $^{elim}t_{1/2}$ divided on rat (2412), human (1261), mouse (116), dog (40), monkey (33), rabbit (21), cattle (14), pig (13), sheep (5), guinea pig (5), hamster (4), horse (2), and goat (1). At least one $^{elim}t_{1/2}$ exists for each of 1407 different substances (MamTKDB 1.0 lists 1481 substances since also 74 pesticides for which no $^{elim}t_{1/2}$ was found are included). An overview is shown in Table A.1 and Fig. A.1 (both in Appendix A). Notably, the collected data have been generated from studies on different substances in different species. Due to little data for some species, mainly human and rat data are further analyzed that contain $^{elim}t_{1/2}$ for 1133 (humans) and 280 (rats) substances, respectively. Only 30 substances had $^{elim}t_{1/2}$ data for both humans and rats. These substances, however, were over-represented by PFAS (known to be much longer in humans than rats), other PBT or POP substances, and on average had 95x longer $^{elim}t_{1/2}$ in humans than rats when comparing data from similar compartments.

The human data display a wider distribution than the rat data, containing plenty of $^{elim}t_{1/2}$ that are both shorter and longer than for rats. Human and rat $^{elim}t_{1/2}$ data are shown as single replicates in Fig. 1 and Table 1. For MamTKDB 1.0, the calculated overall geometric mean $^{elim}t_{1/2}$ for humans is 1.18 vs. 1.20 days for rats, thus slightly shorter in humans. The overall harmonic mean $^{elim}t_{1/2}$ for humans is just 0.011 days, which may relate to the high content of drug $^{elim}t_{1/2}$ in humans having short $^{elim}t_{1/2}$, vs. 0.39 days for rats. Due to species differences (i.e. metabolic rate, metabolism and body size), $^{elim}t_{1/2}$ would have been expected to be longer in humans vs. rats (West and Brown, 2005) had the same substances, compartments and methods been used. A portion of the human data has $^{elim}t_{1/2}$ that are far longer than for rats due to inclusion of a higher content of known bioaccumulating environmental contaminants. In humans $^{elim}t_{1/2}$ for several POP and PBT/vPvB substances are above 10 years (3650 days; the longest for PCB-199/-201 being 84,300 days (231 years)) whereas the rat $^{elim}t_{1/2}$ are rarely over 100 days. Substances identified as bioaccumulating ('B') having a PBT, vPvB or POP label had significantly ($p < .0001$) longer $^{elim}t_{1/2}$ than 'not B' substances both in humans and rats when analyzed using unpaired *t*-tests (two-tailed), Fig. 1. Most human data presently lack specification of males (M) or females (F) and a sensible analysis is not possible. Check of gender differences in rats is described below (section 3.8). Although not in any way approved values, the suggested whole body based 'B' thresholds of 70 (humans) and 17 (rats) days (Goss et al., 2013; Goss et al., 2018) are indicated in Fig. 1 just to have something to refer to. Interestingly, as can be seen, there are numerous 'B' classified chemicals that have short $^{elim}t_{1/2}$ in humans. In rats, numerous 'not B' substances have longer $^{elim}t_{1/2}$ than the discussed threshold of 17 days.

For the rat data, pesticides are the main chemical type. For pesticides used in PPPs, more than 300 DAR dossiers were scrutinized and for 236 pesticides, at least one $^{elim}t_{1/2}$ had been reported. For 74 PPPs, the available DAR dossiers contained no $^{elim}t_{1/2}$. A notice was made in the database for pesticides for which the DARs lacked ADME studies or toxicokinetic information such as $^{elim}t_{1/2}$. The reason for lack of $^{elim}t_{1/2}$ can be several: e.g. that toxicokinetic studies had yet not been performed, that toxicokinetic studies exist but that no $^{elim}t_{1/2}$ had been calculated, or that the toxicokinetic study having an $^{elim}t_{1/2}$ is only available in the original DAR which was not accessible from EFSA's homepage. Out of roughly two dozen scrutinized biocide dossiers,

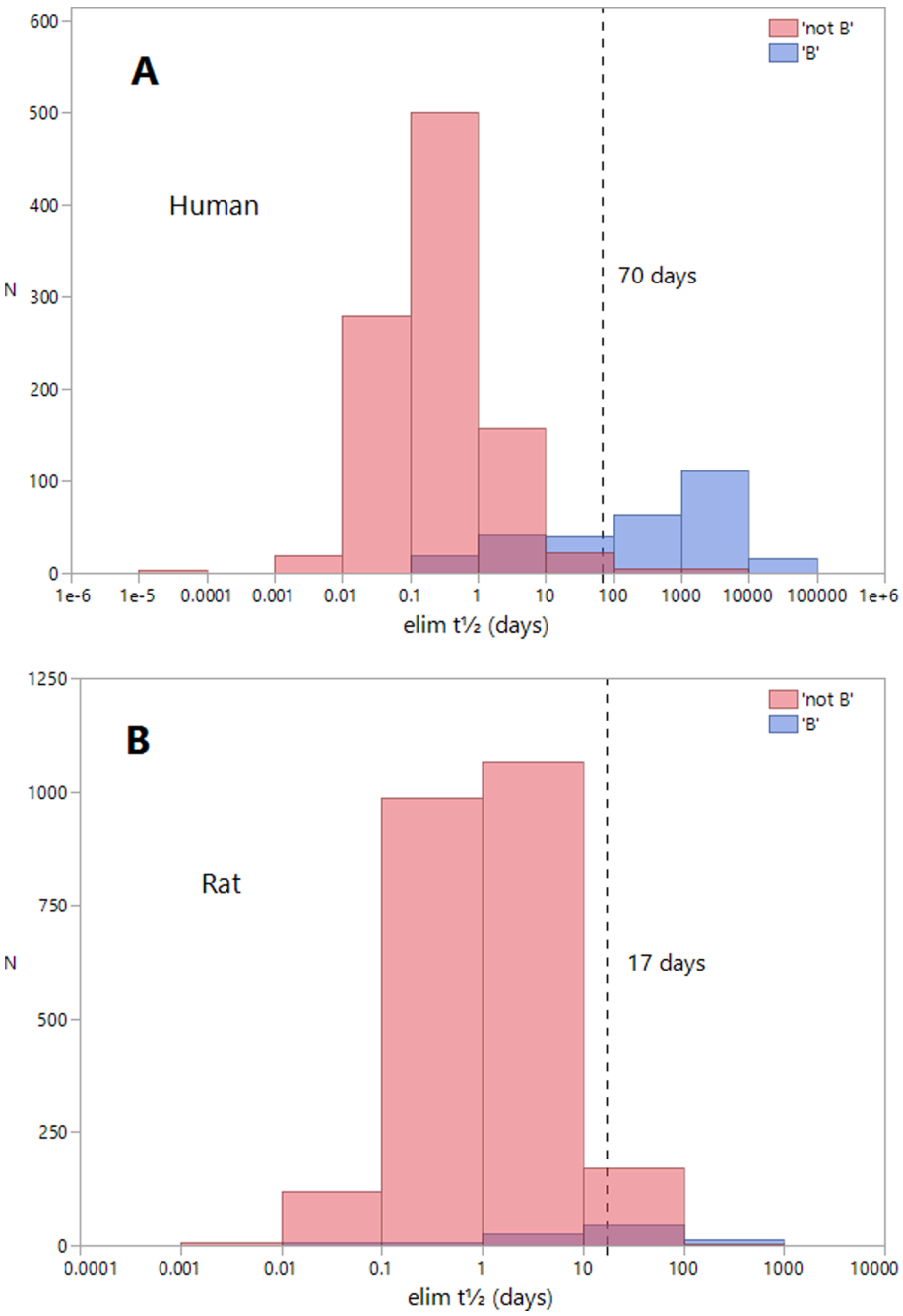

Fig. 1. Distribution of human and rat data in MamTKDB 1.0 separated on identified bioaccumulation properties (B). Bar histograms based on single replicate $^{elim}t_{1/2}$ shown on a logarithmic (base 10) scale (x-axis). A. Human $^{elim}t_{1/2}$ data displaying two distinct peaks for the 'B' (includes vB) and 'not B' data. B. Rat $^{elim}t_{1/2}$ data (all administration types) contain a smaller portion of 'B' substances than the human data. For both humans and rats, $^{elim}t_{1/2}$ for substances having 'B' status were significantly ($p < .0001$) longer than substances without such status (log_{10}-transformed single replicate $^{elim}t_{1/2}$ data analysed using the unpaired two-tailed *t*-test). The discussed thresholds of 70 (human) and 17 (rat) days are indicated with a dashed line. Note the different axis scales. *N* = number of $^{elim}t_{1/2}$.

Table 1
Description of human and rat $^{elim}t_{1/2}$ data in MamTKDB 1.0. Data as single replicates.

