Environmental fate & effects of new generation flame retardants
Waaijers, S.L.

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Chapter 7

Synthesis
Chapter 7 – Synthesis

The aim of the present thesis was to determine the environmental fate and effects of HFFRs that are currently replacing brominated flame retardants in polymers. Reviewing the studies published so far showed that data on the physical-chemical properties, as well as on the persistency, bioaccumulation and (eco)toxicity of HFFRs are scarce and contradictory. Therefore, we set out to develop comprehensive analytical methods for four aromatic organophosphate FRs, and to generate data on their mineralization, biodegradation, and acute and chronic aquatic ecotoxicity to *Daphnia magna*. The present synthesis will focus on the improved understanding of the environmental fate of HFFRs and their biological hazard, offering a perspective for the risk assessment of these new generation FRs and providing support to the application of low-hazard flame retardants.

*Environmental fate of HFFRs*

To predict the fate of a compound in the environment, knowledge of the intrinsic physical-chemical properties is a first prerequisite. Parameters such as Henry’s law constant, water solubility and the \( n \)-octanol/water partition coefficient are required to predict whether a compound will end up in air (volatile compounds), water (soluble compounds) or soil/sediment/biota (hydrophobic compounds) (Schüürmann et al., 2007). This information is also essential to design environmental effect studies. The physical-chemical properties of many HFFRs, were, however, difficult to obtain from the available literature. Moreover, published values of a single property sometimes covered a range of several orders of magnitude (chapter 2) and practical complications such as low water solubilities as well as formation of emulsions complicated the experimental determination of these properties (chapters 3 and 4). To overcome some of these practical complications, the test solutions used in the present study were allowed to equilibrate for at least a week, indeed leading to increased reproducibility and stability of HFFRs concentrations in water, in both the solubility (chapter 3) and ecotoxicity experiments (chapters 4 and 5). Additional filtration or centrifugation removed larger particulates and reduced the formation of emulsions. I recommend that these simple steps should always be included in the experimental testing of compounds with a low water solubility in order to lessen variations in their concentrations in the water, thereby increasing the reliability of the test results.
To accurately determine the concentrations of HFFRs in water, a reliable analytical methodology was needed. The inorganic HFFRs investigated in this thesis could be measured straightforwardly with ICP-AES analysis (chapters 4 and 5). With the exception of TPP, very few publications on the analysis of aromatic OPFR in particular existed (van der Veen and de Boer, 2012) and none were available for DOPO. Therefore, as described in chapter 3, a comprehensive analytical methodology was developed for BDP, DOPO, RDP and TPP. Since several disparities exist in the literature of nominal and actual concentrations in toxicity tests, the presently developed methodology allowed us to generate consistent and more reliable ecotoxicity tests based on measured concentrations. The method also enables studying the fate or potential degradation of these compounds in the environment and it provides a major step towards environmental monitoring of the currently emerging HFFRs.

With the improved experimental set-up and analytical methodology, the fate of BDP, RDP and TPP in activated sludge from a sewage treatment plant could be studied (chapter 4). TPP was hydrolyzed and mineralized within two weeks (chapter 4), whereas RDP was only partly degraded and BDP did not degrade at all. These findings are remarkable since the compounds TPP, RPD and BDP are structurally similar. The question remains therefore what structural difference between TPP, RDP and BDP causes this dissimilar degradability. RDP in fact consists of TPP with an attached DPP moiety and BDP consists of two TPP molecules bound to the middle C-atom of a ‘propane’ (C3) chain. As a result, the structure of BDP can be seen as a derivative of bisphenol A. Although it has been reported that aerobic degradation of BPA is relatively fast in sewage sludge and river waters, it is often not completely mineralized (Kang et al., 2006). The BPA building block of BDP can thus contribute to the lower degradability of the molecule. It may seem counter intuitive that a molecule with a decreased conjugated structure (compared to TPP and RDP) is more stable. Perhaps the additional ‘propane’ group of BDP may hinder electrophilic attacks. BDP has a higher molecular weight (693 g mol⁻¹) than TPP and RDP. Therefore, microorganism might take up the larger BDP molecule more slowly due to limited membrane permeation, as has been observed previously for PAHs (Rostami and Juhasz, 2013). Similarly, a molecular weight cut off has been observed for absorption across
tissues of aquatic organisms for certain hydrophobic organic contaminants (HOCs) (Sijm et al., 2007). However, this phenomenon is still under debate, as some authors argue that it can be ascribed to certain experimental artefacts (Jonker and van der Heijden, 2007). The high hydrophobicity of BDP (log Kow ≥ 4, chapter 2) may contribute as well to its low degradability, since cellular membrane uptake is easier for compounds with a Kow between 1.5 – 4 (Sikkema et al., 1995). Another possible explanation for the slow BDP degradation is that low solubility and/or sorption to organic matter in the sludge itself hamper biodegradation (Schnurer et al., 2006). As a result of one or more of these effects, this larger BDP molecule is less susceptible to degradation than TPP and RDP are. Regardless of the effects of molecular size and bioavailability, it is possible that BDP might break down when studies are performed with an increased time window for adaptation, using primed sludge (as observed for PBBs (Bedard et al., 1998)) or using a high density of a specialized bacterial consortium (e.g. *Pseudomonas* sp. that can breakdown BPA (Kang and Kondo, 2002)).

