Biodegradation in seawater of PAH and alkylphenols from produced water of a North Sea platform

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ABSTRACT

Operational planned discharges of produced water (PW) to the marine environment from offshore oil production installations, contain low concentrations of dispersed oil compounds, like polycyclic aromatic hydrocarbons (PAH) and alkylated phenols (APs). Biotransformation in natural seawater (SW) of naphthalene/PAH and phenol/AP in field-collected PW from a North Sea platform was investigated in this biodegradation study. The PW was diluted in SW from a Norwegian fjord, and the biodegradation study was performed in slowly rotating carousels at environmental conditions (13^0C) over a period of 62 days. Naphthalene/PAH and phenol/AP biotransformation was determined by first-order rate kinetics, after normalization against the recalcitrant biomarker 17α(H),21β(H)-Hopane. The results from this study showed total biotransformation half-lives ranging from 10 to 19 days for groups of naphthalenes and PAH, while half-lives for APs (C0- to C9-alkylated) were 10 to 14 days. Biotransformation half-lives of single components ranged from 8 to >100 days for naphthalenes and PAHs (median 16 days), and from 6 to 72 days (median 15 days) for phenols and AP. Four of the tested PAHs (chrysene, benzo(b)fluoranthene, benzo(e)pyrene, benzo(g,h,i,)perylene) and one AP (4-*tert*-butylphenol) showed biotransformation half-lives >50 days. This is one of a few studies that has investigated the potential for biodegradation of PW in natural SW. Methods and data from this study may be used as a part of Risk Based Approaches (RBA) for assessments of environmental fate of PW released to the marine environment and as part of the persistence related to risk.

1. Introduction

Produced water (PW) from offshore oil and gas production installations is a mixture of formation water and re-injected water produced alongside oil and gas, and is the highest volume of liquid operational discharge generated during oil and gas production process (Neff et al., 2011, NOROG, 2016). The composition of PW can be complex and varies significantly between different oil fields and lifetime of the well (Røe Utvik, 1999; Neff et al., 2011). Before discharge, free oil and larger oil droplets are separated from the waste stream by oil/water separation processes, intended to lower the average concentration of dispersed and dissolved oil to a level permitted by the appropriate regulating authority. In 2015 the average oil concentration in PW released from activities on the Norwegian Continental Shelf (NCS) was 12.3 mg/ L (NOROG, 2016), compared to the discharge limit of 30 mg/L set by the Oslo-Paris Commision (OSPAR) for the Protection of the Marine Environment of the North-East Atlantic (OSPAR, 2001). Once discharged, PW rapidly mixes with natural seawater and undergoes biodegradation, reducing the levels of organic components, thereby also reducing potential exposure levels (Neff et al., 2011; Bakke et al., 2013).

The oil faction of PW, often referred to as "naturally occurring substances" (OSPAR, 2014), contain aromatic compounds of environmental concern, particularly polycyclic aromatic hydrocarbons (PAHs) and alkylated phenols (APs) (Fakhru'l-Razi et al., 2009; Bakke et al., 2013; Zheng et al., 2016). Among these compounds, 2- and 3-ring PAHs and less alkylated (C1-C3) APs are normally quantitatively dominant, whereas the 4- to 6-ring PAHs and C4-C9 APs are present at lower concentrations (Beyer et al., 2012; Bakke et al., 2013). The PAH and AP compounds are primarily distributed as dissolved or oil-associated compounds, depending on their water solubility (Faksness et al., 2004). Some of these compounds may bioaccumulate in organisms, which can cause adverse biological effects (Tollefsen et al.,

2007; OSPAR, 2009; Meier et al., 2011; Beyer et al., 2012). Even though potentially bioaccumulating compounds are usually only present in low concentrations, annual volumes of 130-150 million standard m^3 of PW are released to the sea from offshore installations on the NCS (NOROG, 2016).

However, petrogenic PW compounds discharged to the marine environment are subject to several transformation and depletion processes, including evaporation, photo-oxidation and biodegradation (NRC, 2003). Biodegradation is the only of these processes that have the capacity to completely mineralize these compounds, thus removing these compounds from the environment (Atlas, 1995). Depending on waste treatment technologies at the offshore installation, oil droplets larger than 5 µm may be removed during PW treatment (Nasiri and Jafari, 2017). This is of importance for biodegradation processes, since small oil-droplet dispersions have been shown to result in efficient microbial degradation of oil-associated hydrocarbons, because of the high surface to volume ratios of small oil droplets (Venosa and Holder, 2007; Prince et al., 2013; Brakstad et al., 2015a). Oil compound biodegradation in the marine water column is associated with hydrocarbonoclastic bacteria (Yakimov et al., 2007), and degradation pathways are conducted in successional patterns of microbial communities (Dubinsky et al., 2013; Brakstad et al., 2015b; King et al., 2015).

Despite the vast amount of research on oil biodegradation, few studies have focused on the hydrocarbon biodegradation after release of PW to the marine environment. In this study, we report biodegradation results from field-collected PW samples from a North Sea production platform. In the laboratory, the PW was diluted in natural, uncontaminated seawater from a Norwegian Fjord and incubated in a carousel system developed for biodegradation studies of dispersed oil (Brakstad et al., 2015a).

The purpose of this study was to investigate biodegradation and persistency of PAH and APs in a PW from the NCS. Such investigations can contribute to identifications of

environmental concentrations and persistence of PW compounds. Data can further be used in a Risk Based Approach (RBA) for prioritising mitigation actions on those discharges and compounds that pose the greatest risk to the environment (OSPAR, 2012).

2. Materials and Methods

2.1. Produced water and seawater used in experiments

PW was collected from the Ula Platform in the North Sea (57°6′41”N, 2°50′50”E) on October 5, 2015. The PW was shipped to SINTEF in 5-gallon Teflon liners (Welch Fluorocarbon, Dover, NH) packed in 30-L steel drums with lever locking rings (Air Sea Containers Ltd., Birkenhead, UK). Particle content was measured by Coulter Counter, and the total extractable organic carbon (TEOC) with GC-FID. 50 ml of the PW was centrifuged (3000 rpm; 1 min.) for analysis of dissolved and particulate associated hydrocarbons.

Seawater (SW) was collected from a depth of 80 meter in a Norwegian fjord (Trondheimsfjord; 63°26′N, 10°23′E). The SW is transported through a continuous flow pipeline system to the laboratory facilities of SINTEF Ocean. The inlet of the SW pipeline is below the thermocline, and the water is non-polluted and not influenced by seasonal variations. The salinity of the SW was 34‰, with a water temperature of 6–8°C and dissolved oxygen (DO) of 7-8 mg/L when reaching the laboratory. The SW was acclimated at 13°C for 2 days prior to use and was not amended with nutrients prior to be biodegradation experiment.

2.2 Biodegradation experiment

A biodegradation experiment was performed in a carousel system as described by (Brakstad et al., 2015a). Immediately after arrival to the laboratory, the PW was diluted in

acclimated (13^0C), non-amended SW to a final concentration of 14 mg/L TEOC (see below). The diluted PW was distributed in baked (450°C; 3 hrs.) and autoclaved (121°C; 29 min.) 2 L flasks (Schott). The flasks were filled (no headspace or air bubbles), closed with screw tops having silicon seals (Duran), and mounted on the carousel system at 13°C with slow continuous rotation (0.75 r.p.m). Sterilized controls were prepared by diluting PW in sterile-filtered SW (0.2 µm exclusion limit) and supplied with 100 mg/L (final concentrations) of $HgCl_2$, were also mounted in the carousel system and incubated (13°C). In addition, experimental blanks of acclimated non-amended SW (no PW) were included (experimental blanks). The biodegradation experiment was performed in darkness at 13°C over a period of 62 days. Triplicate samples were sacrificed for analysis after 20 min on the carousel (0-day samples), and after 7, 14, 28, 42, 51 and 62 days of incubation. One experimental blank and one flask with sterilized control were also collected at each sampling day. Sample flask were half-changed with acclimated (13°C) unfiltered or sterilized after sampling at day 7, 14 and 28 of incubation, to avoid anoxic conditions in the system. During the half-changes, the flasks were completely filled to avoid air-bubbles in the systems.

