Institut für Nutzpflanzenwissenschaften und Ressourcenschutz (INRES)

Lehr-und Forschungsbereich Pflanzenernährung

der Rheinischen Friedrich-Wilhelms-Universität zu Bonn

Monitoring of Endocrine Disruptors in Surface Waters of the Mekong Delta in Vietnam

Inaugural – Dissertation

Zur

Erlangung des Grades

Doktor der Agrarwissenschaften

(Dr. agr.)

der

Hohen Landwirtschaftlichen Fakutät

der

Rheinischen Friedrich-Wilhelms-Universität

zu Bonn

Vorgelegt am 03. August 2011

von

Nguyen Thai Hoa

aus Ha Noi, Vietnam

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Referent: PD. Dr. Joachim Clemens
Korreferent: Prof. Dr.-Ing. Armin Rieser

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Summary

This study monitors estrogenic-Endocrine Disruptors (e-EDs) in surface waters in the Mekong Delta in Vietnam, one of the most populated agricultural areas in the world. e-ED is a subclass of Endocrine Disruptors (EDs), which are chemicals with the potential to elicit negative effects on endocrine systems of humans and animals. The feminisation of male fish caused by e-EDs, i.e. 17β-estradiol (E2) and estrone (E1), ethinyl estradiol (EE2), and nonylphenol (NP), is probably the most typical example about the effects of EDs. Since e-EDs can be either natural or synthetic chemicals, their sources to the environment vary differently such as domestic discharge, industrial discharge and agricultural runoff.

A comprehensively review on e-EDs in the literature and related studies in aquatic environment in Vietnam shows that different e-EDs possess different estrogenic potency, and their potency varies widely. The analytical methods used contribute to the variability. However, regardless of the analytical methods estrogens are, in general, estrogenically more potent than xenoestrogens. Therefore, estrogens usually contribute predominantly in the total estrogenic activity of environmental samples although they normally occur at a lower concentration as compared to that of xenoestrogens. In addition, the review shows that in Vietnam although data on estrogens are lacking of, there are some data on xenoestrogens and these chemicals ubiquitously occurred in water bodies at considerable concentrations. In many cases they exceeded the Predicted No Effect Concentration (PNEC) for freshwater life, especially in urban areas. Without contribution from estrogens, estrogenic activity caused by xenoestrogens was generally low due to their weak potency. However, in some cases, their estrogenic activity occurred at considerable levels due to their high concentration in the environment, which may cause an estrogenic risk for aquatic fauna.

The estrogenic activity in the surface waters of Can Tho City (CTC), a mid size city located in the Mekong Delta in Vietnam, was monitored. Water samples from the urban, industrial and suburban areas were analysed by the Yeast Estrogen Screen (YES) assay. Additionally, estrogenic activity was analysed for samples at the upstream and downstream stations out of CTC to estimate the estrogenic discharge of the city. The estrogenic activity was frequently detected (100% in the urban and

industrial areas, 86% in the suburban area). The concentration in the urban (range 0.03 to 33.99 ng E2 equivalent (EEQ)/L, median 0.72 ng EEQ/L) and industrial (range 0.08 to 11.8 ng EEQ/L, median 1.08 ng EEQ/L) areas was significant higher ($p < 0.05$) than the suburban area (range ND (not detected) to 2.99 ng EEQ/L, median 0.256 ng EEQ/L). In the Hau River (Bassac River), the estrogenic activity was lower at the upstream (range 0.015 to 1.09 ng EEQ/L, median 0.333 ng EEQ/L) as compared to the downstream station (range 0.04 to 1.57 ng EEQ/L, median 0.485 ng EEQ/L). In the urban area, 37.5% of the detected samples had a higher concentration than the PNEC of 1 ng/L, wherewas in the industrial and suburban areas; it was 56% and 9%, respectively. The results indicate a potential estrogenic risk for aquatic fauna in all the sampling areas. Using the data, it is estimated that CTC discharged an amount between 44 to 88 g EEQ/day, which is in the same order of magnitude to a modeled result of 37 g EEQ/day. Human and animal discharges seemed to be the major estrogenic sources in this city.

To compare estrogenic activity in the surface waters of different agro-ecosystems in CTC, and Dong Thap Province (DTP), two representative areas in the Mekong Delta in Vietnam, water samples from irrigation canals, agricultural fields, fishponds in the suburban and rural areas in CTC and DTP were analysed on estrogenic activity, respectively. In CTC estrogenic activity (range ND to 3.62 ng EEQ/L, median 0.28 ng EEQ/L) was detected in 68% of all the samples, from those, 17% of the samples had a higher estrogenic activity than the PNEC value. Whereas in DTP estrogenic activity (range ND to 4.97 ng EEQ/L, median 0.2 ng EEQ/L) was detected in 59% of all the samples and it was significantly ($p < 0.05$) lower than in CTC. From those estrogenic activity in 13% of the samples exceeded the PNEC value. Domestic discharge is likely to be the reason for the observed estrogenic activity in both CTC and DTP.

In both areas, in the effluent of fishponds the estrogenic activity (in DTP: range ND to 0.75 ng EEQ/L, median 0.14 ng EEQ/L, in CTC: range 0.05 to 2.66 ng EEQ/L, median 0.69 ng EEQ/L) tended ($p > 0.05$) to be higher as compared to the inflowing water (in DTP: range ND to 1.6 ng EEQ/L, median 0.08 ng EEQ/L, in CTC: range 0.29 to 1.35 ng EEQ/L, median 0.56 ng EEQ/L). Although in DTP the estrogenic activity in agricultural runoff was low (range 0.02 to 0.58 ng EEQ/L, median 0.16 ng EEQ/L), it was elevated in CTC (range ND to 3.6 ng EEQ/L, median 0.3 ng EEQ/L). Direct discharge of domestic wastewater into the fields in CTC could be the reason. Except for the canal category (in DTP: range ND to 2 ng EEQ/L, median 0.19 ng EEQ/L, in

CTC: range ND to 2.99 ng EEQ/L, median 0.256 ng EEQ/L) the estrogenic activity obtained in the fields and the fishponds in CTC was significantly higher than those in their respective categories in DTP ($p < 0.05$).

About one third of the samples caused a cytotoxic effect to the yeast cells in both areas CTC and DTP (in CTC: 35% in urban area; 42% in industrial area; 24.7% in suburban area (canal, fishpond, agricultural field), in DTP: 33.3% (canal, fishpond, agricultural field, national park)). This effect either completely killed the yeast cells or inhibited yeast cell growth; as a consequence, these cytotoxic samples did not generate estrogenic activity.

In CTC, the cytotoxic effect mainly occurred in all sampling categories (i.e. urban area, industrial area, canals and agricultural fields) except in the fishponds. The measured water quality parameters of the cytotoxic samples significantly differed to those of the normal samples, i.e. DO or EC values in the cytotoxic samples were lower or higher than those in the normal samples, respectively. However, the reason for the cytotoxic effect in CTC is unknown. In DTP, this effect mainly occurred in the rice fields and in the canals, and rarely in other sampling categories, i.e. the national park and the fishponds. There was no significant difference between the measured water quality parameters (i.e. DO, EC and pH) of the cytotoxic samples and those of the normal samples. Pesticides could be responsible for the observed cytotoxic effects in DTP although sometimes these effects occurred as well at the upstream station.

In Vietnam, human and animal wastes are not properly treated, and in many cases they are directly discharged into water bodies. Most likely, the rather frequent detection of estrogenic activity in surface waters in the Mekong Delta is related to these discharges. Additionally, elevated estrogenic activity as compared to the PNEC value indicates an estrogenic risk to the aquatic fauna, especially in the urban and industrial areas. Considering these facts, estrogenic activity in the environment in Vietnam certainly deserves further studies to elucidate potential sources and to assess estrogenic risk in detail. In any case, domestic discharges, especially human and animal wastes need to be sufficiently treated in order to reduce estrogenic activity in water bodies in Vietnam.

Zusammenfassung (summary in German)

Mit der vorliegenden Forschungsarbeit wurden die Gehalte von estrogenic-Endocrine Disruptors (e-Eds) im Oberflächenwasser des Mekong Deltas, einer der bevölkertsten landwirtschaftlich genutzten Region der Erde, in Vietnam untersucht. Die Endocrine Disruptors sind chemische Substanzen, die endokrine Drüsen von Menschen und Tieren negativ beeinflussen und somit eine potentielle Störung des Hormonsystems verursachen können. Die e-ED bilden eine Unterklasse der Endocrine Disruptors (Eds). Die Verweiblichung von männlichen Fischen durch e-Eds, und zwar durch 17β-estradiol (E2) und estrone (E1), ethinyl estradiol (EE2), und nonylphenol (NP) ist wahrscheinlich eines der bekanntesten Beispiele für den schädlichen Einfluss von Eds. Da e-Eds sowohl als synthetische wie auch als natürlich vorkommende chemische Substanzen in der Umwelt existieren, ist ihr Ursprung in Gewässern entsprechend unterschiedlich. Potentielle Quellen sind sowohl häusliche und industrielle Abwässer wie auch Abflüsse aus der Landwirtschaft. .

Eine umfangreiche Revision der Literatur über e-Eds und verwandter Studien über aquatische Biotope in Vietnam hat gezeigt, dass unterschiedliche e-Eds stark variierendes östrogenisches Potential besitzen. Zwar beeinflusst die Methode der Analyse den Grad der Unterschiedlichkeit, aber allgemein kann festgestellt werden, dass Östrogene einen größeren potentiellen Einfluss auf das Hormonsystem haben als Xenoöstrogene. Daher sind in Umweltproben die Einflüsse von Östrogenen auf die totale östrogenische Aktivität in der Regel maßgebend, auch wenn sie in geringerer Konzentration als Xenoöstrogene vorkommen. Es mangelt an Studien in Vietnam über Östrogene, aber aus der wenigen Literatur über Xenoöstrogene wird ersichtlich, dass diese allgegenwärtig und in hohen Konzentrationen in vietnamesischen Gewässern vorkommen. In vielen Fällen überschreitet die Konzentration die “Prediced No Effect Concentration“ (PNEC) für Süßwasser Organismen, vor allem in städtischen Gebieten. Auch wenn die östrogenische Aktivität der Xenoöstrogene ohne die Teilhabe von Östrogenen im Allgemeinen wegen ihres schwachen Potentials gering ist, erreichen sie doch in manchen Fällen einen beachtlichen Wert aufgrund ihrer hohen Konzentration. Sie sind somit eine Gefahr für aquatische Organismen.