The present study demonstrated that under identical experimental conditions compounds with apparently similar structural features can vary extensively in biodegradation rate, ranging from full mineralization to non readily degradable. This shows that commonly used methods as read-across and quantitative structure-activity relationships (QSARs) (Raymond et al., 2001; OECD) cannot be used safely to predict the environmental fate of non tested organic HFFRs. The work presented in this thesis shows that degradability decreases with increasing molecular size, but the present data set is too limited to derive a reliable property-activity relationship between the molecular structure of the aromatic OPFRs and their biodegradability. This points again at the tension between the need for extrapolation with its associated uncertainties and the added value of more reliable, though more expensive, time consuming, experimental verification. At this stage, direct verification of the persistence of OPFRs remains essential and extrapolation based on molecular structure is not yet justified.

**Environmental effects of HFFRs**

In order to predict the environmental hazard of a compound, knowledge of its ecotoxicity is needed. The review presented in chapter 2 showed that, in spite of some studies published on the topic, the toxicity of HFFRs is still difficult to evaluate, since frequently important
experimental parameters were not reported or actual concentrations were not measured. In the present thesis, I aimed to circumvent this problem by testing the acute and chronic toxicity of the selected HFFRs in an identical set-up, under the same conditions, measuring the exposure concentrations at regular time intervals, as described in chapters 5 and 6. ATH, ATO, BDP, RDP and TPP showed acute toxic effects to daphnids and APP, ALPI, DOPO, MHO, MPP, ZS and ZHS had no or a low acute toxicity (chapter 5). In this way, the toxicity of the new generation FRs to daphnids could be easily evaluated and compared and the data is ready available for risk assessment purposes.

In the present project one aquatic organism, *D. magna*, was selected to test the aquatic ecotoxicology of HFFRs. Data for a single species are obviously not sufficient for a comprehensive assessment of the environmental hazards of these compounds. This project was part of the European project ENFIRO where also other endpoints (e.g. in vitro toxicity) were studied and socio-economic impact assessments were undertaken (ENFIRO 2009). Nevertheless, the results presented in this thesis showed that seven of the twelve HFFRs either did not exert any acute effect at their water solubilities or had a low toxicity to *Daphnia magna*. On the basis of these findings it is concluded that these seven compounds are suitable replacement candidates for BFRs (Chapter 5). Although it is obviously desirable that other representative species are tested as well, the presented results may be considered as the first step in the search for sustainable HFFRs.

In addition to the acute toxicity tests, chronic life cycle studies were performed with ALPI and DOPO, which are HFFRs exerting a low acute toxicity to *D. magna*. Although again these results reflect the impact on only one species, very relevant findings in terms of environmental hazard were obtained. The tested DOPO concentrations did not cause mortality after 21 days, but the reproduction and population growth rate of the daphnids were clearly affected. These results show that sublethal effects need time to become expressed, underlining the importance of chronic toxicity testing. Sublethal effects, affecting population growth rate, are highly relevant for the field, as they can cascade up to the community and ecosystem level (Hanazato, 2001).

The toxicity of ALPI increased almost six fold when increasing the exposure time from 48 h to 21 days, implying that ALPI could be classified more hazardous according to the European
classification and labeling legislation (chapter 6 and European Union 2008) when judged on the basis of chronic rather than acute toxicity testing. The manufacturer of ALPI states that this HFFR qualifies to be awarded with the EU Ecolabel (Clariant, 2012). This label specifically takes the consumer end product into consideration and sets requirements for the compounds these products contain (European Union, 2011). These requirements also include chronic toxicity studies. The data on the chronic aquatic toxicity of ALPI provided by the manufacturer were, however, previously not available (see also chapters 2 and 6), which hampers an open scientific discussion on the environmental hazard of this compound. Our publicly available data (Waaijers et al. 2013c; chapter 6) show that ALPI is harmful to D. magna in a 21 days life cycle study. These findings seriously question the qualification of ALPI for the Eco label, but also raise some doubt on the environmental safety standards formulated for the Eco labels.

