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Assessing exposure risks for aquatic organisms posed by Tamiflu use under seasonal influenza and pandemic conditions

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ABSTRACT

Environmental pollution by anti-influenza drugs is increasingly recognized as a threat to aquatic environments. However, little is known about empirical data on risk effects posed by environmentally relevant concentrations of anti-influenza drug based on recently published ecotoxicological researches in Taiwan. Here we linked ecotoxicology models with an epidemiological scheme to assess exposure risks of aquatic organisms and environmental hazards posed by antiviral oseltamivir (Tamiflu) use in Taiwan. Built on published bioassays, we used probabilistic risk assessment model to estimate potential threats of environmentally relevant hazards on algae, daphnid, and zerbrafish. We found that Tamiflu use was unlikely to pose a significant chronic environmental risk to daphnia and zebrafish during seasonal influenza. However, the chronic environmental risk posed by Tamiflu use during pandemic was alarming. We conclude that no significant risk to algal growth was found during seasonal influenza and high pandemic Tamiflu use.

1. Introduction

The essential requirements for control of initial influenza outbreaks caused by a new virus are the antiviral drugs. Among antiviral drugs used for the treatment of influenza, the oseltamivir ethylester phosphate (OP) (marketed as Tamiflu®) is the most commonly used and has strongly recommended by the World Health Organization (WHO) (Davies, 2010; Ghosh et al., 2010a,b). Tamiflu is developed based on knowledge of the enzymatic structure (Moscona, 2009). Generally, human uptake Tamiflu and adsorb in gastrointestinal tract in the form of oseltamivir ethylester (OE). Then, hepatic esterases can convert OE into a biochemically active form of oseltamivir carboxylate (OC), a NA inhibitor (He et al., 1999; Straub, 2009). He et al. (1999) indicated that in the body, nearly 80% of OE is metabolized to OC in that OE and OC are excreted mainly by the renal pathway in the ratio of nearly 1:4.

There is evidence both from field observations and experimental studies of significant correlations between increased Tamiflu concentrations in sewage treatment plant (STP) effluents and receiving river waters and influenza epidemic or pandemic conditions (Singer et al., 2007, 2011; Accinelli et al., 2007; Lienert et al., 2007; Ghosh et al., 2010a; Azuma et al., 2012). Singer et al. (2007) reported that Tamiflu and its metabolites might pose a potentially significant, uncharacterized, ecotoxicological risk in affected waterways.

Moreover, OC in river waters might hasten the generation of OC-resistance in wildfowl, but this possibility seems less likely than the potential disruption that could be posed by OC and other pharmaceuticals to the operation of STPs (Fick et al., 2007; Singer et al., 2007). Ghosh et al. (2010a) and Azuma et al. (2012) implicated that OC was present in STP effluents and river waters only during the influenza season in Japan. Singer et al. (2011) suggested that there was a need to harness the empirical data on the effects of Tamiflu on STPs and freshwater ecotoxicity.

Furthermore, there is robust evidence that certain OC concentrations in river waters present an ecotoxicological risk or a pharmacologically relevant risk for enhancing the development of Tamiflu resistance in aquatic organisms (Fick et al., 2007; Söderström et al., 2009; Järhult et al., 2011). Fick et al. (2007) implicated that a ubiquitous use of Tamiflu may result in selection stresses in the environment that favor development of drug-resistance. Söderström et al. (2009) reported that dabbling duck, a natural reservoir of influenza virus, is exposed to Tamiflu that can promote the evolution of viral resistance. Järhult et al. (2011) indicated that environmental OC have been found at concentrations ranging from 58 to 293 ng L^{-1} in rivers and streams during seasonal outbreaks in Sweden. Recently, Azuma et al. (2012) indicated that the highest OC levels measured in STPs and river waters

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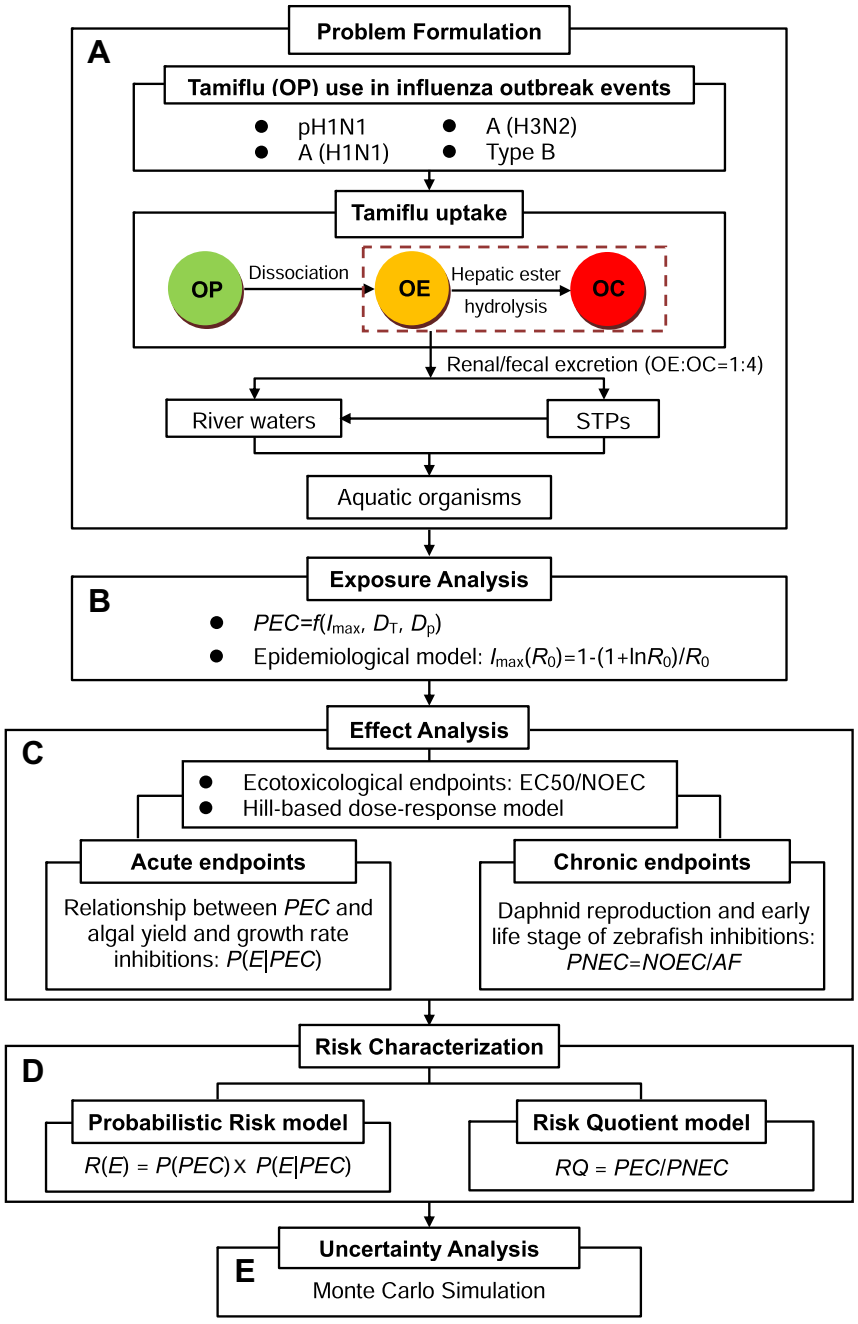