Es wurde die östrogenische Aktivität in Oberflächengewässern von Can Tho City (CTC), einer mittelgroßen Stadt im Mekong Delta, gemessen. Wasserproben aus

städtischen, vorstädtischen und industriellen Gebieten wurden mit der Yeast Estrogen Screen (YES) Test analysiert. Darüber hinaus wurden Proben im oberen und unteren Flusslauf entnommen, um den Östrogengehalt des städtischen Abwassers nachzuweisen. Eine östrogenische Aktivität wurde regelmäßig nachgewiesen (100% in den städtischen und industriellen Gebieten, 86% in den Vorstädten). Die Konzentration in den urbanen (0.03 bis 33.99 ng E2 equivalent (EEQ)/L, Median 0.72 ng EEQ/L) und industriellen (0.08 bis 11.8 ng EEQ/L, Median 1.08 ng EEQ/L) Gebieten war signifikant höher ($p < 0,05$) als in den Vorstädten (ND (in german?) bis 2.99 ng EEQ/L, Median 0.256 ng EEQ/L). Im Fluss Hau (Bassac) war die östrogenische Aktivität stärker an der im unteren Flusslauf gelegenen Station (0.015 bis 1.09 ng EEQ/L, Median 0.333 ng EEQ/L) verglichen zu der Station im oberen Flusslauf (0.04 bis 1.57 ng EEQ/L, Median 0.485 ng EEQ/L). Im urbanen Gebiet hatten 37,5% der Proben, in denen östrogenische Aktivität nachgewiesen wurde, eine Konzentration höher als die PNEC von 1 ng/L. 56% in den Industriegebieten und 9% in den Vorstädten überstiegen die PNEC von 1 ng/L. Die Ergebnisse weisen auf ein potentielles Östrogen Risiko für aquatische Fauna in allen Untersuchungsgebieten hin. Anhand der Daten wurde geschätzt, dass CTC täglich 44 bis 88 g EEQ in die Gewässer leitet. Das entspricht derselben Größenordnung des durch ein Model ermittelten Ergebnisses von 37 g EEQ pro Tag. Menschliche und tierische Fäkalien scheinen die Hauptquelle für Östrogene in der Stadt zu sein.

Es wurden in zwei für das Delta repräsentativen Gebieten, CTC und Dong Thap Provinz (DTP), Wasserproben auf östrogenische Aktivität untersucht. Die Proben wurden aus Bewässerungskanälen, von bewirtschafteten Feldern, sowie Fischteichen in ländlichen als auch vorstädtischen Gebieten entnommen, um die östrogenische Aktivität in verschiedenen landwirtschaftlichen Ökosystemen zu vergleichen. In CTC wurde die östrogenische Aktivität (ND (in german ?) bis 3.62 ng EEQ/L, Median 0.28 ng EEQ/L) in 68% aller Proben gefunden, wobei von diesen 17% eine höhere östrogenische Aktivität aufwiesen als der PNEC Wert. Im Vergleich wurde in DTP östrogenische Aktivität (ND bis 4.97 ng EEQ/L, Median 0.2 ng EEQ/L) in 59% aller Fälle nachgewiesen, 13% von diesen mit höheren PNEC Wert. DTP zeigte somit einen signifikant ($p < 0.05$) geringeren Wert als CTC. Man kann davon ausgehen, dass in CTC und DTP häusliche Abwässer die Quelle für die gefundene östrogenische Aktivität sind.

In beiden Gebieten tendierte ($p > 0.05$) die östrogenische Aktivität im abfließenden Wasser von Fischteichen höher zu sein (in DTP: ND bis 0.75 ng EEQ/L, Median 0.14 ng EEQ/L, in CTC: 0.05 bis 2.66 ng EEQ/L, Median 0.69 ng EEQ/L) als im einfließenden Wasser (in DTP: ND bis 1.6 ng EEQ/L, Median 0.08 ng EEQ/L, in CTC: 0.29 bis 1.35 ng EEQ/L, Median 0.56 ng EEQ/L). Die östrogenische Aktivität in Abwässern der Landwirtschaft war in DTP gering (0.02 bis 0.58 ng EEQ/L, Median 0.16 ng EEQ/L) und im Vergleich dazu höher in CTC (ND bis 3.6 ng EEQ/L, Median 0.3 ng EEQ/L). Der Grund dafür könnte das direkte Einleiten von häuslichen Abwässern auf die Felder in CTC sein. Die östrogenische Aktivität in den Feldern und Fischteichen war in CTC signifikant ($p < 0.05$) höher als in DTP mit Ausnahme von Messungen in den Kanälen (in DTP: ND bis 2 ng EEQ/L, Median 0.19 ng EEQ/L, in CTC: ND bis 2.99 ng EEQ/L, Median 0.256 ng EEQ/L).

Ungefähr ein Drittel aller Proben aus beiden Gebieten wirkten cytotoxisch auf Hefezellen (in CTC: 35% im ländlichen Raum, 42% in Industriegebieten und 24,7% in den Vorstädten, gemessen in Kanälen, Fischteichen und landwirtschaftlichen Feldern; in DTP: 33,3% gemessen in Kanälen, Fischteichen, landwirtschaftlichen Feldern und im Nationalpark). Zellen starben entweder gänzlich ab oder ihr Wachstum wurde unterdrückt. Als Folge entwickelten diese Proben keine östrogenische Aktivität.

In CTC trat eine cytotoxische Wirkung in allen Bereichen auf, in denen Proben entnommen wurden (also im ländlichen Raum, im Industriegebiet, in Kanälen und Feldern) mit Ausnahme von den Fischteichen. Die gemessenen Parameter von Wasserqualität der cytotoxischen Proben unterschieden sich signifikant von denen der normalen Proben, dass heißt die DO und EC Werte waren jeweils geringer und größer in den cytotoxischen Proben als in den normalen Proben. Der Grund für die cytotoxische Wirkung in CTC ist nicht bekannt. In DTP wurde die gleiche Wirkung in den Proben der Reisfelder und Kanälen nachgewiesen aber kaum in denen der anderen Bereiche, also im Nationalpark und den Fischteichen. Es gab auch keinen signifikanten Unterschied zwischen den gemessenen Parametern der Wasserqualität (d.h. DO, EC und pH) der cytotoxischen und der normalen Proben. Pestizide könnten für die beobachtete cytotoxische Wirkung in DTP verantwortlich sein, allerdings wurde dieselbe Wirkung auch manchmal an der Messstation im Oberlauf des Flusses nachgewiesen.

Menschliche und tierische Fäkalien werden in Vietnam nicht sachgerecht aufgearbeitet, sondern in vielen Fällen direkt in die Gewässer geleitet. Der regelmäßige Nachweis von östrogenischer Aktivität im Oberflächenwasser des Mekong Delta ist sehr wahrscheinlich auf diese Abwässer zurückzuführen. Hinzukommt, dass erhöhte östrogenische Aktivität im Vergleich zum PNEC Wert auf ein Östrogen Risiko für die Fauna der Gewässer, vor allem der Vorstädte und Industriegebiete, schließen lässt. Ausgehend von diesen Befunden verdient die östrogenische Aktivität in der Umwelt Vietnams sicherlich weitergehender Untersuchung, um die potentiellen Quellen und das Östrogen Risiko im Detail besser verstehen zu lernen. In jedem Fall müssen menschliche und tierische Fäkalien hinreichend aufgearbeitet werden, um die östrogenische Aktivität in Vietnams Gewässern zu verringern.

Erklärung (Declaration)

Ich versichere, dass ich diese Arbeit selbständig verfaßt habe, keine anderen Quellen und Hilfsmateralien als die angegebenen benutzt und die Stellen der Arbeit, die anderen Werken dem Wortlaut oder dem Sinn nach entnommen sind, kenntlich gemacht habe. Die Arbeit hat in gleicher oder ähnlicher Form keiner anderen Prüfungsbehörde vorgelegen.

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This thesis would have never been in this shape without the different kinds of help and support of many people and organizations. First of all, I would like to thank the German Federal Ministry of Education and Research (BMBF) and the Vietnamese Ministry of Science and Technology (MOST) for supporting this research through the project Water Related Information System for the Sustainable Development of the Mekong Delta in Vietnam (WISDOM).

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Dedication

This thesis is dedicated to the following persons:

Nguyen Phuong Thao

Nguyen Gia Linh

Ngo Thi Thu Thuy

Nguyen Liem

Nguyen Thi Lan Anh

Nguyen Van Troi

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List of Abbreviations

BPA	Bisphenol A
CTC	Can Tho City
DO	Dissolved Oxygen
DONRE	Department Of Natural Resources and Environment
DTP	Dong Thap Province
E1	Estrone
E2	17β-estradiol
E3	Estriol
EC	Electrical Conductivity
EDCs	Endocrine Disrupting Compounds or Chemicals
EDs	Endocrine Disruptors
EE2	17α-ethynylestradiol
e-EDs	estrogenic-Endocrine Disruptors
EEF	Estrogenic Equivalent Factor (estrogenic potency)
EEQ	E2 equivalent (estrogenic equivalent or estrogenic activity)
GSO	General Statistics Office
HCMC	Ho Chi Minh City
HNC	Ha Noi City
LOEC	Lowest Observed Effect Concentration
MONRE	Ministry Of Natural Resources and Environment
NOEC	No Observed Effect Concentration
NP	Nonylphenol
OCPs	Organochlorine Pesticides
OP	Octylphenol
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PNEC	Predicted No Effect Concentration
USEPA	The US Environmental Protection Agency
YES	Yeast Estrogen Screen

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1. General introduction

1.1 Background

Endocrine Disrupting Compounds (EDCs) or Endocrine Disruptors (EDs) are chemicals with the potential to elicit negative effects on endocrine systems of humans and wildlife (Campbell et al., 2006; Liu et al., 2009a). The concept of EDC was first introduced by Colborn et al. (1993). EDCs can be either natural or synthetic chemicals (Giesy et al., 2002), therefore, they had been occurring in the environment for many decades before this term was being introduced. The US Environmental Protection Agency (USEPA) defines an EDC as: “An exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior” (USEPA, 1997)

It became widely accepted that a wide range of diverse chemicals known to be present in the environment possessed endocrine disruption. This is a broad class of chemicals included several sub-classes such as estrogenic chemicals, anti-estrogenic chemicals, androgenic chemicals, and anti-androgenic chemicals. Perhaps one of the most common phenomena related to EDC is the incidence of intersexuality in fish, and particularly the possibility of feminisation of male fish by estrogenic chemicals was seriously considered. Feminisation of wild fish has been reported considerably more frequently than masculinisation, and appears to be more widespread (Sumpter, 2005). Research focused on the identification of the chemicals causing the observed feminisations, and the results were clear and surprising: the estrogenic chemicals, particularly the natural steroidal estrogens 17β-estradiol (E2) and estrone (E1), and the synthetic steroidal estrogen ethinyl estradiol (EE2) appeared to be the causative chemicals (Purdom et al., 1994; Desbrow et al., 1998) although industrial estrogenic chemicals (so called xenoestrogens or anthropogenic estrogenic chemicals) such as nonylphenol (NP) can induce intersexuality in fish, and even complete feminisation (Sumpter and Johnson, 2008).