2.3 Analyses

2.3.1 Oil droplet analyses

Particle concentrations and size distributions were determined by Coulter Counter measurements (Beckman Multisizer 4; Beckman Coulter Inc., Brea, CA, U.S.A) fitted with either 100 µm or 280 µm apertures, for measurement of droplets within a diameter range of 2-60 µm or 5.6-100 µm, respectively. Filtered (0.22 µm) SW was used as electrolyte. All droplet sizes reported here are expressed as median droplet diameter if not otherwise mentioned.

2.3.2 Chemical analyses

Samples were solvent-solvent extracted with dichloromethane (DCM) for measurements of semivolatile organic compounds (SVOC) by gas chromatographic methods. TEOC analyses were performed by a gas chromatograph coupled to a flame ionization detector (GC-FID; Agilent 6890N with 30 m DB1 column; Agilent Technologies). Target analyses of 29 naphthalenes/PAH, 36 phenol/APs, and the biomarker 17α(H),21β(H)-Hopane (30ab Hopane) were performed by a gas chromatograph coupled to a mass spectrometer (GC-MS; Agilent 6890 plus GC coupled with an Agilent 5973 MSD detector, operated in Selected Ion Monitoring [SIM] modus; Agilent Technologies), as previously described (Brakstad et al., 2014; Brakstad et al., 2015a). The target compounds are shown in Table S1A and B. The response values for individual target analytes were determined, with a signal-to-noise ratio of 10 as the lower detection limit, and a lower limit of detection (LOD) of 0.01 µg/L was defined for individual oil compounds.

2.3.3 Microbial analyses

Microbial cells were enumerated in all samples using epifluorescence microscopy. Samples were stained with the nucleic acid stain 4,6- diamidino-2-phenylindol (DAPI) (Porter and Feig, 1980).

Concentrations of viable heterotrophic microbes (HM) and oil degrading microbes (ODM) were determined in the dispersions by most-probable number (MPN) quantification in 24-well cell culture plates, using a modified version of sheen-screen method by Brown and Braddock (1990). MPN enumeration of HM were preformed using a Marine Broth 2216 medium (Difco). Concentrations of ODM were determined in marine Busnell-Haas broth

supplemented with NaCl (30g/L), 20 µl crude Ula oil were added to each well. Plates were incubated at 13°C for 7 days for HM and 14 days for ODM.

2.3.4 Other analyses

Dissolved oxygen (DO) was measured by a dissolved oxygen meter (YSI, Inc., Yellow Springs, OH).

2.4 Calculations and statistics

For determination of biotransformation, concentrations of targeted compounds/compound groups were normalized against 30ab Hopane, as recommended by Prince et al. (1994). Depletion was determined as a) the percentage normalized concentrations at each sample of the mean normalized concentration at start of the experiment (C_0), or b) as percentage normalized concentration in unfiltered SW of normalized concentration in sterilized SW sampled at the same day. Biotransformation kinetics of normalized data were determined by non-linear regression analyses, using the option "plateau followed by one-phase exponential decay" in GraphPad Prism vs. 6.0 (GraphPad Software Inc., La Jolla, CA), including lag-periods (plateau) and rate coefficients ($k1$). Biotransformation total half-lives ($t_{1/2}$) were determined as the sum of the lag-periods and the half-lives determined from the rate coefficients ($0.693/k1$).

3. Results and Discussion

3.1 PW characteristics

PW from the Ula platform was analysed for particle size distribution and concentration by Coulter Counter (CC) analyses and GC-FID. CC measurements showed a particle concentration of 14.1±2.2 mg/L, with a median size of 7.4±0.3 µm. GC-FID analyses of solvent extracts showed a TEOC concentrations of 24.44 mg/L in the PW (Table 1). GC-MS analyses of the PW extracts showed that measured aromatic compounds constituted 23.1 % of the TEOC, distributed between 3.73 % naphthalenes, 1.12 % 2-6-ring PAH, 18.09 % C0-C3 APs and 0.12% C4-C9 APs (Table 1). These measurements showed that the relative concentrations of the quantified component groups were in the same order of magnitude as previously observed in PW from other oil fields at the NCS (Røe Utvik, 1999). The high content of APs, primarily as C0-C3 APs in PW has been reported in several studies (Boitsov et al., 2007, Røe Utvik, 1999). The major part of the TEOC not quantified by the GC-MS analyses, was probably constituted by semivolatile saturates. In addition, PW usually contains high concentrations of small organic acids and monoaromatic hydrocarbons (Røe Utvik, 1999; Neff et al., 2011) that were not quantified as part of this study.

Centrifugation of the PW (3000 r.p.m.) revealed a pelleted fraction, showing that the dispersion was not predominated by oil droplets, but mainly by small mineral particles originating from the reservoir (Fig. 1). Comparison of particle and TEOC concentrations inferred that a considerable part of the extractable organic material was not present as oil droplets but associated with particles. Ula is a sandstone reservoir with micro-quartz grain coating of very small grain size, in addition to clay grain coats like illite and chlorite (Niazi, 2011). GC-FID chromatograms of the pellet and supernatant after centrifugation also showed that low-boiling point compounds, were dissolved in the water fraction, while most of the

poorly water-soluble *n*-alkanes were present in the pellet fractions attached to the sedimented particles (Fig. 1).

Oil droplets can interact with mineral particles by adsorption of hydrocarbons, or by direct aggregation between oil and the particles, where less soluble oil compounds have stronger sorption affinity (Gong et al., 2014; Yu et al., 2006). Thus, low solubility *n*-alkanes will have better affinity for the mineral particles than the more soluble naphthalenes and small PAHs.

3.2 Aggregation of PW particles in natural seawater

During the biodegradation experiment in the carousel system the sizes of particles within the Coulter Counter measuring range (2-100 μm) and were found to increase gradually during incubation from 4.7±0.22 μm (day 14) to 15.0±6.58μm (day 51). The size distribution (3.7 to 4.66 μm) was maintained in sterilized controls throughout the experiment (Fig. S1). From day 14 aggregation of dispersed PW particles was observed. Aggregates appeared in a granular macroscopic form in the natural unfiltered SW, but not in the sterilized controls (Fig. S2). These macroscopic aggregates showed high densities, settled rapidly, and persisted throughout the experiment. Microscope analyses revealed microbial attachment to the aggregates (Fig. 2), and increasing numbers of microbes attached to the aggregates by time. Aggregation also appeared late in the 62-day experiment in sterilized control, but to a lesser extent, generating smaller particles with lower sinking velocities than in natural SW. This result reveals that aggregation was mainly related to biological activities.

Since a significant part of the oil was attached to the PW particles (Fig. 1), we assume that the macroscopic, fast sinking, aggregations observed in our experiments were related to the presence of small oil-mineral aggregates (OMA; Stoffyn-Egli and Lee, 2002). Due to the

slower formation of macroscopic aggregate formation in the sterilized controls (Fig. S2), and the attachment of microbes to the aggregates in biotic samples (Fig. 2), it is likely that formation of large aggregates is mediated by microbial activity, rather than physical interactions between oil and mineral particles alone. Water turbulence (wave actions and water currents) and low to intermediate salinity water may promote OMA generation, reduce re-coalescence and increase biodegradation (Lee et al., 1996). Suspended minerals, like illite, are known to be incorporated into organic aggregates working as ballast increasing the settling speed of the structures (Passow and De La Rocha, 2006). Whether the aggregation observed in our studies is a laboratory phenomenon or not, is not known. PW rapidly dilutes after discharge in the environment, and sediment particle concentrations of at least 10 mg/L has been suggested for significant oil deposition in the environment (Boehm et al., 1987).

