Journal of Hazardous Materials 187 (2011) 480–487

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Health risk characterisation for environmental pollutants with a new concept of overall risk probability

Qiming Cao [a,b,*], Qiming Yu [a], Des W. Connell [c]

[a] Griffith School of Engineering, Nathan Campus, Griffith University, 170 Kessels Road, Nathan, QLD 4111, Australia
[b] POPs Research Centre, Tsinghua University, Beijing 100084, China
[c] Griffith School of Environment, Nathan Campus, Griffith University, 170 Kessels Road, Nathan, QLD 4111, Australia

ARTICLE INFO

Article history:
Received 27 October 2010
Received in revised form 7 January 2011
Accepted 12 January 2011
Available online 18 January 2011

Keywords:
Health risk assessment
Risk characterisation
Overall risk probability
Endocrine disrupting chemicals
Estrogen

ABSTRACT

In health risk assessment, risk is commonly characterised by calculating a simple hazard quotient (HQ), which cannot reflect the actual distribution of exposure and health effect values. This study aimed to develop a new risk characterisation method, the overall risk probability (ORP) method based on probabilistic techniques. Exposure exceedence values were calculated to obtain an exposure exceedence curve (EEC). The area under the EEC was calculated as the ORP value to represent the risk. This method was demonstrated by a case study for two steroidal EDCs, 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) for fish in surface water. It was found that the risk probability of fish exposed to E2 (ORP, 8.1%) and EE2 (ORP, 27%) were both above the reference value of 2.5%, which was consistent with the results of HQ method. Assuming independent action of individual EDCs, a combined risk probability of 33% was obtained for the mixture effects of E2 and EE2. Our results implicated that the adverse health effects imposed by E2 and EE2 were significant for fish in surface water worldwide.

1. Introduction

Each year, large quantities of chemicals are released into the environment contaminating land, water, air and food sources. As a result, various adverse health effects such as cancers, birth defects and reproductive abnormalities have been observed in many wildlife species and humans. For example, a particular group of pollutants termed as endocrine disrupting chemicals (EDCs) is able to cause endocrine disruption in living organisms. Evidence of this includes increased vitellogenin (VTG) levels in male and juvenile female fish, reproductive abnormalities, altered sexual ratios and neuroendocrine disruption in some aquatic species [1–5]. Research has also revealed possible links between EDCs (e.g., DDT and DES) and adverse human health effects such as female breast cancers, male testicular and prostate cancers [6–13].

With more evidences on adverse health effects appeared in the scientific communities and public media, the health risks of emerging or existing environmental pollutants are subjected to close scrutiny by many regulatory authorities. Thus, the assessment of these health risks becomes a crucial step for any further regulatory actions. The principle goal of a risk assessment is to define a 'safe' exposure level, which can protect the majority of organisms at most of the time with minimum costs. In this context, the concept of 'risk' generally has three core elements, exposure, adverse effects and likelihood or probability of adverse effects. The risk will be zero without any of these three elements [14,15]. Risk assessment using probabilistic techniques will enable the risk assessor to express the risk in terms of probability distribution, rather than the traditional deterministic methods using a single-point risk estimation approach.

Historically, probabilistic techniques have been applied to engineering problems since the 1970s, such as the estimation of seismic risk and assessment of nuclear power plant safety [16,17]. Recently it has been applied to assess the risk of environmental pollutants [18–22]. In this method, the exposure and effect values are expressed in cumulative probability distributions (CPD) and plotted in the same diagram. For simple risk estimation, the risk can be expressed as a hazard quotient (HQ) value (also referred to as risk quotient), which is the ratio of an exposure concentration to an adverse effect concentration [19]. The estimated HQ values are compared with a reference value of one to show whether the risk caused by the target pollutant is significant or not. However, this risk characterisation method is a single-point risk estimation method, which cannot reflect the actual shape and distribution of the CPD curves.

The aim of this article was to propose a new health risk characterisation method by using the concept of overall risk probability (ORP), which is capable of reflecting the shape and distribution of

* Corresponding author at: Griffith School of Engineering, Nathan Campus, Griffith University, 170 Kessels Road, Nathan, QLD 4111, Australia. Tel.: +61 7 3735 5289; fax: +61 7 3735 7459.
E-mail address: qimingcao@mail.tsinghua.edu.cn (Q. Cao).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.01.054

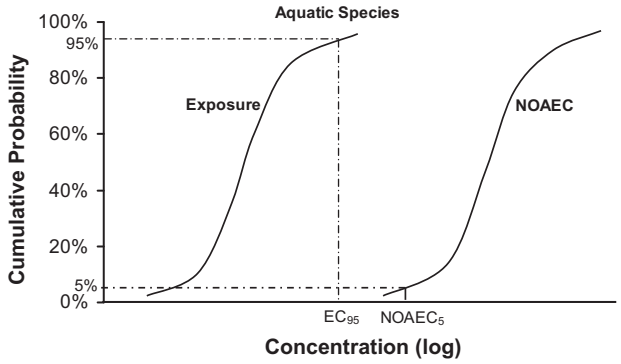


Fig. 1. Cumulative probability distribution of exposure and NOAEC values for aquatic species.

CPD curves. As an example, a case study of health risk characterisation with this new concept was conducted for two typical steroidal EDCs, 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) for fish in surface water.

2. Methodology

2.1. Risk assessment using probabilistic techniques

The use of probabilistic techniques in risk assessment has gained increasing popularity in the area of environmental science, which proved its usefulness and applicability. A detailed description of conducting probabilistic risk assessment was published in two US EPA guidance documents [23,24]. Its application was also demonstrated elsewhere in a number of case studies [18,20,21]. Briefly, the exposure and adverse effect values (measured or simulated) are cumulatively distributed in the same plots, which were illustrated in Fig. 1 for aquatic species and Fig. 2 for humans and mammals. In Fig. 1, aqueous phase concentration is used for the measure of exposure, whereas in Fig. 2, daily dose of exposure is used. The risk is evaluated from the overlapped region between the exposure and effects CPD curves. Generally, the closer the two CPD curves, the higher is the level of risk.

It is important to note that, in the calculation of cumulative probabilities for exposure values, those values that are reported to be below the detection limit should also be counted as part of the total number of exposure values. Solomon et al. [19] assigned a dummy value of zero for these values to obtain the correct position of each point in the CPD curve. Cao [25] suggested using random values between zero and the detection limit to simulate random sampling, which was regarded as an improvement in data treatment.

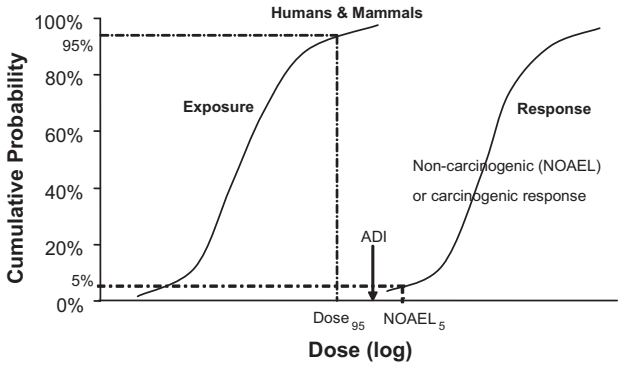


Fig. 2. Cumulative probability distribution of exposure and NOAEL values for humans and mammals.