Most of examples of endocrine disruption were found with aquatic animals, and surface water appears to be an important exposure environment. EDCs have been

reported in different environmental samples (Shore and Shemesh, 2003; Campbell et al., 2006; Liu et al., 2009a), and aquatic environment seems to be the major sink of these compounds. It is obvious that the total estrogenic activity of a surface water sample is estrogenically contributed by different estrogenic chemicals in the sample. Therefore, the estrogenic effects are probably a consequence of exposure to mixtures of chemicals, but not a single chemical. Due to the co-occurrence of these chemicals in the environment, the estrogenic activity of a sample is usually reported as total estrogenic activity, and usually relative to E2 equivalent (EEQ). It is very clear that different estrogenic chemicals possess different estrogenic potency. Among reported estrogenic chemicals, E2 and EE2 are the most potent estrogens. Most, and perhaps all, xenoestrogens such as alkylphenols, PCBs, PAHs, BPA, Phthalates and pesticides are much less potent, usually by a few orders of magnitude (three or four), but sometimes even more (Sumpter and Johnson, 2005).

Although the contribution of estrogenic chemicals in the total estrogenic activity depends on the specific location, the natural (E2, E1) and synthetic (EE2) estrogens seem to be the significant contributors to the overall estrogenic activity of wastewater treatment effluents and rivers in many places around the world (Sumpter and Johnson, 2008). In addition, it should be noted that there can be local differences in the composition of the estrogenic chemicals in the water bodies. For example, in the River Aire in the U.K. high concentrations of NP and other alkylphenolic chemicals may be the most significant estrogenic contributors. Another example is the Kanzaki River in Japan, in which genistein, an isoflavone of plant origin, is the main estrogenic chemical (Sumpter and Johnson, 2008). The potency of an estrogenic chemical seems to be a main factor in endocrine disruption since E2 and EE2 usually occur in the environment at very low concentration (several nanogram/L) while other xenoestrogens occur at higher concentration (higher nanogram level or microgram level) (Campbell et al., 2006; Ying et al., 2009; Wang et al., 2010).

While steroidal estrogens such as E1, E2 and E3 are mainly excreted by humans and animals, xenoestrogens are excreted during industrial processes, and they can occur in household cleaners, plastics, agrochemicals, and as a consequence in the municipal and industrial wastewaters. Although wastewater treatment plants remove around 60-90% of the steroid estrogens from the effluents of wastewater treatment plants (Sumpter and Johnson, 2008), these effluents were still reported as the major

source for estrogenic chemicals (Campbell et al., 2006), and endocrine disruption in aquatic animals are usually recorded around or downstream of these effluents (Folmar et al., 1996; Jobling et al., 1998; Gross-Sorokin et al., 2006). The actual impact on aquatic animals would probably depend on the dilution factor of the effluents; however, it is clear that steroid estrogens can cause endocrine disruption in these species at very low concentration (Sumpter and Johnson, 2008). Due to a variety of estrogenic chemicals, their sources to the environment may vary differently from point sources such as municipal effluent and industrial effluent to non-point sources such as agricultural runoff and discharge from aquaculture activities (Campbell et al., 2006).

Unlike persistent xenoestrogens such as PCBs, DDTs, PAHs, the natural estrogens are well degraded in river water, with half lives of around a few days (Ying et al., 2002; Sumpter and Johnson, 2008). However, the discharges of estrogens in rivers are not one-off occasional events; they are consistently being discharged into the environment every day of the year. This discharging characteristic of estrogens has justified these chemicals the term ‘pseudo-persistence’ in the environment (Sumpter and Johnson, 2008). It is clear that continuous exposure to estrogenic chemicals via the water can cause the endocrine disruption effects, even when the actual concentration of these chemicals is low. Moreover, it is likely that estrogenic chemicals can cause an additional effect even if each chemical is present at a concentration below that which causes an effect (Sumpter and Johnson, 2005). Therefore, aquatic fauna may be vulnerable to estrogenic chemicals due to continuous exposure is the common exposure scenario for them, and they are usually exposed simultaneously to different estrogenic chemicals in water bodies.

Due to the occurrence of estrogenic chemicals, especially steroid estrogens in the environment at very low concentration, it is difficult, time-consuming work to monitor target chemicals in the environment. Moreover, analysis of these chemicals in complex samples such as wastewater and sediment is a challenging task. The recent interest in EDC has promoted the development of different analytical methods, including chemical and biological methods. Although the biological method or the chemical method has its own advantages and disadvantages, the biological method may provide major advantages by estimating the total estrogenic activity or the effect of many estrogenic compounds in the samples or in the environment (Campbell et al.,

2006). Another advantage of biological methods such as cellular bioassay (E-Screen, YES, ER-CALUX) is a high throughput, cost effectiveness, and suitability for large scale screening to give first evidence of the occurrence of estrogenic contaminants in ecosystem. Among established in vitro assays, the YES assay has been used in many studies as a cost effective, robust, simple in vitro screening system tool for assessing estrogenic activities in environmental samples (Beck et al., 2006; Ma et al., 2007). Another approach to obtain estrogenic concentration in the environment is modeling. One example is the model developed by Johnson and Williams, (2004) which predicts the input and effluent concentrations for specific chemicals from a wastewater treatment plant. The model requires only the head of population and flow from the wastewater treatment plant. This model can be combined with other hydrological models and chemical process models to predict the chemical concentration in the water catchments (Sumpter and Johnson, 2005).

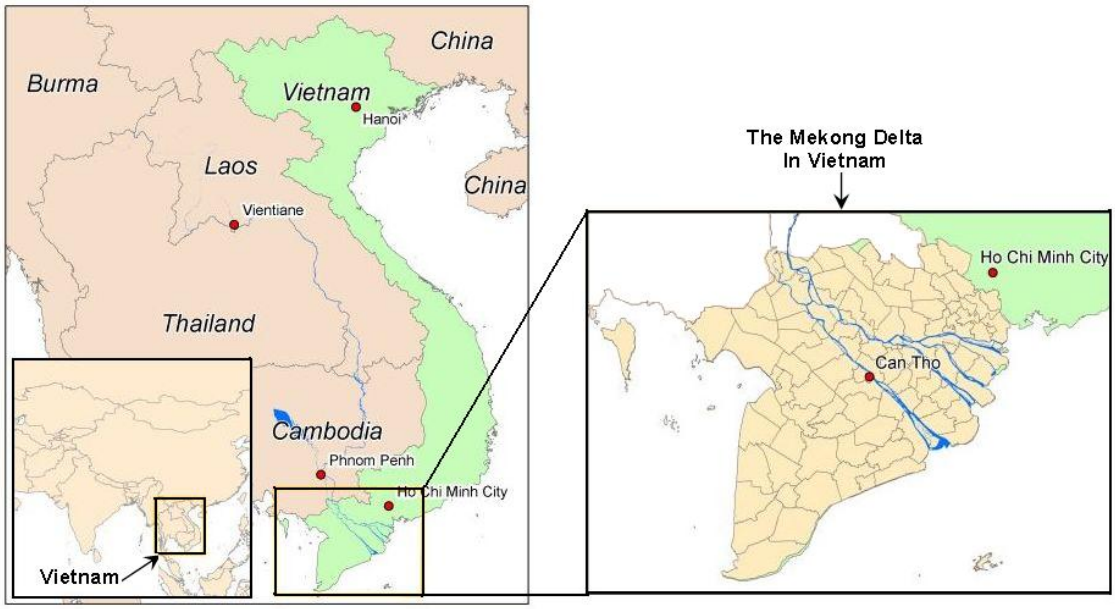

Figure 1.1: Map of Vietnam in Asia, and the Mekong Delta in Vietnam

Vietnam, a developing country, is located in tropical monsoon area in Southeast Asia (Figure 1.1) and occupies about 330,000 square kilometers. The country has a north to south distance of 1,600 kilometers and a coastal of 3,200 kilometers. Its population is about 84 millions people, of which approximately 70% live and rely heavily on agricultural areas where major crop is rice farming. On the one hand, the rapid urbanisation and industrialisation process taking place in Vietnam is accompanied by

an improvement of the socio-economic conditions. On the other hand, this process also increases environmental pollution from the uncontrolled usage of toxic chemicals such as pesticides, untreated domestic and industrial waste discharges.

Surface and ground water pollution in Vietnam is becoming more seriously, especially for small rivers and canals located inside the urban areas. There is about 4% of industrial wastewater being treated according to Vietnamese standard. In total, an estimated amount of 3,110,000 m^3 domestic, industrial and hospital wastewater are daily discharged into surface water without any treatment in Vietnam. Of which, domestic wastewater is the largest proportion, accounted for 64% (2,010,000 m^3). The remaining is industrial wastewater (32%; 980,000 m^3) and hospital wastewater (4%; 120,000 m^3) (MONRE, 2005).