	Human, all data	Human, 'not B'	Human, 'B'	Rat, all data	Rat, 'not B'	Rat, 'B'
N of $^{elim}t_{1/2}$	1261	979	282	2412	2330	82
Median (25/75% percentile), days	0.40 (0.10/2.50)	0.24 (0.08/0.69)	520 (39.2/2683)	1.12 (0.49/2.92)	1.07 (0.47/2.60)	18.5 (3.54/36.7)
Geometric mean (95% CI[1]), days	1.18 (0.97–1.43)	0.26 (0.23–0.29)	235 (164–338)	1.20 (1.13–1.27)	1.10 (1.04–1.17)	12.1 (7.96–18.2)
Harmonic mean (95% CI), days	0.011 (0.0001–0.0043)	0.008 (0.0001–0.0033)	3.67 (2.62–6.11)	0.39 (0.35–0.45)	0.38 (0.34–0.44)	1.33 (0.77–5.03)
Arithmetic mean(95% CI), days	565 (392–738)	9.15 (0.80–17.5)	2494 (1761–3227)	4.25 (3.65–4.85)	2.98 (2.73–3.23)	40.3 (26.0–54.6)

[1] CI = Confidence interval.

twelve contained $^{elim}t_{1/2}$ data. When looking up mentioned original publications, $^{elim}t_{1/2}$ was found also for other related biocides, so that in total $^{elim}t_{1/2}$ data have been included for 24 biocides (11 of these are also pesticides). For veterinary medicines, 45 EPMARs were scrutinized and at least one $^{elim}t_{1/2}$ had been reported for 21 medicines. Scientific publications often contained more study details than study summaries (e.g. GLP following OECD TG 417) in reports or dossiers. Cases with a tri-compartment model were limited to few cases: examples of models yielding half-lives for α-, β-, and γ-phases are hexachlorobenzene (HCB) studies in Beagle dogs (Sundlof et al., 1982) and Rhesus monkeys (Yang

et al., 1978), as well as some of the studies of the PPPs Topramezone (BAS 670H), Glufosinate, and Triazoxide (both three- and four-compartment models used) in rats. Then, $^{elim}t_{1/2}$ for the slowest (γ- or δ-) phase is used.

3.2. Observation of possible study limitations

In the animal ADME studies that were reviewed here, revealing bioaccumulation was not always a focus, e.g. analyzing target tissues/organs for lipophilic substances such as white fat, brain and skin was often omitted and autoradiography checking was not regularly performed. The study summaries sometimes contained unclarities particularly regarding blood related parameters, e.g. which specific compartment the $^{elim}t_{1/2}$ was for (whole blood, serum, plasma, blood cells or else) as the experimental procedures (e.g. use of blood clotting prevention agent or not, centrifugation steps, etc.) were poorly described. Whole blood contains plenty of white and red (dominant) cells in addition to platelets that potentially can bind chemicals, but only occasionally the study summaries reported that blood had been perfused away from tissues by thoroughly rinsing blood vessels. Although the full studies were not available, it seems likely that this is rarely done, possibly since it is difficult and time consuming.

3.3. Efficiency of the screening criteria log $K_{ow} > 2$ and log $K_{oa} > 5$

Of all substances in MamTKDB 1.0 having an $^{elim}t_{1/2}$, 97.4% have log $K_{oa} > 5$ and 66.1% have log $K_{ow} > 2$ (KoaWIN and KOWWIN values used); 64.9% have both log $K_{ow} > 2$ and log $K_{oa} > 5$ (i.e. screening criteria for bioaccumulation potential in air-breathing organisms). In both humans and rats most of the substances having long $^{elim}t_{1/2}$ do have log $K_{ow} > 2$ and log $K_{oa} > 5$, see 3D scatterplots in Fig. A.2. These criteria therefore generally seem relevant for selecting substances for further bioaccumulation assessment and/or testing. PFAS, however, differ in this respect. While most PFAS have log $K_{ow} > 2$ (PFBS has 1.82), most of them have log $K_{oa} < 5$ (PFBS has 5.05): PFHxS (4.95), PFHpS (4.89), PFOS (4.84), PFBA (4.45), PFHxA (4.35), PFHpA (4.30), PFOA (4.24), PFNA (4.19), PFDA (4.14), PFUnDA (4.09). Thus, several PFAS known to bioaccumulate in humans would not be subject to bioaccumulation assessment for air-breathing organisms based on the present criteria. At physiological pH, PFAS are often ionized and their log K_{ow} and log $D_{7.4}$ values often differ considerably. In rats there are also some chemicals in the range log $K_{ow} = 0$ to 2 that have relatively long $^{elim}t_{1/2}$, see Fig. A.2. For both the human and rat data there are many chemicals with log $K_{ow} > 2$ that also have intermediate or short $^{elim}t_{1/2}$.

3.4. Effect of PFAS' molecular weight on $^{elim}t_{1/2}$ in humans, mice, monkeys, pigs and rats

The influence of PFAS' molecular weight (MW) and type (PFCAs and PFSAs, both are PFAAs and negatively charged at pH 7.4) on $^{elim}t_{1/2}$ was checked by plotting the MW of all available PFAS in MamTKDB 1.0 against $^{elim}t_{1/2}$ for humans, mice, monkeys, pigs and rats separately, Fig. 2 (A-E). Data are for blood plasma/serum or whole body ($^{elim}t_{1/2}$ for individual organs/tissues have yet not been collected for PFAS). In most species and for both types of PFAS, an increased MW was generally associated with an increased $^{elim}t_{1/2}$ (only in rats, $^{elim}t_{1/2}$ were slightly shorter for PFAS around 300 than at around 200 g/mol). As shown, PFCAs and PFSAs of similar MW have similar $^{elim}t_{1/2}$. The human data (PFCAs and PFSAs together) could be fitted with a second order polynomial curve with goodness of fit $R^2 = 0.84$, Fig. 2A. The discussed 'B' threshold of 70 days corresponds to PFAS around 310 g/mol based on this curve. In rats and monkeys, PFAS around 300 g/mol have short $^{elim}t_{1/2}$. To test if data for other species resemble those in humans, also their data were fitted with second order polynomial curves with reasonable success (notably, there are fewer data for mice, monkeys and pigs and some gaps in the curves). When considering the very long $^{elim}t_{1/2}$ for large PFAS in

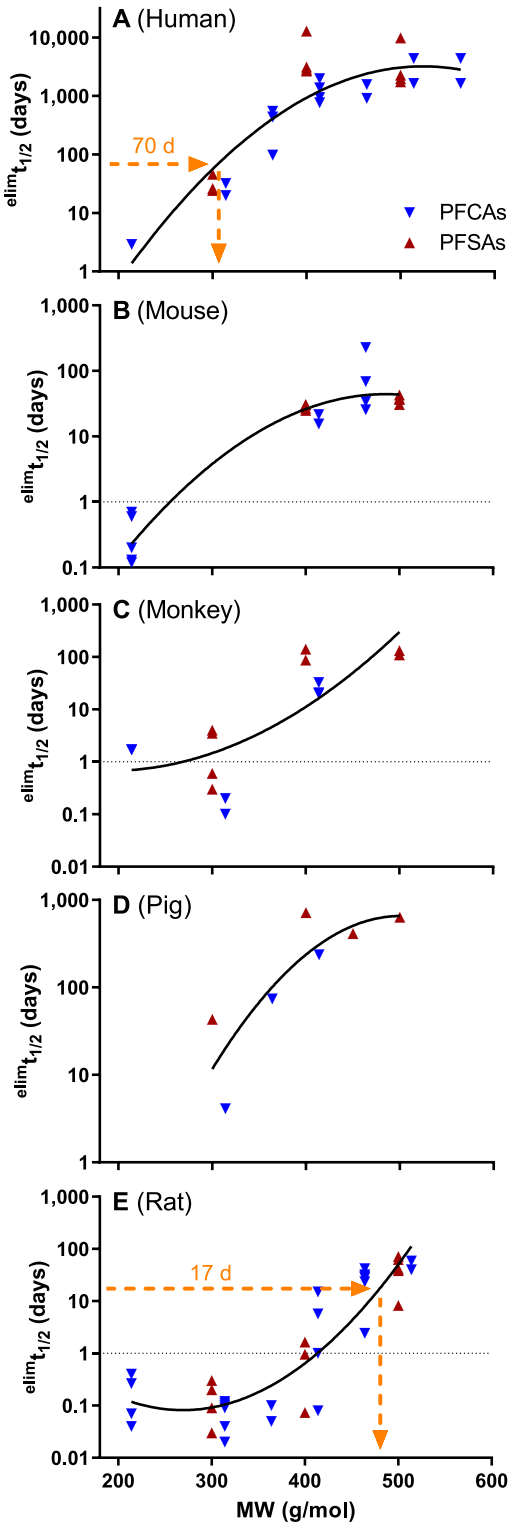

Fig. 2. Effect of the MW of PFAS on $^{elim}t_{1/2}$ in humans, mice, monkeys, pigs and rats. PFCAs (▼) and PFSAs (▲) $^{elim}t_{1/2}$ data (single replicates, $^{elim}t_{1/2}$ shown on $\log_{10}$ scale) together were fitted with second order polynomial curves in all species. A. Human. Goodness of fit $R^2 = 0.84$ ($N = 28$ data points). B. Mouse. $R^2 = 0.94$ ($N = 20$). C. Monkey. $R^2 = 0.63$ ($N = 15$). D. Pig. $R^2 = 0.73$ ($N = 7$). E. Rat. $R^2 = 0.80$ ($N = 42$). Human and rat data are for whole body and blood plasma or serum. Pig, monkey and mouse data are for blood serum only. Discussed whole body 'B' thresholds in humans (70) and rats (17) are indicated in orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

humans as well as the curve shape, data for pigs (and possibly mice for which there is a data gap between 200 and 400 g/mol, Fig. 2B) resemble those of humans, whereas data for rats and monkey do not (their curves also bend in the opposite direction). Notably, as PFAS' MW increase, so does the log K_{ow} (or log P) and log $D_{7.4}$ (plotting these instead of MW against $^{elim}t_{1/2}$ yields similar curves).