In the assessment of adverse effects for aquatic organisms (e.g., fish), the *no-observed-adverse-effects-concentration* (NOAEC) values are collated and ranked to calculate cumulative probabilities (CP) (Fig. 1). Similarly in dose–response assessment for humans and mammals, the *no-observed-adverse-effects-level* (NOAEL) values are used for non-carcinogenic effects, whilst other indicative levels can be used for carcinogenic effects (Fig. 2). Due to experimental difficulties and sensitive ethical issues [26,27], NOAEL values are not always available for humans. Thus, NOAEL values obtained in animal studies can be used to extrapolate NOAEL values for humans with appropriate methods. Currently, there are three interspecies extrapolation methods: extrapolation based on caloric demand, body weight and body surface area [29]. These methods have been reviewed and compared by several authors [29–32]. The body surface area method has been recommended by the US Food and Drug Administration [33], which is described in Eq. (1).

$$\mathrm{NOAEL_{HED}} = \mathrm{NOAEL_{animal}} \times \frac{K_{\mathrm{m\ animal}}}{K_{\mathrm{m\ human}}} \quad (1)$$

where $\mathrm{NOAEL_{HED}}$ is the human equivalent daily dose (ng kg $\mathrm{BW^{-1}\ d^{-1}}$), $\mathrm{NOAEL_{animal}}$ is the animal dose (ng kg $\mathrm{BW^{-1}\ d^{-1}}$), K_m is a factor calculated as the body weight (BW) divided by body surface area ($\mathrm{m^2}$). Some typical values of K_m were set by the US Federal Drug Administration for humans and some common mammals used in laboratory studies [33].

The measured or extrapolated human NOAEL values can be used to determine a reference dose value (RfD) or an *acceptable daily intake* (ADI) by dividing a safety factor ranging from 10 to 1000 (Fig. 2). Due to experimental difficulties in the determination of NOAEL and NOAEC values, Bailer and Oris [34] suggested that the *lowest-observed-adverse-effects-level* (LOAEL) values or the *lowest-observed-adverse-effects-concentration* (LOAEC) values can also be used in effects assessment in the absence of NOAEL and NOAEC values.

2.2. Risk characterisation by single-point methods

Commonly, risk is characterised by the hazard quotient ($\mathrm{HQ_{95/5}}$) method for non-carcinogenic effects and the 'slope factor' method for carcinogenic effects. The $\mathrm{HQ_{95/5}}$ method is a single-point comparison between exposure and non-carcinogenic effect values, which is generally expressed as an exposure value divided by an effects value [35]. For the protection of the majority of population under most exposure conditions, a $\mathrm{HQ_{95/5}}$ value is calculated as an exposure value at 95% of CP divided by an adverse effects value at 5% of CP, which is described by Eqs. (2) and (3).

$$\mathrm{HQ_{95/5}} = \frac{\mathrm{EC_{95}}}{\mathrm{NOAEC_5}} \quad \text{(Aquatic species)} \quad (2)$$

where $\mathrm{EC_{95}}$ is the exposure concentration at 95% of CP and $\mathrm{NOAEC_5}$ is the adverse effects concentration at 5% of CP.

$$\mathrm{HQ_{95/5}} = \frac{\mathrm{Dose_{95}}}{\mathrm{NOAEL_5}} \quad \text{(Humans and mammals)} \quad (3)$$

where $\mathrm{Dose_{95}}$ is human or mammals daily dose at 95% of CP and $\mathrm{NOAEL_5}$ is the adverse effects level at 5% of CP.

The $\mathrm{HQ_{95/5}}$ value of one can be regarded as a reference value to assess whether a significant level of health risk occurs or not. If $\mathrm{HQ_{95/5}}$ is less than 1, it means that less than 5% of organisms will be affected by 95% of exposure concentrations, or the majority of exposure concentrations will affect only a minority of the population. If $\mathrm{HQ_{95/5}}$ is larger than 1, it means that more than 5% of fish will be affected by 95% of exposure concentration, or the population affected by most exposure concentrations is significant (>5%).

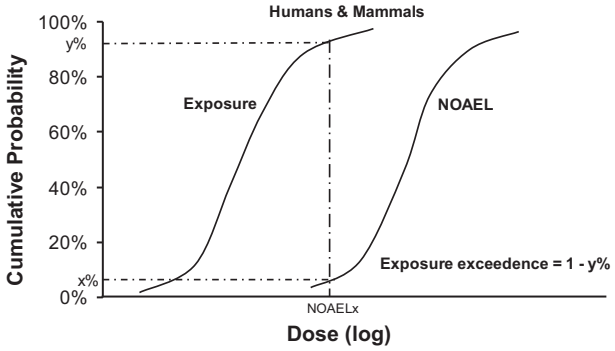


Fig. 3. The calculation of exposure exceedence value at any cumulative probability of affected samples.

For carcinogenic effects, the US EPA [36] set the guidelines to calculate the carcinogenic risk by introducing a slope factor:

$$CR = SF \times CDI \quad (4)$$

where CR is the carcinogenic risk, SF is the slope factor and CDI is the chronic daily intake. Most SF values can be found in the integrated risk information system (IRIS) database of US EPA (www.epa.gov/iris), or it can be derived from the slope by linear extrapolating from the high dose range to low dose range in the dose-response curve.

2.3. Risk characterisation by overall risk probability

In contrast to the above single-point methods, the ORP method is a multipoint risk characterisation method, which can be applied to both non-carcinogenic and carcinogenic effects. The ORP method is based on the use of an exposure exceedence curve (EEC) [37]. The EEC is the plot of exposure exceedence values against CP values for given affected samples. The calculation of exposure exceedence values can be illustrated as shown in Fig. 3. For example, at any *x*% of affected samples indicated in the effects CPD curve, it corresponds to an effect level of NOAEL*x* (or a dose level related to a certain carcinogenic effect). Within the overlapped region, this NOAEL*x* value also corresponds to an exposure CP value of *y*%. The CP of exposure level higher than this NOAEL*x* value, $(1 - y\%)$ is calculated as the exposure exceedence value. Thus, an EEC can be obtained by plotting the CP of affected samples against exposure exceedence values, which is shown as curve A in Fig. 4.

The risk can be qualitatively characterised by the relative distance between the EEC and the origin of the axes (Fig. 4). A larger distance implies higher risk. Therefore, the EEC provides a tool which is able to compare the risk level among different EDCs. Interestingly, the area under the EEC increases when the curve moves away from the origin. Thus, the area can be used to quantify the risk. This area is obtained as the product of the two probabilities in the CPD curves for exposure and effects values. This is referred as the ORP value here ranging from 0 to 1.