In addition, solid waste is also a source significantly causing water pollution in Vietnam. It is reported that in 2003, 15,400,000 tons of solid waste were generated from domestic and industrial activities in Vietnam. From those, about 80% originated from domestic activities (approximately 50% from urban areas and 50% from rural areas). The remaining 20% was from industrial and hospital activities. Moreover, the amount of solid waste has increased at the rate from 10 to 16% every year. However, there are only 70% and 20% of total solid waste were collected in urban and rural areas, respectively. The remaining amount is not collected and treated, but instead burned, disposed of at road sites, trenches, or even to rivers, lakes and streams nearby. Concerning the collected solid waste, it is mainly dumped in the landfills which are mostly not properly designed, managed or even open dump sites. In the whole country, Vietnam has about 80 waste landfills that are in operation. However, among those, only eight are considered as sanitary and hygienic landfills. Poorly and improperly designed landfills have caused seriously environmental problem for the surrounding communities, including contamination of ground and surface water by untreated leachage, emission of airborne pollutants (MONRE, 2004; MONRE, 2005).

Although EDCs have been monitored in America, Europe and other Asian countries, there is still lack of information on estrogenic activity in other parts of the world such as tropical developing countries (Duong et al., 2010). To the author's knowledge, this is the first time estrogenic activity is comprehensively studied in Vietnam.

1.2 Hypothesis and Objectives

With a population of about 20 million people, the Mekong Delta in Vietnam, the last part of the Mekong River (Figure 1.1), is one of the highest productive agricultural areas in the world. The area is characterized by a dense system of rivers and canals, where households are mainly located along these rivers and canals. Water from the Mekong River is the main water resource for irrigation, domestic, industrial and service, and aquaculture in the region. In this area, the sanitation system is mainly comprised of septic tanks in the urban areas (Yen Phi et al., 2010), and latrines in the rural areas. It is reported that the percentage of household using latrines that directly drained to the open river, fishpond is more than 80% in most of provinces in this region (Soussan et al., 2005).

It is hypothesized that ubiquitous occurrence and high contamination of estrogenic activity in surface waters of the Mekong Delta in Vietnam may be expected due to its high population density, poorly designed sanitation infrastructures, and the intensive agronomic activities, especially in urban areas. Therefore, as a consequence local aquatic fauna may be at the risk of estrogenic activity.

In order to test this hypothesis, the following two major objectives were formulated accordingly:

1. To comprehensively review endocrine disruptors that was reported in sediment and surface water environment in Vietnam.
2. To monitor estrogenic activity in different surface water categories including urban area, industrial area, suburban area, rural area, agricultural fields and fishponds in the Mekong Delta in Vietnam.

1.3 Outline of the thesis

This thesis is structured into six chapters. This chapter provides the general introduction and justification for the study. Chapter 2 reviews the occurrence and

concentration of estrogenic endocrine disruptors in sediment and surface water environment in Vietnam. Chapter 3 presents the concentration and occurrence of estrogenic activity in the surface waters of urban, industrial, and suburban areas in Can Tho City. Chapter 4 addresses the estimation of estrogenic discharge from CTC obtained, and discusses several potential sources. Chapter 5 presents the concentration and occurrence of estrogenic activity in the surface waters of different agro-ecosystems including agricultural fields, fishponds, irrigation canals in CTC and DTP. Finally, chapter 6 provides general conclusions and highlights future research needs.

2. Estrogenic endocrine disruptors and their occurrence in surface water and sediment environment in Vietnam

Abstract

Estrogenic Endocrine Disruptors (e-EDs), a subclass of Endocrine Disruptors (EDs), are environmental contaminants that may be hormonally active at low concentration and are emerging as a major concern for water quality. This study summarizes and highlights some commonly reported e-ED groups and their estrogenic potency, e.g. anthropogenic estrogenic groups such as alkylphenols, Polychlorinated Biphenyls (PCBs), Organochlorine Pesticides (OCPs), Polycyclic Aromatic Hydrocarbons (PAHs), phthalates and Bisphenol A (BPA), and estrogens such as estrone (E1), 17β-estradiol (E2) and 17α-ethynylestradiol (EE2). In general, regardless of analytical method used, estrogens are estrogenically more potent than anthropogenic chemicals, the estrogenic potency of which varies widely from two to eight orders of magnitude lower than E2. A review of the occurrence and concentration of some of these e-EDs reported in surface waters and sediments in Vietnam showed that anthropogenic estrogenic chemicals were ubiquitously present in water bodies at considerable concentrations, especially in urban areas. In general, these anthropogenic chemicals exhibited relatively low estrogenic activity because of their weak potency. However, in some cases, especially in urban areas, their estrogenic activity was at considerable levels due to their high concentrations in the environment, indicating insufficiently treated or untreated municipal discharges as a source. Moreover, the high concentrations of estrogens detected in surface water influenced by municipal discharges suggest that human and animal wastes are not sufficiently treated before being discharged into the environment. Although information on e-EDs is still limited, the estrogenic activity originating from some of these compounds reported in Vietnam indicates possible effects on local aquatic fauna, and certainly justifies further studies in order to identify potential sources and to assess estrogenic risk. In any case, municipal discharges, especially human and animal wastes, need to be sufficiently treated in order to reduce estrogenic activity in water bodies in Vietnam.

2.1 Introduction

Since the 1950s, there has been an increasing number of reports on abnormal reproductive functions and behaviors, demasculinisation or feminisation of males, and decreased hatching success in wild life (Colborn et al., 1996). These phenomena could be related to chemicals that disrupt or affect the endocrine system.

The effects of endocrine disruptors (EDs) are to: (1) mimic the effects of endogenous hormones; (2) antagonise the effects of endogenous hormones; (3) disrupt the synthesis and metabolism of endogenous hormones; and (4) disrupt the synthesis and metabolism of hormone receptors (Sonnenschein and Soto, 1998). Diverse effects of EDs on animal health have been reported, such as masculinisation of female mosquito fish (Parks et al., 2001), compromised of the reproductive success of roach (Jobling et al., 2002b), altered sexual maturation and gamete production in wild roach (Jobling et al., 2002a), increased in uterine weight in pre-pubertal rats (Laws et al, 2000), feminisation of male fish (Gercken and Sordyl, 2002), vitellogenin induction in male fish (Panter et al., 2000; Thorpe et al., 2003; Hutchinson et al., 2006; Bogers et al., 2007), high plasma vitellogenin (female egg yolk) concentrations (Purdom et al., 1994), incidence of inter-sexuality in fish (Jobling et al., 1998; Vermeirssen et al., 2005; Sumpter, 2005), irreversible deleterious effects in birds and rodents (Zacharewski et al., 1997; Sonnenschein and Soto, 1998), and reproductive abnormalities in amphibians and birds (Singleton and Khan, 2003). EDs are also suspected to decrease male reproductive capacity, harm the male and female reproductive system, and induce breast cancer and testicular cancer in men and other adverse consequences (Colborn et al., 1993; Colborn et al., 1996; Safe, 2000; Andersson et al., 2001; Waring and Harris, 2005; Aksglaede et al., 2006).

Among EDs, the e-ED group together with its contamination in water bodies has received considerable attention due to their potential disruption of the endocrine system of many species at low concentrations (Ying et al., 2002a; Hanselman et al., 2003; Campbell et al., 2006; Khanal et al., 2006; Liu et al., 2009a). One of the main e-ED subgroups, steroidal estrogens are mainly excreted by humans and animals (Ying et al., 2002a; Hanselman et al., 2003; Campbell et al., 2006), and are ultimately found in the effluent of wastewater treatment plants. Such plants are the main source of e-

EDs (Kirk et al., 2002; Coors et al., 2004; Campbell et al., 2006). In addition, industrial effluents and agricultural runoff are reported to be considerable sources of e-EDs (Hurst and Sheahan, 2003; Xie et al., 2005; Campbell et al., 2006; Lavado et al., 2009)

Vietnam is a predominantly agricultural country located in a tropical region. Its population is about 84 millions, of which approximately 70% live in and rely heavily on agricultural areas (Tra, 2003). As Vietnam is a developing country, rapid urbanisation and industrialisation processes are taking place, accompanied by an improvement in socio-economic conditions and by increasing environmental pollution from untreated domestic and industrial waste discharges, pesticides and poorly uncontrolled usage of toxic chemicals. There is currently a lack of information on e-EDs in the environment in Vietnam.

The objectives of this review chapter are: (1) to summarise and highlight some commonly reported e-ED groups, their estrogenic potency, and their respective sources; and (2) to review the occurrence and concentrations of some e-EDs reported in sediment and surface water environments in Vietnam.

2.2 Commonly reported estrogenic endocrine disruptors and their sources

e-EDs are a broad class of chemicals, comprising probably a hundred compounds (Campbell et al., 2006), including both natural and man-made (anthropogenic) types. In terms of natural estrogenic chemicals, the most commonly reported compounds are estrogens, including natural estrogens such as estrone (E1), 17β-estradiol (E2) and estriol (E3), and synthetic estrogens such as 17α-ethynylestradiol (EE2). The most common anthropogenic estrogenic compounds are alkylphenols, Bisphenol A (BPA), Polychlorinated Biphenyls (PCBs) and Organochlorine Pesticides (OCPs), Polycyclic Aromatic Hydrocarbons (PAHs), and phthalates.

Natural estrogens such as E1, E2 and E3, and synthetic estrogens such as EE2 and mestranol (MeEE2), have estrogenic activity and can be found ubiquitously in the

environment (Ying et al., 2002a; Labadie et al., 2007). They are either secreted by the adrenal cortex, testis, ovary and placenta in humans and animals, or are fed to humans and animals as contraceptives or animal growth promoters (Lange et al., 2002). They are reported to be the agents leading to feminisation of fish (Sumpter and Johnson, 2008). It has been confirmed in both laboratory and field studies that an extremely low concentration (1-10 ng/L for E2 and even at low as 0.1 ng/L for EE2) can cause abnormal responses in fish (Desbrow et al., 1998; Routledge et al., 1998; Witters et al., 2001; Shore and Shemesh, 2003; Vine et al., 2005; Chimchirian et al., 2007). In addition, it has been demonstrated in different studies that e-EDs are able to act together to produce significant effects when combined at individual concentrations below their No Observed Effect Concentrations (NOECs) (Silva et al., 2002; Filby et al., 2007).

Estrogenic activity of PAHs has been observed in several biological experiments such as the yeast assay system, MCF-7 cells and the yeast two-hybrid assay system (Wang et al., 2004; Hayakawa et al., 2007). Many of the PAHs are carcinogenic to humans and laboratory animals, and the principal concern regarding exposure to PAHs is that they increase the risk of cancer (Hayakawa et al., 2007). PAHs are ubiquitous environmental pollutants. They are formed through incomplete combustion of fossil fuel, wood and other organic materials, including automobile exhausts, domestic heating and industrial processes.