Fig. 1. Schematic illustration of the probabilistic risk assessment framework and the algorithm used in this present study: (A) problem formulation, (B) exposure analysis, (C) effect analysis, (D) risk characterization, and (E) uncertainty analysis (see text for the symbol descriptions).

were 827 and 288 ng L^{-1}, respectively, during a seasonal influenza outbreak in Japan.

Singer et al. (2008) suggested that an environmental risk assessment protocol could be used to assess the risk for OC-contaminated river waters generating OC-resistant viruses in aquatic organisms and to develop the realistic worst-case exposure scenarios. Straub (2009) and Hutchinson et al. (2009) performed an environmental risk assessment for Tamiflu use and concluded that Tamiflu posed no significant risk to surface waters, sewage works or coastal marine compartment during regular seasonal and high pandemic use conditions in Europe and USA.

Tamiflu is widely used in Taiwan (up to 60,000 box treatments) in the event of influenza epidemics and pandemics during January–April, 2011 (Centers for Disease Control, Taiwan, http://www.cdc.gov.tw). Little is known, however, about the empirical data on risk effects by environmentally relevant concentrations of anti-influenza drug built on recently published ecotoxicological researches in Taiwan.

The purpose of this paper was to assess the potential exposure risk of aquatic organisms and environmental hazards posed by antiviral drug Tamiflu use under seasonal influenza and pandemic conditions in Taiwan. An ecotoxicological model with an epidemiological scheme was employed to compute Tamiflu residues and treatment dosage. A probabilistic risk assessment model was used to estimate risks posed by environmentally relevant hazards. The potential control measures on reducing Tamiflu residues in STPs were also discussed. Our study could lend a contribution to ecotoxicological research on assessing how anti-influenza drug

residues frequently found in aquatic systems may have a direct or indirect impact on growth, fecundity, and survival of aquatic organisms (Graham et al., 2010).

2. Materials and methods

Here we developed risk estimations of potential exposures and environmental hazards posed by Tamiflu use during an influenza outbreak event in a probabilistic risk assessment framework (schematically illustrated in Fig. 1) that combines the ecotoxicological models with an epidemiology consideration and is described in details in the subsequent sections.

2.1. Problem formulation

Here infected patients administrate OP orally. Then OP dissociates in the digestive tracts to a well-absorbed form of OE in that OE is rapidly metabolized to OC. Over time, OE and OC are excreted by the renal and fecal pathways in the ratio of 1:4. The mixture of OE + OC enters water bodies by way of treated waste water effluents in that aquatic organisms experience adverse effects.

To place our results in a more realistic context, we chose New Taipei City, a metropolitan city in north Taiwan, with average 3,830,040 ± 65,301 (mean ± sd) populations in the period 2005–2011 as our study site. New Taipei City had stockpiled Tamiflu in preparation for an influenza pandemic. Tamiflu is available to treat symptomatic cases with a stockpile for up to 30% of population (Taiwan CDC, 2009). On the other hand, these may be used prophylactically to reduce transmission. The treatment of cases will reduce morbidity and mortality and has been shown to be cost-effective for high risk patients.

Three influenza sub(type) viruses of A (H1N1), A (H3N2), and type B as well as the emerging pandemic H1N1 2009 (pH1N1) were taken into account. The global distribution of pH1N1 strain prompted the WHO to declare the first influenza pandemic of the 21st century in June 2009.

2.2. Exposure analysis

The environmental concentration of Tamiflu residues in surface waters and sewage works under an event of seasonal influenza and pandemic use conditions for a virus-specific influenza can be predicted as (Ghosh et al., 2010a),

$$PEC = \frac{1}{W}[I_{\max} \times D_T + (1 - I_{\max}) \times p \times D_P] \times 10^3, \quad (1)$$

where PEC is the predicted environmental concentration of Tamiflu residues (μg L^{-1}), I_{max} is the maximum infectious fraction of population; D_T is the daily average Tamiflu treatment dosage for one confirmed case (mg $person^{-1}$ d^{-1}), p is the proportion of antiviral prophylaxis population based on the total medical personnel proportion of the total population and can be estimated to be 0.0075 ± 0.0009 (mean ± sd) (National Statistics, ROC; http://www.stat.gov.tw), D_P is the Tamiflu prophylaxis dosage for an adult with a value of 75 mg $person^{-1}$ (US FDA, 2009), and W is the average per capita water use in New Taipei City and is estimated to be 291 (95% CI: 287–295) L $person^{-1}$ d^{-1} (Water Resources Agency, 2012, Ministry of Economic Affairs, ROC).