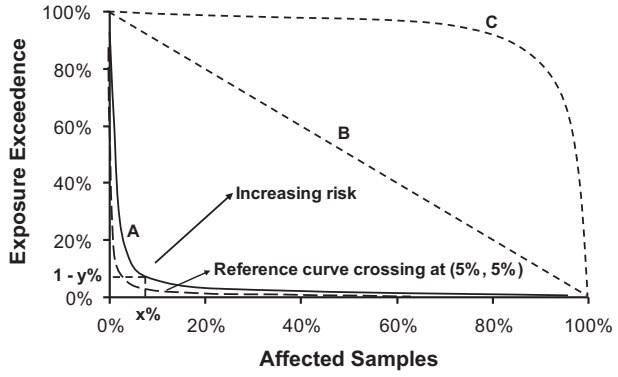


Fig. 4. Exposure exceedence curve derived from the cumulative probability distributions of exposure and effects values.

It is observed that the relative position between the exposure and effect CPD curves can affect the shape of the EEC. Usually, the exposure CPD curve is on the left hand side of effects CPD curve and the shape of EEC is concave (curve A in Fig. 4). However, if the two CPD curves are overlapped with each other, the EEC became a straight line and the corresponding ORP value is 0.50 (diagonal line B in Fig. 4). In some unusual situations, such as accidental industrial spills, the exposure CPD curve is on the right hand side of effects CPD curve, the EEC will be convex (curve C in Fig. 4).

In correspondence to the reference value of one in the $HQ_{95/5}$ method, a reference value for the ORP method can also be developed. This value is calculated as the area under a reference curve crossing the point of (5%, 5%) as shown in Fig. 4. The point of (5%, 5%) corresponds to the reference value of one in the $HQ_{95/5}$ method. By taking half of the total area of the small rectangular and two triangulars in Fig. 4, the reference value obtained for the ORP method is 2.5%. With this reference value, it enables the risk assessor to judge whether a significant level of risk is imposed or not. For example, if the obtained EEC for a particular EDC is above the reference curve, or if the ORP value calculated is larger than 2.5%, the risk is considered as significant.

2.4. Considerations for mixture of environmental pollutants

In practical situations, human and animals are usually exposed to a mixture of pollutants, instead of a single pollutant. These pollutants may impose antagonistic, additive or synergistic effects on the exposed organisms [28,38–42]. Although the combined mixture effects have been assessed by several concentration addition models [38–40,43,44], the lack of information on exposure scenarios makes health risk assessment difficult for mixtures [42]. With the concept of ORP proposed in this work, this issue can be resolved by assuming each pollutant acts independently. For each individual pollutant in a mixture, the probability of having certain adverse health effects is calculated as ORP_i ($i = 1, 2, \ldots, n$) according to the method described in Section 2.3. Thus, the probability of no adverse health effects for this pollutant is $(1 - ORP_i)$. For the mixture, the probability of no certain adverse health effects is the product of all $(1 - ORP_i)$ values for individual pollutant. Therefore, the probability of having adverse health risk for the mixture can be calculated as:

$$ORP_{mixture} = 1 - \prod_{i=1,2,\ldots,n} (1 - ORP_i) \quad (5)$$

where $ORP_{mixture}$ is the ORP of mixture, ORP_i is the ORP of individual pollutant.

2.5. Comparison between $HQ_{95/5}$ and ORP method

The major difference between the $HQ_{95/5}$ method and the ORP method is that the former is a single-point risk estimation method, whilst the latter takes into account of all points in exposure and effects CPD curves. In other words, the information of the shape of the CPD curves is taken into account by the ORP method. Generally, there is a good agreement between these two methods. For example in Fig. 5a, both $HQ_{95/5}$ and ORP values decrease when the effects CPD curve moves from B to C. In the other two examples illustrated in Fig. 5b and c, both $HQ_{95/5}$ and ORP values increase when the slope of exposure or effects CPD curve decreases.

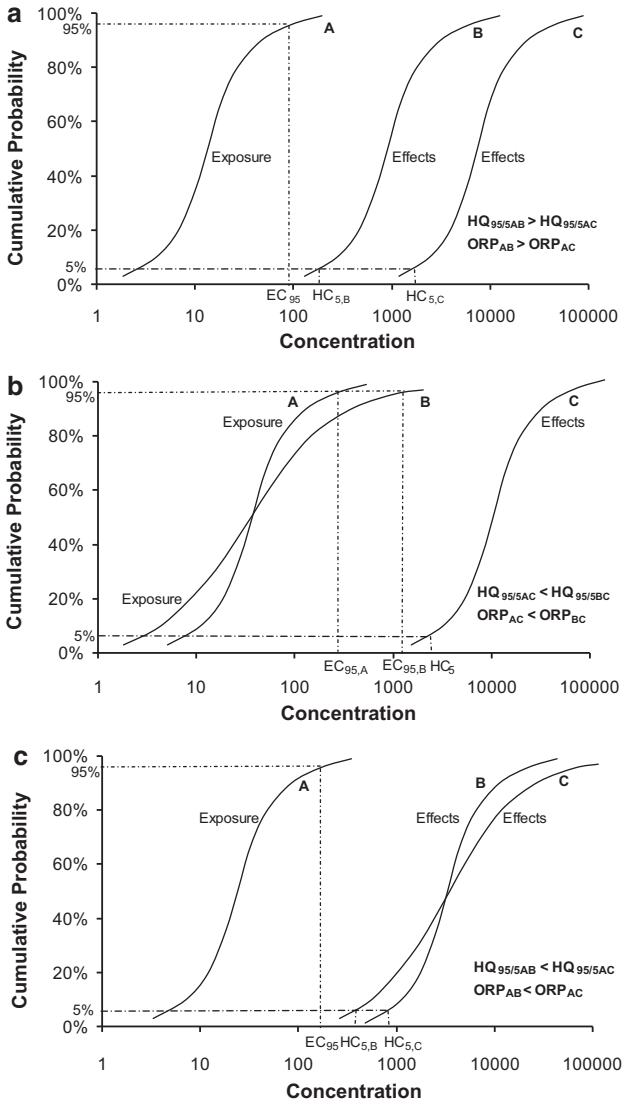


Fig. 5. (a) Agreement between the $HQ_{95/5}$ and ORP method when effects CPD curve moves from B to C. (b) Agreement between the $HQ_{95/5}$ and ORP method when the slope of exposure CPD curve decreases from A to B. (c) Agreement between the $HQ_{95/5}$ and ORP method when the slope of the effects CPD curve decreases from B to C.