The increased toxicity with increased exposure time raises concerns about the potential chronic toxicity of the other compounds as well. The HFFRs that did not show acute effects (chapter 5) were APP, MHO, MPP, ZHS and ZS. To identify possible sublethal toxicity to daphnids, also chronic toxicity data for these compounds are required, but these data are missing for APP, ZHS and ZS (chapter 2). MHO is not expected to exert chronic toxicity as magnesium is an essential metal and a major component of natural waters (European Chemicals Bureau, 2000), but this has not been confirmed to our knowledge by any toxicity study. Concerning MPP, chronic toxicity tests with Daphnia published previously showed an increase in toxicity of melamine from LC₅₀ >2000 mg L⁻¹ to about 60 mg L⁻¹ (draft study (U.S. EPA 2008; no experimental details were provided), yielding a high ACR of at least 33. This high ACR for MPP might indicate a specific mode of action (chapter 6 and Länge et al. 1998, Roex et al. 2000, Marinkovic et al., 2011). In the present project, increasing the exposure time manifested “hidden” toxicity and therefore it is concluded that, although time consuming and expensive, experimental verification is the only reliable way to obtain insight in the chronic, sublethal effects of compounds.

Under the European chemical legislation, REACH, it is already mandatory to test short-term ecotoxicity to invertebrates (preferably Daphnia species) as well as growth inhibition of an
aquatic plant (preferably algae), for compounds produced, imported or used in the European Union in quantities above than 1 tonne per year (Annex VII, (European Union, 2006)). These are minimum requirements and with increasing production volumes (1-10, 10-100, 100-1000, >1000 tonnes) additional short- or long-term toxicity studies are required (e.g. on fish, plants, terrestrial invertebrates and birds) (Annex VII-X, (European Union, 2006)). The REACH requirements for aquatic toxicity testing do not have to be met if a substance is very poorly soluble in water or very unlikely to cross biological membranes. In the study described in chapter 5, however, no evidence was obtained that either high molecular weight or low water soluble HFFRs are excluded from exerting aquatic toxicity. This clearly shows that even when a compound may be considered poorly water soluble or if its ability to cross a biological membrane is open for debate (Sijm et al., 2007; Jonker and van der Heijden, 2007), this does not mean that a compound will not show toxic effects. The present thesis shows that waiving the minimal required ecotoxicity tests (REACH legislation, > 1 tonne compounds, (European Union, 2006)) is not always justified for poorly soluble compounds, as seen here for the HFFRs studied. Experimentally testing the environmental fate and effects of the heterogeneous group of these HFFRs is the only safe, but time consuming and costly approach. Since the HFFRs are a relatively new group of FRs, reported data on the biodegradation and ecotoxicity of HFFRs are scarce and dominated by grey literature such as safety data sheets or non-refereed reports from manufacturers (chapter 2). During this project (Chapter 2) it became apparent that in some cases (eco)toxicity and biodegradation studies did exist (personal communications with industry), but that these were not publicly available. As experiments are expensive and time consuming and competition is fierce, companies are reluctant to hand out this data. To date, i.e. three years later, these compounds have been registered, but some of this data are still not accessible by searching on CAS number on the ECHA website (2014). Because of this concealed data, experiments have been repeated unintentionally, which is not only expensive, but also unethical considering the animal testing needed. This problem is, however, not restricted to industry alone; scientists too may be unaware of how to make their data more valuable. Open scientific literature can often not be used for risk assessment as not
all relevant data are published, which is needed to assess the reliability of the study (Moermond et al., 2012). Furthermore, to fully benefit from all available studies, provided that they are reliable and relevant, regulatory evaluation criteria have to offer enough flexibility to include toxicity data obtained using non-standard test species for which no OECD guidelines are available (Agerstrand et al., 2011). Although the documentation of experimental detail and creating transparency may offer challenges, the information is essential for proper hazard evaluation under the REACH legislation. The discrepancies between publicly unavailable data provided by manufacturers and publicly available data provided by scientists hampers a transparent, objective hazard evaluation, especially of new emerging compounds.

In summary, structurally similar HFFRs exhibiting different biodegradation potential showed that commonly used methods as read across and QSARs cannot be used safely to predict the environmental fate of untested HFFRs. At this stage, direct verification of the persistence of OPFRs remains essential. Toxicity tests with daphnids showed that ATH, ATO, BDP, RDP and TPP are acutely toxic and that APP, ALPI, DOPO, MHO, MPP, ZS and ZHS have no or a low acute toxicity. In chronic toxicity tests DOPO affected population growth, while the toxicity of ALPI increased with increased exposure time, potentially leading to a shift in classification from low to moderate toxicity. Several chapters of this thesis showed the dilemma between the need for extrapolation with its associated uncertainties and the need for more reliable, though expensive and time consuming, experimental verification. The new insight in the obtained present work provides further support to the application of low-hazard flame retardants.