The D_T in Eq. (1) can be calculated as

$$D_T = 0.7 \times 2 \times \sum_{j=1}^{7}[D_j \times P_j], \quad (2)$$

where D_j is the recommended treatment dosage (RTD) for age group j (mg $person^{-1}$), 0.7 represents one patient will be taking the RTD for 5 days in one week, 2 represents one patient will be taking the RTD twice daily in treatment period, j is the age group, and P_j is the proportion of confirmed cases for age group j. Eq. (1) assumed that all influenza confirmed cases were treated with Tamiflu with zero degradation of Tamiflu residues in surface waters or sewage works (Straub, 2009; Ghosh et al., 2010a).

To estimate RTD of Tamiflu for age-specific pediatric patients (1–12 years old) in Taiwan, we appropriately transformed RTD from weight-specific into age-specific based on data of body weight-specific dosage (US FDA, 2009) and age-specific body weight (Taiwan DOH, 2008). The confirmed cases of influenza in Taiwan can be classified into seven age groups: <3, 3–5, 6–11 months, 1–2, 3–6, 7–10, and ≥11 years old. The proportion of each age group confirmed cases for pH1N1, influenza A (H1N1), A (H3N2), and type B were estimated from Taiwan CDC in the period 2005–2009, by which D_T can be estimated.

The age group-specific RTD of Tamiflu (D_j) are suggested by US FDA (2009) as: (*i*) adult and adolescent (>12 years old) patients were 75 mg twice daily for 5 days, (*ii*) 1–12 years old patients were 30, 45, 60, and 75 mg twice daily for 5 days for body weight ≤15, 16–23, 24–40, and >40 kg, respectively, and (*iii*) <3, 3–5, and 6–11 months old patients were 12, 20, and 25 mg twice daily for 5 days, respectively.

To take into account the toxicologically and epidemiologically relevant factors in aquatic environments, we constructed the relationship between *PEC* and influenza transmission potential. In the epidemiology scheme, we usually used the basic reproduction number, R_0, to quantify the disease transmissibility (Anderson and May, 1991). R_0 is defined as the average number of infections generated by an infectious individual during infectious period in a wholly susceptible population.

When $R_0 > 1$ it implies that the epidemic is spreading within a population and incidence is increasing, whereas $R_0 < 1$ means that the disease is dying out. An average R_0 of 1 means the disease is endemic equilibrium within the population. The R_0 essentially determines the rate of spread of an epidemic and how intensive a policy will need to be to control the epidemic (Ferguson et al., 2003).

In the absence of an intervention, the maximum infectious fraction of population (I_{max}) during the epidemic is seen to depend only on R_0 and can be expressed as (Anderson and May, 1991),

$$I_{\max} = 1 - \frac{1}{R_0}(1 + \ln R_0), \quad (3)$$

that increases with increasing of R_0. Eq. (3) is based on the theoretical relationship between epidemic and R_0 assuming a homogeneous and unconstrained epidemic size. Thus *PEC* in Eq. (1) can be obtained by incorporating with Eqs. (2) and (3). The R_0 estimates for pH1N1, A (H1N1), A (H3N2), and type B can be adopted from published data (Chen and Liao, 2010; Tsai et al., 2010). Eqs. (1) and (3) reveal that the concentrations of Tamiflu residues in river waters or sewage works under seasonal influenza and pandemic use conditions could be characterized by an epidemiological determinant R_0.

2.3. Effect analysis

There were two effects concerning acute and chronic endpoints that were reanalyzed in our study. First, to construct the dose–response relationship between concentrations of Tamiflu residues and acute adverse effect of aquatic organisms, we adopted the toxicity data of Tamiflu residues on green algae (*Pseudokirchneriella subcapitata*), daphnid (*Daphnia magna*), and zebrafish (*Danio rerio*) in early life stage from Straub (2009). Straub (2009) used ratio of 1:4 mixture of OE and OC (i.e., OE + OC 1:4) as the surrogate concentrations of Tamiflu residues.

Briefly, for acute green algae bioassays, Straub (2009) carried out the experiments to determine the growth rate inhibition for green algae exposed to waterborne OE + OC 1:4 of 1, 3.2, 10, 32, and 100 mg L^{-1} for 72 h. Straub (2009) found that: (*i*) 72-h effective concentrations of yield inhibition (EC_Y) at 50%, 20%, and 10% were 60.3 (95% CI: 52.3–66.6), 9.49 (7.45–17.9), and 6.08 (5.17–7.24) mg L^{-1}, respectively, (*ii*) estimated 72-h effective concentrations of growth rate inhibition (EC_G) at 50%, 20%, and 10%, were 463, 66.4 (55.9–70.3), 37.2 (29.1–42.7) mg L^{-1}, respectively, and (*iii*) no observed effect concentration of growth rate inhibition ($NOEC_G$) was found at 10 mg L^{-1}.

For chronic daphnid bioassays, Straub (2009) carried out the experiments to determine the survival, length, and dry weight for parental daphnids and the number of offspring per parental female for the less than 24-h-old young daphnid exposed to waterborne OE + OC 1:4 of 0, 10, 32, 100, 320, and 1000 μg L^{-1} for 21 days. The result indicated that the chronic NOEC estimate for any of these endpoints was found at ≥1000 μg L^{-1}.