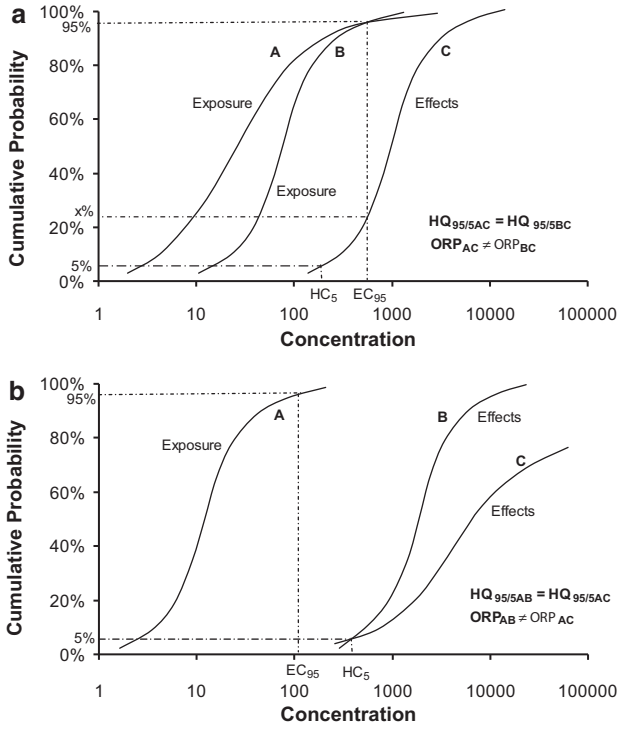


Fig. 6. (a) Disagreement between the $HQ_{95/5}$ and ORP method when two exposure CPD curves overlapped at EC_{95}. (b) Disagreement between the $HQ_{95/5}$ and ORP method when two effects CPD curves overlapped at HC_5.

However in other cases, disagreement also exists between these two methods. For example, in Fig. 6a and b, $HQ_{95/5}$ values calculated from the exposure and effect CPD curves are the same, because overlap occurs at the EC_{95} or HC_5 for different exposure or effects CPD curves. Apparently, the ORP values calculated from their exceedence curves will be different, depending on the slope changes of the exposure or effects curve. This was explained in detail in the following example.

In Fig. 6a, the slope of exposure CPD curve A is smaller than curve B, resulting in two different exceedence curves as shown in Fig. 7. These two exceedence curves intersect at the point of (x%, 5%) with x% being the cumulative probability of the effects CPD curve at EC_{95}. The relative size of the area under these two exceedence curves depends on the position of this overlapped point of (x%, 5%). The value of ORP_{AC} equals the value of ORP_{BC} only at a particular point when the size of those two shaded areas is the same. Similar analysis could be done with the example in Fig. 6b, which also shows that $HQ_{95/5}$ method cannot reflect the shape of CPD curves.

In some rare situations, the CPD curve of exposure or effects is close to a vertical line because of extremely small standard deviation compared with the other CPD curve with large standard deviation. If the exposure CPD forms a vertical line, a 'Z' shaped exposure exceedence curve is obtained with a rectangular area of x% (or ORP = x%). Similarly, if the effect CPD forms a vertical line, a mirror 'Z' shaped exceedence curve is obtained. The area under the exceedence curve will be $(1-x\%)^2$. From the above analysis, it again indicates that the ORP method reflects more information regarding the CPD curve of exposure and effect data. Therefore, the ORP method is regarded as an improvement in risk characterisation. It has been further proof-tested with seven EDCs, which all showed good agreement with the HQ method. As an example, the risk characterisation for two steroidal EDCs was presented in the following case study.

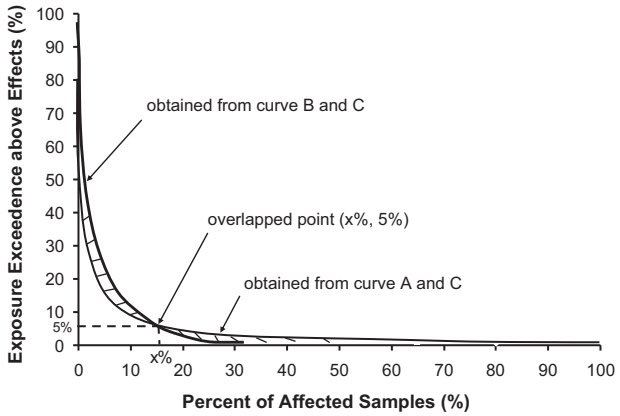
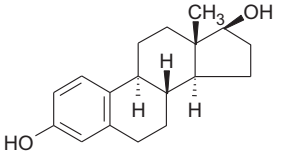
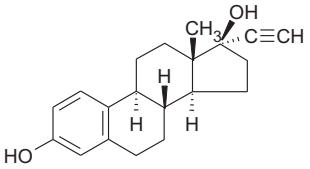


Fig. 7. Comparison of $HQ_{95/5}$ and ORP values when the exposure CPD curves overlapped at the same EC_{95} point (exceedence curves derived from CPD curves in Fig. 6a).

Table 1
Physicochemical properties and chemical structure of 17β-estradiol (E2) and 17α-ethinylestradiol (EE2).[a]

EDC	MW (g mol^{-1})	MP (°C)	S (mg L^{-1})	log K_{ow} (–)	EEF (–)	Chemical structure
E2	272	222	3.9	3.57	1	
EE2	296	183	9.7	3.67	1.2	

Data adapted from [89,90].
[a] MW – molecular weight, MP – melting point, *S* – aqueous solubility, log K_{ow} – octanol/water partition coefficient, and EEF – E2 equivalent factor.

2.6. A case study of E2 and EE2

The steroidal EDC, 17β-estradiol (E2) is a naturally occurring estrogen produced in human and animal body, but it can also be synthesised from other steroids [45,46]. In contrast, 17α-ethinylestradiol (EE2) is a synthetic steroidal EDC and mainly used for contraceptive purposes. The physicochemical properties listed in Table 1 indicate that E2 and EE2 are hydrophobic and can easily partition into organic matter or fat tissues. After metabolism in the body, E2 and EE2 are excreted as sulphate and glucuronide conjugates. Conjugation makes estrogens more water soluble and biologically inactive [47,48]. Once entering the sewer system and wastewater treatment plant (WWTP), the conjugated E2 is completely deconjugated into its free form by sewage bacteria [49–52].

Although the majority of E2 and EE2 can be removed in WWTPs with activated sludge process, they can be detected in WWTP effluents with concentration from below detection limit to several ng L^{-1}. Recent studies indicated that these remaining steroidal EDCs in effluent can still be a threat to aquatic organisms in surface water [1,52–54]. Due to bioconcentration and biomagnification mechanisms, birds or humans that consume the contaminated fish may also be under health threat. Therefore, it is necessary to conduct health risk assessment for aquatic organisms exposed to steroidal EDCs in surface water.

In this study, the measured exposure concentration values of E2 and EE2 were derived from published scientific literature from 15 countries worldwide, such as Germany, France, Italy, the Nederland, UK, the USA, Canada, Japan and China [49,55–73]. With effects assessment, the induction of significant level of vitellogenin (VTG), a yolk protein, has been commonly used as a biomarker for adverse health effects for fish. In this study, NOAEC values on VTG induction for fathead minnow, brown trout, rainbow trout, Japanese medaka, and zebrafish were derived from numerous published studies [1,53,54,74–87].