3.5. Relation of PFAS' protein binding and lipophilic properties to $^{elim}t_{1/2}$ in humans and rats in comparisons to non-PFAS substances

In Fig. 3, log K_a(HAS) and log $D_{7.4}$ for all collected PFAS (divided into PFCAs and PFSAs) were plotted against $^{elim}t_{1/2}$ in humans and rats and were also compared to all other non-PFAS in MamTKDB 1.0. As can be seen in Fig. 3C, PFAS stand out in humans, having very long $^{elim}t_{1/2}$ despite their low-intermediate lipophilicity in comparison to non-PFAS. Analysed PFCAs have somewhat higher log $D_{7.4}$ than PFSAs. In rats, PFAS have similar $^{elim}t_{1/2}$ as other non-PFAS, again suggesting that the rat poorly resemble humans in terms of PFAS. The human non-PFAS data show "hockey stick" patterns and were fitted by piecewise linear regression with calculation of breakpoints using JMP; found to be log K_a(HAS) = 4.750 (Fig. 3A) and log $D_{7.4}$ = 3.994 (Fig. 3C), respectively, using repeated (automatic) iterations. Non-PFAS substances above these breakpoints having the longest $^{elim}t_{1/2}$ in humans include highly chlorinated PCBs, PCDDs and PCDFs.

3.6. Effect of PCB chlorination degree on $^{elim}t_{1/2}$ in humans

Strongly chlorinated PCBs have among the longest $^{elim}t_{1/2}$ known in humans. When the number of chlorine atoms in the respective PCBs were plotted against their $^{elim}t_{1/2}$, a strong association between an increased $^{elim}t_{1/2}$ with increased degree of PCB chlorination was observed, Fig. 4. For other species including rats, no $^{elim}t_{1/2}$ for PCBs have yet been collected into MamTKDB 1.0.

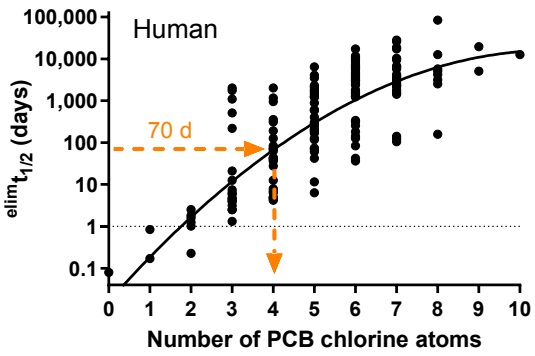

Fig. 4. Effect of PCB chlorination degree on $^{elim}t_{1/2}$ in humans. All available $^{elim}t_{1/2}$ for PCBs in MamTKDB 1.0 were included. Many $^{elim}t_{1/2}$ for PCBs were taken from Arnot 2014 (Arnot et al., 2014), for which the dominant compartment is whole body. One data point for the related non-chlorinated 1,1′-biphenyl (not a PCB) is included at 0 chlorine atoms. Data ($N = 207$ single replicate $^{elim}t_{1/2}$ on log_{10} scale) could be fitted with a second order polynomial curve with goodness of fit $R^2 = 0.65$. The discussed human whole body B threshold of 70 days is indicated in orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.7. Binding to blood cells

When going through the various studies it became clear that quite a few substances exhibited binding to blood cells mainly composed of RBCs (red blood cells). Some examples are mentioned here. A metabolite of Musk xylene was found to covalently bind (adduct formation) to human RBC haemoglobin (Riedel et al., 1999). In rats, 72 h after being orally administrated Metobromuron (^{14}C-labeled), erythrocytes contained 2–3 times the amount of radioactivity in plasma and 56–90% of the radioactivity was associated with haemoglobin (DAR for Metobromuron). Administration of Epoxiconazole (^{14}C-labeled) to rats

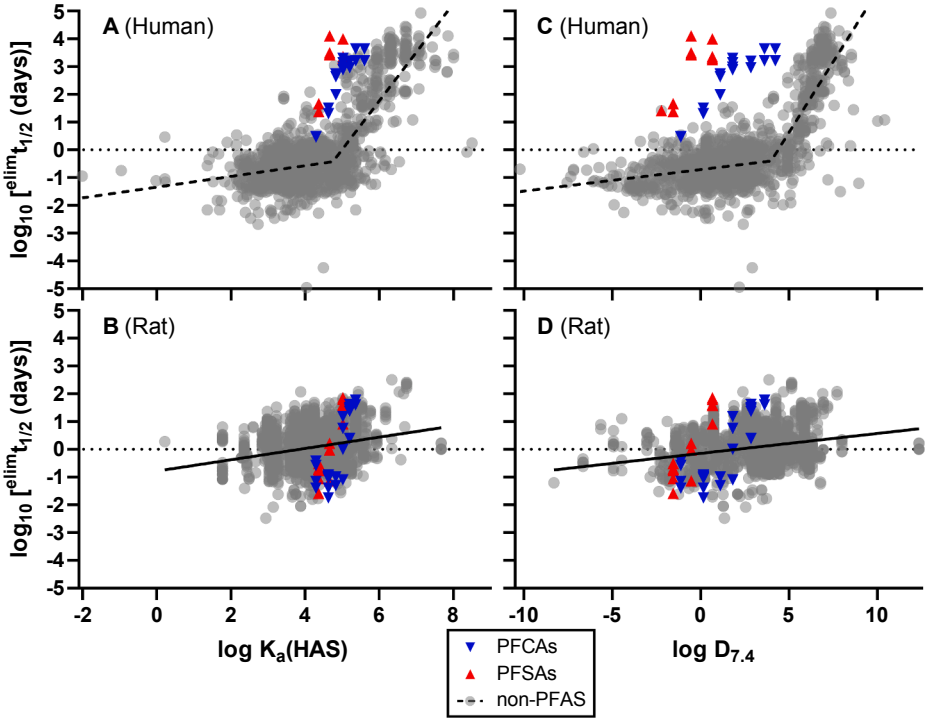

Fig. 3. Influence of PFAS' log K_a(HAS) and log $D_{7.4}$ on $^{elim}t_{1/2}$ in humans and rats and comparison with non-PFAS substances. The influence of PFAS' serum albumin affinity (log K_a(HAS)) on $^{elim}t_{1/2}$ is shown for humans (A) and rats (B). The PFCAs (▼) and PFSAs (▲) have log K_a(HAS) in a similar range which is relatively narrow in comparison to non-PFAS substances (●). The influence of PFAS' lipophilicity (log $D_{7.4}$) on $^{elim}t_{1/2}$ is shown for humans (C) and rats (D). Data are shown as log_{10}-transformed single replicate $^{elim}t_{1/2}$ for all compartments (PFAS data are mainly for blood plasma, blood serum and whole body). The regression lines (all are based on non-PFAS data) show that as log K_a(HAS) and log $D_{7.4}$ increase, so does $^{elim}t_{1/2}$ in both humans and rats.

resulted in indication of binding of parent or metabolite to blood cells, this since whole blood and spleen levels were declining more slowly than blood plasma (DAR for Epoxiconazole). Tri-allate or its metabolites (Tri-allate was extensively metabolized) showed high affinity for blood cells (the authors presume covalent binding to haemoglobin). At rat sacrifice, 240 h (10 days) after oral administration of Tri-allate (^{14}C- and ^{13}C-labelled), blood cells displayed the highest radioactivity, followed by spleen, kidney and thereafter other organs. The blood cell fraction had 60–110 times the radioactivity compared to blood plasma (DAR for Tri-allate). In rats, Triflumuron (^{14}C-labeled) displayed the longest $^{elim}t_{1/2}$ (17.38 days after single administration) in erythrocytes out of 15 compartments studied, and a follow-up study showed that most of the erythrocyte-bound radioactivity was found in the globin fraction (76.2%) in the form of a metabolite (DAR for Triflumuron). In rats, Dimethachlor bound covalently to haemoglobin inside RBCs and whole blood and spleen displayed a considerably longer $^{elim}t_{1/2}$ than blood plasma. However, it is assumed that binding of Dimethachlor to human haemoglobin is unlikely (or much less) due to differences in the structure of rat and human haemoglobin (rat haemoglobin has a reactive cysteine residue (Cys β-125) in the β-chain that humans lack) (DAR for Dimethachlor). In rats, Fenbuconazole (^{14}C-labeled) had much longer $^{elim}t_{1/2}$ in whole blood than in blood plasma (single administration, the same study). This indicated preferential partitioning into RBCs, stated to be a property of chemicals with a chlorophenol moiety such as fenbuconazole (DAR for Fenbuconazole).

3.8. Rat MamTKDB 1.0 data

For rats, 2412 $^{elim}t_{1/2}$ were collected for 280 substances. The number of studies, administration types, number of compartments presented with $^{elim}t_{1/2}$ data as well as level of details (ages and sex, etc.) presented in the dossier summaries and publications varied considerably. Rat data are dominated by pesticides. Single, preconditioning and repeated studies had been performed for 274, 37, and 48 of the 280 substances, respectively, see Table 2, thus single administration was by far the most common type. The preconditioning and repeated studies were nearly always accompanied by single administration studies, but for two substances only a preconditioning study existed and for three substances only repeated studies existed. For five substances all three administration types existed.