For zebrafish development in the early life stage bioassays, Straub (2009) carried out the experiments to determine the successful hatching rate, the number of deformed or abnormal behavior larvae, the post-hatch survival, and the length and dry weight of the surviving fishes at the end (over 32 days) of test for the fertilized zebrafish eggs exposed to waterborne OE + OC 1:4 of 10, 32, 100, 320, and 1000 μg L^{-1} for 32 days. The chronic NOEC estimate for these endpoints of zebrafish was found at ≥1000 μg L^{-1}.

Here a 2-parameter Hill-based dose–response equation was used to fit the acute data of algal yield and growth rate inhibitions exposed to waterborne Tamiflu residues concentrations,

$$E(C_w) = \frac{1}{1 + \left(\frac{EC50}{C_w}\right)^n}, \quad (4)$$

where $E(C_w)$ is the adverse effect depending on the OE + OC 1:4 concentration C_w (mg L^{-1}) and n is the fitted Hill coefficient which is a measure of cooperativity. We treated EC50 probabilistically to account for the inherent uncertainty that arises from a number of sources, including the limited number of observations and limited sample size within bioassays.

2.4. Risk characterization

Risk characterization is the phase of risk assessment where the results of the exposure and quantitative effect assessments are integrated to provide an estimate of risk for the population under study. Applying the Hill-based dose–response models in Eq. (4), the cumulative distribution function (cdf) of predicted algal adverse effects (% effect, E) for a given *PEC* can be expressed mathematically as the conditional cdf of $P(E_Y|PEC)$ and $P(E_G|PEC)$, for yield and growth rate inhibitions, respectively.

The probability density function (pdf) of R_0-based *PEC* of Tamiflu residues ($P(PEC)$) can be estimated by Eqs. (1)–(3). Thus, followed by the Bayesian inference,

the exposure risk for algae (the posterior probability) can be calculated as the product of $P(PEC)$ (the prior probability) and the conditional probability of $P(E_Y|PEC)$ or $P(E_G|PEC)$ (the likelihood). It results in a joint probability function (JPF) or exceedance profile, which describes the probability of exceeding the concentration associated with a particular degree of effect.

This can be expressed mathematically as a probabilistic risk model as,

$$R(E_Y) = P(PEC) \times P(E_Y|PEC), \quad (5a)$$

$$R(E_G) = P(PEC) \times P(E_G|PEC), \quad (5b)$$

where $R(E_Y)$ and $R(E_G)$ are the cumulative distribution functions describing the exposure probabilistic risks of algal yield inhibition and growth rate inhibition, respectively. Graphic display of Eq. (5) provides a means of assessing how alterations in environmental concentrations due to management efforts would affect the risk assessment. Thus, the exceedance risk profile can be obtained by $1 - R(E)$.

To assess chronic reproduction and development endpoints, we employed a risk quotient model to determine the potential exposure risk for daphnid and zebrafish as (US EPA, 2000),

$$RQ = PEC/PNEC, \quad (6)$$

where RQ is the risk quotient (−) and $PNEC$ is the predicted no-effect concentration (μg L^{-1}) that can be calculated by chronic NOEC (i.e., 1000 μg L^{-1}) for the adverse effect of daphnid reproduction test and the adverse effect of zebrafish development in early life stage test divided by the assessment factor (AF) of 10 based on Technical Guidance Document on Risk Assessment (European Commission, 2003) and European Agency for the Evaluation of Medicinal Products (EMEA, 2006).

For RQ value larger than 1, some potential for inhibiting reproduction and development can be inferred. RQ value less than 1 indicates the Tamiflu residues concentration poses no chronic environmental risk to aquatic organisms.

2.5. Uncertainty analysis

To quantify the uncertainty and its impact on the estimation of expected risk, a Monte Carlo (MC) simulation was implemented that included input distributions for the parameters of the derived dose–response function, as well as for estimated exposure parameters. Largely because of limitations in the data used to derive model parameters, inputs were assumed to be independent. The result shows that 10,000 iterations are sufficient to ensure the stability of results.

Moreover, a MC technique with 10,000 iterations (stability condition) was also performed to generate 2.5 and 97.5 percentiles as the 95% confidence interval (CI) for fitted dose–response model. The Crystal Ball® software (Version 2000.2, Decisioneering, Denver, Colorado, USA) was used to implement the MC simulation. TableCurve 2D (Version 5.01, AISN Software Inc., Mapleton, OR, USA) was used to perform the model fittings.