Both exposure and effects values were cumulatively distributed in the same plots and shown in Fig. 8a and b. With these plots, a risk characterisation was conducted by the $HQ_{95/5}$ and ORP method respectively. The $HQ_{95/5}$ values were obtained by simply using Eq. (2), which gave values of 16 and 250 for E2 and EE2 respectively. It should be noted that the EC_{95} and $NOAEC_5$ values estimated from Fig. 8 in logarithm were converted into their original values before they can be calculated with Eq. (2). Compared with the $HQ_{95/5}$ method, exposure exceedence values had to be calculated and plotted against the CP of affected fish to obtain the EEC, which were shown in Fig. 9 for E2 and EE2 respectively. The area under each EEC was calculated numerically to be 8.1% for E2 and 27% for EE2. Assuming independent action, the ORP value calculated by Eq. (5)

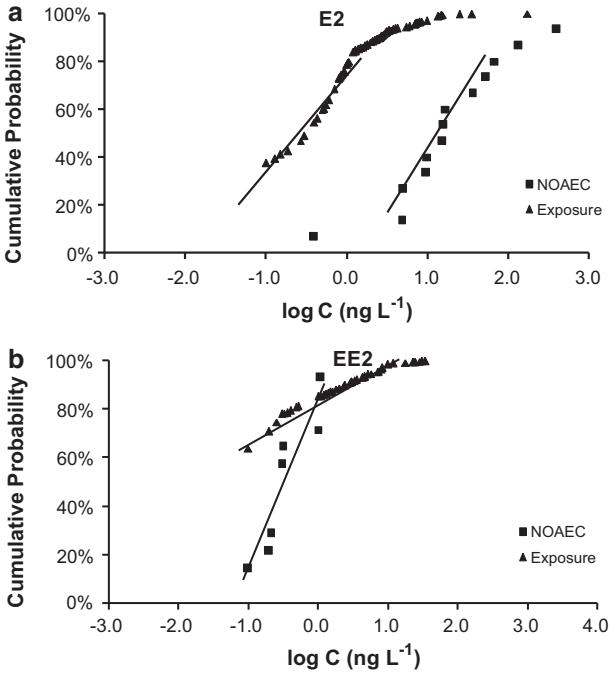


Fig. 8. (a) The characterisation of measured exposure and effects concentration values of E2 in surface water worldwide for fish. (b) The characterisation of measured exposure and effects concentration values of EE2 in surface water worldwide for fish.

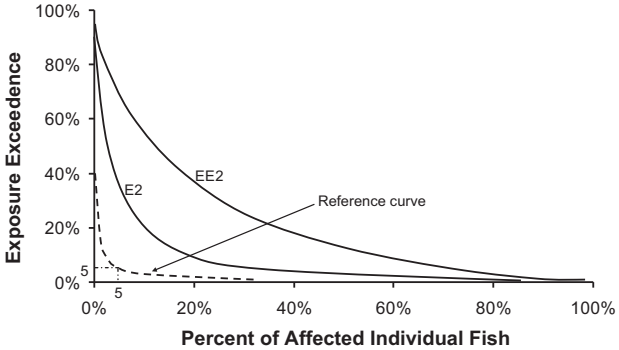


Fig. 9. Exposure exceedence curve of E2 and EE2 in surface water for fish.

for the mixture effects was 33%, which was higher than the ORP value of individual estrogens.

3. Discussion

The probabilistic techniques used in risk assessment conveniently provided either qualitative or quantitative observations regarding the health risk. With the CPD curves plotted in Fig. 8, a significant level of health risk was observed with fish exposed to E2 and EE2, because the exposure and effects CPD curves were almost completely overlapped. The slop of CPD curves indicates the range or standard deviation of exposure and effects values. This is particularly important for effects assessment, because larger slope corresponds to a narrower range of NOAEC values, which implies fish is very sensitive to these pollutants. It was also noticed in Fig. 8b that a large proportion of exposure values for EE2 were below the detection limit. This reflected the fact that the concentration of EE2 in wastewater effluent is generally lower than E2. Although the half-life of EE2 is much longer than E2 in activated sludge and natural environment [88], its potential for adsorption to organic matter is higher than E2 due to larger log K_{ow} values (Table 1). As a result of these factors, the concentration of EE2 in surface water is generally lower than E2.

A distinct difference in the effects assessment between E2 and EE2 is that the NOAEC values of EE2 were lower than those of E2. The median effects concentration of 14 and 0.3 ng L^{-1} at 50% of CP were estimated from the effects curves for E2 and EE2 respectively. These two median concentrations are only general reflections of their strength of biological activity (or potency) and should be distinguished from the EEF values listed in Table 1, which used the same bioassay for comparison (E-screen or MVLN cells).

The $HQ_{95/5}$ values obtained for E2 (16) and EE2 (250) were well above the reference value of one, showing significant level of risk for fish. This result implies a global pollution of surface water by these two EDCs, because the exposure and effects data were derived from numerous sources from 15 countries worldwide. Therefore, the removal of E2 and EE2 has to be improved for current WWTPs. This issue however is problematic for many WWTPs, as their principle goal is to remove most common pollutants to meet local effluent discharge requirements. In addition, there is a lack of legislative actions on the emerging issue of EDC pollution. As a result, the discharge limit of EDCs is not specified in the license of most WWTPs.

Although the $HQ_{95/5}$ values indicated a significant level of risk, the results were difficult to interpret. With the obtained $HQ_{95/5}$ values, the risk assessor only knows that the health risk of EE2 is higher than E2, but the probabilities of health risk were difficult to quantify. This weakness, however, was addressed by the ORP method proposed in this work. With the plotted EEC in Fig. 9, it was straight forward to observe the different level of risk between these two EDCs. Apparently, EE2 exhibit much higher level of health risk for fish. The area under the EEC was easy to calculate, which gave ORP value of 8.1% for E2 and 27% for EE2. The results can be generally interpreted that there were 8.1% and 27% of probability that fish were at risk. Both ORP values were above the reference value of 2.5%, showing significant level of risk. This was also consistent with the results obtained using the $HQ_{95/5}$ method. In addition, the ORP for the case of E2 and EE2 presented as a mixture was also easily quantified as 33%. This quantification with the $HQ_{95/5}$ method whereas is not feasible.

4. Conclusions

This study developed a more detailed risk characterisation method, the overall risk probability (ORP) method. Although this method had some degree of consistency with the commonly used hazard quotient ($HQ_{95/5}$) method, it showed a clear advantage of being able to reflect the shape and actual distribution of exposure and effects values. This method also enables the comparison of risk level among different pollutants either qualitatively by the exposure exceedence curve (EEC) or quantitatively by the ORP value. With this method, the obtained ORP values for E2 and EE2 in the case study were all above the reference value implicating the adverse effects on fish are significant in a global scale. Particularly the synthetic EDC of EE2 imposed much higher health risk for fish than the natural EDC of E2. Therefore, the removal of these two pollutants in WWTPs needs to be improved. In addition, the mixture effects were also easily quantified by the ORP method whereas this quantification is not feasible with the $HQ_{95/5}$ method.

Acknowledgements

This work was supported in part by a Research Grant from Australia Research Council.