Whereas preconditioning studies in general only reported $^{elim}t_{1/2}$ for blood plasma (or serum), whole blood and/or the whole body (based on faeces and/or urine excretion), repeated studies commonly reported $^{elim}t_{1/2}$ for multiple organs/tissues in addition (see Fig. 7 below). For preconditioning, 61 studies reported 81 $^{elim}t_{1/2}$ for 37 substances. The most common length of daily dosing with a non-labelled substance was 14 consecutive days (91.8% of the studies) that was followed with dosing of a labelled substance on the next day, i.e. on day 15. Other preconditioning dosing periods with a non-labelled substance were: 7 (1.6% of the studies), 9 (1.6%), 10 (1.6%) and 21 (3.3%) consecutive days that were followed with dosing of a labelled substance on the next day, i.e. on day 8, 10, 11 and 22. The time to establish steady state will differ dependent on the substance and dose. For repeated administration, 64 studies reported 332 $^{elim}t_{1/2}$ for 48 substances. Out of these, 57 studies (for 41 substances) administrated a labelled substance and 7 studies (for 6 substances) a non-labelled substance. Dosing periods were (for studies using both non-labelled and labelled substances together): 7 (20.6% of the studies), 10 (1.6%), 14 (49.2%), 20 (1.6%), 21 (1.6%), 28 (9.5%), 42 (3.2%), 49 (3.2%), 56 (1.6%), 70 (6.3%), and 119 (1.6%) consecutive days, respectively. One of the two 49-day studies only performed dosing on working days (5 out of 7 days per week) and one of the repeated studies that administrated a labelled substance did not specify the length of the dosing period in the available DAR.

Table 2
Description of rat $^{elim}t_{1/2}$ data for each administration type in MamTKDB 1.0. Data as single replicates.

Administration type:	Single	Preconditioning	Repeated	All
N of $^{elim}t_{1/2}$ in rats	1999	81	332	2412
Median (25/75% percentile), days	0.92 (0.42/2.12)	1.13 (0.51/1.78)	6.25 (2.31/12.7)	1.12 (0.49/2.92)
Geometric mean (95% CI[1]), days	0.93 (0.88–0.99	0.99 (0.81–1.22)	5.51 (4.84–6.28)	1.20 (1.13–1.27)
Harmonic mean (95% CI), days	0.34 (0.30–0.40)	0.66 (0.54–0.85)	2.11 (1.60–3.11)	0.39 (0.35–0.45)
Arithmetic mean (95% CI), days	3.40 (2.73–4.08)	1.49 (1.15–1.84)	10.0 (8.70–11.3)	4.25 (3.65–4.85)
Number of substances studied in rats	274	37	48	280

[1] CI = Confidence interval.

For the rat data there are somewhat more males (M: 57.4%) than females (F: 42.6%) $^{elim}t_{1/2}$ data. Geometric means for single replicate $^{elim}t_{1/2}$ for M were somewhat longer than F for single (0.98 vs. 0.89 days), preconditioning (1.11 vs. 0.91 days) and for repeated (6.76 vs. 4.59 days) administration as well as for all three administration forms together (1.27 vs. 1.14 days). However, the effect was only significant for repeated administration ($p = 0.031$; single replicate $\log_{10}$-transformed $^{elim}t_{1/2}$ data analysed using two-tailed non-paired *t*-tests), but a careful analysis revealed that different substances had often been studied for repeated administration among the two sexes which could explain this effect. Thus, we cannot conclude whether there are any significant gender effects overall (gender effects may exist for individual substances).

Single high dose administration sometimes resulted in a quite longer $^{elim}t_{1/2}$ than for low dose, e.g. for Cyprodinil (DAR for Cyprodinil). The opposite was seldom observed, but similar $^{elim}t_{1/2}$ for low and high dose was common. However, this seems to be substance dependent and this eventual effect was not further investigated. Single doses (low and high) used also vary considerably in-between studies.

3.9. Does the type of administration affect $^{elim}t_{1/2}$ for the collected rat data?

Collected rat $^{elim}t_{1/2}$ are shown as single replicates in Fig. 5. Some of the longest $^{elim}t_{1/2}$ were from single administration of rodenticides, often for liver. As shown in Fig. 5B, $\log_{10}$ transformation increased data normality. Analyses of $\log_{10}$-transformed data using one-way ANOVA with Tukey's post-test identified that repeated administration gave significantly ($p < .001$) longer $^{elim}t_{1/2}$ than for single or preconditioning. No difference was found between single and preconditioning administration. Importantly, these findings (that $^{elim}t_{1/2}$ from repeated studies are longer) should be regarded as a strong trend only since the substances and compartments studied differ among the three administration types. However, Fig. 6 below compares $^{elim}t_{1/2}$ from different administration types for the very same compartment and chemical, confirming that $^{elim}t_{1/2}$ from repeated studies are longer.

Rat $^{elim}t_{1/2}$ for substances that had been studied in the very same compartment (various) by more than one administration type were analysed using paired *t*-tests (two-tailed), Fig. 6. First, arithmetic means for each substance, compartment and administration type were calculated. Distributions (same administration type, different substances) did not pass the D'Agostino and Pearson normality test and data were $\log_{10}$-transformed to attain normal distribution. Whereas preconditioning had no effect on $^{elim}t_{1/2}$ versus single administration ($p = .354$, $N = 50$ data pairs) (Fig. 6A), repeated administration significantly prolonged $^{elim}t_{1/2}$ in comparison to single ($p < .0001$, $N = 165$ data pairs) (Fig. 6B). Also

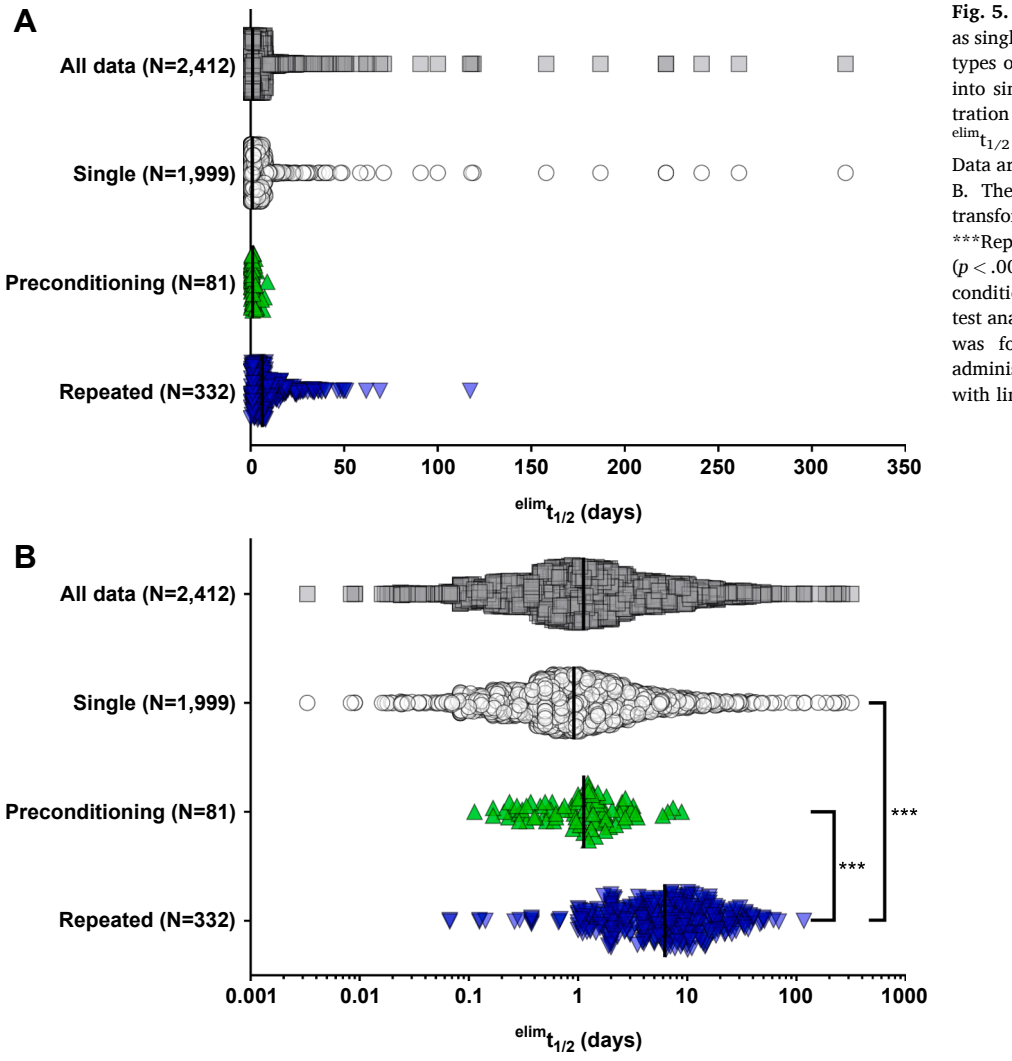

Fig. 5. All rat $^{elim}t_{1/2}$ data in MamTKDB 1.0 shown as single replicates. A. Data distribution for all three types of administration together as well as divided into single, preconditioning and repeated administration types. *N* is the number of single replicate $^{elim}t_{1/2}$ data points for each administration type. Data are right-skewed and not normally distributed. B. The same data shown on log_{10} scale. Log_{10} transformations considerably improved normality. ***Repeated administration resulted in significantly ($p < .001$) longer $^{elim}t_{1/2}$ than single and preconditioning by one-way ANOVA with Tukey's post test analyzing log_{10}-transformed data. No difference was found between single and preconditioning administration. The median values are indicated with lines.