Alkylphenols have been reported to be e-EDs (Soto et al., 1995; Routledge and Sumpter, 1997; Tabira et al., 1999; Solé et al., 2000; Johnson and Sumpter, 2001; Ying et al., 2002b; Paris et al., 2002; Shioji et al., 2006). These chemicals give rise to a number of effects, such as increasing the incidence of abnormalities in offspring and possibly inducing vitellogenin production in rainbow trout and in rainbow trout hepatocytes and carp; directly mimicking endogenous hormones; antagonising the natural actions of endogenous hormones or changing the rate of synthesis and metabolism of natural hormones; altering hormone receptor levels; inducing proliferation in the luminal epithelium of the endometrium in ovariectomised rats; causing a reduction in testicular weight by adulthood; and decreasing in testicular weight, leading to a 10-21% reduction in daily sperm production (Markey et al., 2002). Alkylphenols are intermediates of the mineralisation of Alkylphenol Polyethoxylates (APEs). APEs comprise two major groups, nonylphenol polyethoxylate and

octylphenol polyethoxylate. Nonylphenol polyethoxylate is the most prevalent, representing 80% of all APEs. Octylphenol polyethoxylate makes up most of the remaining 20% of APEs (Sonnenschein and Soto, 1998; Markey et al., 2002). APEs are used in industry as industrial detergents, emulsifiers in the production of liquid pesticides, commercial vehicle and metal cleaners, and in floor care and commercial laundry products. They are also used in personal care products such as shampoo, conditioners and hair colouring agents in households. The main source of alkylphenols in water is from direct discharge or via sewage.

BPA has been demonstrated to be an e-ED in a variety of in vitro and in vivo assays (Vinggaard et al., 2000; Arukwe et al., 2000; Markey et al., 2002; Rankouhi et al., 2002; Li et al., 2004). The effects reported include: inducing cell proliferation, up-regulation of the expression of vitellogenin mRNA in primary hepatocytes from male *Xenopus laevis*, maternal toxicity, a reduction in the number of pregnant rats, a reduction in the number of live foetuses born, decreased in maternal weight, increased in maternal liver weight and maternal mortality, and decreased foetal body weight and uterine weight (Markey et al., 2002). BPA is a synthetic substance widely used to produce polycarbonated plastics for food and drink containers, the lining of tin cans, toys, baby bottles, dental sealants, flame-retardants and plastic wrap.

PCBs have been reported to be estrogenic (Soto et al., 1995; Arukwe et al., 2000; Layton et al., 2002; deCastro et al., 2006). Background levels of some of the compounds in this group may be causally related to decreased male reproductive capacity, increased incidence of breast cancer and neurodevelopmental defects in children (Safe, 2002). PCBs can also directly interact with receptors and produce estrogenic effects at very low concentrations in rainbow trout hepatocytes (Layton et al., 2002), and have been associated with reproductive abnormalities in animals (deCastro et al., 2006). PCBs are mainly used for industrial purposes such as dielectric fluids in transformers and other electrical equipment. They are persistent environmental contaminants that bio-accumulate in wildlife and humans (Tanabe and Minh, 2010).

OCPs and their metabolites have been detected in environmental samples and food products, and are reported to be estrogenic (Soto et al., 1995; Klotz et al., 1996; Arnol et al., 1996; Zaroogian et al., 2001; De Boever et al., 2001; Safe, 2002; Gordon et al.,

2004; Kojima et al., 2005). They can cause anti-estrogenic responses in rodent uterus models, synergistic estrogen receptor binding and transactivation responses, and affect male rat sexual differentiation (Safe, 2002). OCPs are synthetic organic insecticides that are highly persistent in organisms and the environment because of their low water solubility (Keithmaleesatti et al., 2009). OCPs such as 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (p,p'-DDT), 2-(p-chlorophenyl)-2-(o-chlorophenyl)-1,1,1-trichloroethane (o,p'-DDT), 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE) and related compounds have been extensively used for pest control. The scientific evidence that OPCs damage wildlife and humans (Cohn et al., 2007; Tanabe and Minh, 2010) has led to restrictions on use or the banning of many of these bioaccumulative compounds.

Phthalates are e-EDs (Soto et al., 1995; Harris et al., 1997) and they can cause adverse affects on rat testes size and sperm production, and reproductive toxicity in rats (Harris et al., 1997). Moreover, they can cause adverse reproductive development in the offspring of adult mammals such as testicular toxicity, effects on semen quality, decreased fertility in female Wistar rats and decreased sperm quality in males, and the modification of steroid concentrations in female rats (Harris and Sumpter, 2002). Phthalates are synthetic substances added to plastics to make them softer, more flexible, resilient and long-lasting. They are found in vinyl flooring, glues, inks, pesticides, detergents, plastic bags, food packaging, children's toys, shower curtains, soaps, shampoos, perfumes, hair spray and nail polish (Harris and Sumpter, 2002).

Since e-EDs can be of varying chemical composition and are either naturally occurring or man-made, their sources for the environment are also diverse and include discharges from domestic sewage, industry, agricultural land use, wastewaters from dairies and aquaculture and even atmospheric deposition. Spawning fish may locally increase the estrogen concentrations in river water. Livestock feedlots have also been demonstrated to be potential sources of estrogenic compounds from excretion of hormones in manure and urine (Campbell et al., 2006).

In summary, the main e-ED sources reported are discharges from domestic and industrial activities (Desbrow et al., 1998; Routledge et al., 1998; Lange et al., 2002; Ying et al., 2002a; Shore and Shemesh, 2003; Shore et al., 2004; Campbell et al.,

2006; Fernandez et al., 2007). These discharges contain different e-EDs, e.g. alkylphenol, PCBs, PAHs, phthalates, BPA, natural hormones and synthetic estrogens from contraceptives (Muthumbi et al., 2003; Furuta et al., 2004; Owens et al., 2007), and contribute to the pollution of surface waters. In addition to domestic and industrial discharges, discharges from agricultural activities are reported to be a source of e-ED (Shore et al., 1993; Lange et al., 2002; Ying et al., 2002a; Hurst and Sheahan, 2003; Shore and Shemesh, 2003; Shore et al., 2004; Campbell et al., 2006). Besides natural estrogens, runoff from agricultural fields contains pesticides, fertilisers and other anthropogenic estrogenic chemicals such as nonylphenol ethoxylates OCPs, DDT, dieldrin, lindane, atrazine and permethrin (Shore et al., 2004; Campbell et al., 2006). The occurrence of e-EDs has been extensively reported in surface water, groundwater, drinking water and sediments (Witters et al., 2001; Ying et al., 2002a; Campbell et al., 2006; Isobe et al., 2006; Pojana et al., 2007; Viganò et al., 2008; Liu et al., 2009b).

2.3 Estrogenic potency

Among natural estrogens, E2 is estrogenically the most potent compound and thus the estrogenic potency (EEF) of a substance or a sample is usually given in relation to E2. Other natural estrogens (E1, E3) and anthropogenic estrogenic compounds (alkylphenol, PCBs, PAHs, OCPs, BPA and phthalates) are generally from a few up to eight orders of magnitude less potent than E2 (Soto et al., 1995; Zacharewski, 1997; Legler et al., 2002a; Legler et al., 2002b; Rutishauser et al., 2004; deCastro et al., 2006) (Table 2.1).

The estrogenic potency of a sample is usually referred to its estrogenic activity or E2 equivalent (EEQ) (Eq 1).

$$EEQi = Ci \times EEFi, \text{ and } EEQt = \sum EEQi \qquad (1)$$

Where:

EEQt: Total estrogen equivalent

i: The compound i in a mixture

Ci: The concentration of compound i in a mixture

EEQi: Total estrogen equivalent of compound i in a mixture

EEFi: Estrogenic potency of compound i in a mixture (Campbell et al., 2006)

Depending on the analytical methods used, the estrogenic potency is sometimes referred to as binding affinity, e.g. in estrogen receptor binding assays (Blair et al., 2000; Fang et al., 2000), or as proliferative effect in E-Screen assay (Soto et al., 1995; Fang et al., 2000). In this paper, the author only used the term estrogenic potency. Values of the estrogenic potency of different chemicals reported in the literature are collated and summarised in Table 2.1 and Figure 2.1.

Table 2.1: Estrogenic potency of different groups of estrogenic chemicals analysed by different methods: Letter superscripts in bracket indicate the reference; number superscripts in bracket indicate the methods used to analyse estrogenic potency

Chemical	EEF	Chemical	EEF
Estrogens			
17β-Estradiol (E2)	1	Estrone (E1)	2.4×10^{-2} (b2)
			0.38 (d1)
			1.0×10^{-2} (e2)
			5.6×10^{-2} (k3)
			0.1 (k1)
			7.0×10^{-2} (k6)
			7.3×10^{-2} (y6)
17α-Estradiol (17α-E2)	2.0×10^{-2} (b2)	Estriol (E3)	5.4×10^{-2} (b2)
	0.1 (e2)		2.4×10^{-3} (d1)
	1.6×10^{-2} (k3)		0.1 (e2)
	1.0×10^{-2} (k1)		5.0×10^{-3} (n1)
	0.11 (k6)		9.7×10^{-2} (y6)
	2.1×10^{-2} (n1)		