References

[1] C.E. Purdom, P.A. Hardiman, V.J. Bye, N.C. Eno, C.R. Tyler, J.P. Sumpter, Estrogenic effects of effluents from sewage treatment works, J. Chem. Ecol. 8 (1994) 275–285.
[2] S. Jobling, M. Nolan, C.R. Tyler, G. Brighty, J.P. Sumpter, Widespread sexual disruption in wild fish, Environ. Sci. Technol. 32 (1998) 2498–2506.
[3] M.A. Rempel, J. Reyes, S. Steinert, W. Hwang, J. Armstrong, K. Sakamoto, K. Kelley, D. Schlenk, Evaluation of relationships between reproductive metrics, gender and vitellogenin expression in demersal flatfish collected near the municipal wastewater outfall of Orange County, California, USA, Aquat. Toxicol. 77 (2006) 241–249.
[4] F. Gagne, C. Blaise, C. Andre, C. Gagnon, M. Salazar, Neuroendocrine disruption and health effects in *Elliptio complanata* mussels exposed to aeration lagoons for wastewater treatment, Chemosphere 68 (2007) 731–743.
[5] A.M. Vajda, L.B. Barber, J.L. Gray, E.M. Lopez, J.D. Woodling, D.O. Norris, Reproductive disruption in fish downstream from an estrogenic wastewater effluent, Environ. Sci. Technol. 42 (2008) 3407–3414.
[6] R.M. Sharp, N.E. Skakkebaek, Are oestrogens involved in the falling sperm counts and disorders of the male reproductive tract? Lancet 341 (1993) 1392–1395.
[7] A.S. Whittemore, Prostate cancer, Cancer Surv. 19–20 (1994) 309–322.
[8] M.F. Fernandez, V. Pedraza, N. Olea, Estrogens in the environment: is there a breast cancer connection? Cancer J. 11 (1998) 11–17.
[9] J. Toppari, N.E. Skakkebaek, Endocrine disruption in male human reproduction, in: L.J. Guillette, D.A. Grain (Eds.), Environmental Endocrine Disrupters: An Evolutionary Perspective, Taylor and Francis, London, 2000, pp 269.
[10] P.A. Fowler, D.R. Abramovich, N.E. Haites, P. Cash, N.P. Groome, A. Al-Qahtani, T.J. Murray, R.G. Lea, Human fetal testis Leydig cell disruption by exposure to the pesticide dieldrin at low concentrations, Hum. Reprod. 22 (2007) 2919–2927.
[11] C. Brisken, Endocrine disruptors and breast cancer, Chimia 62 (2008) 406–409.
[12] A.C. Gore, Developmental programming and endocrine disruptor effects on reproductive neuroendocrine systems, Front. Neuroendocrinol. 29 (2008) 358–374.
[13] T.J. Woodruff, A. Carlson, J.M. Schwartz, L.C. Giudice, Proceedings of the summit on environmental challenges to reproductive health and fertility: executive summary, Fertil. Steril. 89 (2008) 281–300.
[14] D.J. Paustenbach, Human and Ecological Risk Assessment, John Wiley and Sons, New York, 2007, 1556.
[15] T. Beer, F. Ziolkowski, Environmental Risk Assessment: An Australian Perspective, Australia EPA, Canberra, 1995, p. 136.
[16] S.H. Hanauer, Probabilistic risk assessment in nuclear regulatory process in United States history and perspective, Trans. Am. Nucl. Soc. 22 (1975), 455–455.
[17] D.H. Weichert, W.G. Milne, Canadian methodologies of probabilistic seismic risk estimation, Bull. Seismol. Soc. Am. 69 (1979) 1549–1566.
[18] K.R. Solomon, D.B. Baker, R.P. Richards, D.R. Dixon, S.J. Klaine, T.W. LaPoint, R.J. Kendall, C.P. Weisskopf, J.M. Giddings, J.P. Giesy, L.W. Hall, W.M. Williams, Ecological risk assessment of atrazine in North American surface waters, Environ. Toxicol. Chem. 15 (1996) 31–74.
[19] K. Solomon, J. Giesy, P. Jones, Probabilistic risk assessment of agrochemicals in the environment, Crop Prot. 19 (2000) 649–655.
[20] J.O. Straub, K.M. Stewart, Deterministic and probabilistic acute-based environmental risk assessment for naproxen for western Europe, Environ. Toxicol. Chem. 26 (2007) 795–806.
[21] D. Djohan, J. Yu, D. Connell, E. Christensen, Health risk assessment of chlorobenzenes in the air of residential houses using probabilistic techniques, J. Toxicol. Environ. Health Part A 70 (2007) 1594–1603.
[22] N. Hamidin, Q.J. Yu, D.W. Connell, Human health risk assessment of chlorinated disinfection by-products in drinking water using a probabilistic approach, Water Res. 42 (2008) 3263–3274.