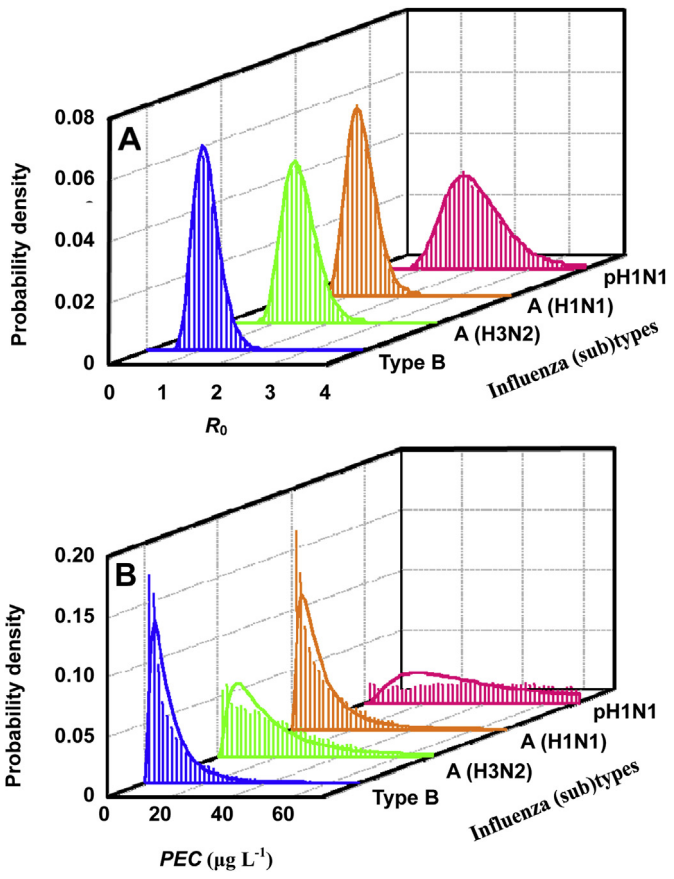

Fig. 2. Probabilistic density distributions of (A) basic reproduction number, R_0 and (B) predicted environmental concentration, PEC of Tamiflu residues for influenza sub(type) viruses of A (H1N1), A (H3N2), and type B, and pH1N1.

3. Results

3.1. R_0-based PEC estimates

We reanalyzed the adopted R_0 data from published literature. The results indicated that lognormal distribution with a geometric mean (gm) and a geometric standard deviation (gsd) (LN(gm, gsd)) best described R_0 distributions of LN(1.89, 1.34) for pH1N1, LN(1.19, 1.25) for A (H1N1), (1.42, 1.24) for A (H3N2), and LN(1.08, 1.25) for type B (Fig. 2A).

Our results showed that D_T values were 94.13, 82.47 ± 22.48 (mean ± sd), 84.83 ± 6.24, and 81.41 ± 23.77 mg person^{-1} for pH1N1, A (H1N1), A (H3N2), and type B, respectively, based on adjusted age-specific dosages and virus-specific proportion of confirmed cases (Table 1).

Given the estimated virus-specific R_0 distributions, the maximum infectious fraction (I_{max}) can also be estimated via Eq. (3). We thus incorporated estimated I_{max} and D_T distributions into Eq. (1) to estimate PEC distributions, resulting in the best-fitted lognormal models with gm of 36.52, 7.31, 13.64, and 6.13 μg L^{-1} and gsd of 2.38, 2.46, 2.48, and 2.37 for pH1N1, A (H1N1), A (H3N2), and type B, respectively (Fig. 2B).

3.2. Dose-response analysis

Here the dose–response profiles describing the relationships between OE + OC 1:4 concentration and algae yield and growth rate inhibitions can be reconstructed based on a Hill model (Fig. 3). Our results showed that the Hill model was best-fitted to the published data, resulting in an EC50 = 60.30 ± 6.53 mg L^{-1} (mean ± se) and $n = 1.0012 \pm 0.112$ for yield inhibition ($r^2 = 0.94$) (Fig. 3A) and EC50 = 463 ± 111 mg L^{-1} and $n = 1.0019 \pm 0.168$ for growth rate inhibition ($r^2 = 0.86$) (Fig. 3B).

The U.S. EPA (2000) recommended that effective concentration inducing 10% inhibition (EC10) could be used as a surrogate threshold for a regulatory endpoint in probabilistic ecological risk

Table 1
Daily average Tamiflu treatment dosage (D_T) calculation (Eq. (2)) based on age-specific dosages and influenza virus-specific proportions of confirmed case in Taiwan in the period of 2005–2009.

Age group[a]	Dosage[a] (D_j, mg person^{-1})	Age-specific proportion of confirmed cases (P_j)[b]			
		pH1N1	A (H1N1)	A (H3N2)	Type B
<3 mon	12	0.002	0.003 ± 0.006[c]	0.008 ± 0.008	0.003 ± 0.002
3–5 mon	20	0.004	0.012 ± 0.008	0.011 ± 0.003	0.002 ± 0.002
6–11 mon	25	0.004	0.006 ± 0.008	0.022 ± 0.006	0.010 ± 0.008
1–2 year	30	0.021	0.058 ± 0.020	0.095 ± 0.008	0.064 ± 0.011
3–6 year	45	0.086	0.302 ± 0.108	0.216 ± 0.037	0.285 ± 0.061
7–10 year	60	0.246	0.231 ± 0.099	0.102 ± 0.014	0.299 ± 0.136
≥11 year	75	0.637	0.386 ± 0.186	0.547 ± 0.053	0.337 ± 0.197
D_T (mg person^{-1})		94.13	82.47 ± 22.48	84.83 ± 6.24	81.41 ± 23.77

[a] Age-specific dosage is adjusted by combining both data of body weight-specific dosage (US FDA, 2009) and age-specific body weight (Taiwan DOH, 2008).
[b] Estimated from Taiwan CDC.
[c] Mean ± sd.