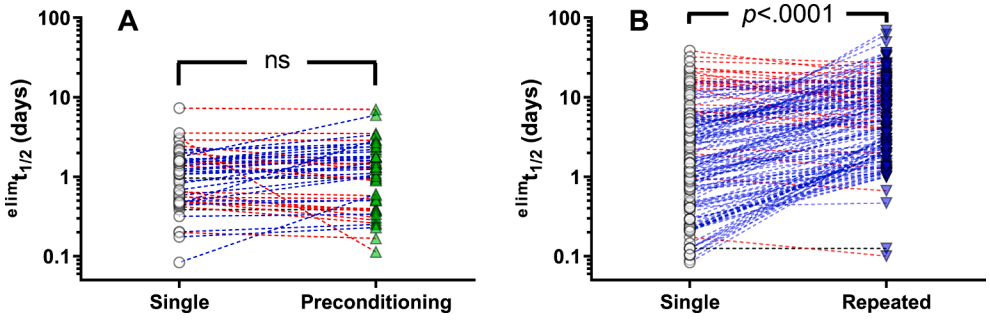

Fig. 6. Effect of administration type on $^{elim}t_{1/2}$ for substances for which there were both single, preconditioning or repeated administration data for the same compartment. After log_{10}-transformation (to obtain normally distributed data, data are shown untransformed but on a log_{10} scale), matching $^{elim}t_{1/2}$ data for two different administration types (same substance and compartment) were analysed using paired *t*-tests (two-tailed). A. Single vs. preconditioning data were not different ($p = .354$, $N = 50$ data pairs). B. Single vs. repeated data were significantly different ($p < .0001$, $N = 165$ data pairs). Data points shown as calculated arithmetic means for each compartment and substance, with connecting lines in-between two administration types for the same substance and compartment type. Lines having positive slopes are blue, decreasing red, and horizontal black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

for the blood plasma/serum compartment alone, repeated administration significantly prolonged $^{elim}t_{1/2}$ in comparison to single ($p = .0002$ (two-tailed), $N = 23$ data pairs for 23 substances), data not shown. Thus, the trend that $^{elim}t_{1/2}$ from repeated studies are longer (Fig. 5) is shown to be true in general, although this likely differs from substance to substance. Moreover, the slopes of the connecting lines in Fig. 6B indicate that there is no clear trend that chemicals having short $^{elim}t_{1/2}$ in single studies also should have short $^{elim}t_{1/2}$ in repeated studies or that chemicals having long $^{elim}t_{1/2}$ in single studies should have extra long $^{elim}t_{1/2}$ in repeated studies. Rather, this seems to vary from substance to substance. For preconditioning vs. repeated administration comparisons there were too few matching data points to perform a meaningful analysis.

3.10. Comparison of $^{elim}t_{1/2}$ among rat compartments

Collected rat $^{elim}t_{1/2}$ were categorized into 38 main compartments, thus for about half of the ≈70–80 organs/tissues in the body no $^{elim}t_{1/2}$ had been reported. The number of single replicates that make up each calculated mean $^{elim}t_{1/2}$ is specified in supplementary Table A.2 which also describes how pooling of sub-compartments was made, number of data points and substances. Blood plasma and blood serum were pooled but blood cells and whole blood are here treated as separate compartments. Table A.3 shows median values, geometric means with 95% CIs for each of the 38 compartments and administration types (based on calculated arithmetic means for each substance, compartment and administration type). As some compartments had little data, only 21 compartments that had sufficient $^{elim}t_{1/2}$ were analyzed further, for which geometric means and 95% CIs (for geometric means) are shown in Fig. 7.

For single administration, blood cells (RBCs or total) surprisingly had the longest geometric mean (2.43 days) although data consists of a relatively small number ($N = 17$) of substances. The second longest geometric mean was for white fat (1.84 days, $N = 38$ substances),

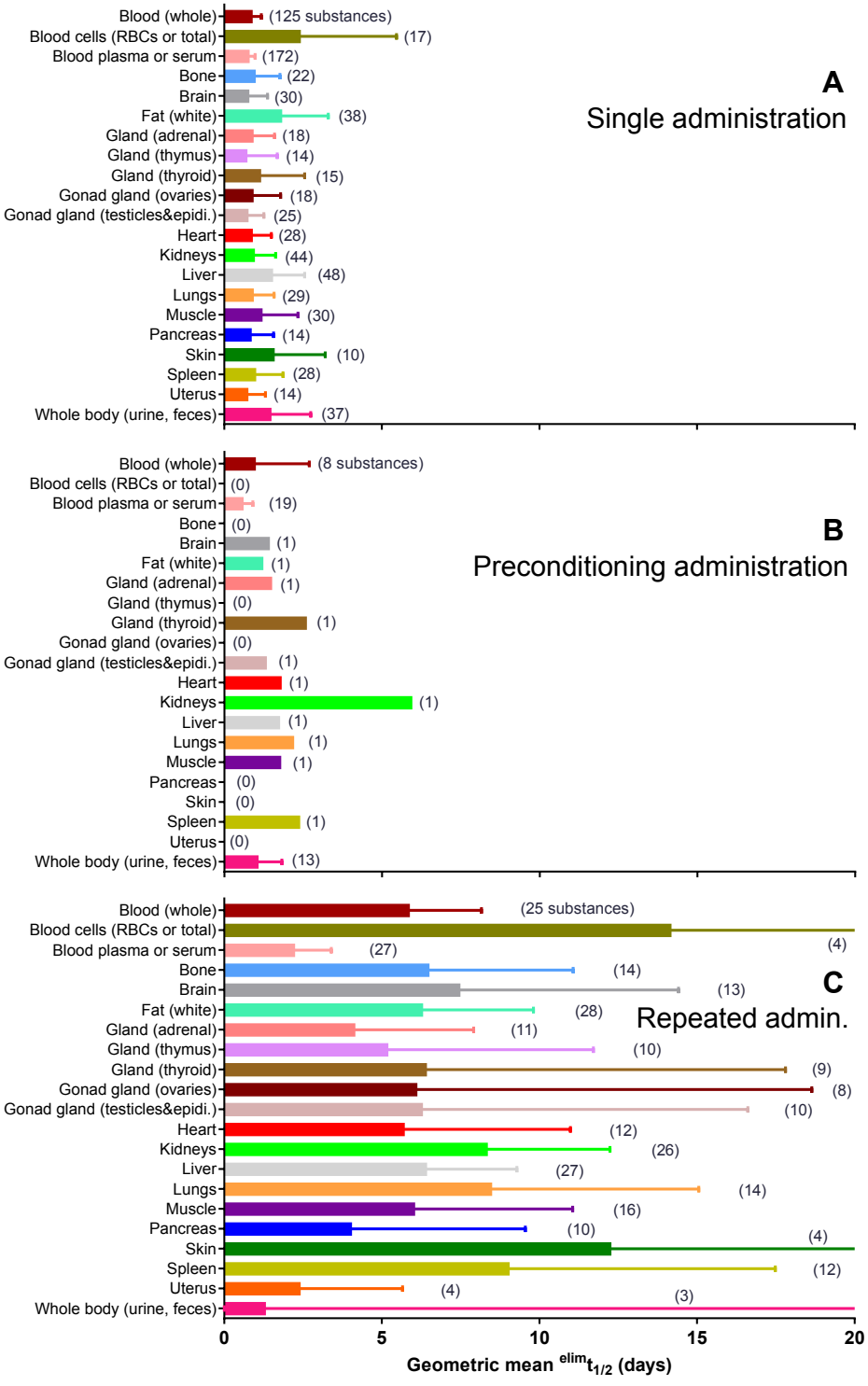

Fig. 7. MamTKDB 1.0 rat $^{elim}t_{1/2}$ data shown for the three administration types as geometric means with 95% CI for the 21 compartments having the most data. Single (A), preconditioning (B) and repeated administration (C), respectively. Geometric means are based on arithmetic means for each substance (the number of substances making up each bar is shown within parenthesis). For preconditioning some compartments had very few, or even lacked, data. Same scale for all graphs shown up to 20 days (95% CI can be very large when N is small).

followed by skin (1.60 days, $N = 10$), liver (1.55 days, $N = 48$), and whole body (urine/faeces excretion) (1.50 days, $N = 37$). Noticeably, the geometric mean for blood plasma/serum was relatively short (0.81 days, $N = 172$) and was shorter than for blood cells (RBCs or total) and whole blood. For preconditioning the number of substances making up each bar were often too few for sensible comparisons between compartments, but available $^{elim}t_{1/2}$ were often in the same range as for single administration. For repeated administration, the longest geometric mean was for blood cells (RBCs or total) (14.2 days, $N = 4$) followed by skin (12.3 days, $N = 4$), spleen (9.06 days, $N = 12$), lungs (8.50 days, $N = 14$) and kidneys (8.37 days, $N = 26$). The geometric mean for blood plasma/serum was relatively short also for repeated administration (2.25 days, $N = 27$). Drastically shorter blood plasma/serum $^{elim}t_{1/2}$ vs. other organs/tissues was noticed for Benzovinviflupyr, Metalaxyl-M and Thiabendazole (based on DARs for each substance). Whole body (urine/faeces excretion/egestion) had the shortest geometric mean (1.32 days, $N = 3$), although very few data points. Since the substances often differ between the administration types and as $^{elim}t_{1/2}$ from repeated administration are scarce, no statistical analysis was performed.

