Preliminary aquatic toxicity hazard evaluation of DOPO-HQ as a potential alternative to PBDEs

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Introduction

With the gradual ban of PBDEs, the application of phosphorus flame retardants (PFRs) greatly increased and became the main flame retardants in various products. However, they had similar properties or were of more concern due to their toxicity for halogenated PFR including aquatic toxicity properties [1] and potentially carcinogenic property [2,3]. Recent studies have suggested bioaccumulative properties [4], as well as their persistence and long-range transport [5]. In 2009, Environment Canada and Health Canada proposed a risk management action for TCEP, with the objective to reduce exposures by eliminating the compound from furniture, electronic products (e.g., televisions and computers) and so on [6]. The European Community (EC) has proposed to restrict the use of halogen flame retardants [7]. For this reason, halogen-free flame retardants for polymeric materials have attracted increasing attention in recent years.

Recent, more and more alternatives are applied in the market. But comprehensive information on their possible toxicological effects are lacking. Several countries and organization performed risk assessment for alternatives to decaBDE [8-11]. These reports evaluated the main types of brominated flame retardant and some alternatives options. They included that halogenated PFRs and TPP are potentially problematic for replacement of decaBDE for their environmental and human hazard, while RDP and BDP are potential alternatives to decaBDE based on the present information [8-11].

DOPO is a novel phosphorus-containing reaction intermediate, DOPO and its derivatives has attracted intensive attention due to high flame retardant efficiency and thermal properties. It has less halogen and toxins and are less likely to divert have been widely used in a variety of polymer as flame retardants in recent years. According to the evaluation of the European Flame Retardants Association, it was thought to be the most successful solution to flame retardants. It has a low bioaccumulation potential, are not persistent, were tested negatively for a series of toxic effects including neurotoxicity and showed moderate to zero leaching from various polymers [12]. The ENFIRO hazard assessment showed to have less issues of toxicity concern for DOPO [9].

DOPO-HQ was synthesized through the reaction of DOPO and p-benzoquinone. It has been also used as a reactive flame retardant [13]. Owing to the introduction of the rigid structure of DOPO-HQ and the pendant phosphorus-containing group, the resultant resins provided not only better flame retardant properties [14], but also higher thermal stability and glass transition temperature [15]. It has been applied in

PU foams and epoxy resin for printed circuit boards. But there is very lack the comprehensive information on their possible toxicological effects and environmental impacts.

In the present work, the aquatic toxicity of DOPO-HQ was investigated including acute and chronic aquatic toxicity compared comprehensively with those of decaBDE and its typical alternatives.

Materials and methods

Chemical

DOPO-HQ ($C_{18}F_{14}PO_4$, CAS 88208-50-1) was obtained from the China Flame Retardant Society.

Aquatic toxicity test

The test was performed according to OECD202, OECD203 and OECD211[16-18]. According to the result of pre-test, it wasn't observed obvious acute toxicity for *Daphnia* and fish. Therefore, both limit tests were performed as follows.

For *Daphnia* acute immobilization test, a control group and one treatment group with saturation solution were prepared respectively in 60 mL of reconstituted test water. Every group contained four replicates. Each replicate was comprised of five *Daphnia magna*. The test conditions were as follows: 48 h with 16 h: 8 h (light: dark) photoperiod; 19.9-21.6 °C; pH, 6.25-6.47; and dissolved oxygen, 8.12-8.48 mg/L. *Daphnia magna* were not fed during the test. At 24 h and 48 h, the numbers of immobilized *Daphnia magna* were observed.

For fish acute toxicity test, a control group and one treatment group with saturation solution were set in tap water. Every group contained three replicates, and each replicate contained ten *Gobiocypris rarus*. The test conditions were as follows: 96 h with a 12 h: 12 h (light:dark) photoperiod; 23.2-24.5 °C; pH, 6.77-7.35; and dissolved oxygen; 68.0-93.5% ASV. Mortality or any abnormalities of fish were observed daily.