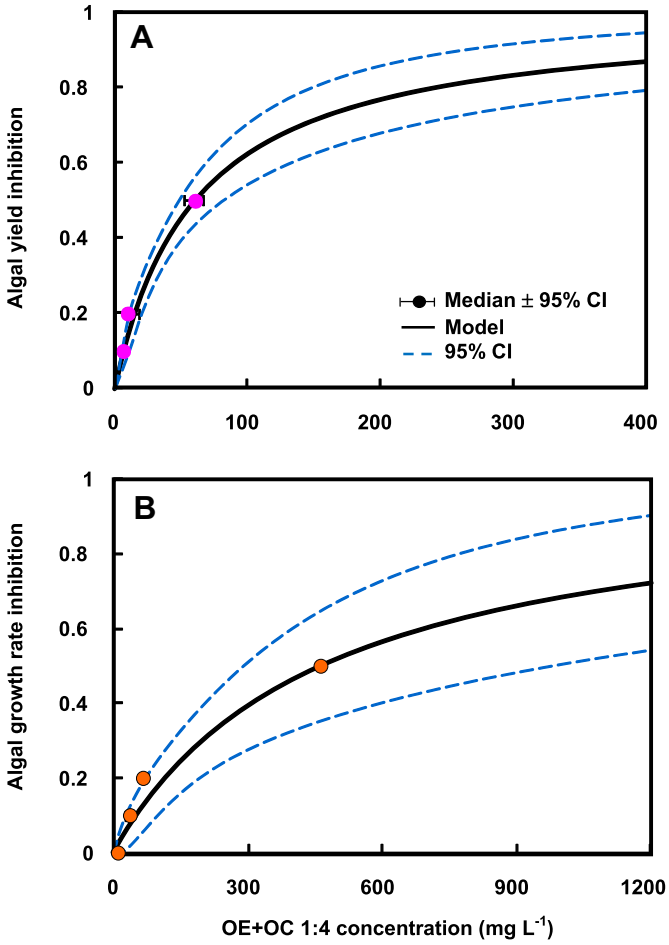

Fig. 3. Optimal fit of 2-parameter Hill equation to experimental data of (A) yield inhibition and (B) growth rate inhibition of algae versus OE + OC 1:4 concentrations based on the published 72-h acute toxicity bioassay.

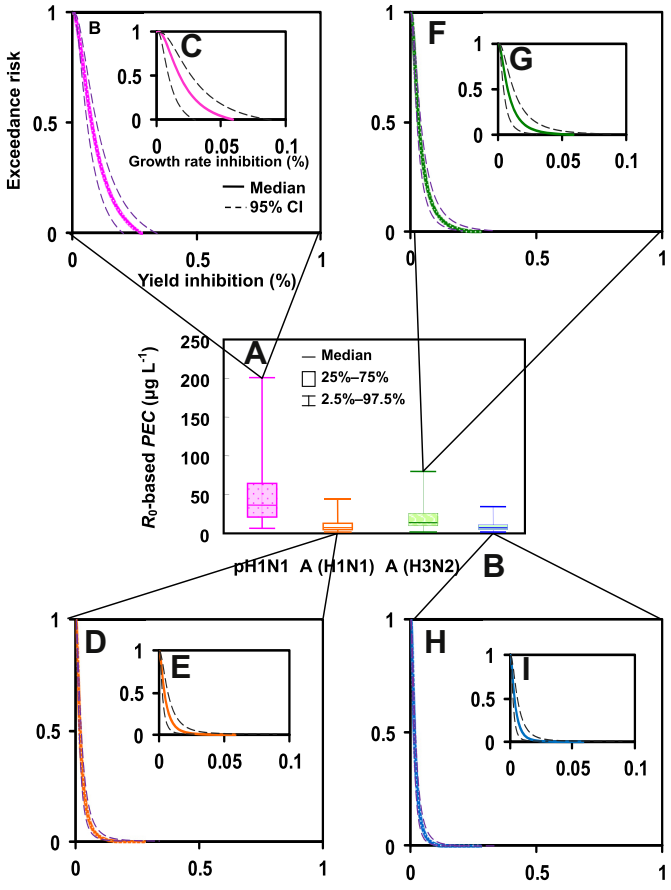

Fig. 4. (A) Box-and-whisker plot represents the distribution of R_0-based *PEC*s of Tamiflu residues in surface waters and sewage works and the uncertainty in *PEC* estimates for three influenza (sub)type viruses and pH1N1. Exceedance risk probability distributions of (B, D, F, H) yield inhibition and (C, E, G, I) growth rate inhibition of algae.

assessment. It can be seen from Fig. 3 that the calculated EC10 values were 7.04 (95% CI: 5.23–10.69) and 52 (27–98) mg L^{-1} compared with EC50 estimates of 60.30 (95% CI: 48.13–81.56) and 463 (290–979) mg L^{-1} for algal yield and growth rate inhibitions, respectively.

3.3. Risk assessment

Given the conditional dose–response distributions $P(E_Y|PEC)$ and $P(E_G|PEC)$ (Fig. 3) and R_0-based *PEC* distributions for influenza (sub) type viruses (Fig. 4A); the exceedance risk probability of algal yield and growth rate inhibitions can then be estimated by Eq. (4) (Fig. 4B–I). Fig. 4A shows that pH1N1 experiences a highest *PEC* of 36.02 (95% CI: 6.65–200.27) μg L^{-1} compared with other influenza (sub)types with average *PEC*s ranging from 6.18 to 13.47 μg L^{-1}.

A similar fashion of exceedance risk profiles exists in algal yield and growth rate inhibitions subjected to virus-specific *PEC* of OE + OC 1:4 (Fig. 4). Our results indicated that the probabilities that 50% or more of the algal yield and growth rate inhibited (risk = 0.5) ranged from 10^{-3}% to 10^{-2}%, i.e., the probability is 50% that only 10^{-3}%–10^{-2}% of algae will be affected, indicating no significant adverse effect for algae exposed to waterborne concentrations of OE + OC 1:4 (Table 2).

On the other hand, the estimated *RQ* values were 0.36 (95% CI: 0.07–2) for pH1N1, 0.07 (0.01–0.44) for A (H1N1), 0.13 (0.02–0.79) for A (H3N2), and 0.06 (0.01–0.34) for type B viruses (Fig. 5). Generally, the results indicated that OE + OC 1:4 concentration seems unlikely to result in a significant chronic environmental risk to daphnia reproduction and zebrafish development under seasonal influenza condition. Yet, the chronic environmental risk posed by Tamiflu use under pH1N1 condition was alarming based on a conservative point of view with a 97.5th-tile of $RQ = 2$ (Fig. 5).