Chemical	EEF	Chemical	EEF
	3.1×10^{-2} (y6)		
17α-Ethinylestradiol (EE2)	1.19 (d1)	Mestranol (MES)	1.3×10^{-2} (d1)
	0.7 (d7)		1.0×10^{-4} (n1)
	1.62 (d8)		2.2×10^{-2} (y6)
	1.2 (k3)		
	1.2 (k1)		
	0.8 (k6)		
	1 (n1)		
B-Estradiol-17-valerate (E2-VAL)	0.21 (d1)	Moxestrol (MOX)	10 (e2)
			0.14 (y6)
16-Hydroxyestrone	1.0×10^{-3} (e2)	Diethylstilbestrol (DES)	10 (e2)
			2 (n1)
			3.99 (y6)
cis-Tamoxifen	1.0×10^{-5} (e2)	Indanestrol	0.1 (e2)
Metabolite from tamoxifen	1.0×10^{-5} (e2)	Indenestrol-A	1 (e2)
R26008	1.0×10^{-3} (e2)	Indenestrol-B	0.1 (e2)
11β-Chloromethylestradiol	10 (e2)	Pseudo-DES	1.0×10^{-3} (e2)
Pseudo-DES-e	0.1 (e2)	d-Equilenin	1.0×10^{-2} (e2)
Pseudo-DES-z	0.1 (e2)	Coumestrol	1.0×10^{-5} (e2)
Zearalenol	1.0×10^{-2} (e2)	Ethynyl-estradiol	1 (e2)
			1.9 (y6)
Zearalenone	1.0×10^{-2} (e2)	B-Estradiol 3-benzoate	8.3×10^{-3} (n1)
Hexestrol	9.1×10^{-2} (n1)	Dienestrol	9.1×10^{-2} (n1)
			0.37 (y6)
19-Nortestosterone	$<1.7 \times 10^{-6}$ (n1)	4-Hydroxyestrone (4OHE1)	4.8×10^{-3} (n1)
2-Hydroxyestradiol (2OHE2)	7.1×10^{-3} (n1)	4-Hydroxyestradiol (4OHE2)	3.0×10^{-3} (n1)

Chemical	EEF	Chemical	EEF
2-Hydroxyestrone (2OHE1)	2.6×10^{-4} (n1)	17β-Estradiol-17-glucuronide	3.7×10^{-4} (b2)
17β-Estradiol-3-glucuronide	1.3×10^{-3} (b2)	Estrone-3-glucuronide	2.9×10^{-5} (b2)
Estriol-3-glucuronide	5.5×10^{-4} (b2)	β-Estradiol 17-β-D-glucuronide	$<1.1 \times 10^{-4}$ (n1)
β-Estradiol 3-β-D-glucuronide	5.0×10^{-4} (n1)	17β-Estradiol-3-sulphate, 17-glucuronide	1.7×10^{-5} (b2)
Estrone β-D-glucuronide	$<4 \times 10^{-6}$ (n1)	Estradiol-3-sulphate	5.5×10^{-4}(b2)
17α-Estradiol-3-sulphate	3.6×10^{-5} (b2)	Estrone-3-sulphate	1.2×10^{-5} (b2) $<1.1 \times 10^{-5}$ (n1)
17β-Estradiol-3-sulphate	2.6×10^{-3} (b2)	β-Estradiol-3-sulphate	2.3×10^{-4} (n1)
17β-Estradiol-3,17-disulphate	1.2×10^{-5} (b2)	1,3,5(10)-Estratrien-3, 6α, 17β-triol	7.1×10^{-3} (y6)
Estra-1,3,5(10)-trien-3-ol	1.8×10^{-3} (y6)	3-Hydroxyestra-1,3,5(10)-trien-16-one	5.1×10^{-3} (y6)
3-Deoxyestradiol	5.0×10^{-3} (y6)	16β-Hydroxy-16-methyl-3-methylether 17β estradiol	3.3×10^{-4} (y6)
3-Methylestriol	2.2×10^{-4} (y6)	3-Deoxyestrone	6.0×10^{-5} (y6)
Meso-hexestrol	3 (y6)	Diethylstilbestrol monomethyl ether	0.2 (y6)
3,3'-Dihydroxyl hexestrol	0.15 (y6)	Dimethylstilbestrol	0.14 (y6)
2,6-Dimethyl hexestrol	0.13 (y6)	Hexestrol, mono methyl ether	9.0×10^{-2} (y6)
p-(α,β-Diethyl-p-methyl phenethyl)-meso-phenol	4.0×10^{-2} (y6)	DL-hexestrol	3.6×10^{-2} (y6)
α,α-Dimethyl-β-ethyl	9.5×10^{-3} (y6)	Diethylstilbestrol dimethyl	5.6×10^{-4} (y6)

Chemical	EEF	Chemical	EEF
allenolic acid		ether	
Doisynoestrol	2.0×10^{-5} (y6)	Norethynodrel	2.2×10^{-3} (y6)
5α-Androstane-3β, 17β-diol	1.2×10^{-3} (y6)	5α-Androstane-3α, 17β-diol	2.0×10^{-5} (y6)
Polycyclic aromatic hydrocarbons and related compounds			
Benzo(a)anthracene (BAA)	5.7×10^{-7} (z10)	Dibenz(a,h)anthracene (DBA)	8.8×10^{-7} (z10)
Pyrene (Py)	4.0×10^{-3} (p6)	Fluoranthene (Frt)	5.0×10^{-3} (p6)
Benz(a)anthracene (BaA)	5.0×10^{-3} (p6)	Chrysene (Ch)	3.0×10^{-3} (p6)
Benzo(c)phenanthrene (BcPh)	2.0×10^{-3} (p6)	9-Hydroxybenzo[*b*] fluoranthene (9-OHBbFR)	0.205 (p6)
2-Hydroxyfluorene (2-OHFl)	5.6×10^{-4} (p9) 0.128 (p6)	10-Hydroxybenzo[*b*] fluoranthere (10-OHBbFR)	0.015 (p6)
2-Hydroxyphenanthrene (2-OHPh)	7.5×10^{-4} (p9) 0.232 (p6)	3-Hydroxybenzo[*k*] fluoranthere (3-OHBkFR)	1.3×10^{-4} (p9) 0.15 (p6)
3-Hydroxyfluoranthere (3-OHFR)	4.2×10^{-4} (p9) 0.207 (p6)	1-Hydroxybenzo[*a*] pyrene (1-OHBaP)	0.187 (p6)
1-Hydroxypyrene (1-OHPy)	2.4×10^{-4} (p9) 0.11 (p6)	3-Hydroxybenzo[*a*] pyrene (3-OHBaP)	0.394 (p6)
1-Hydroxybenzo[*a*] anthracene (1-OHBaA)	0.047 (p6)	8-Hydroxybenzo[*a*] pyrene (8-OHBaP)	0.376 (p6)
2-Hydroxybenzo[*a*] anthracene (2-OHBaA)	0.137 (p6)	4-Hydroxybenzo[*e*] pyrene (4-OHBeP)	4.2×10^{-4} (p9) 0.117 (p6)
3-Hydroxybenzo[*a*] anthracene (3-OHBaA)	4.2×10^{-3} (p9) 0.228 (p6)	11-Hydroxybenzo[*g*] chrysene (11-OHBgCh)	0.197 (p6)
4-Hydroxybenzo[*a*] anthracene (4-OHBaA)	7.5×10^{-3} (p9) 0.294 (p6)	13-Hydroxybenzo[*g*] chrysene (13-OHBgCh)	0.135 (p6)

Chemical	EEF	Chemical	EEF
5-Hydroxybenzo[*a*] anthracene (5-OHBaA)	0.058 (p6)	5-Hydroxybenzo[*c*] phenanthrene (5-OHBcPh)	0.044 (p6)
9-Hydroxybenzo[*a*] anthracene (9-OHBaA)	0.41 (p6)	1-Hydroxychrysene (1-OHCh)	4.2×10^{-4} (p9) 0.126 (p6)
10-Hydroxybenzo[*a*] anthracene (10-OHBaA)	3.2×10^{-3} (p9) 0.295 (p6)	2-Hydroxychrysene (2-OHCh)	4.2×10^{-3} (p9) 0.406 (p6)
11-Hydroxybenzo[*a*] anthracene (11-OHBaA)	0.027 (p6)	3-hydroxychrysene (3-OHCh)	0.138 (p6)
1-Hydroxybenzo[*c*] phenanthrene (1-OHBcPh)	1.0×10^{-3} (p6)	4-Hydroxychrysene (4-OHCh)	0.012 (p6)
2-Hydroxybenzo[*c*] phenanthrene (2-OHBcPh)	0.185 (p6)	6-Hydroxychrysene (6-OHCh)	0.09 (p6)
3-Hydroxybenzo[*c*] phenanthrene (3-OHBcPh)	0.345 (p6)	4-Hydroxybenzo[*c*] phenanthrene (4-OHBcPh)	0.21 (p6)
12-Hydroxybenzo[*b*] fluoranthene (12-OHBbFR)	1.0×10^{-4} (p9)		
Alkylphenols and BPA			
4-Nonylphenol (4-NP)	2.3×10^{-5} (a3) 3.0×10^{-5} (e2) 2.3×10^{-5} (k3) 5.7×10^{-4} (k1) 5.0×10^{-4} (k6) 3.7×10^{-4} (y6)	Nonylphenol (NP)	2.5×10^{-5} (d1) 7.5×10^{-5} (d7) 1.1×10^{-3} (d8)

Chemical	EEF	Chemical	EEF
	3.5×10^{-4} (y6)		
	3.1×10^{-4} (y6)		
	2.9×10^{-4} (y6)		
	1.9×10^{-4} (y6)		
4-Cctylphenol (4-OP)	1.4×10^{-6} (a3)	Octylphenol (OP)	7.8×10^{-6} (d1)
	1.4×10^{-6} (k3)		3.3×10^{-5} (d7)
	1.0×10^{-5} (k1)		1.3×10^{-3} (d8)
	5.0×10^{-5} (k6)		
	2.0×10^{-4} (m1)		
	5.0×10^{-5} (y6)		
Bisphenol-A (BPA)	5.0×10^{-4} (c4)	5-Octylphenol	3.0×10^{-4} (e2)
	9.0×10^{-5} (c1)		
	1.1×10^{-4} (d1)		
	3.6×10^{-5} (d7)		
	2.5×10^{-3} (d8)		
	1.0×10^{-4} (m1)		
	8.0×10^{-5} (y6)		
Nonylphenol monoethoxylate (NP1E0)	3.8×10^{-6} (a3)	4-sec-Butylphenol	3.0×10^{-6} (e2)
			4.3×10^{-6} (y6)
Nonylphenol diethoxylate (NP2E0)	1.1×10^{-6} (a3)	4-tert-Butylphenol	3.0×10^{-6} (e2)
			3.3×10^{-7} (m1)
			2.4×10^{-6} (y6)
Nonylphenol tetraethoxylate (NP4E0)	1.1×10^{-7} (a3)	4-Isopentylphenol	3.0×10^{-6} (e2)
Nonylphenol ethoxylates (NPEO)	3.8×10^{-6} (k3)	4-tert-Pentylphenol	3.0×10^{-6} (e2)
	4.0×10^{-6} (k1)		
	1.0×10^{-5} (k6)		
Octylphenol ethoxylates	$<6 \times 10^{-7}$ (k3)	4-Chloro-3,5-	1.1×10^{-6} (m1)