A control group and one treatment group with saturation solution were prepared in an 80 ml M_4 solution. The solution was replaced on the 2^{nd}, 5^{th}, 7^{th}, 9^{th}, 12^{th}, 16^{th} and19^{th} days of the test, and the old and new media were analyzed the concentration by HPLC. There were ten replicates for each group including the treatment group and control group. A replicate contained one *Daphnia magna*. The test conditions were as follows: 21 d with a 16 h: 8 h light: dark photoperiod; illumination, 1147 to 1362 lux, 20.2-20.7 °C; pH, 7.68-8.31; and dissolved oxygen, 7.36- 9.30 mg/L. *Daphnia magna* were fed five times per week during the test. The numbers of living offspring, aborted eggs, and dead offspring were recorded and removed from the first brood. Mortality of the parent individuals was also recorded.

Chemical and Statistical analysis

For studies, the concentrations of the test samples from aquatic toxicity test were confirmed by HPLC analysis. The recoveries of spiked samples (n=3) of 1mg/L ranged from 89.2 to 105%, with RSD below 7.49%.

Statistical analysis was conducted using the TOXCALC (v5.0.26) software.

Results and Discussion

The concentrations of treat group were analyzed by HPLC. However, it couldn't be detected with concentration below the detection of 0.08mg/L.

The aquatic acute toxicity test was performed at three trophic levels including green algae (*Pseudokirchneriella subcapitata*), invertebrates (*Daphnia magna*) and native fish (*Gobiocypris rarus*). No immobilisation or death was observed for *Daphnia magna and* fish respectively in both control group and the treat group. 48 h-EC_{50} for *Daphnia magna* and 96h-LC_{50} for *Gobiocypris rarus* were greater than the saturation solution.

The chronic toxicity was evaluated by the reproduction of *Daphnid magna*. During the test, no parent mortality was observed in the control group, and no aborted eggs or dead offspring were recorded. One parent was dead on the first day in the treat group during the test. The estimation of the 21 days cumulative adult deaths of the treatment groups to *Daphnia magna* was analyzed by the Steel's Many-One Rank Test compared with the control. There was no difference in the statistics. It may be an accident and could be permitted. The mean number of offspring per parent that survived to the end of the test in the control group was 101 with a CV of 16.2%, correspondingly, the average value of offspring per parent was 106 with a CV of 10.9%. The reproduction of the treatment groups to *Daphnia magna* was analyzed by the Wilcoxon Rank Sum Test compared with the control. There was also no difference in the statistics, therefore, 21 days NOEC for the reproduction of *Daphnia magna* is greater than the saturation water solubility.

Whether acute or chronic toxicity to aquatic organisms for DOPO-HQ, no effects were observed at the saturation water solubility. According to classification criteria of GHS, the environmental hazard classification was based on the aquatic toxicity issued by UN [19]. When a long-term NOEC was above the water solubility, hazard classification can be negated. It meant that DOPO-HQ doesn't show the aquatic hazard based on the present data.

There are many alternatives to PBDEs. The five most widely commercial alternatives were selected including TBBPA, TPP, TCEP, TCPP and TDCPP. In addition, RDP and BADP, as two new and potential alternatives were also selected. Such above alternatives and decaBDE are selected as reference substances in comparison of ecotoxicity to DOPO-HQ.

TCPP has a documented 96h-LC_{50} value of 51 mg/L for fathead minnow (*Pimephales promelas*) and a NOEC of 9.8 mg/L [3,11]. A 21-day reproduction test using *Daphnia magna* showed a NOEC of 32 mg/L for TCPP based on adult mortality [3]. The corresponding LC_{50} for bluegill sunfish (*Lepomis macrochirus*) was 180 mg/L [3]. In addition, TCPP is considered to be potentially carcinogenic [2,3].

