Chemosphere 154 (2016) 34–39

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Predicting changes in aquatic toxicity of chemicals resulting from solvent or dispersant use as vehicle

Mikio Kikuchi [a,*], Masamitsu Nakagawa [b], Suguru Tone [b], Hotaka Saito [c], Tatsuhiro Niino [b], Natsumi Nagasawa [a], Jun Sawai [a]

[a] Kanagawa Institute of Technology, 1030 Shimo-Ogino, Atsugi, Kanagawa 243-0292, Japan
[b] LSI Medience Co., 1000 Kamoshida-cho, Aoba-ku, Yokohama, Kanagawa 227-0033, Japan
[c] Life Science Institute, Inc., 13-4, Uchikanda 1-chome, Chiyoda-ku, Tokyo 101-0047, Japan

HIGHLIGHTS

- The changes were studied on chemicals with a non-specific mode of action.
- Use of DMF or HCO-40 at 20 mg/L does not affect on the toxicity.
- 100 mg/L of HCO-40 may alter the toxicity of chemicals of high log P_{ow} values.
- The adsorption changes by HCO-40 was closely correlated with the changes in toxicity.
- The adsorption test can predict toxicity changes by using vehicle.

ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form
4 March 2016
Accepted 8 March 2016
Available online 31 March 2016

Handling Editor: A. Gies

Keywords:
Aquatic toxicity
Daphnia magna
Dispersant
log P_{ow}
Silicone
Adsorption

ABSTRACT

The influence of two vehicles (*N*,*N*-dimethylformamide [DMF] as solvent and polyoxyethylene hydrogenated castor oil [HCO-40] as a dispersant) on the acute toxicity of eight hydrophobic chemicals with a non-specific mode of action to *Daphnia magna* was investigated according to the OECD Guidelines for the Testing of Chemicals, No. 202. An increased 48-h EC_{50} value for *D. magna* or reduced toxicity resulting from the addition of HCO-40 to the test medium was observed for five of the eight chemicals examined.

Each of eight chemicals was dissolved in water at a concentration of either 10 mg/L or 1.0 mg/L, with or without DMF or HCO-40. Silicone film as a model of a biological membrane was then immersed in each solution, and the concentration of each chemical in the water was monitored until equilibrium was reached for each test substance, after which the adsorbed amount of each chemical was determined. The amounts of *p*-pentylphenol and four other substances with log P_{ow} (1-octanol/water partition coefficient) values greater than 3.4 adsorbed onto the silicone film decreased with increasing concentrations of HCO-40. However, 3-chloro-4-fluoronitrobenzene and two other substances with log P_{ow} values less than 2.6 demonstrated no changes in adsorption with either increasing HCO-40 concentration or the addition of DMF.

The reduced adsorption in the presence of a vehicle on the silicone film correlated closely with changes in toxicity. These results indicate that the methodology developed in this study enables the prediction of changes in toxicity resulting from the addition of vehicles to a test system.

1. Introduction

Aquatic toxicity testing of chemicals using fish or daphnids is performed to assess water quality in order to protect aquatic ecosystems. In aquatic toxicity testing of poorly water-soluble substances, it can be difficult to prepare a concentrated stock solution and maintain a constant exposure concentration in the water. Modifications to media preparation protocols may be required in cases where the exposure concentration of a test substance is likely to decline significantly over the test period. These cases typically involve preparation of a concentrated stock solution of the test substance in a water-miscible organic solvent or surface-active

* Corresponding author.
E-mail address: miki.kikuchi2006@gmail.com (M. Kikuchi).

http://dx.doi.org/10.1016/j.chemosphere.2016.03.030
0045-6535/

dispersant as a vehicle or carrier. It is necessary to select an appropriate type and concentration of solvent or dispersant prior to aquatic toxicity testing, as a substance's toxicity measured using a solvent or dispersant can differ from that of the substance alone (Weyman et al., 2012).

The Organization for Economic Cooperation and Development (OECD) Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (2000) states that "The use of chemical dispersants or emulsifying agents is not generally advocated because of the potential for physical-chemical interactions influencing the apparent toxicity of the test substance" (Organization for Economic Co-operation and Development, 2000). The inactivation of preservatives such as paraben by nonionic surfactants in cosmetics and pharmaceuticals is well known (Kabara, 1984). However, Yakata et al. (2006) reported that dispersants do not affect evaluations of the bioconcentration potential of test substances. Scientific evidence regarding the effects of solvents or dispersants at concentrations of 100 mg/L or lower remains limited in aquatic toxicology.