3.3 Dissolved oxygen

The carousel system used in the current study was designed for maintaining dispersions or suspensions during long-term biodegradation periods (Brakstad et al., 2015a). By using flasks without headspace, oil droplets will not surface braking the droplets, and evaporation of organic analytes or dissolution of oxygen is avoided. DO was depleted in the flasks with PW diluted in natural SW with a measured TEOC concentration of 6.86 ± 0.47 mg/L, but not in sterilized and blank controls (Fig. S3). Because of the rapid DO depletion, SW half-changes were performed after 7, 14 and 28 days of incubation, to replenish consumed DO. Between days 0 and 7, DO concentrations were reduced by 71%. DO concentration were again reduced by 95% between the half-change at day 7 and sampling at day 14 (Fig. S3). In the same time period the TEOC concentrations were reduced to 2.80±0.21 mg/L. Previous biodegradation studies with the same Trondheimsfjord seawater inoculum

with dispersed crude oil (2-4 mg/L) resulted in 40-60 % DO saturation at the end of 64-day long experiments (Brakstad et al., 2015a). However, the repeated DO depletion experienced during the PW biodegradation experiment probably resulted from rapid biodegradation of small organic acids. They are known to rapidly biodegradable in natural sea water, and may exceed TEOC concentrations by 10 to >20 times (Røe Utvik, 1999). To correct for the repeated SW dilution, biotransformation was determined by normalization of targeted PAH and AP compounds against the recalcitrant biomarker 30ab Hopane (Prince et al., 1994). Biodegradation of volatile monoaromatic hydrocarbons may also have contributed to the observed rapid oxygen consumption, since these may appear in similar or higher concentrations to PAH (Neff et al., 2011) with comparable biodegradation rates to naphthalenes and 2- to 3-ring PAH (Brakstad and al., 2015a).

3.4 Microbial concentrations and PW compound group depletion

The particle attachment of the microbial cells (Fig. 2) complicated total cell counts by epifluorescence microscopy, and microbial concentrations were therefore determined by MPN counts. The results showed that concentrations of both heterotrophic and oil-degrading microbes increased rapidly in PW water samples and peaked after 14 days of incubation (Fig. 3), with factors of more than 1000 times higher concentrations than in SW blanks (Fig. S4A).

Although depletion of TEOC (49%) and aromatics (62%; sums of naphthalenes, PAH and AP) was also found in sterilized controls at the end of the experiment, depletion of TEOC and aromatics were much faster in unfiltered SW (Fig. S4 B and C). The depletion of these compound groups in the natural SW was therefore the result primarily of biotransformation. This was further substantiated by correcting depletion in natural SW for the depletion in sterilized controls, resulting in similar results for non-corrected and corrected TEOC and

aromatic depletion (Fig. S4 B and C). The reason for the decline of TEOC and aromatics measured in sterilized controls is not known. Hydrocarbons may attach to glass walls, and glass walls and seals were rinsed by solvent (DCM) to include material attached to glass flask surfaces in the analyses (Brakstad et al., 2015a).

Comparison of depletion of PW component groups and MPN quantification showed that the peak HM and ODM concentrations were associated with the early biodegradation of TEOC and aromatics. Normalized concentrations of aromatics were decreased from start of the experiment (day 0) by 5±13% at day 7 to 73±5 % after day 14. This was the period when the MPN concentrations increased most (Fig. 3). These data are in agreement with recent studies of biodegradation of several chemically dispersed oils at 13°C, in which microbial stimulation between days 7 and 14 coincided with >80 % *n*-alkane and PAH biotransformation in the same period (Brakstad et al., 2018).

3.5 Biotransformation of PW groups

Since potential environmental impacts from naturally occurring organic compounds in PW has been associated with PAH and AP (OSPAR, 2014), we focused on biotransformation of these targeted semivolatile aromatic compounds. To determine biotransformation, the depletion in the natural SW was not corrected for the non-biotic depletion in sterilized SW, since this had only negligible impacts on the depletion, as shown for TEOC and aromatics (Fig. S4 B and C). Both phenols and naphthalenes/PAHs showed non-responsive lag-periods before biotransformation (Fig. 4). The total half-lives determined (sum of lag-periods and half-lives determined from rate coefficients) of the PAH increased with higher molecular size of the compounds. However, for the phenol groups, the half-lives were relatively short, ranging from 10 to 14 days (Fig. 4). Correspondingly, naphthalene half-lives were in the same

range as for the phenols (approximately 10 days), while 2- to 3-ring and 4- to 6-ring PAH half-lives were 17-43 days (Fig. 4). Lag periods, half-lives and biotransformation rate coefficients of the different phenol and naphthalene/PAH groups are listed in Table S2. The PW naphthalene and PAH biotransformation lag-periods, rate coefficients and half-lives determined in this study were comparable to previously reported experimental biodegradation data on small-droplet dispersions of crude oil at low concentrations (2-3 mg/L oil) in natural SW from the Trondheimsfjord at 4-13°C, or in Gulf of Mexico deep water (Brakstad et al., 2015a; Brakstad et al., 2018; Wang et al., 2016). However, corresponding biotransformation studies and data for fractions of alkylated phenols are not available in the literature to our knowledge, since phenols are only present in very low concentrations in crude oils.

3.6 Biotransformation and persistence of targeted PW compounds

Of 65 targeted naphthalene/PAH and phenol/AP compounds/compound groups measured by GC-MS analyses, 10 compounds (4 PAH and 6 AP) were detected below the LOD of 0.01µg/L in the PW (Table S1). In addition, concentrations of 8 compounds (1 PAH and 7 APs; Table S1) were reduced below LOD after 7-14 days of incubation, and these were not included for determination of biotransformation.

Biotransformation rate coefficients and half-lives were determined for 46 naphthalene/PAH (n=23) and phenol/AP (n=23) compounds/compound groups in the PW diluted in SW. Lag-periods, biotransformation rate coefficients and half-lives are shown in Table S3A and B. The total half-lives for these compounds are shown in Fig. 5, in which the lag-periods (X_0) are included. Total half-lives varied from 8 to >100 days (median 16 days) for naphthalenes/PAH, whereas AP half-lives ranged from 5 to 70 days (median 15 days). For comparison, total half-life of TEOC (dispersed oil) was calculated to be 18 days (Fig. 5, Table

316 S2). For 2- to 3-ring aromatic compounds, the increases of half-lives correlated with alkyl
317 substitution, as shown with naphthalenes, phenanthrenes and dibenzothiophenes. Several
318 biodegradation studies of dispersed crude oils in natural SW also have shown that PAH
319 biodegradation decreased by increased alkyl substitution (Brakstad et al., 2015a; Brakstad et
320 al., 2018; Douglas et al. 1996; Prince et al., 2013; Venosa and Holder, 2007; Wang et al.,
321 1998, Wang et al., 2016). Half-lives increased also with higher aromatic ring numbers; 8 days
322 for naphthalene, 12 days for phenanthrene, and 65 days for chrysene, while total half-lives of
323 benzo(b)fluoranthene, benzo(e)pyrene and benzo(g,h,i)perylene were judged to be >100
324 days. The naphthalene/PAH half-lives in PW were mainly comparable to total half-lives
325 determined for dispersed crude oils in experiments performed in natural Norwegian coastal or
326 Gulf of Mexico deep water at 13 or 5°C (Brakstad et al., 2015a, Brakstad et al, 2018; Wang et
327 al., 2016). The fact that the PAH biodegradation half-lives were comparable to results from
328 previous studies with low oil concentrations (2-3 mg/L), and with oxygen-saturated SW
329 throughout the experimental period, strongly indicate that the temporary oxygen depletions
330 experienced in the current study did not affect the PW compound biodegradation rates.

331 For the APs, relations between half-lives and alkyl substitution were not shown to be
332 as pronounced as for the naphthalenes/PAH. While half-lives of 2-methylphenol and 4-
333 methylphenol were 14 and 8 days, respectively, half-lives of 4-*n*-hexylphenol and 4-*n*-
334 heptylphenol were 5-8 days (Fig. 5B; Table S3). However, half-lives were increased for 4-*n*-
335 octylphenol (33 days) and 4-*n*-nonylphenol (24 days). The half-lives were longer for C4- to
336 C7- *tert*- substituted APs (19 to 70 days) than the C4- to C7- APs with linear alkyl chains
337 (total half-lives of 5 to 14 days), due to the steric hindrances by the quaternary carbon atoms
338 on initial beta-oxidation and subsequent lipid catabolism (Wang and Stout, 2010).

339 While coastal SW was used in these experiments, we have previously determined
340 biodegradation rates of *para*-cresol and 2,4-dimethylphenol, using surface SW from the Ula

field, showing biotransformation half-lives close to 7 days for both AP compounds (Brakstad and Almås, unpublished). This is mainly in agreement with the data in the current study, and we therefore do not consider the use of the Trondheimsfjord SW to result in significant biotransformation differences compared to SW from the original PW source.