3.11. How well do $^{elim}t_{1/2}$ between rat compartments correlate?

For substances having $^{elim}t_{1/2}$ from more than one compartment, associations between the 21 most data-rich compartments were analysed for (data as calculated arithmetic means) using Spearman rank correlations and linear (x-y) regressions. The three administration types were analysed together. As we show above, $^{elim}t_{1/2}$ from repeated administration studies are somewhat longer than from single or preconditioning, but which is often the case for both compartments for each association tested. Repeated data would be too few on their own. Analyses were limited to a minimum of ≥11 observations to obtain reliable Spearman rank correlations, which reduced the number of compartments to 20 since whole body (based on urine/faeces excretion/egestion) $^{elim}t_{1/2}$ data seldom were accompanied with $^{elim}t_{1/2}$ data from other compartments. The reason could be that to obtain whole body (urine/faeces) $^{elim}t_{1/2}$ the animals must be kept alive whereas to obtain tissue $^{elim}t_{1/2}$ the animals need to be sacrificed, thus different study designs. The 20 compartments allowed 380 (19×20) tests for associations. In general, $^{elim}t_{1/2}$ data for the 20 compartments were relatively well correlated as indicated by high Spearman (ρ) values and low p-values. A few examples of tests for correlations and associations are shown in Fig. 8 and all results are shown as a corrgram in Table A.4. The correlation between white fat and brain ($\rho = 0.782$, $p = .0001$), both fatty tissues, was weaker than most other correlations among the various compartments and could be due to the blood brain barrier (BBB) having tight junctions, but the blood perfusion rate is also higher in brain. The correlation between white fat (visceral) and skin (both have adipose tissue) was slightly stronger ($\rho = 0.846$, $p = .0003$). Uterus stuck out being the compartment that correlated the least with other compartments and its correlation towards white fat ($\rho = 0.492$, $p = .045$) was the weakest of all tested correlations. The second weakest correlation was between blood plasma/serum vs. blood cells ($\rho = 0.594$, $p = .0058$).

For linear regression analyses, untransformed $^{elim}t_{1/2}$ data were nearly always not normally distributed, displayed low goodness-of-fit (R^2) and a few outliers often had a very strong influence on the slopes (β). Plotting data on $\log_{10}$ scales, however, considerably limited the influence of outliers on slopes and increased goodness of fit (R^2), Fig. 8. Therefore, prior to linear regression analyses, all data were first $\log_{10}$-transformed (slopes for $\log_{10}$-transformed data are called β').

Plasma/serum correlated relatively poorly with most other compartments and generally had shorter $^{elim}t_{1/2}$ than other compartments as seen from β'.

3.12. Examples of substances having long $^{elim}t_{1/2}$ in rats

Thirty substances in MamTKDB 1.0 have at least one $^{elim}t_{1/2}$ longer in one or several compartments than the discussed whole body based threshold of 17 days for rats, see Table A.5. Of these, seven are POPs, six have PBT status (are included in the REACH Candidate List) out of which three are rodenticides (designed to be toxic and have long $^{elim}t_{1/2}$), leaving seventeen substances out of which several are PPPs ($^{elim}t_{1/2}$ collected from pesticide DARs) that potentially could be problematic in terms of bioaccumulation in air-breathers if used. Noticeable, many of these $^{elim}t_{1/2}$ are for single administration and would most likely have been even longer had the administration been repeated.

4. Discussion

The toxicokinetic database MamTKDB 1.0 was shown useful when analyzing mammalian $^{elim}t_{1/2}$ data and is a promising tool to support the assessment of bioaccumulation potential of chemicals. To our knowledge, MamTKDB 1.0 is the first publicly available collection of $^{elim}t_{1/2}$ for substances in air-breathing terrestrial mammals, which in addition are coupled to predicted physicochemical data. Gathering of study details was in nearly all cases possible for the animal and human $^{elim}t_{1/2}$ collected by us (Hofer et al., 2021). However, for the included human OECD Toolbox $^{elim}t_{1/2}$ data that are stated to be for whole body (Arnot et al., 2014) (thus they are likely based on blood plasma or urinary/faecal excretion/egestion since collection of human organs is often not possible), specific details are often lacking and there is no reference to the original study (for drugs, references could be available in a follow-up paper (Lombardo et al., 2018)). More details on those studies can hopefully be included in future versions of MamTKDB; we included physiochemical data also for these substances. During our data collection, AUC, C_{max}, and T_{max} values were occasionally recorded but AUC values appeared challenging to interpret and use in a comparative way (e.g. arbitrary AUC values can drastically differ in numbers and different units had sometimes been used in the studies). Before eventual use, AUC data would likely require normalization somehow.

It is evident from Fig. 7 (supported by Figs. 5 and 6) that in general (not necessarily the case for each substance), repeated administration results in considerably longer $^{elim}t_{1/2}$ than from single or preconditioning administration. According to OECD TG 417, single administration may be adequate to determine accumulation and persistence potential (OECD, 2010). In agreement, for e.g. rodenticides (are often vitamin K antagonists that cause prevention of blood coagulation) that accumulate in liver and have very long liver $^{elim}t_{1/2}$, then single administration can be enough. However, our analyses show that for substances with short or intermediate $^{elim}t_{1/2}$ after single administration, then repeated administration is necessary to generate representative $^{elim}t_{1/2}$. As an example, repeated (14-day, 1 mg) administration of Benzovindiflupyr (pesticide) in rats resulted in considerably longer $^{elim}t_{1/2}$ for several organ/tissue (except blood plasma) compartments than after single administration despite giving a higher dose (40 mg). For repeated Benzovindiflupyr administration, various organs/tissues had $^{elim}t_{1/2}$ in the range 17.3–69.1 days whereas corresponding single administration $^{elim}t_{1/2}$ were just 2.4–8.7 days. For Benzovindiflupyr, blood plasma had an $^{elim}t_{1/2}$ of only 2.49 days (repeated administration) which was close to the $^{elim}t_{1/2}$ of 1.8 days obtained after single administration, illustrating (as one example of several) that blood plasma/serum $^{elim}t_{1/2}$ often under-represent $^{elim}t_{1/2}$ in organs/tissues. Repeated (compared to single or preconditioning) dosing should better ascertain a high radiolabeled substance load into peripheral organ/tissue compartments (some large and/or deep with slow influx rates due to little blood perfusion, unfavourable partitioning, little active or passive transport through the cell membrane or else) and establishment of steady state (Birkett and Prescriber, 2009; OECD, 2010; Toutain and Bousquet-Melou, 2004). With preconditioning, the non-labelled substance may have reached steady state, but not the radiolabelled one (measured) given only the last