Chronic toxicity data of 21 d determined for *Daphnia magna* were 0.05 mg/L [20]. The 96h-LC_{50} for rainbow trout (*Oncorhynchus mykiss*) was 1.1 mg/L for TDCPP (Amgard TDCP) and the NOEC 0.56 mg/L. The 96h-LC_{50} for killifish (*Oryzias latipes*) and goldfish (*Carassius auratus*) were reported to be 3.6 and 5.1 mg/L, respectively. LC_{50} of TCEP reported for fish (96 h) by Fisk et al. varied from 6.3 to 250 mg/L [11]. Sasaki et al. reported 96h-LC_{50} values for killifish (*Oryzias latipes*) and goldfish (*Carassius auratus*) of 210 and 90 mg/L, respectively. Killifish exposed to TCEP at 200 mg/L for 72 h showed spinal deformities. Yoshioka et al. reported an LC_{50} for the killifish (*Oryzias latipes*) of 251 mg/L. 96h-LC_{50} for rainbow trout (*Oncorhynchus mykiss*) was 249 mg/L [3].

A number of studies have been performed and summarized on the toxicity of TPP [10]. The acute toxicity of TPP for fish (96h-LC_{50}) reached 0.4-0.85 mg/L [10]. The acute toxicity index of TPP for fish (96h-LC_{50}) ranges from 0.36 mg/L in rainbow trout [1]. 48h-EC_{50} of *daphnia* was 1.35 mg/L. For the chronic toxicity, LOEC of 0.037 and 0.23 mg/L was found for survival and growth of fish, and an estimated NOEC for *daphnia* of 0.1 mg/L was found [1].

It gave a LC_{50} of 12.3 mg/L for fish, and an EC_{50} of 0.76 mg/L for *daphnia* immobilization when exposed on RDP [10]. For *Daphnia magna*, 21d-NOEC and 21d-EC_{50} are 0.021 mg/Land 0.037 mg/L [10]. However, the exposed concentration was higher than the water solubility of 0.11μg/L [1]. It is thought that the toxicity may be a result of the presence of undissolved test substance [10]. It also may be due to the amount of TPP present in the technical products. Therefore, it is suggested that no effects at saturation based on the only few toxicity data are available for RDP. Experimental data for BDP for fish, daphnia, and algae indicate above 100mg/L, which is more than water solubility. It may be nominal concentration. Therefore, there aren't aquatic effects up to the limits of the water solubility [10].

For TBBPA, an overall 21-day LOEC and NOEC of 0.98 mg/L and 0.30 mg/L, respectively, were measured in the *Daphnia magna*, based on significantly reduced reproduction [20]. The 35-day LOEC and NOEC for fathead minnow (*Pimephales promelas)* were 0.31 mg/L and 0.16 mg/L, respectively, based on significantly reduced embryo and larval survival.

Based on the available data, decaBDE appears to have a very low toxicity in acute tests, with no effects being seen up to the substances water solubility [10,21]. A chronic dietary fish study identified decreased thyroid hormone, deiodinase activity and gonad size, plus increased mortality, after dietary exposure to part per billion doses of decaBDE [10].

Compared the ecotoxicity of DOPO-HQ with those of decaBDE and its alternatives, the aquatic toxicity are lower than Cl-containing PFRs, TPP and TBBPA, no effect was observed at saturation as the same as RDP and BDP.

Conclusion

The aquatic toxicity of DOPO-HQ is evaluated and showed low acute and chronic aquatic toxicity. Compared comprehensively the ecotoxicity of DOPO-HQ with those of decaBDE and its typical alternatives, it is lower than those of Cl-containing PFRs, TPP and TBBPA, those are the potentially problematic alternatives. DOPO-HQ shows no effect at the saturation which is similar to those of decaBDE, RDP and BDP since all of them are insolubility. Therefore, DOPO-HQ could be a potential alternative to decaBDE from environmental perspective.

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References

[1] van der Veen, Jacob DB. 2012. Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. *Chemosphere* 88:1119-1153.

[2] Ni Y, Kumagai K and Yanagisawa Y. 2007. Measuring emissions of organophosphate flame retardants using a passive flux sampler. *Atmospheric Environment* 41:3235-3240.

[3] WHO.1998. Flame retardants: tris(chloropropyl) phosphate and tris(2-chloroethyl) phosphate. *Environmental Health Criteria 209,* Geneva. Switzerland.