Operational discharges to the North Sea from the offshore industry are regulated by the OSPAR, and persistent compounds are defined by half-lives in water of >50 days (OSPAR, 2005). The results of Fig. 5 showed that four 4- to 6-ring PAH (chrysene, benzo(b)fluoranthene, benzo(e)pyrene, benzo(g,h,i)perylene) were biotransformed with half-lives longer than 50 days, but only one AP (4-tert-butylphenol). However, it must be emphasized that the OSPAR criteria are based on mineralization (OSPAR, 2005; OECD, 2006), while our results are provided as biotransformation data. However, biotransformation of PAH and APs will results in degradation products with increased polarity. This will reduce the *n*-octanol-water partition coefficients (LogPow), compared to the original compounds. Thus, the risk of accumulation in marine organisms and the marine food web are reduced after the onset of biodegradation of most PW compounds. Increased polarity and reduced LogPow are also associated with reduced acute toxicity of PW compounds in the marine environment (French-McCay, 2002).

4. Conclusions

This is one of a few studies that have investigated biodegradation and persistency of oil compounds from PW diluted in SW. Concern has been raised that PW compounds may persist in the marine environment after discharge to SW. The results of his study also showed that naphthalene and PAH biotransformation rates in PW are comparable to measurements made in dispersed crude oil, where studies showed that half-lives increase with higher

numbers of aromatic rings and more complex alkyl-substitution. Potential environmental impacts from oil compounds from PW may therefore be primarily related to a restricted suite of high-molecular weight PAH compounds, or to APs with branched alkyl-substitution.

Since PAH and APs from PW have been associated with environmental concern, the biodegradation data presented will be a part of the fate processes defining the fate of these compounds related to risk. Biodegradation is the only process that may completely remove hazardous organic compounds through complete mineralization. Although only biotransformation was measured here, the initial degradation step results in increased polarity of the parent compounds, resulting in reduced logKow values of intermediates. Acute effect concentrations for individual compounds, described by EC_{50} or LC_{50} values, are often determined using regressions between (logKow) and $LogLC_{50}$ (French-McCay, 2002), resulting in reduced logKow and decreased acute toxicity during biodegradation. However, in some cases, metabolites of some PAHs and APs may be more toxic or persistent than the parent compound, emphasising the importance of more in-depth studies of biodegradation of environmental pollutants, with the focus of identifying such metabolites.

The aggregation effect shown in our laboratory study, which may contribute to transport of particles with attached oil compounds was possibly the result of processes similar to oil-mineral aggregation (OMA; Stoffyn-Egli et and Lee, 2002), which may further promote oil constituent biodegradation. Whether this aggregation was a laboratory phenomenon or not, could be further studied with dilution experiments, simulating the PW dilutions in SW after release.

The results from this study may be used in RBA for comparison of environmental concentrations to potential effect concentrations such as in the calculation of marine environmental impact analyses (Johnsen et al., 2000). Our new PW studies have demonstrated

389 the suitability of the carousel biodegradation technique, which is an advanced biodegradation
390 method (Brakstad et al., 2016), to quantify dispersed oil biodegradation rates and to generate
391 empirical data (e.g. biodegradation rates) on persistency. The biodegradation rates generated,
392 using this technique, can be implemented in regulatory models (e.g. DREAM; Reed and Rye,
393 2011). The biodegradation method described in the current study, using a whole effluent
394 approach for biodegradation measurements, may also be an important supplement to
395 standardized respirometric methods, determining mineralization of single compounds relevant
396 PW discharges. The method described may also be used as an assessment tool for the testing
397 of the persistence of PW substances. Taken into account the heterogeneity of PWs (Neff et al.,
398 2011; Røe Utvik, 1999), biodegradation studies of other PWs should be investigated to
399 strengthen the data of this study, and to include the other PW compounds not included in this
400 study (organic acids, saturates and volatile compounds).

401

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Tables

Table 1. Composition of TEOC, naphthalenes, PAH and phenols in Ula PW.

Component group	Concentration (mg/L)	% of total dispersed oil
TEOC	24.44	
Naphthalenes	0.911	3.73
PAH 2-3 rings	0.269	1.10
PAH 4-6 rings	0.005	0.02
Phenol C0-C3	4.420	18.09
Phenol C4-C5	0.026	0.11
Phenol C6-C9	0.003	0.01
		23.05

Figures

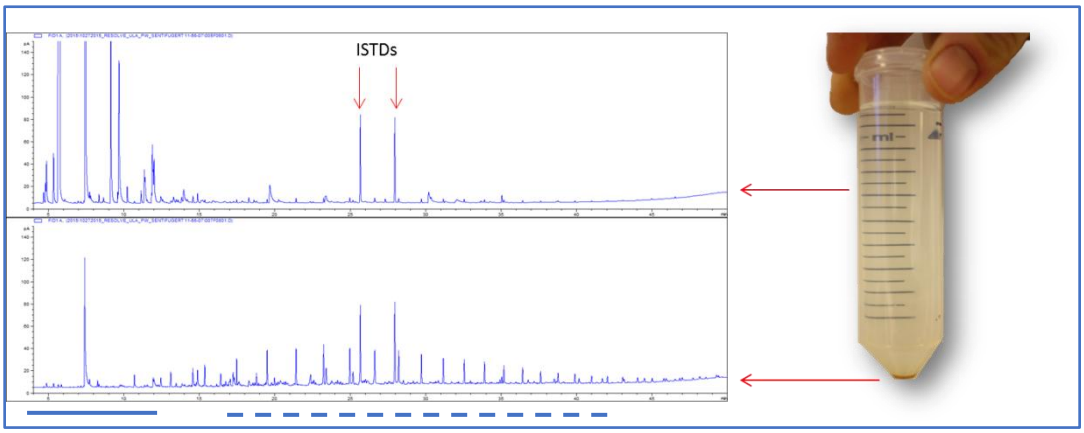

Fig. 1 Distribution of TEOC in the water and particle phase of Ula produced water. The unbroken line describes the chromatogram retention time (RT) associated with small extractable aromatic compounds (liquid phase), and the broken line the RT where *n*-alkanes are abundant as separate peaks (precipitate).

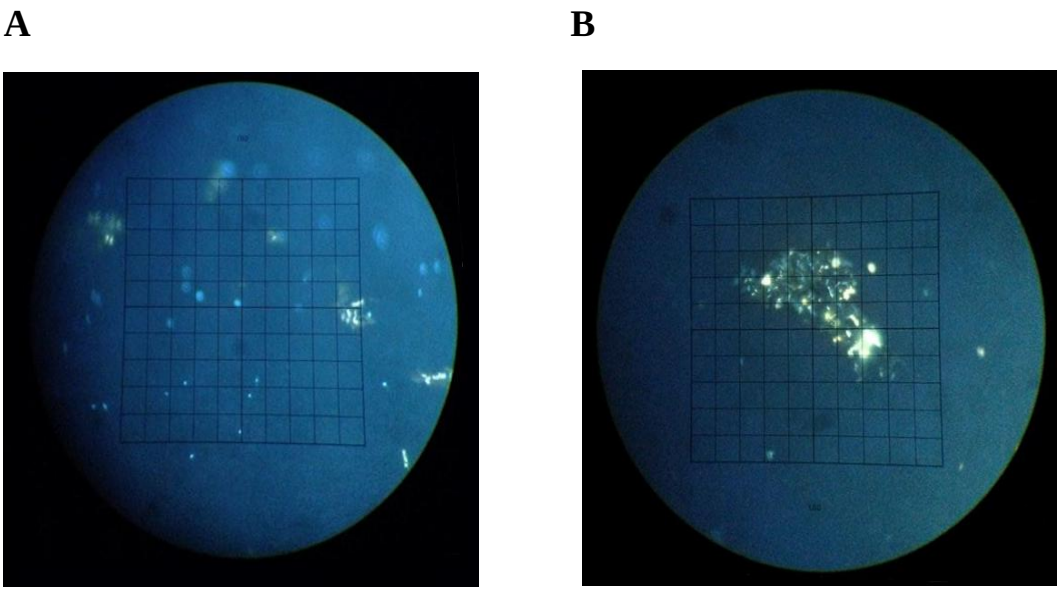

Fig. 2 Aggregates of microorganisms observed after day 7 (A) and day 14 (B) of the experiment. Microorganism are stain with DAPI, and illuminated using EPI fluorescence microscopy.