4. Discussion

4.1. Ecotoxicology perspectives

In our study, the average *PEC* of Tamiflu residues under a pandemic condition was nearly 36 μg L^{-1} that posed no significant threat on algae yield and growth. However, from a long-term ecological hazard point of view, Tamiflu use during pandemic is

Table 2
Acute algal yield and growth rate inhibitions at exceedance risk of 0.5 in response to environmental Tamiflu concentration during virus-specific seasonal influenza and pandemic conditions.

Influenza	Yield inhibition (%)	Growth rate inhibition (%)
pH1N1	0.081 (0.045–0.148)[a]	0.018 (0.012–0.027)
A (H1N1)	0.0178 (0.01–0.032)	0.0043 (0.0028–0.0065)
A (H3N2)	0.033 (0.019–0.061)	0.0078 (0.005–0.012)
Type B	0.012 (0.008–0.027)	0.0036 (0.0024–0.0054)

[a] Median (95% CI).

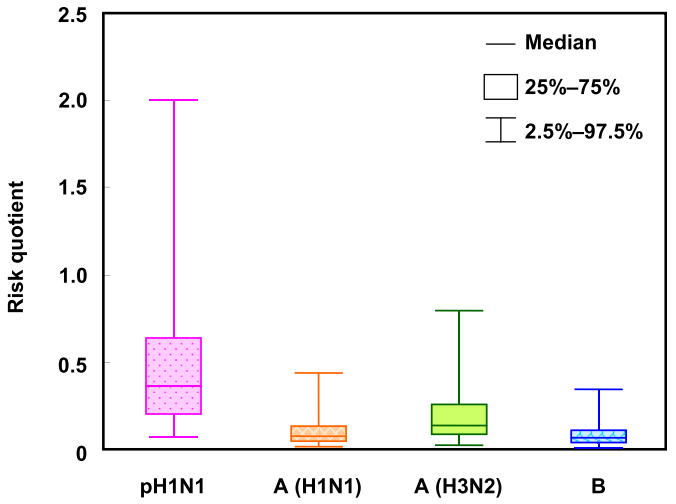

Fig. 5. Box-and-whisker plot of environmental risk quotients for influenza (sub)type viruses, A (H1N1), A (H3N2), and type B, and pH1N1.

alarming. In Sweden, environmental OC concentrations were expected to reach ~100 ppb during pandemics (Järhult et al., 2011). In England, the *PEC* of Tamiflu residues during pandemic in the Thames River Basin ranged from 0.027 to 21.3 μg L^{-1} (Singer et al., 2011). In Japan, the highest OC concentrations were detected ranging from 0.29 to 0.83 and 0.19–0.29 μg L^{-1} at STPs and receiving river waters, respectively, during a seasonal influenza outbreak (Ghosh et al., 2010a; Azuma et al., 2012). Hutchinson et al. (2009) indicated that Tamiflu use did not pose the significant exposure risks for marine organisms, such as annelids, echinoderm, and mollusk etc., under pandemic influenza conditions.

In light of a conservative assessment on the exposure risks for aquatic organisms associated with Tamiflu use under seasonal influenza and pandemic conditions, our study assumed that 100% antiviral drug Tamiflu coverage of the infection population and Tamiflu residues were not dissipated and biodegraded at STPs. This assumption may cause a significant difference between the *PEC* estimates at ppb level and the detected concentrations at ppt level.

Ghosh et al. (2010b) indicated that the OC removal efficiencies were 4.3 ± 2.06 (mean ± sd), 24.3 ± 7.28, and 92.9 ± 0.78% for primary, secondary, and tertiary (with ozone) treatment processes in STPs in Japan during 2008–2010, respectively. Prasse et al. (2010) also indicated that the OC removal efficiency of tertiary (with chemical phosphorus removal) treatment process was 59%. Matsuo et al. (2011) indicated that OE removal efficiency of secondary treatment process was 6–44%. Azuma et al. (2012) also indicated that OE removal efficiencies were 3–14% and 90% for secondary and tertiary (with ozone) treatment processes, respectively. Moreover, similar OE and OC removal efficiencies were found among different levels of treatment processes in STPs (Ghosh et al., 2010b; Prasse et al., 2010; Matsuo et al., 2011; Azuma et al., 2012). Recently, Singer et al. (2013) suggested that effective drug compliance may lead to less mis- and un-used Tamiflu and less wastage based on a waste water epidemiology approach.

Here when we used removal efficiencies of 0, 4, 24, 59, and 93% to reduce Tamiflu residues in STPs, *PEC* estimates were ranging from 6.18 to 36.02, 5.91 to 34.47, 4.68 to 27.27, 2.53 to 14.77, and 0.44 to 2.56 μg L^{-1}, respectively, under seasonal influenza and pandemic conditions. On the other hand, 97.5th percentile *RQ* for daphnid and zebrafish can be calculated to be 2, 1.92, 1.52, 0.82, and 0.14 at Tamiflu residues removal efficiencies of 0, 4, 24, 59, and 93%, respectively. These results show that there are no significant risks ($RQ < 1$) for daphnid and zebrafish when Tamiflu residues removal efficiency in STPs is greater than or equal to 59%. Thus, we suggest that tertiary treatment (e.g., ozonation) need to be taken into account in sewage treatment process under an influenza pandemic condition for reducing the exposure risks of Tamiflu residues for aquatic organisms (Mestankova et al., 2012).