[23] US EPA, Guidelines for Ecological Risk Assessment, US EPA, 1998 (accessed 12.04.09) http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12460.
[24] US EPA, Risk Assessment Guidance for Superfund: Volume III – Part A, Process for Conducting Probabilistic Risk Assessment, US EPA, 2001 (accessed 21.05.2010) www.epa.gov/superfund/RAGS3A/index.htm.
[25] Q. Cao, Behaviour and health risk assessment of endocrine disrupting chemicals from wastewater, a PhD thesis, available from Griffith University, Brisbane, Australia, 2010, pp. 85–87.
[26] T. Damstra, S. Barlow, A. Bergman, R. Kavlock, G. Van Der Kraak, Global assessment of the state-of-the-science of endocrine disruptors, WHO/IPCS/EDC/02/2 (2002), pp. 2–3.
[27] European Commission, Technical Guidance Document on Risk Assessment, European Chemicals Bureau, Institute for Health and Consumer Protection, 2003, p. 311.
[28] E. Diamanti-Kandarakis, J.P. Bourguignon, L.C. Giudice, R. Hauser, G.S. Prins, A.M. Soto, R.T. Zoeller, A.C. Gore, Endocrine-disrupting chemicals: an endocrine society scientific statement, Endocr. Rev. 30 (2009) 293–342.
[29] T. Vermeire, H. Stevenson, M.N. Pieters, M. Rennen, W. Slob, B.C. Hakkert, Assessment factors for human health risk assessment: a discussion paper, Crit. Rev. Toxicol. 29 (1999) 439–490.
[30] K. Watanabe, F.Y. Bois, L. Zeise, Interspecies extrapolation – a reexamination of acute toxicity data, Risk Anal. 12 (1992) 301–310.
[31] S.C. Gad, Statistics and Experimental Design for Toxicologists and Pharmacologists, 4th ed., CRC Taylor & Francis, Boca Raton, 2006.
[32] S. Reagan-Shaw, M. Nihal, N. Ahmad, Dose translation from animal to human studies revisited, FASEB J. 22 (2008) 659–661.
[33] FDA, Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, Food and Drug Administration, U.S. Department of Health and Human Services, 2005 (accessed 09.06.10) http://www.fda.gov/cder/guidance/index.htm.
[34] A.J. Bailer, J.T. Oris, Estimating inhibition concentrations for different response scales using generalized linear models, Environ. Toxicol. Chem. 16 (1997) 1554–1559.
[35] US EPA, Final Report: Endocrine Diruptor Screening and Testing Advisory Committee, US EPA, Washington, USA, 1998.
[36] US EPA, Guidelines for Carcinogen Risk Assessment, EPA/630/P-03/001F, US EPA, Washington, USA, 2005.
[37] K. Solomon, P. Takacs, Probabilistic risk assessment using species sensitivity distributions, in: L. Posthuma, G.W. Suter, T.P. Traas (Eds.), Species Sensitivity Distributions in Ecotoxicology, Lewis Publishers, Boca Raton, 2002, p. 616.
[38] E. Silva, N. Rajapakse, A. Kortenkamp, Something from "nothing" – eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects, Environ. Sci. Technol. 36 (2002) 1751–1756.
[39] J.P. Sumpter, A.C. Johnson, R.J. Williams, A. Kortenkamp, M. Scholze, Modeling effects of mixtures of endocrine disrupting chemicals at the river catchment scale, Environ. Sci. Technol. 40 (2006) 5478–5489.
[40] K.L. Thorpe, M. Gross-Sorokin, I. Johnson, G. Brighty, C.R. Tyler, An assessment of the model of concentration addition for predicting the estrogenic activity of chemical mixtures in wastewater treatment works effluents, Environ. Health Perspect. 114 (2006) 90–97.
[41] P.J. Chen, E.J. Rosenfeldt, S.W. Kullman, D.E. Hinton, K.G. Linden, Biological assessments of a mixture of endocrine disruptors at environmentally relevant concentrations in water following UV/H_2O_2 oxidation, Sci. Total Environ. 376 (2007) 18–26.
[42] A. Kortenkamp, Low dose mixture effects of endocrine disrupters: implications for risk assessment and epidemiology, Int. J. Androl. 31 (2008) 233–237.
[43] K.L. Thorpe, T.H. Hutchinson, M.J. Hetheridge, M. Scholze, J.P. Sumpter, C.R. Tyler, Assessing the biological potency of binary mixtures of environmental estrogens using vitellogenin induction in juvenile rainbow trout (*Oncorhynchus mykiss*), Environ. Sci. Technol. 35 (2001) 2476–2481.
[44] A.J. Murk, J. Legler, M.M.H. van Lipzig, J.H.N. Meerman, A.C. Belfroid, A. Spenkelink, B. van der Burg, G.B.J. Rijs, D. Vethaak, Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays, Environ. Toxicol. Chem. 21 (2002) 16–23.
[45] C.G.D. Brook, J. Nicholas, Essential Endocrinology, 4th ed., Blackwell Publishing, Oxford, 2001.
[46] T. Gracia, P.D. Jones, E.B. Higley, K. Hilscherova, J.L. Newsted, M.B. Murphy, A.K.Y. Chan, X.W. Zhang, M. Hecker, P.K.S. Lam, R.S.S. Wu, J.P. Giesy, Modulation of steroidogenesis by coastal waters and sewage effluents of Hong Kong, China, using the H295R assay, Environ. Sci. Pollut. Res. 15 (2008) 332–343.
[47] R.G. Kozak, I. D'Haese, W. Verstraete, Pharmaceuticals in the environment: focus on 17α-ethinyloestradiol, in: K. Kummerer (Ed.), Pharmaceuticals in the Environment, Sources, Fate, Effects and Risks, Springer-Verlag, Berlin, Germany, 2001, pp. 49–65.
[48] T. de Mes, G. Zeeman, G. Lettinga, Occurrence and Fate of Estrone, 17β-estradiol and 17α-ethynylestradiol in STPs for domestic wastewater, Rev. Environ. Sci. Biotechnol. 4 (2005) 275–311.
[49] A.C. Belfroid, A. Van der Horst, A.D. Vethaak, A.J. Schafer, G.B.J. Rijs, J. Wegener, W.P. Cofino, Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands, Sci. Total Environ. 225 (1999) 101–108.
[50] G.H. Panter, R.S. Thompson, N. Beresford, J.P. Sumpter, Transformation of a non-oestrogenic steroid metabolite to an oestrogenically active substance by minimal bacterial activity, Chemosphere 38 (1999) 3579–3596.
[51] A.C. Johnson, J.P. Sumpter, Removal of endocrine-disrupting chemicals in activated sludge treatment works, Environ. Sci. Technol. 35 (2001) 4697–4703.
[52] G. D'Ascenzo, A. Di Corcia, A. Gentili, R. Mancini, R. Mastropasqua, M. Nazzari, R. Samperi, Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities, Sci. Total Environ. 302 (2003) 199–209.
[53] P.D. Hansen, H. Dizer, B. Hock, A. Marx, J. Sherry, M. McMaster, C. Blaise, Vitellogenin – a biomarker for endocrine disruptors, Trends Anal. Chem. 17 (1998) 448–451.
[54] E.J. Routledge, D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock, J.P. Sumpter, Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach, Environ. Sci. Technol. 32 (1998) 1559–1565.
[55] A. Lagana, A. Bacaloni, I. De Leva, A. Faberi, G. Fago, A. Marino, Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters, Anal. Chim. Acta. 501 (2004) 79–88.
[56] H.M. Kuch, K. Ballschmiter, Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range, Environ. Sci. Technol. 35 (2001) 3201–3206.
[57] P. Adler, T. Steger-Hartmann, W. Kalbfus, Distribution of natural and synthetic estrogenic steroid hormones in water samples from Southern and Middle Germany, Acta Hydroch. Hydrob. 29 (2001) 227–241.
[58] M. Cargouet, D. Perdiz, A. Mouatassim-Souali, S. Tamisier-Karolak, Y. Levi, Assessment of river contamination by estrogenic compounds in Paris area (France), Sci. Total Environ. 324 (2004) 55–66.
[59] J.K. Fawell, D. Sheahan, H.A. James, M. Hurst, S. Scott, Oestrogens and oestrogenic activity in raw and treated water in severn trent water, Water Res. 35 (2001) 1240–1244.
[60] X.-Y. Xiao, D.V. McCalley, J. McEvoy, Analysis of estrogens in river water and effluents using solid-phase extraction and gas chromatography–negative chemical ionisation mass spectrometry of the pentafluorobenzoyl derivatives, J. Chromatogr. A 923 (2001) 195–204.
[61] T. Isobe, H. Shiraishi, M. Yasuda, A. Shinoda, H. Suzuki, M. Morita, Determination of estrogens and their conjugates in water using solid-phase extraction followed by liquid chromatography–tandem mass spectrometry, J. Chromatogr. A 984 (2003) 195–202.
[62] A.D. Vethaak, J. Lahr, S.M. Schrap, A.C. Belfroid, G.B.J. Rijs, A. Gerritsen, J. de Boer, A.S. Bulder, G.C.M. Grinwis, R.V. Kuiper, An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of The Netherlands, Chemosphere 59 (2005) 511–524.
[63] G. Pojana, A. Gomiero, N. Jonkers, A. Marcomini, Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon, Environ. Int. 33 (2007) 929–936.
[64] H. Noppe, T. Verslycke, E. De Wulf, K. Verheyden, E. Monteyne, P. Van Caeter, C.R. Janssen, H.F. De brabander, Occurrence of estrogens in the Scheldt estuary: a 2-year survey, Ecotoxicol. Environ. Saf. 66 (2007) 1–8.
[65] Y. Zuo, K. Zhang, Y. Deng, Occurrence and photochemical degradation of 17[alpha]-ethinylestradiol in Acushnet River Estuary, Chemosphere 63 (2006) 1583–1590.
[66] J.L. Zhao, G.G. Ying, L. Wang, J.F. Yang, X.B. Yang, L.H. Yang, X. Li, Determination of phenolic endocrine disrupting chemicals and acidic pharmaceuticals in surface water of the Pearl Rivers in South China by gas chromatography–negative chemical ionization-mass spectrometry, Sci. Total Environ. 407 (2009) 962–974.
[67] M.J. Benotti, R.A. Trenholm, B.J. Vanderford, J.C. Holady, B.D. Stanford, S.A. Snyder, Pharmaceuticals and endocrine disrupting compounds in US drinking water, Environ. Sci. Technol. 43 (2009) 597–603.
[68] M. Williams, M. Woods, A. Kumar, G.G. Ying, A. Shareef, M. Karkkainen, R. Kookana, Endocrine Disrupting Chemicals in the Australia Riverine Environment: A Pilot Study on Estrogenic Compounds. A Joint Project Between CSIRO and Land & Water Australia, Land & Water Australia, Canberra, 2007, p. 105.
[69] B. Lei, S. Huang, Y. Zhou, D. Wang, Z. Wang, Levels of six estrogens in water and sediment from three rivers in Tianjin area, China, Chemosphere 76 (2009) 36–42.
[70] E.P. Kolodziej, D.L. Sedlak, Rangeland grazing as a source of steroid hormones to surface waters, Environ. Sci. Technol. 41 (2007) 3514–3520.
[71] G. Morteani, P. Moller, A. Fuganti, T. Paces, Input and fate of anthropogenic estrogens and gadolinium in surface water and sewage plants in the hydrological basin of Prague (Czech Republic), Environ. Geochem. Health 28 (2006) 257–264.
[72] S.A. Snyder, T.L. Keith, D.A. Verbrugge, E.M. Snyder, T.S. Gross, K. Kannan, J.P. Giesy, Analytical methods for detection of selected estrogenic compounds in aqueous mixtures, Environ. Sci. Technol. 33 (1999) 2814–2820.
[73] J.H. Shen, B. Gutendorf, H.H. Vahl, L. Shen, J. Westendorf, Toxicological profile of pollutants in surface water from an area in Taihu Lake, Yangtze Delta, Toxicology 166 (2001) 71–78.
[74] G.H. Panter, R.S. Thompson, J.P. Sumpter, Adverse reproductive effects in male fathead minnows (*Pimephales promelas*) exposed to environmentally relevant concentrations of the natural oestrogens, oestradiol and oestrone, Aquat. Toxicol. 42 (1998) 243–253.
[75] P. Bjerregaard, P.R. Hansen, K.J. Larsen, C. Erratico, B. Korsgaard, H. Holbech, Vitellogenin as a biomarker for estrogenic effects in Brown trout, *Salmo trutta*: laboratory and field investigations, Environ. Toxicol. Chem. 27 (2008) 2387–2396.
[76] F. Brion, C.R. Tyler, X. Palazzi, B. Laillet, J.M. Porcher, J. Garric, P. Flammarion, Impacts of 17 beta-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in zebrafish (*Danio rerio*), Aquat. Toxicol. 68 (2004) 193–217.