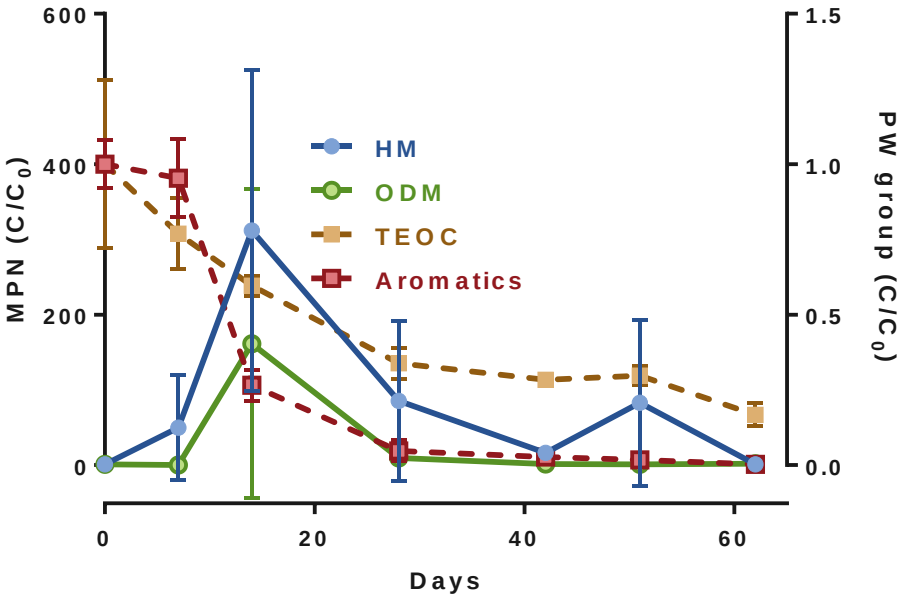

Fig. 3 MPN (HM and ODM), TEOC and aromatics determined as ratios between concentrations at different sampling dates (C) and day 0 (C_0). TEOC and aromatic concentrations were normalized against 30ab Hopane.

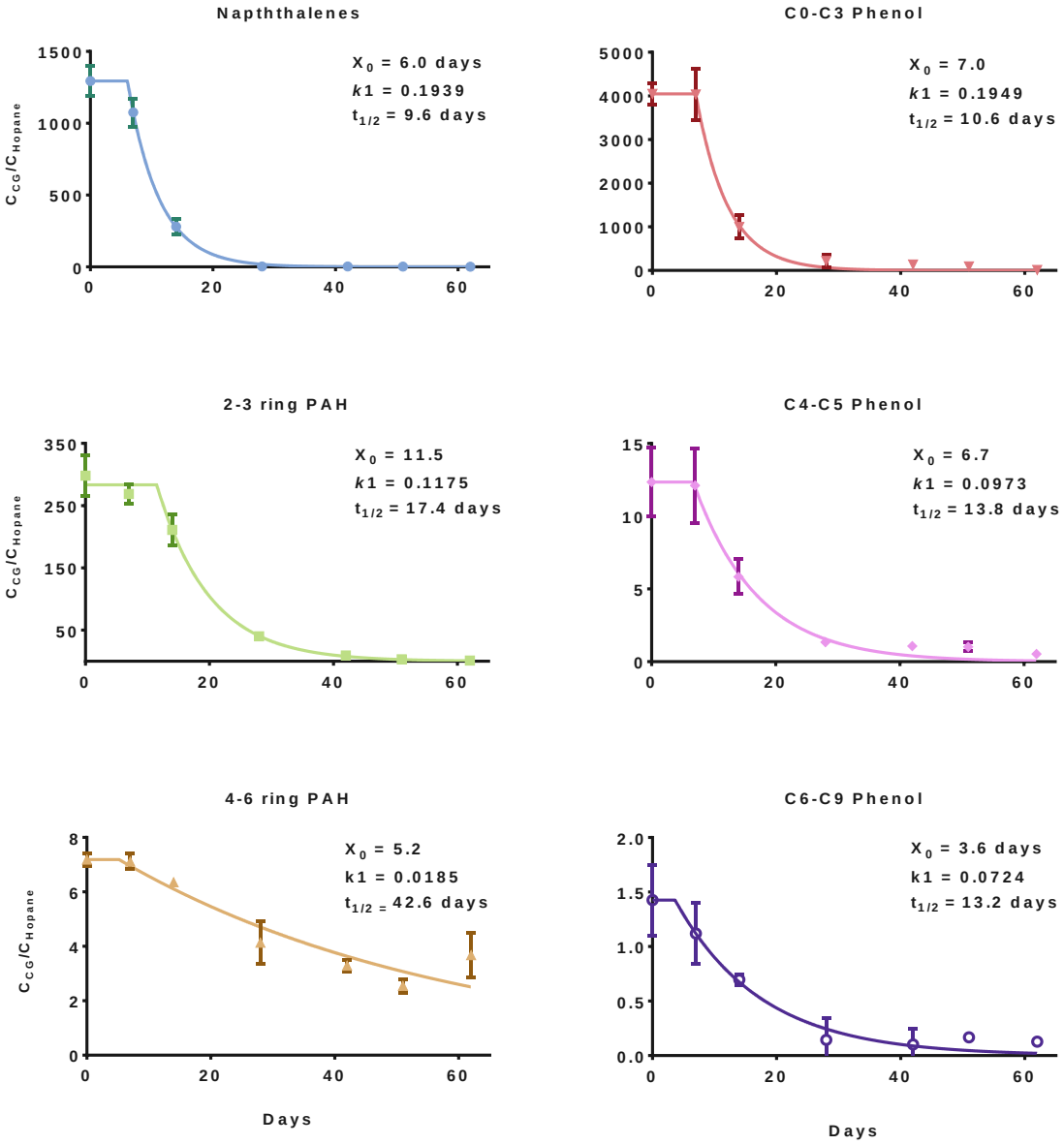

Fig. 4 First-order rate biotransformation curves of different component groups with lag-periods included. The results are shown as the ratios between compound groups (CG) concentrations and 30ab Hopane concentrations (C_{GC}/C_{Hopane}). The half-lives ($t_{1/2}$) shown are the sum of the lag-periods (X_0) and half-lives determined from the first-order rate coefficients ($k1$). Additional data are shown in Table S2.