[4] Leonards P, Steindal EH, van der Veen, I, Berg V, Bustnes JO, van Leeuwen S. 2010. Screening of organophosphorus flame retardants. SPFO-Report 1091/2011. TA-2786/2011.

[5] de Wit CA, Herzke D, Vorkamp K. 2010. Brominated flame retardants in the Arctic environment-trends and new candidates. *Sci Total Environ* 408:2885-918.

[6] Government of Canada, 2009. Proposed risk management approach for ethanol, 2-chloro-, phosphate (3:1) or tris (2-chloroethyl) phosphate (TCEP) chemical abstracts service registry number (CAS RN): 115–96-8. Environment Canada Health Canada. Ottawa, Canada.

[7] Lu S Y, Hamerton I. 2002. *Prog. Polym. Sci.* 27: 1661–1712.

[8] Pakalin S, Cole T, Steinkellner J, Nicolas R, Tissier C, Munn S, Eisenreich S. 2007. European Report EUR 22693 EN. Review on production processes of decabromodiphenyl ether (decaBDE) used in polymeric applications in electrical and electronic equipment, and assessment of the availability of potential alternatives to decaBDE. Brussel, Belgium.

[9] EU, 2013. Final report summary-enfiro(Life cycle assessment of environment of environment-compatible flame retardants(prototypical case study)). ENFIRO, Amsterdam, Netherlands.

[10] US EPA, 2014. An alternatives assessment for the flame retardant decabromodiphenyl ether (DecaBDE). US Environmental Protection Agency, Washington, DC.

[11] Fisk PR, Girling, AE, Wildey, RJ, 2003. Prioritisation of Flame Retardants for Environmental Risk Assessment. Environment Agency, United Kingdom, London.

[12] Brandsma, S.H., 2014. Occurrence and fate of alternative flame retardants in the environment. PhD thesis. VU University, Amsterdam, Netherlands.

[13] Wang CS, Lin CH. 1999. Synthesis and properties of phosphorus containing polyarylates derived from 2-(6-oxido-6H-dibenz [c, e][1,2]oxaphosphorin-6-yl)-1,4-dihydroxy phenylene. *Polymer 40*: 4387-4398.

[14] Wang CS, Shieh JY. 1998. Synthesis and properties of epoxy resins containing 2-(6-oxid-6H-dibenz[c,e][1,2] oxaphosphorin-6-yl)1,4-benzenediol. *Polymer 39*, 5819–5826.

[15] Liu YL, Chiu, YC. 2003. Novel approach to the chemical modification of poly

(vinyl alcohol): phosphorylation. *J. Polym. Sci., Part A: Polym.Chem. 41*: 1107-1113.
[16] OECD, 2004. OECD guidelines for the testing of chemicals, *daphnia* sp., acute immobilisation test, OECD202, Organization for Economic Cooperation and Development, Paris, France.
[17] OECD, 1992. OECD guidelines for the testing of chemicals, fish, acute toxicity test, OECD 203, Organization for Economic Cooperation and Development, Paris, France.
[18] OECD, 2008. OECD guidelines for the testing of chemicals, *daphnia magna* reproduction test, OECD211, Organization for Economic Cooperation and Development, Paris, France.
[19] UN. 2007. The Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Part 4 Environmental Hazards. New York and Geneva, UN.
[20] Government of Canada, 2013. Screening Assessment Report, Phenol, 4,4'-(1-methylethylidene) bis[2,6-dibromo-Chemical Abstracts Service Registry Number 79-94-7, Ethanol, 2,2'-[(1-methylethylidene)bis [(2,6-dibromo-4,1-phenylene)oxy]] bis Chemical Abstracts Service Registry Number 4162-45-2 Benzene, 1,1'-(1-methylethylidene)bis[3,5-dibromo-4-(2-propenyloxy) -Chemical Abstracts Service Registry Number 25327-89-3, Environment Canada Health Canada, Environment Canada Health Canada. Ottawa, Canada.
[21] European Union, 2002. Risk Assessment Report, bis(pentabromophenyl) ether, CAS No: 1163-19-5, EINECS No: 214-604-9, risk assessment, Italy.