Chemical	EEF	Chemical	EEF
(OPEO)	4.0×10^{-6} (k1) 4.0×10^{-6} (k6)	dimethylphenol	
4-tert-Amylphenol	5.0×10^{-6} (m1) 5.0×10^{-6} (y6)	2,6-Di-t-butylphenol	5.0×10^{-8} (m1)
4-Chloro-3-methylphenol	3.3×10^{-7} (m1) 4.2×10^{-6} (y6)	4-Dodecylphenol	1.9×10^{-4} (y6)
4-tert-Octylphenol	1.5×10^{-4} (y6)	4-n-Nonylphenol	3.2×10^{-5} (y6)
2-sec-Butylphenol	2.9×10^{-6} (y6)	2-Chloro-4-methylphenol	2.2×10^{-6} (y6)
3-Ethylphenol	1.4×10^{-6} (y6)	4-Chloro-2-methylphenol	2.1×10^{-6} (y6)
4-Ethylphenol	7.0×10^{-7} (y6)	2,2'-Methylenebis (4-chlorophenol)	4.0×10^{-5} (y6)
2,2-Bis-(4-hydroxyphenyl)-butane (bisphenol B)	8.6×10^{-4} (y6)	BIS (4-hydroxyphenyl)-methane	9.0×10^{-6} (y6)
4,4'-Sulfonyldiphenol	9.0×10^{-6} (y6)	Diphenolic acid	7.0×10^{-6} (y6)
4,4'-Dihydroxystilbene	2.8×10^{-3} (y6)	2,2;,4,4'-Tetrahydroxybenzil	2.1×10^{-3} (y6)
4,4'-Ethylene diphenol	3.7×10^{-4} (y6)	4-Phenethylphenol	2.0×10^{-5} (y6)
4-Phenylphenol	1.0×10^{-5} (y6)	3-Phenylphenol	4.0×10^{-6} (y6)
Polychlorinated biphenyls			
4-Hydroxybiphenyl	3.0×10^{-6} (e2) 1.0×10^{-4} (m1)	2,2',3,5' Tetrachlorobiphenyl	1.1×10^{-5} (g2)
2-Hydroxybiphenyl	5.0×10^{-7} (m1)	2,2',4,5' Tetrachlorobiphenyl	1.4×10^{-5} (g2)
4,4'-Dihydroxybiphenyl	3.0×10^{-6} (e2)	2',3',4',5'-Tetrachloro-4-hydroxybiphenyl	1.0×10^{-5} (e2)
2',5',-Dichloro-3-hydroxybiphenyl	1.0×10^{-6} (e2)	2,3',4,4' tetrachlor-Biphenyl	8.0×10^{-6} (g2)
4,4'-Dihydroxybiphenyl	1.1×10^{-4} (m1)	2,4,4',5 tetrachlor-Biphenyl	7.0×10^{-6} (g2)
2',5',-Dichloro-2-	1.0×10^{-6} (e2)	2,3,4,5-	1.0×10^{-6} (e2)

Chemical	EEF	Chemical	EEF
hydroxybiphenyl		Tetrachlorobiphenyl	
2',5',-Dichloro-4-hydroxybiphenyl	1.0×10^{-5} (e2)	2,4,4',6-Tetrachlorobiphenyl	1.0×10^{-6} (e2)
2,3,4-Trichlorobiphenyl	1.0×10^{-6} (e2)	2,2',3,3',4 Pentachlorobiphenyl	1.4×10^{-5} (g2)
2',2',5-Trichloro-4-hydroxybiphenyl	1.0×10^{-5} (e2)	2,2',4,4',5 Pentachlorobiphenyl	8.0×10^{-6} (g2)
2,2',4 Trichlorobiphenyl	4.0×10^{-5} (g2)	2,2',4,5',6 Pentachlorobiphenyl	1.5×10^{-5} (g2)
2,2',5 Trichlorobiphenyl	4.0×10^{-5} (g2)	2,3,3',4',6' Pentachlorobiphenyl	7.0×10^{-6} (g2)
2',4',6'-Trichloro-4-hydroxybiphenyl	1.0×10^{-4} (e2)	2,2',3,3',6,6'-Hexachlorobiphenyl	1.0×10^{-6} (e2)
2,4,6 Trichlorobiphenyl	4.0×10^{-5} (g2)	2,2',3,3',4,4' Hexachlorobiphenyl	7.0×10^{-6} (g2)
2,2',4,5-Tetrachlorobiphenyl	1.0×10^{-6} (e2)	2,2'3,3',5,6,6' Hexachlorobiphenyl	1.4×10^{-5} (g2)
2',3',4',5'-Tetrachloro-3-hydroxybiphenyl	1.0×10^{-6} (e2)	2,4'-Dichlorobiphenyl	2.0×10^{-6} (y6)
2',3',4',5'-Tetrachloro-4-biphenylol	2.3×10^{-3} (y6)	2',5'-Dichloro-4-biphenylol	3.6×10^{-4} (y6)
4-Chloro-4'-biphenylol	7.0×10^{-5} (y6)	2-Chloro-4-biphenylol	2.0×10^{-5} (y6)
3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	1.0×10^{-5} (y6)		
Pesticides			
o,p'-Dichlorodiphenyl trichloroethane	9.1×10^{-6} (a3)	Dieldrin	1.0×10^{-6} (e2)
	1.0×10^{-6} (e2)		1.12×10^{-6} (h5)
(o,p'-DDT)	1.0×10^{-5} (y6)		2.4×10^{-7} (k3)
p,p'-Dichlorodiphenyl trichloroethane	1.0×10^{-6} (e2)	α-Endosulfan	1.0×10^{-6} (e2)
	5.28×10^{-6} (h5)		

Chemical	EEF	Chemical	EEF
(p,p'- DDT)			
β-Endosulfan	1.0×10^{-6} (e2)	Kepone	1.0×10^{-6} (e2)
			1.13×10^{-5} (h5)
			1.3×10^{-4} (y6)
o,p-Dichlordiphenyldichloroethylene (o,p-DDE)	2.3×10^{-5} (a3)	Methoxychlor	1.0×10^{-6} (e2)
			4.48×10^{-6} (h5)
			1.0×10^{-6} (k3)
Endosulfan	1.0×10^{-6} (k3)	Chlordane	9.6×10^{-7} (k3)
Dichlorodiphenyl dichloroethane (DDD)	9.06×10^{-6} (h5)	1-Hydroxychlordene	1.0×10^{-6} (e2)
p,p'- Dichlorodiphenyl dichloroethylene (p,p'-DDE)	2.3×10^{-6} (h5)	Toxaphere	1.0×10^{-6} (e2)
o,p'- Dichlorodiphenyl dichloroethylene (o,p'-DDE)	2.3×10^{-6} (k3)	Ψ-Chlordane	3.19×10^{-6} (h5)
Fenarimol	1.92×10^{-6} (h5)	2,4,5-Trichlorophenoxyacetic Acid	1.29×10^{-6} (h5)
a-Chlordane	1.17×10^{-5} (h5)	Linuron	1.07×10^{-6} (h5)
Phthalates			
Diethyl phthalate (DEP)	3.2×10^{-8} (a3)	Diisobutyl phthalate (DIBP)	1.0×10^{-7} (i1)
	5.0×10^{-7} (i1)		
	5.0×10^{-7} (k1)		
	5.0×10^{-7} (k6)		
	2.0×10^{-5} (x6)		
Dibutyl phthalate (DBP)	1.8×10^{-8} (a3)	Ditridecyl phthalate (DTDP)	1.0×10^{-7} (i1)
	1.0×10^{-7} (i1)		
	3.6×10^{-4} (x6)		
	2.0×10^{-5} (x6)		

Chemical	EEF	Chemical	EEF
Butylbenzyl phthalate (BBP)	1.4×10^{-6} (a3) 1.0×10^{-6} (i1) 2.8×10^{-4} (x6) 7.0×10^{-3} (x6) 2.0×10^{-5} (x6)	Dimethyl phthalate (DMP)	1.1×10^{-5} (k3) 1.0×10^{-6} (k1) 0 (k6)
Di-2-ethylhexyl phthalate (DEHP)	$<6 \times 10^{-7}$ (k3) 2.0×10^{-5} (x6)	Dioctyl phthalate (DOP)	$<6 \times 10^{-7}$ (k3)
Miscellaneous chemicals			
3-Phenoxybenzyl alcohol 3	5.0×10^{-5} (f1)	Benzophenone-2	1.4×10^{-4} (m1)
3-Phenolxybenzaldehyde 4	7.0×10^{-5} (f1)	Benzophenone-3	1.0×10^{-5} (m1)
3-(4-Hydroxy-phenoxy)benzyl alcohol 6	5.0×10^{-5} (k3)	Benzophenone-6	5×10^{-8} (m1)
2,4,2',4'-Tetrabromodiphenylether (BDE47)	2.0×10^{-7} (k3)	Benzophenone-7	3.3×10^{-6} (m1)
2,3,4,2',4'-Pentabromodiphenylether (BDE85)	2.0×10^{-7} (k3)	Phenyl salicylate	3.3×10^{-6} (m1)
2,4,5,2',4'-Pentabromodiphenylether (BDE99)	2.0×10^{-7} (k3)	Benzyl salicylate	1.7×10^{-6} (m1)
2,4,6,2',4'-Pentabromodiphenylether (BDE100)	2.0×10^{-5} (k3)	Menthyl salicylate	5×10^{-6} (m1)
1,3,5,3'-Tetrabromo-4'-hydroxydiphenylether (T3-like OH-BDE)	1.0×10^{-4} (k3)	Ethylhexyl salicylate	5×10^{-7} (m1)
4,4'-	2.5×10^{-5} (m1)	Resorcinol	1.25×10^{-5} (m1)