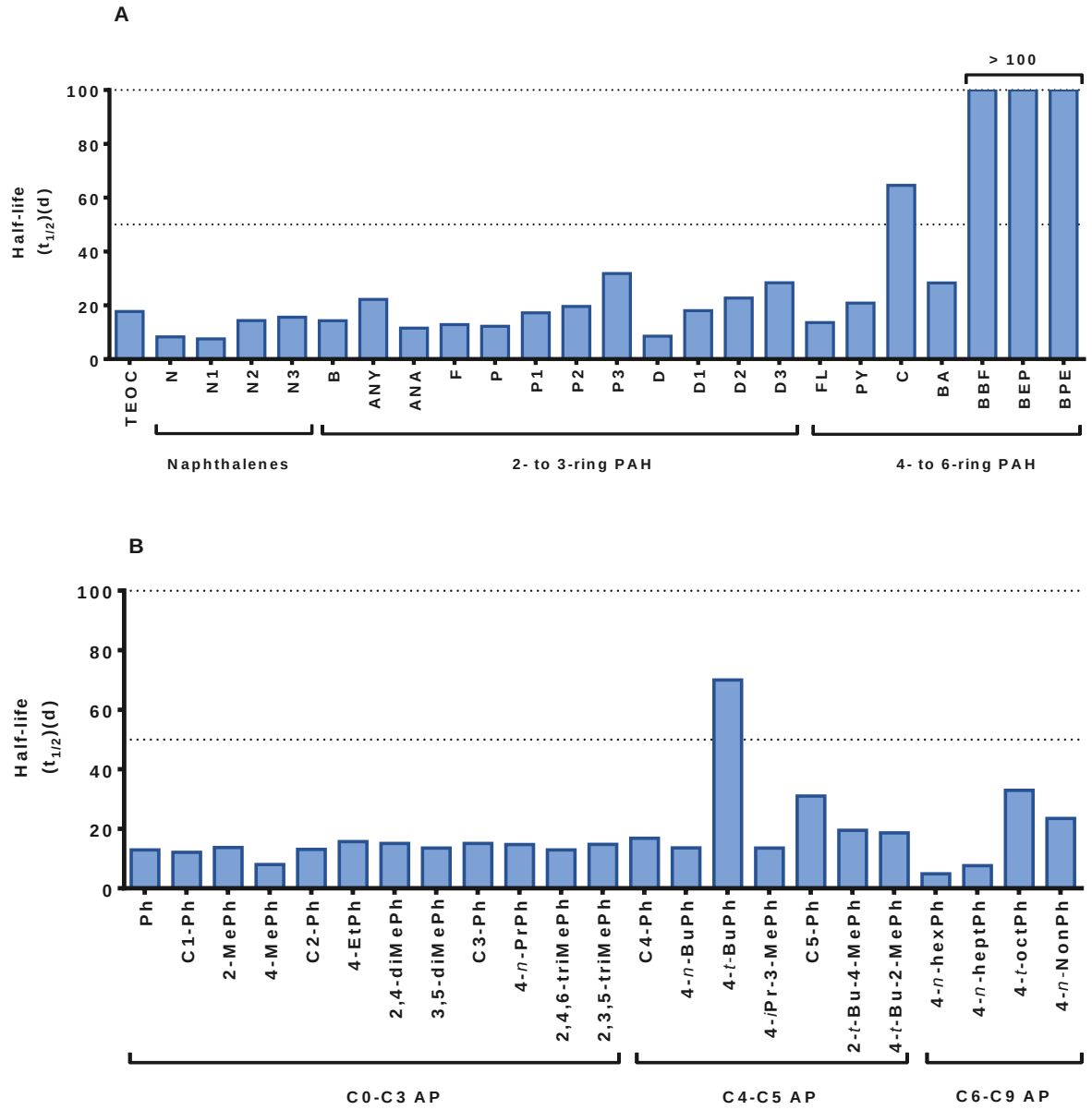

Fig. 5 Total biotransformation half-lives (including lag-periods) for TEOC, naphthalenes and PAH (A), and phenol and alkylated phenols (B), see also Table S3. The non-responsive lag-period (X_0) is included in the half-lives. Abbreviations are shown in Table S1.

Figure 1

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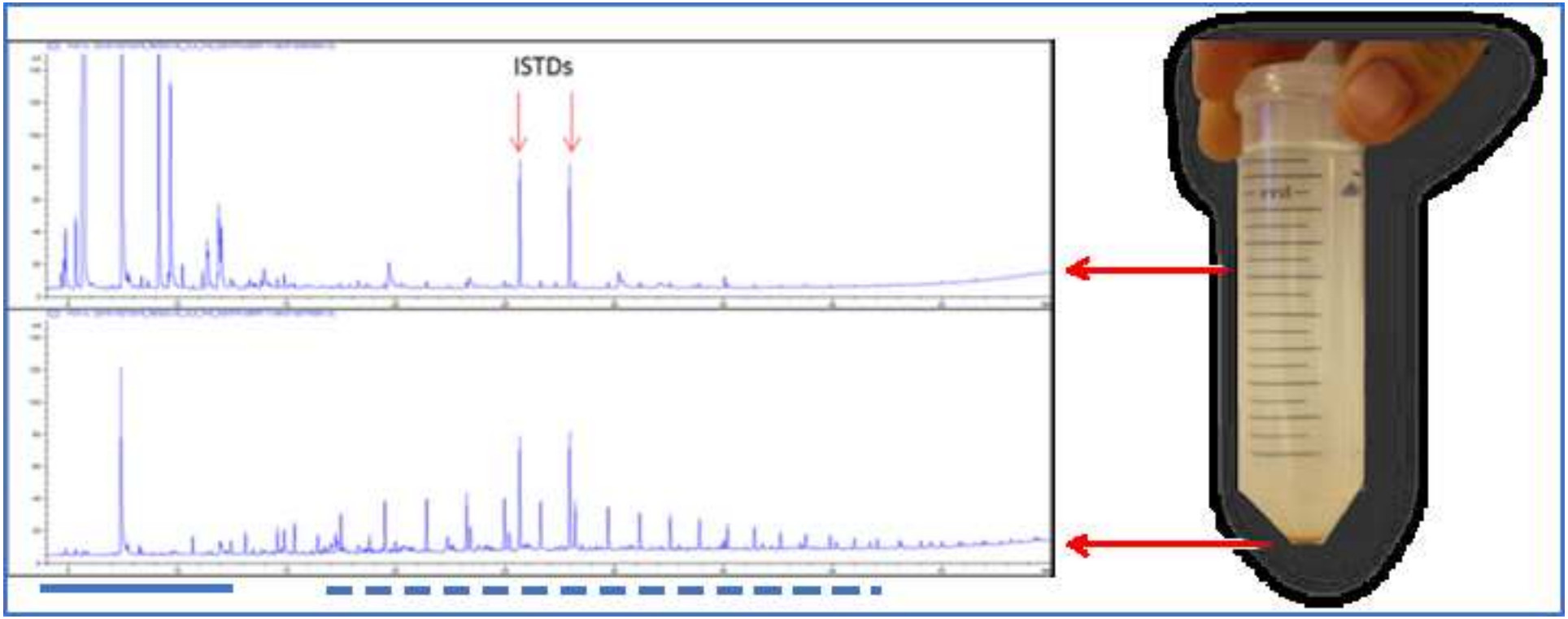

Figure 2

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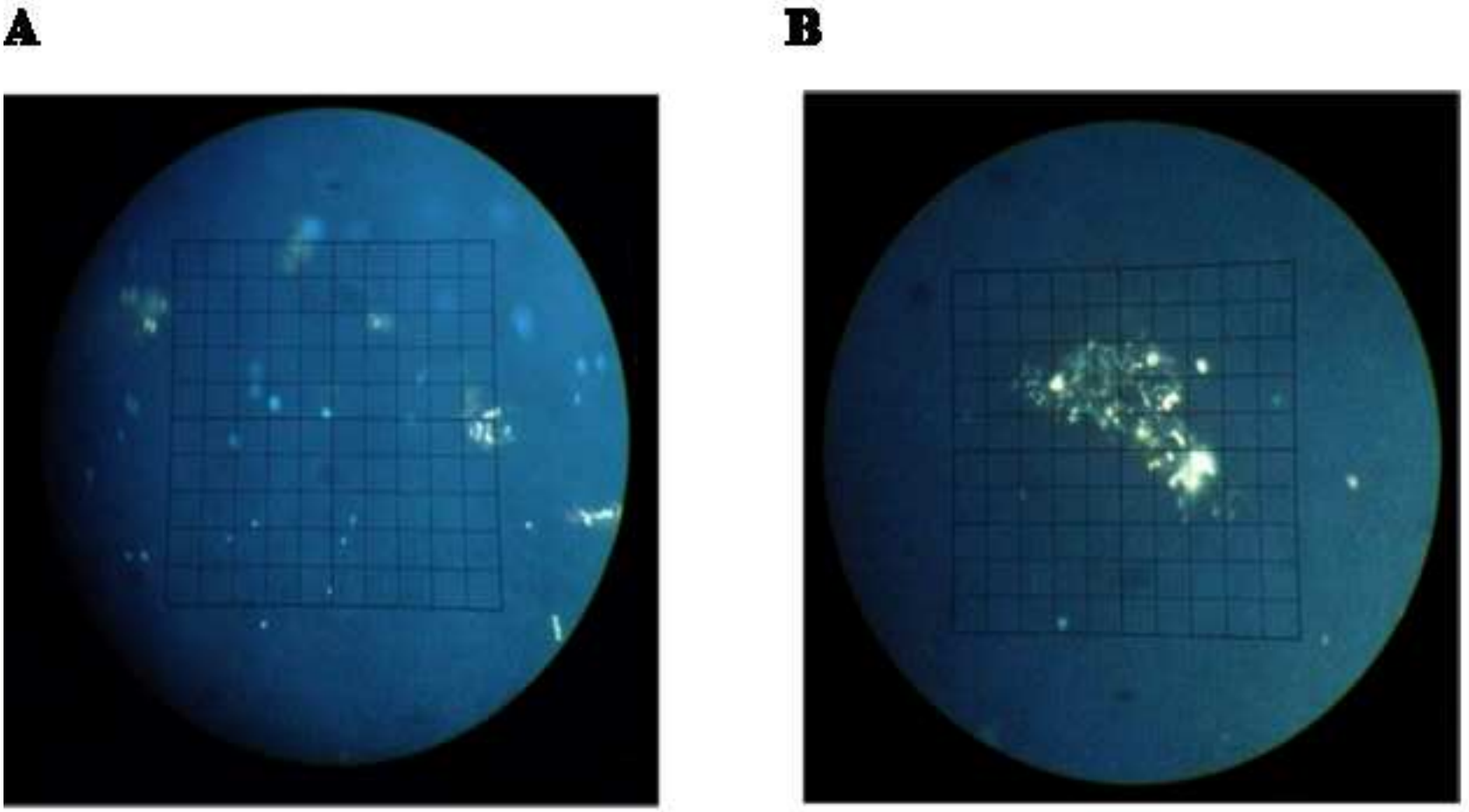