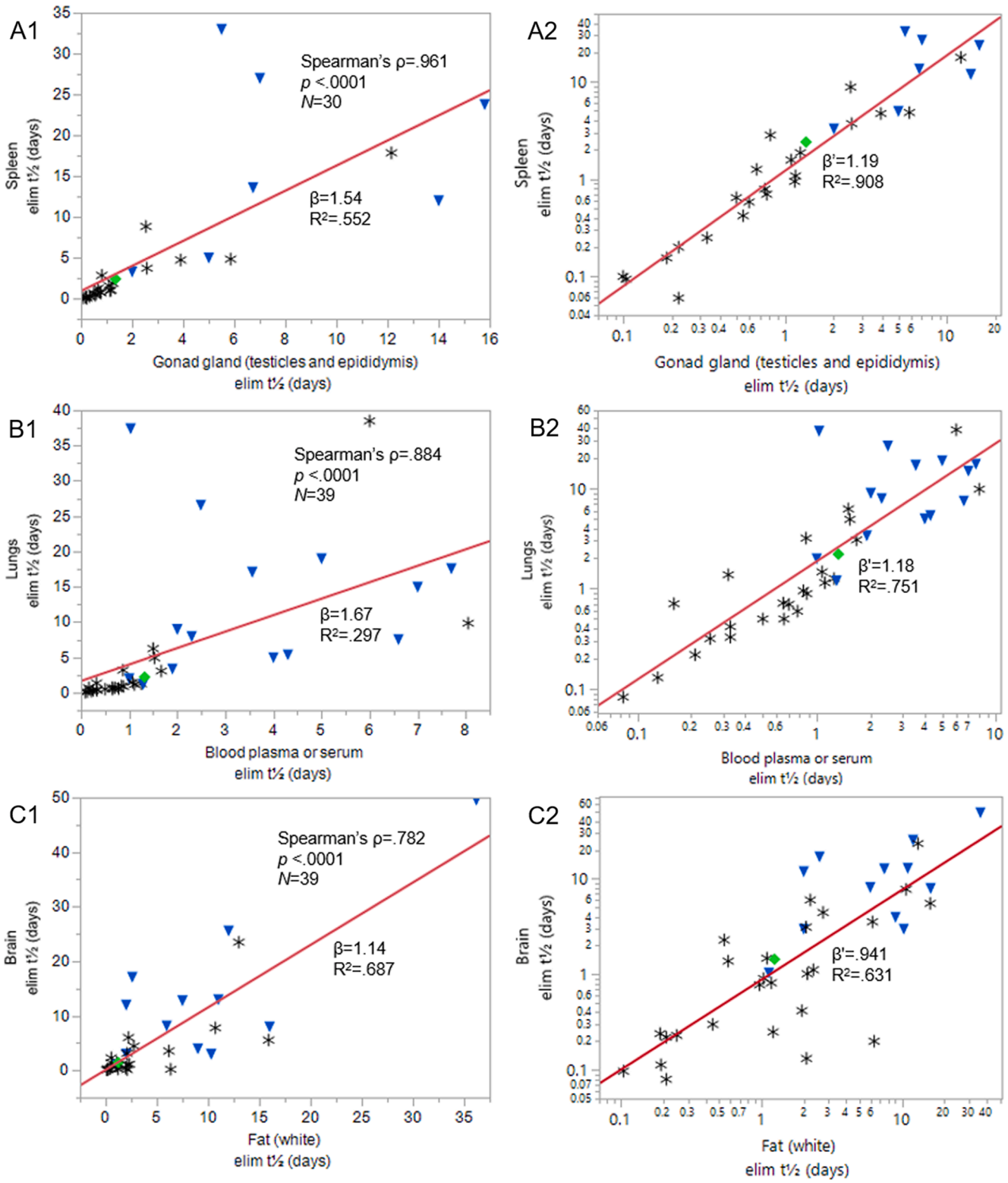

Fig. 8. Examples of tests for correlations and associations between two different rat compartments and the effect of $\log_{10}$-transformation. Spearman's rank tests of correlation and linear regressions (β and β' describe slopes of regression lines; R^2 is goodness-of-fit) describe relationships between two compartments. Left side: correlations shown for untransformed data. A1. Gonad gland (testicles and epididymis) vs. spleen. B1. Blood plasma or serum vs. lungs. C1. Fat (white) vs. brain. Right side: the same compartments but with data shown on $\log_{10}$ scale in A2, B2 and C2. In general, regression line slopes (β) for untransformed data (left side) were strongly dependent on a few outliers (as can be seen) and were considered unreliable. Log_{10}-transformations produced more normally distributed data (was always the case) with more robust linear regression slopes (β') and in most cases also reduced data scattering and increased R^2. Data shown as calculated arithmetic mean $^{\text{elim}}t_{1/2}$ for each substance and compartment. Single (✱), preconditioning (◆) and repeated (▼) administration types are analysed together.

day. With preconditioning, the total dose radiolabelled substance administrated is also just a fraction of that given during repeated administration. With prolonged dosing, V_d will successively increase as tissues are loaded. Another example that plasma $^{elim}t_{1/2}$ does not always correlate well with $^{elim}t_{1/2}$ in tissues comes from the veterinary medicine Clodronic acid (inhibitor of osteoclast-mediated bone resorption, a biphosphonate). The EPMAR report mentions that in mice, radioactivity was seen to persist in the bone, spleen, thymus and small intestine for up to one year ($^{elim}t_{1/2}$ were not provided for these compartments), whereas the plasma $^{elim}t_{1/2}$ was just 1–2 h. The situation is stated to be similar in rats. Bisphosphonates physically bind to bone and accumulate over time.

Our MamTKDB 1.0 analyses show that $^{elim}t_{1/2}$ data for humans and rats, the two species having the most $^{elim}t_{1/2}$ data, differ in many ways, e.g. in that different types of substances have been studied, that metabolites are often followed in rats (fate of ^{14}C-label is followed) but rarely or less extensively (e.g. just one metabolite and only in blood plasma) in humans, that the type and length of administration is often better controlled in rats, and that rat data are composed of a higher content of $^{elim}t_{1/2}$ for various tissue/organ compartments. The resulting $^{elim}t_{1/2}$ can be very different if only disappearance or excretion of parent compound vs. parent and metabolites is followed. Interestingly, some substances (e.g. Thiabendazole) have much longer $^{elim}t_{1/2}$ in rats (various compartments) than in measurable human compartments (often blood plasma) which could possibly be due to formation of metabolites that are retained, but only followed in rats. Whereas human data in MamTKDB 1.0 (mainly composed of drugs and environmental contaminants) range from minutes to over 100 years, the longest rat data (mainly composed of pesticides) are just above 100 (longest was 318) days. Drugs often have short $^{elim}t_{1/2}$ and for 670 of the drugs (single i.v. injections) included, $^{elim}t_{1/2}$ ranged from 4 min to just 50 days, the median value was 4.1 h (mean was 18 h) and only 12% (82 drugs) had $^{elim}t_{1/2} > 1$ day (24 h) (Obach et al., 2008). The included environmental chemicals, on the other hand, often have very long half-lives, e.g. ranging from hundreds to thousands of days in humans (Arnot et al., 2014) and were often generated at steady state like conditions (repeated exposures). In comparison to rat data, the human data is based on more substances with B/vB identified properties. Substances identified as bioaccumulating (PBT or vPvB) have often received this status based on aquatic (e.g. fish) and, due to species differences, may potentially not bioaccumulate (or have long $^{elim}t_{1/2}$) in land living mammals. Although B/vB substances generally had longer $^{elim}t_{1/2}$ than substances without such status (Fig. 1), some also had relatively short $^{elim}t_{1/2}$. PAHs, despite being bulky and lipophilic and that bioaccumulate in marine invertebrates (Meador et al., 1995) generally have short $^{elim}t_{1/2}$ both in rats and humans (exemplified by pyrene), likely as they undergo metabolic conversion by cytochrome P450 enzymes. PCBs are considered 'B' as substance group, although low chlorinated PCBs can have short $^{elim}t_{1/2}$. On the other hand, there are also 'not B' substances that had $^{elim}t_{1/2}$ well above the suggested thresholds of 17 (rats) and 70 (humans) days (Goss et al., 2013; Goss et al., 2018), that can become subjects for future regulations. This number would have been even higher had there been more repeated administration studies in animals (in MamTKDB 1.0 most rat $^{elim}t_{1/2}$ are from single administration). Another difference is that most human studies are on adults at various ages, whereas young adult rats (that may have little body fat) are recommended according to OECD TG 417.

After that the rat-based edition of OECD TG 417 came out in 2010 (OECD, 2010), there is a noticeable shift from using various animal species to mainly study rats. For comparisons, setting thresholds, use of toxicokinetic data for analysis and predictions, it may be good to focus on just a few animal species. If significant differences in toxicity/toxicokinetics between humans and the rat (standard toxicity test model) are known for a substance, other species can still be considered that better resemble humans (OECD, 2010).

One endogenously produced compound known to bind certain xenobiotics is melanin (different types in different tissues) present e.g. in skin melanocytes, and results in pigmented animals can differ from those in albino (OECD, 2010; Ono et al., 2003). In general, melanins (negatively charged polyanions) bind lipophilic, basic compounds such as amines and positively charged metal ions. For brain studies, it should be noted that the neuromelanin (dark pigment) content increases inside human brain catecholaminergic neurons with age (ubiquitous, but particularly in substantia nigra and locus coeruleus). However, rodents generally lack neuromelanin, mice are devoid but rats seem to form a small amount at high age (Barden and Levine, 1983; DeMattei et al., 1986; Marsden, 1961), but are often studied at young age.

For any set of positive numbers such as $^{elim}t_{1/2}$, the harmonic mean $\leq$ geometric mean $\leq$ arithmetic mean. Thus, in terms of bioaccumulation, harmonic means are the least conservative. In Tables 1 and 2, geometric means best match medians. Individual studies differ in types of mean $^{elim}t_{1/2}$ reported, and which type of mean to be used in a particular situation likely needs to be clarified, see e.g. (Martinez and Bartholomew, 2017).

Since MamTKDB 1.0 contains plenty of $^{elim}t_{1/2}$ data for PFAS (various species) and PCBs (only human data included so far), it was natural to check them for some trends. We did not try to collect all $^{elim}t_{1/2}$ for PFAS and PCBs in the open literature, there are probably still more to be collected. PFAS in MamTKDB 1.0 (all are PFAAs) have a negatively charged functional group and proteinophilic properties (binding to proteins and/or fatty acids depend on type of functional group(s) as well as chain length) and they commonly bind to serum and liver proteins, and undergo extensive reabsorption at the hepatic, intestinal and renal level in humans (EFSA et al., 2020). Our analyses show that pigs resemble humans more than rodents in terms of toxicokinetics for negatively charged PFAAs. It is possible that pigs (e.g. mini-pigs) can be suitable for ADME studies also for other substances, but might not be the right choice for substances undergoing sulfation (Dalgaard, 2015). Pigs also lack neuromelanin (monkeys, dogs, cats, sheep and goat contain more) (Marsden, 1961; Nielsen et al., 2009) which may be important for accumulation of chemicals in brain. PFCAs and PFSAs (both charged) are not metabolized (EFSA et al., 2020) and our MW-based comparison of $^{elim}t_{1/2}$ for PFCAs to those of PFSAs shows similar values in the species analyzed when similar MW. PFSAs tend to have longer $^{elim}t_{1/2}$ than PFCAs when of the same (fluorinated) chain length. In a recent review, $^{elim}t_{1/2}$ were reported to decrease in the order of PFHxS > PFOS > PFOA > PFBS > PFBA, and further, in the order of humans > monkeys > rodents (Pizzurro et al., 2019) which is in line with our results. Still, although non-human primates (monkeys) are closest to humans in terms of genetic homology, $^{elim}t_{1/2}$ for PFAS can drastically differ between monkeys and humans (EFSA et al., 2020). For ADME studies on non-persistent substances, species specific metabolic differences should be taken into account, e.g. in cytochrome P450 liver enzymes (Bogaards et al., 2000). The trend observed in humans that an increased degree of PCB chlorination extends $^{elim}t_{1/2}$ is likely due to increased resistance towards metabolic conversion. Proper choice of species is relevant also for toxicodynamics, if the target is not available there is often no toxicity.