[77] C.D. Metcalfe, T.L. Metcalfe, Y. Kiparissis, B.G. Koenig, C. Khan, R.J. Hughes, T.R. Croley, R.E. March, T. Potter, Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (*Oryzias latipes*), Environ. Toxicol. Chem. 20 (2001) 297–308.
[78] K. Van den Belt, R. Verheyen, H. Witters, Comparison of vitellogenin responses in zebrafish and rainbow trout following exposure to environmental estrogens, Ecotoxicol. Environ. Saf. 56 (2003) 271–281.
[79] H. Holbech, K. Kinnberg, G.I. Petersen, P. Jackson, K. Hylland, L. Norrgren, P. Bjerregaard, Detection of endocrine disrupters: evaluation of a fish sexual development test (FSDT), Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 144 (2006) 57–66.
[80] V.J. Kramer, S. Miles-Richardson, S.L. Pierens, J.P. Giesy, Reproductive impairment and induction of alkaline-labile phosphate, a biomarker of estrogen exposure, in fathead minnows (*Pimephales promelas*) exposed to waterborne 17 beta-estradiol, Aquat. Toxicol. 40 (1998) 335–360.
[81] I.J. Kang, H. Yokota, Y. Oshima, Y. Tsuruda, T. Yamaguchi, M. Maeda, N. Imada, H. Tadokoro, T. Honjo, Effect of 17 beta-estradiol on the reproduction of Japanese medaka (*Oryzias latipes*), Chemosphere 47 (2002) 71–80.
[82] M. Seki, S. Fujishima, T. Nozaka, M. Maeda, K. Kobayashi, Comparison of response to 17 beta-estradiol and 17 beta-trenbolone among three small fish species, Environ. Toxicol. Chem. 25 (2006) 2742–2752.
[83] K.L. Thorpe, R.I. Cummings, T.H. Hutchinson, M. Scholze, G. Brighty, J.P. Sumpter, C.R. Tyler, Relative potencies and combination effects of steroidal estrogens in fish, Environ. Sci. Technol. 37 (2003) 1142–1149.
[84] C.R. Tyler, R. van Aerle, T.H. Hutchinson, S. Maddix, H. Trip, An in vivo testing system for endocrine disruptors in fish early life stages using induction of vitellogenin, Environ. Toxicol. Chem. 18 (1999) 337–347.
[85] L.T. Xie, K. Thrippleton, M.A. Irwin, G.S. Siemering, A. Mekebri, D. Crane, K. Berry, D. Schlenk, Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay, Toxicol. Sci. 87 (2005) 391–398.
[86] Y.X. Jin, R.J. Chen, L.W. Sun, H.F. Qian, W.P. Liu, Z.W. Fu, Induction of estrogen-responsive gene transcription in the embryo, larval, juvenile and adult life stages of zebrafish as biomarkers of short-term exposure to endocrine disrupting chemicals, Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 150 (2009) 414–420.
[87] E. Thomas-Jones, K. Thorpe, N. Harrison, G. Thomas, C. Morris, T. Hutchinson, S. Woodhead, C. Tyler, Dynamics of estrogen biomarker responses in rainbow trout exposed to 17 beta-estradiol and 17 alpha-ethinylestradiol, Environ. Toxicol. Chem. 22 (2003) 3001–3008.
[88] Q. Cao, Q. Yu, D.W. Connell, Degradation rate constants of steroids in sewage treatment works and receiving water, Environ. Technol. 29 (2008) 1321–1330.
[89] R.L. Gomes, M.D. Scrimshaw, J.N. Lester, Determination of endocrine disrupters in sewage treatment and receiving waters, Trends Anal. Chem. 22 (2003) 697–707.
[90] Q. Cao, V. Garib, Q. Yu, D.W. Connell, M. Campitelli, Quantitative structure–property relationships (QSPR) for steroidal compounds of environmental importance, Chemosphere 76 (2009) 453–459.