Figure 3

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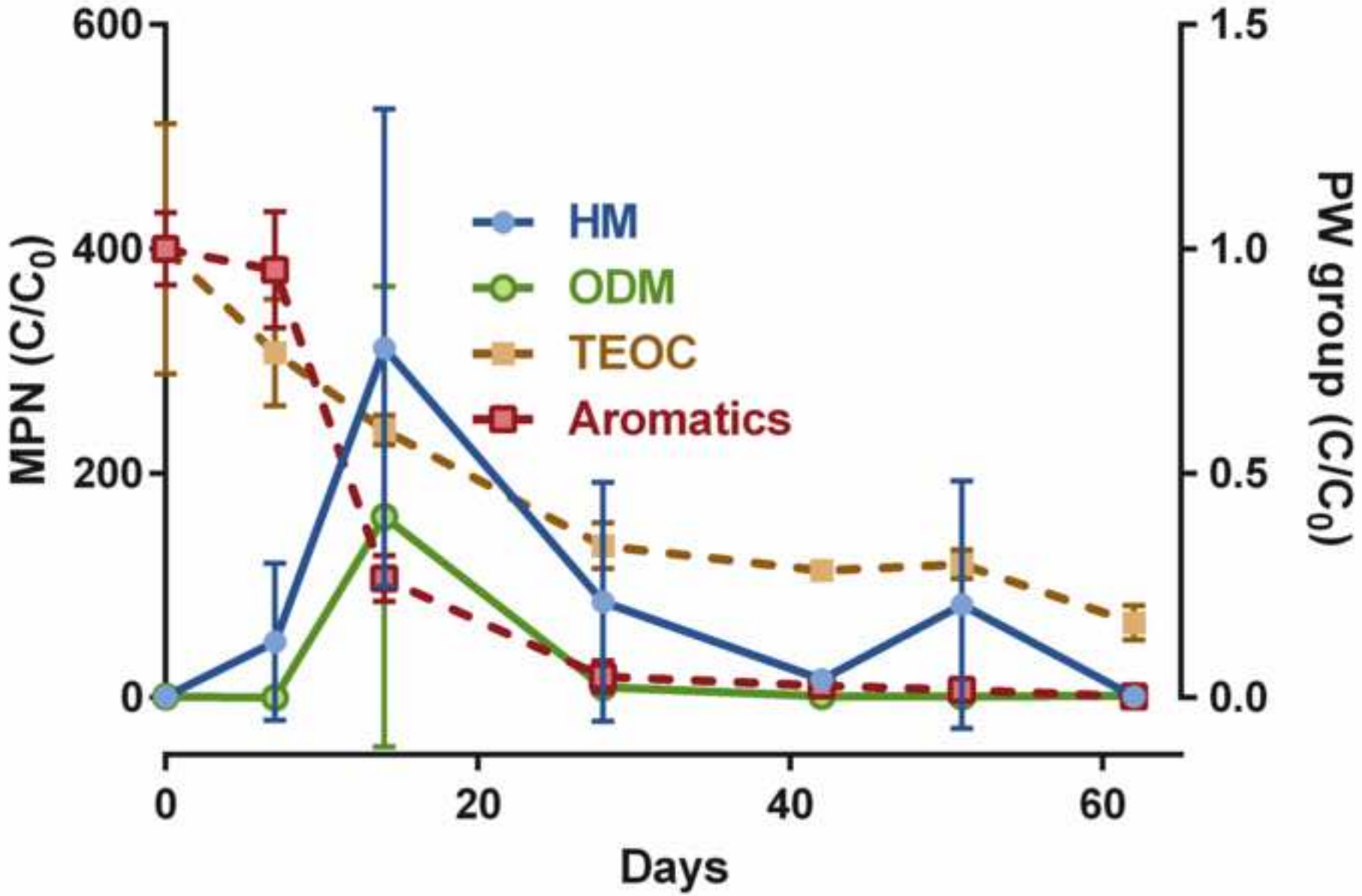

Figure 4

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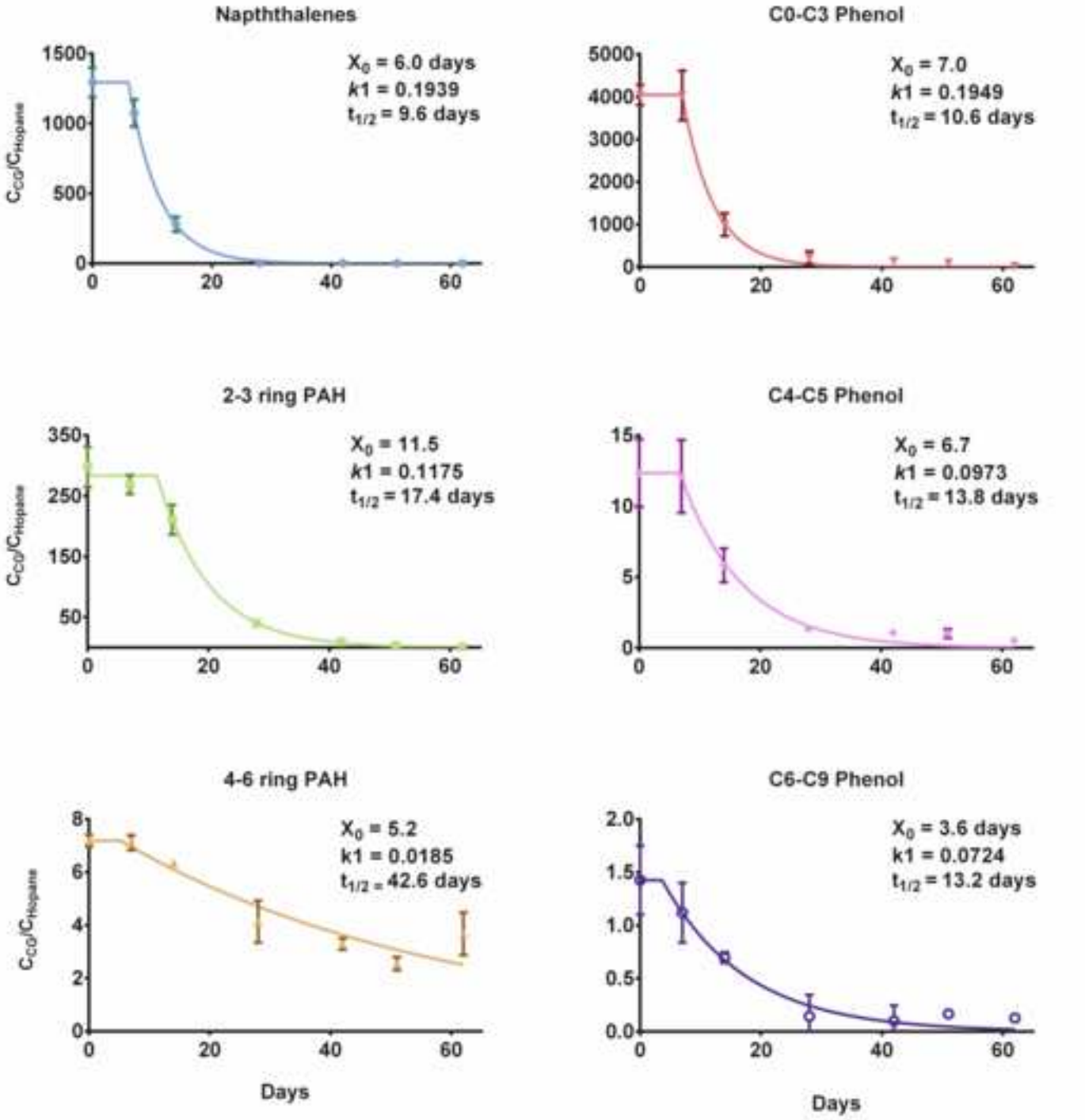