For several pesticides there were no or few toxicokinetic data in the DARs, or when available, the ADME summaries sometimes have limited useful information related to bioaccumulation, e.g. no $^{elim}t_{1/2}$ calculated or that $^{elim}t_{1/2}$ is available just for one or a limited number of compartments. This is not a desirable situation since active substance residues from pesticides can constitute a major human xenobiotic source through foods despite being checked for maximum residue levels (MRLs). In the EU, toxicokinetic and metabolism studies are at present not required in REACH at any tonnage. For active substances (pesticides) in PPPs, however, such studies are and have since long been required according to Regulation (EU) No 283/2013, Regulation (EU) No 544/2011, and Council Directive 91/414/EEC. Chemicals including pesticides not any longer allowed in the EU may still be used elsewhere.

A surprisingly high number of substances interacted with (bound to) blood cells that are dominated by haemoglobin-rich red blood cells

(RBCs), resulting in a long $^{elim}t_{1/2}$. Blood plasma/serum often had shorter $^{elim}t_{1/2}$ than blood cells and other tissues/organs, Fig. 7 and Table A.4. If substances or their metabolites bind covalently to haemoglobin, $^{elim}t_{1/2}$ of RBCs or whole blood can be expected to be proportional to the RBC lifespan of approximately 60–65 days in rats (Derelanko, 1987) and 120 days in humans (Drenckhahn, 1988). Covalent binding to haemoglobin is known to occur also for other substances, e.g. acrylamide, glycidamide and Ethylene oxide. In fact, haemoglobin adducts are used as a biomonitoring marker of human acrylamide exposure (Vikstrom et al., 2012). Thus, haemoglobin appears to function as a scavenger for reactive substances.

One limitation with our study is that the substances making up the $^{elim}t_{1/2}$ data for one compartment (or administration) type vs. another type were necessarily not the same. For example, one substance may have been studied in just one or two compartments, whereas another substance was studied in a dozen different compartments (such a substance will contribute more in some types of analyses). No substance was studied in all compartments. With a high number of data points, however, this uncertainty decreases.

In the REACH legislation in the EU, the thresholds log $K_{ow} > 2$ and log $K_{oa} > 5$ are initial lipophilicity and volatility based screens for air-breathing organisms (ECHA Guidance R.11) (ECHA, 2017), initiating further bioaccumulation assessment. After experimental testing in aquatic species, substances with a BCF > 2000 are considered bioaccumulative (B) and with a BCF > 5000 very bioaccumulative (vB). REACH allows use of other information such as toxicokinetic data (various species) in B assessment. Since our analysis shows that 64.9% of the substances in MamTKDB 1.0 have log $K_{oa} > 5$ and log $K_{ow} > 2$ values (present B screening criteria) it is possible that these thresholds are somewhat too inclusive, meaning that few chemicals are removed by performing this screen. It has previously been pointed out that there are thousands of chemicals fulfilling these criteria (Gobas et al., 2009; Gobas et al., 2003). What can be proper criteria is complicated by that there are substances (e.g. PFAS out of which many have log $K_{oa} < 5$) known to bioaccumulate in humans that are not highly lipophilic and do not bioaccumulate in fish or rodents. Rather than using log K_{ow} as one of the criteria, log $D_{7.4}$, which takes ionization affected substances into account (log K_{ow} is for un-ionized chemicals), could be considered. For substances that ionize, the pH can affect their charge and thereby their lipophilicity, as well as interaction with membrane transporters. However, for PFAS, setting appropriate thresholds based on log $D_{7.4}$ is also challenging as shown in Fig. 3.

The hereby discussed whole body-based B thresholds of 70 (humans) and 17 (rats) days have not been approved in any way and there are presently no accepted thresholds. For discussion of eventual thresholds, our compartmental analyses indicate that if the blood plasma/serum (or whole body excretion based) $^{elim}t_{1/2}$ would be 17 (rat) or 70 (human) days, then other compartments can be expected to have even longer $^{elim}t_{1/2}$ due to entrapment inside cells/organelles and binding to their components (i.e. membranes and proteins). Thus, it is possible that eventual future tissue/organ/cell-based B thresholds shall be allowed to be longer than for blood plasma/serum or whole-body excretion.

Guaranteeing no bioaccumulation in rats requires that 100% of the administrated radio-labelled dose is excreted/egested/exhaled within a reasonable timeframe. However, the reported elimination degree (%) of absorbed substance/metabolites from the body can, after having gone through several studies, often be somewhat uncertain as sometimes excretion of 105–110% of the administrated dose is claimed (which is impossible). This is possibly due to long homogenization, dilution or uncertain quantification e.g. of ^{14}C-radioactivity. Thus, statements that ">95% was excreted and no bioaccumulation can be assumed" shall be taken with some precaution as this may mean that ≈5–10% of the dose not accounted for remains somewhere in the body.

Bioaccumulation (when an organism absorbs a substance at a rate faster than that at which the substance is eliminated by metabolism or excretion/exhalation) of a parent substance or its eventual metabolites formed inside the body may not be occurring despite long $^{elim}t_{1/2}$ if the exposure or absorption/bioavailability (F) is low. Also, some xenobiotics (e.g. antioxidants) may act protective while others can be toxic, $^{elim}t_{1/2}$ is not a toxicity potency parameter.

For the future, a more systematic calculation (and reporting) of compartmental $^{elim}t_{1/2}$ in toxicokinetic studies will help building the database and allow better predictions of bioaccumulation potential of untested substances. When scrutinizing study summaries, we often encountered expressions like "the substance was excreted rapidly" without specifying an $^{elim}t_{1/2}$. Calculating $^{elim}t_{1/2}$ for the compartments studied should be encouraged or even required. We plan on integrating MamTKBD into the OECD QSAR Toolbox which supports read-across and quantitative structure analysis relationships (QSAR). The database can also be expanded with other relevant parameters such as CL, V_d and affinities for various macromolecules. In this first version of MamTKDB, no literature searches (e.g. for bioaccumulating substances) were performed. Thus, there are plenty of $^{elim}t_{1/2}$ still to be collected. Examples of other classes (in addition to PCBs and PFAS) of chemicals suspected of bioaccumulation are various polyhalogenated chemicals such as polychlorinated naphthalenes (POPs), paraffins, dechloranes, as well as lipophilic organo-metals.

There is always a risk that substances accumulate in tissues that are normally not analyzed (e.g. the eye or in an endocrine organ) in ADME studies. If all organs/tissues need to be checked if mother substance and metabolites remain in them, then autoradiography may be suitable. Homogenization of all ≈70–80 organs/tissues in the body followed by LSC analysis is cumbersome and can also be difficult for tough tissues such as bone. Along with that repeated administration studies often are more appropriate than single (for which often both low and high dose studies are performed) or preconditioning, an alternative way to assure that bioaccumulation does not occur in any of the many organs/tissues could be to only perform one repeated low/medium dose administration study using just a few animals of each sex with a ^{14}C-labeled substance and check the tissue radioactivity after a certain time (e.g. after 14 days) post-dosing using quantitative whole-body autoradiography. This would allow faster investigation of more tissues. To check that the methodology is working, one animal could be given a positive control ^{14}C-labeled substance known to bioaccumulate in a parallel experiment. To obtain an $^{elim}t_{1/2}$, however, some animals would also need to be sacrificed during at least two time-points post-dosing. For repeated administration studies, a faster establishment of steady state in blood plasma could possibly be achieved by giving a proper loading dose based on V_d (can be calculated from a single bolus i.v. dose) and the body weight (Birkett and Prescriber, 2009).

Measuring xenobiotics in tissue/organs from donors or deceased individuals of various ages is rarely done (few studies available) although this could demonstrate bioaccumulation in humans. Organ/tissue levels in wild predatory terrestrial mammals can also be relevant.

5. Conclusion

For most substances, single or preconditioning administration is insufficient in terms of assessing bioaccumulation potential in animals and repeated dose studies are necessary. Following declining concentrations in organs/tissues is often more relevant than blood plasma/serum which often underrepresents $^{elim}t_{1/2}$ in organs/tissues and provides no information over from which tissue(s) the substance (or radiolabelled metabolites) are released, nor what their concentrations are or have been (high concentrations may be required for toxicity). However, following the elimination in blood is relevant for substances with a high blood distribution such as PFAS. Rat, the standard animal model, is not always appropriate for ADME studies. Assessment of the bioaccumulation potential in air-breathing mammals will benefit from an integration of various lines of evidence, and $^{elim}t_{1/2}$ data should be considered as one of them.

CRediT authorship contribution statement

Tim Hofer: Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Oddvar Myhre:** Data curation, Writing - review & editing. **Johanna Peltola-Thies:** Project administration, Writing - review & editing. **Doris Hirmann:** Data curation, Project administration, Writing - review & editing.

Declaration of Competing Interest

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

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Disclaimer

The views expressed in this article are solely those of the authors and the content of the paper does not represent the views or positions of ECHA.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106592.

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