4.2. Epidemiology perspectives

Järhult et al. (2011) indicated that oseltamivir resistance developed through the acquisition of H247Y mutation in NA gene when mallards (*Anas platyrhynchos*) infected by influenza A (H1N1) virus with an exposure OC concentration of 1 μg L^{-1}. The viral genotypes in water sample were detected containing a mixture combination of both wild-type and H274Y strains and, consequently, the H247Y mutants rapidly became predominate among the viral population at OC levels of 80 μg L^{-1}. Therefore, this study revealed that the median *PEC* estimates, ranging from 6.18 to 36.02 μg L^{-1}, may cause the occurrence of an environmental oseltamivir resistance.

Singer et al. (2007) used D_T of 75 mg twice daily to estimate the *PEC*. On the other hand, Ghosh et al. (2010a) used 85% adult (75 mg twice daily) and 15% children (45 mg twice daily) to estimate D_T value. However, they did not consider the proportion of age-specific confirmed cases for various influenza (sub)type viruses for estimating the *PEC*. Hence, it might lead to the *PEC* overestimated, due to the recommended treatment dosages for pediatric patients of less than 75 mg. This study was taken into account the proportion of age-specific confirmed cases that were used to estimate D_T for pH1N1, A (H1N1), A (H3N2), and type B viruses. Thus, the *PEC* estimates at STP effluents should be more accurate as in the real situations.

In recent decades, mathematical modeling of infectious diseases dynamics has grown substantially and been gaining certain momentum. Models could be used to address public concerns relating to an ever-expanding number of emerging diseases and to explore the importance of biological and ecological characteristics on disease transmission (Anderson and May, 1991; Keeling and Rohani, 2008). The most well-known susceptible–infectious–recovered (SIR) model is a potentially powerful tool for modeling transmission dynamics of diseases. The use of the SIR model in disease transmission dynamics should only increase in the future. The SIR model can provide a basic description of the transmission dynamics of pandemic influenza by using a simple parameterized set of ordinary differential equations. In the future work we may incorporate the epidemiological SIR modeling into the ecotoxicological models to assess the ecotoxicological risks of antiviral drug use during an influenza outbreak of varying severity within a probabilistic assessment framework.

4.3. Implications for ecological risk assessment

This study provided an approach for assessing the exposure risks of aquatic organisms in response to environmental anti-influenza drug metabolites based on epidemiological and ecotoxicological modeling and can be applied for future environmental risk assessment on antiviral drug exposures. Moreover, there is a need for empirical data on the effects of antibiotics and antiviral drugs on STPs and freshwater ecotoxicity (Singer et al., 2011). Therefore, the effects of aquatic organisms exposed to mixed concentrations of antiviral and antibiotic drugs can also be taken into account in future environmental risk assessment.

In the future work we may focus on developing the environmental quality criteria (EQC) for Tamiflu residues. US EPA (1995) indicated that EQC plays a pivotal role in protecting ecosystems from undesirable effects of chemicals as it is an essential part of both source- and effects-oriented management for chemical

substances. A major complication in deriving EQC for aquatic organisms is the high degree of uncertainty resulting from the lack of dose–response information and the large environmental variability in exposures among individuals. We can choose an appropriate risk criteria value based on a 10% probability of exceedance the effect concentration affecting 10% (EC10) of sensitive aquatic organisms as suggested by US EPA (1995).

Suggestions have been made that the EC5 would be more protective of ecosystem structure and function than EC10 or EC50 (Van der Hoeven et al., 1997; Moore and Caux, 1997). Versteeg et al. (1999) and Van der Brink et al. (2002) also suggested that the selection of a hazard external effect concentration (EEC) protecting 95% of the single-specific sensitivity distribution (i.e., EEC5) appears to provide an appropriate level of protection when compared to multispecies tests or field studies. One reason is that if concentrations of this compound are below the EEC5, more than 95% of the biological species set considered will not display effects as determined by the chronic toxicity tests. In our study, the EC5 estimates are 3.52 (95% CI: 2.61–5.39) and 25 (11–97) mg L^{-1} for algal yield and growth rate inhibitions, respectively.

Because chronic tests are more lengthy and the endpoints are somewhat subjective, it is not surprising that more often chronic and standards are based on the acute-to-chronic ratio (ACR) (US EPA, 1985; Ford, 2001). The ACR is the ratio of acute toxicity value of environmental concentration to its chronic toxicity values. The ACRs can be used to estimating the chronic toxicity based on the acute toxicity data and vice versa. The ACRs are derived on a species-by-species basis, ideally with both the acute and chronic toxicity data developed from the same test. The ACR values are typically greater than one, reflecting the fact that chronic toxicity typically occurs at lower levels than dose acute toxicity. US EPA (1985) suggested that the ACR approach can be served as the basis of the chronic criteria in that the ACR is the geometric mean of the ratio of acute to chronic values. In our case, the estimated ACR is ~57 for algal yield inhibition during Tamiflu use under seasonal influenza and pandemic conditions.

5. Conclusions

We linked relevant principles of ecotoxicology and epidemiology to assess the potential exposure risks of aquatic organisms posed by residues induced from antiviral drug Tamiflu use during seasonal influenza and pandemic conditions. We used a probabilistic risk assessment model to estimate potential threats of environmentally relevant hazards based on published acute and chronic bioassays. Our results indicated that concentration of Tamiflu residues was unlikely to pose a significant chronic environmental risk to daphnia reproduction and zebrafish development during seasonal influenza. However, the chronic environmental risk posed by Tamiflu use during the pH1N1 condition was alarming. On the other hand, no significant risk to algal yield and growth rate was found during regular seasonal influenza and high pandemic use of Tamiflu.

Moreover, because anti-influenza drugs are projected to increase and become consequently more available for the growing population, it is expected that the concentrations of anti-influenza drug residues in aquatic environments should be increased. Our results highlight ecotoxicologically important and call for a probabilistic risk assessment framework to examine the full environmental impact of anti-influenza drug residues.

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