Figure5A

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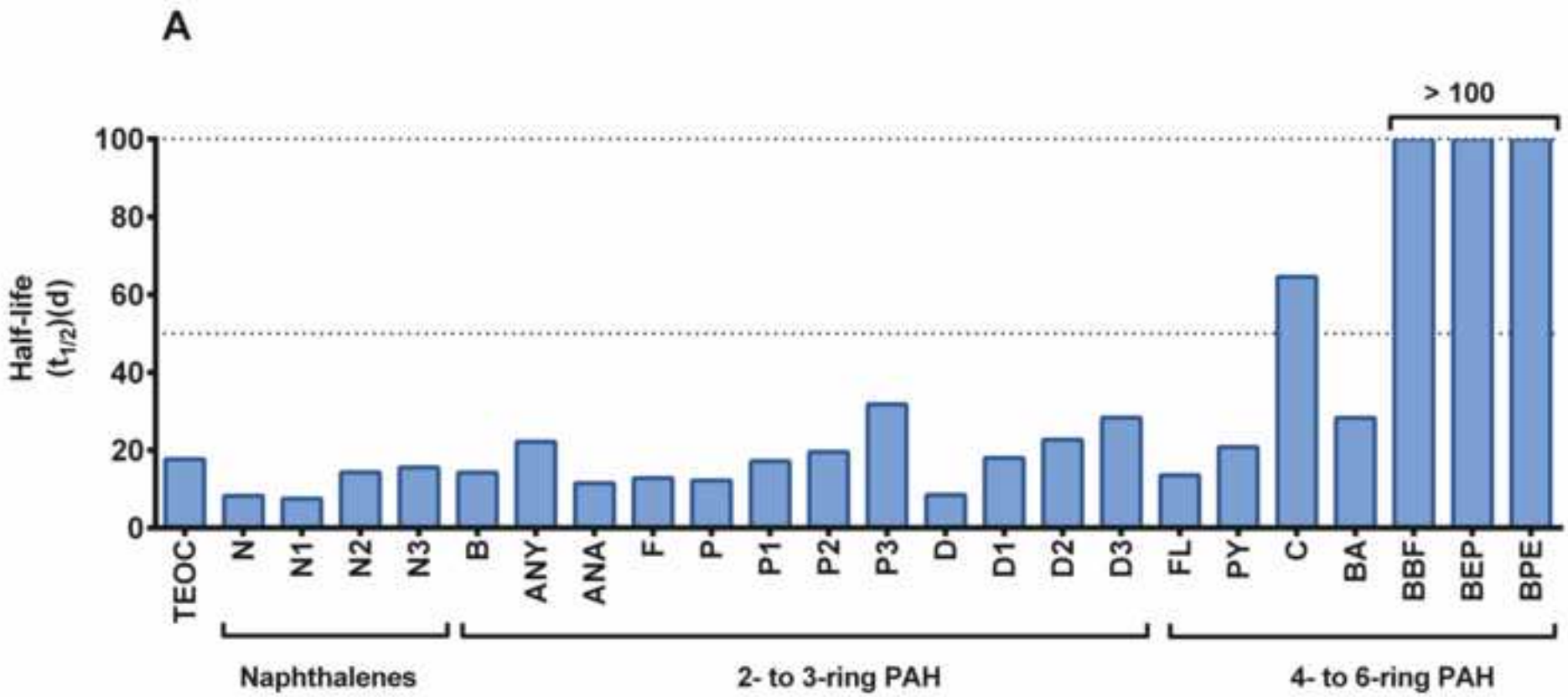

Figure5B

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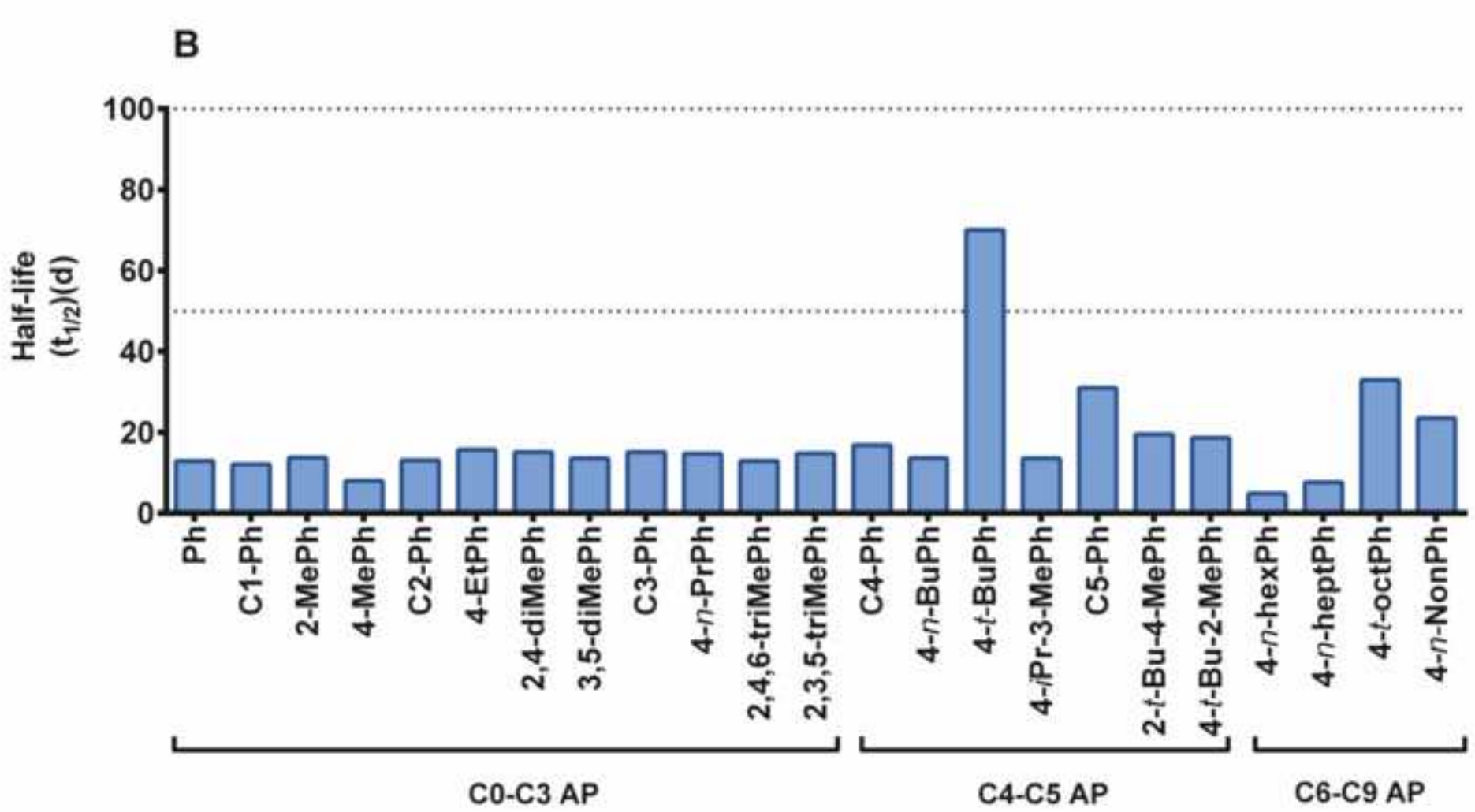

Supplementary Material

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