The purposes of this study were to clarify how the use of a solvent or dispersant as a vehicle affects the toxicity of a test substance and to develop a methodology for predicting the influence of vehicles on toxicity to perform simple, reliable, and affordable aquatic toxicity testing. Two vehicles, a water-miscible organic solvent and a surface-active dispersant, were evaluated for their effect on the acute toxicity of eight poorly water-soluble hydrophobic chemicals with a non-specific mode of action to *Daphnia magna*. For the second purpose, the effect of these vehicles on the adsorption of various test chemicals to silicone film as a model biological membrane was also determined. The relationship between changes in toxicity and adsorption associated with the addition of vehicles was analyzed, as vehicle-induced changes in the amount of a test substance adsorbed to a biological membrane over time can affect its toxicity.

2. Materials and methods

2.1. Chemicals

Eight test substances were evaluated in the present study: 4-pentylpheno1 (4-PP), 2,3,4,6-tetrachlorophenol (TCP), 2-methylnaphthalene (2-MN), 1,3-dimethylbenzene (*m*-xylene, MXY), 2-acetylbenzo[*b*]thiophene (ABT), 3-chloro-4-fluoronitrobenzene (CFNB), 4-chlorotoluene (ChTo), and 4-ethyl-1,1-biphenyl (ETBP). The log P_{ow} (1-octanol/water partition coefficient) values, and water solubility of the test substances are shown in Table 1. Two types of vehicle were used: *N*,*N*-dimethylformamide (DMF) as an organic solvent, and polyoxyethylene hydrogenated castor oil (HCO-40) as a dispersant with surface-active property. Both agents exhibit low toxicity to aquatic organisms, and hence DMF is commonly used and HCO-40 was often used in aquatic toxicity testing of water insoluble substances. HCO-40 was purchased from Nikko Chemicals Co., Ltd. (Tokyo, Japan), whereas the other chemicals were obtained from Kanto Chemical Co., Inc. (Tokyo), Tokyo Chemical Industry Co., Ltd. (Tokyo), Kishida Chemical Co., Ltd. (Osaka, Japan), or Junsei Chemical Co., Ltd. (Tokyo). Silicone film was obtained from AS ONE Corporation (Osaka).

2.2. Measurement of P_{ow}

The P_{ow} of the chemicals was determined using a high-performance liquid chromatography (HPLC) method with UV detection (JIS Z 7260-117, 2006). The HPLC mobile phase was methanol/Tris-HCl buffer 0.01 mol/L (pH = 7.6) (75/25).

2.3. Vehicle concentrations

The vehicle concentrations were as follows. In case A, no vehicle was used in the preparation of the test solutions. In case B, 100 μL/L DMF was used, and in cases D and E, HCO-40 was used at 100 and 1000 mg/L, respectively. In case C of the toxicity test, HCO-40 was used at ≤20 mg/L, and DMF or 2-methoxyethanol was used at ≤20 μL/L to prepare stable solutions. In case C of the adsorption test, HCO-40 was used at 20 mg/L. No case A testing of 4-ethyl-1,1-biphenyl was carried out, as the water solubility of this compound is 1.22 mg/L, and therefore, a test solution could not be prepared without the use of a vehicle.

2.4. Acute toxicity test

Acute immobilisation testing was performed according to the OECD Guidelines for the Testing of Chemicals, No. 202, "*Daphnia* sp., Acute Immobilisation Test". M4 medium was used for dilution water. At least five test concentrations were prepared in a geometric series. The 48-h median effect concentration, 48-h EC_{50}, was calculated based on mean measured concentration at the beginning of exposure and the end of exposure (48-h) by HPLC with UV detection.

Daphnia magna was used as the test organism and was obtained from the National Institute for Environmental Studies, Japan, and has been maintained in parthenogenetic culture in our laboratory (LSI Medience Co.) since 1995. Daphnids were reared in Elendt M4 medium at 20 ± 1 °C under a 16/8-h light/dark cycle. The medium was replaced three times per week, and juveniles were removed every day. Daphnids were fed daily with living unicellular green algae, *Chlorella vulgaris*.

For the *D. magna* acute immobilisation test, young female daphnids less than 24 h old were used. Twenty daphnids were exposed to the test solution (5 daphnids/100 mL, 4 replicates) for 48 h under static or semi-static conditions. The surface of the test solution was covered with a Teflon sheet to minimize evaporation of the culture medium and test substance. All experiments were

Table 1
Names and properties of chemicals used.

Chemicals	Abbr.	CAS No.	Water solubility [a] mg/L	log P_{ow}
4-pentylphenol	4-PP	14938-35-3	84.8	3.43
3-chloro-4-fluoronitrobenzene	CFNB	350-30-1	380	2.61
4-chlorotoluene	ChTo	106-43-4	370	3.49
m-xylene	MXY	108-38-3	200	3.57
2-methylnaphthalene	2-MN	91-57-6	25.4	3.97
2-acetylbenzo[*b*]thiophene	ABT	22720-75-8	46	2.29
4-ethyl-1,1-biphenyl	ETBP	5707-44-8	1.22	4.99
2,3,4,6-tetrachlorophenol	TCP	58-90-2	23	2.56

[a] ChemIDplus