Chemical	EEF	Chemical	EEF
Dihydroxybenzophenone		monobenzoate	
Benzylparaben	2.5×10^{-4} (m1)	Chlorothymol	2.5×10^{-6} (m1)
Butylparaben	1.25×10^{-4} (m1)	Bis(4-hydroxyphenyl)methane	1.1×10^{-4} (m1)
Propylparaben	3.3×10^{-5} (m1)	Nordihydroguaiaretic acid	1.7×10^{-6} (m1) 3.1×10^{-4} (y6)
Ethylparaben	5.0×10^{-6} (m1)	Butylated hydroxytoluene	1.25×10^{-7} (m1)
Methylparaben	3.3×10^{-7} (m1)	4-t-Butylcatechol	3.3×10^{-6} (m1)
Benzophenone-1	3.3×10^{-4} (m1)	Butylated hydroxyanisole	5.0×10^{-7} (m1)
2-Ethylhexyl 4-hydroxybenzoate	1.8×10^{-4} (y6)	Heptyl 4-hydroxybenzoate	8.0×10^{-5} (y6)
Benzyl 4-hydroxybenzoate	3.0×10^{-5} (y6)	Butyl 4-hydroxybenzoate	9.0×10^{-6} (y6)
Propyl 4-hydroxybenzoate	6.0×10^{-6} (y6)	Ethyl 4-hydroxybenzoate	6.0×10^{-6} (y6)
Methyl 4-hydroxybenzoate	4.0×10^{-6} (y6)	4,4'-dihydroxybenzophenone	3.0×10^{-5} (y6)
2,4-Dihydroxybenzophenone	2.0×10^{-5} (y6)	4-Heptyloxyphenol6	1.3×10^{-5} (y6)
4-Benzyloxyphenol	3.6×10^{-6} (y6)	Phenolphthalein	1.3×10^{-4} (y6)
Phenol red	1.0×10^{-5} (y6)	Phenolphthalin	2.0×10^{-6} (y6)
Aurin	3.2×10^{-4} (y6)		

a: Legler et al. (2002a)

b: Gadd et al. (2010)

c: Li et al. (2004)

d: Rutishauser et al. (2004)

e: Soto et al. (1995)

1: Yeast estrogen screen (YES) assay

2: E-Screen assay

3: Estrogen receptor-mediated luciferase reporter gene (ER-CALUX) assay

4: Enzyme-linked receptor assay (ELRA)

f: McCarthy et al. (2006)

g: deCastro et al. (2006)

h: Gordon et al. (2004)

i: Harris et al. (1997)

k:de Voogt and van Hattum (2003)

m: Miller et al. (2001)

n: Bovee et al. (2004)

p: Hayakawa et al. (2007)

x: Harris and Sumpter (2002)

y: Blair et al. (2000)

z: Villeneuve et al. (2002)

5: LUMI-CELL assay

6: Estrogen receptor (ER) binding

7: Production of zona radiate proteins in trout hepatocytes (ZRP)

8: Induction of reporter gene expression in the transfected rainbow trout gonad cell line (RTG-2)

9: Yeast Two-Hybrid Assay (Two-Hybrid)

10: MVLN assay

Estrogenic potency is dependent on the target compound (Table 2.1). However, in general, the estrogens are the most potent group (median EEF 10^{-2}), regardless of analytical method, while the anthropogenic estrogenic groups are the least potent (with potency values generally ranging from two to eight orders of magnitude lower than E2 (Figure 2.1). Among these anthropogenic groups, regardless of analytical method, the PAHs have the highest median EEF (10^{-4}), followed by the three groups alkylphenols, PCBs and miscellaneous (median EEFs 10^{-5}). The phthalates and pesticides groups seem to be less potent, with median EEFs six and seven orders of magnitude lower than that of E2 (data not shown in Figure 2.1).

Estrogenic potency of a chemical also varies depending on the analytical method used (e.g. E1, E3, EE2, NP, OP, BPA) and therefore the sensitivity of these analytical methods is different for test chemicals (Table 2.1; Rutishauser et al., 2004). In addition to limited number of compounds being analysed by different methods, the potency of estrogenic compounds varies when analysed by different methods and also varies depending on the target compounds (Table 2.1), it is difficult to draw any firm conclusions on which method is estrogenically more or less sensitive than others. However, for some specific chemicals, several studies have reported the differences among in vitro assays used for analyzing their estrogenic potency. Legler et al. (2002a) concluded that the ER-CALUX assay is six to 20 times estrogenically more sensitive to some compounds (E2, NP and o,p'-DDT) than the YES assay. Those authors listed some possible reasons for this difference, including differences in

permeability between the yeast and mammalian cell membranes, differences in cellular transcription factors, multiple drug resistance, endogenous yeast estrogen binding proteins, and a difference in the solubility and availability of compounds in the assays. In addition, in the study by Li et al. (2004), the ELRA assay was found to be several to ten times more sensitive to E2, BPA and resveratrol than the YES assay. Furthermore, Rutishauser et al. (2004) reported that among three in vitro assays (YES, ZRP, RTG-2), the ZRP assay was the least sensitive to the steroid compounds tested (E2, EE2), and the RTG-2 assay was above an order of magnitude more sensitive than the other two assays for the anthropogenic chemicals (BPA, NP, OP).

Based on the data summarised in Table 2.1, the author compared differences between analytical methods within an estrogenic group, regardless of the target compound/s. A statistically significant difference (Kruskal-Wallis Test) between the methods was obtained for some groups, e.g. alkylphenols ($p = 0.03$), pesticides ($p = 0.002$), phthalates ($p = 0.04$) and PAHs ($p = 0.000$). In contrast, there was no significant difference (Kruskal-Wallis Test) for PCBs ($p = 0.079$), estrogens ($p = 0.24$) and miscellaneous chemicals ($p = 0.22$).

The methods used to analyse EEFs presented in Figure 2.1 are either cellular bioassays (YES, E-Screen, ER-CALUX, LUMI-CELL, ZRP, RTG-2, Yeast Two-Hybrid and MVLN) or non-cellular assays (ELRA and ER-binding). The cellular assays use yeast cells (YES, Yeast Two-Hybrid), human cells (E-Screen, ER-CALUX, LUMI-CELL, MVLN) or fish cells (ZRP). The cells may need to be bioengineered (e.g. the yeast cells) so that an estrogen binding to the estrogen receptors (ER) will produce a dimer able to bind to and stimulate an estrogen response element (ERE) that promotes the expression of a measurable protein (Campbell et al., 2006). More information about these assays can be found in different reports, e.g. Oppen-Berntsen et al. (1994) for ZRP, Soto et al. (1995) for E-Screen, Routledge and Sumpter (1996) for YES, Legler et al. (1999) for ER-CSLUX, Seifert et al. (1999) for ELRA, Blair et al. (2000) for ER-binding, Nishihara et al. (2000) for Yeast Two-Hybrid, Rodgers and Denison (2000) for LUMI-CELL; Villeneuve et al. (2002) for MVLN and Rutishauser et al. (2004) for RTG-2.

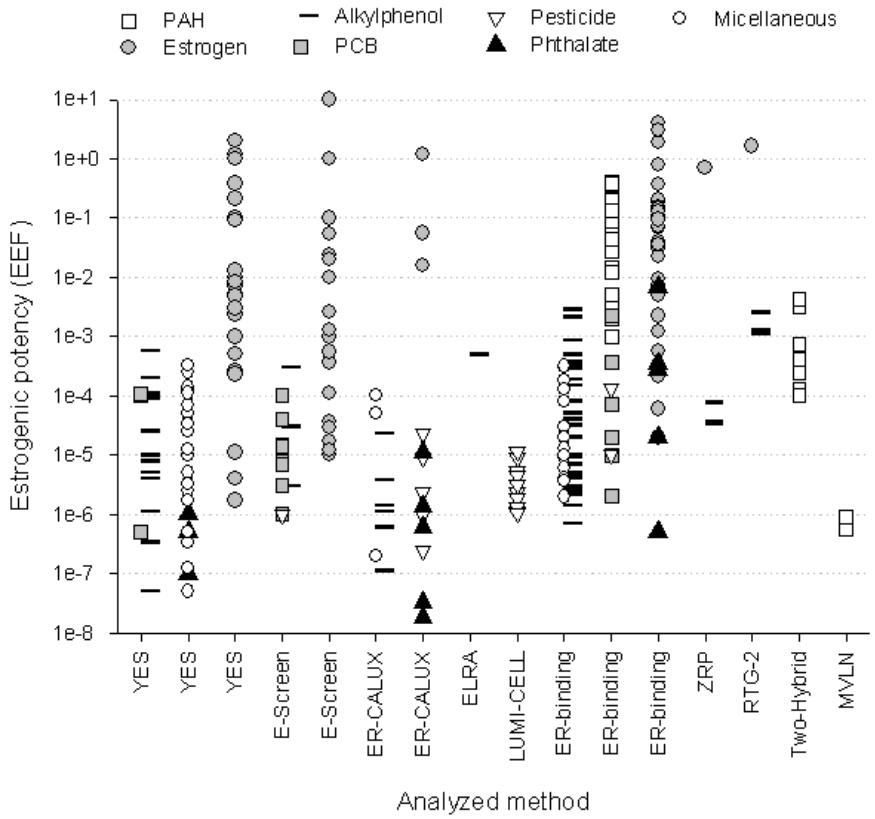

Figure 2.1: Estrogenic potency analysed by different methods for different estrogenic groups: alkylphenol (—); PCB (□); pesticide (▽); PAH (□); phthalate (▲); estrogen (○); miscellaneous (○). YES (yeast estrogen screen assay); E-Screen (E-Screen assay); ER-CALUX (estrogen receptor-mediated luciferase reporter gene assay); ELRA (enzyme linked receptor assay); LUMI-CELL (LUMI-CELL assay); ER binding (estrogen receptor binding assay); ZRP (production of zona radiate proteins in trout hepatocytes assay); RTG-2 (the induction of reporter gene expression in the transfected rainbow trout gonad cell line assay); Two-Hybrid (yeast Two-Hybrid assay); MVLN (MVLN assay)

An estrogenic chemical is able to diffuse into the target cells, where it binds to ER to form an estrogen-receptor complex. In addition, the prevailing model suggests that this complex then interacts with an ERE of a target gene and with the transcriptional machinery (Fang et al., 2000). Therefore, the main difference between the cellular and non-cellular assays mentioned above is that they measure the end points at different levels of biological complexing of estrogenic action, i.e. receptor binding, expression of a reporter gene and cell proliferation (Fang et al., 2000). The non-cellular assays (ELRA, ER-binding) measure the binding affinity of a chemical for ER, the very first

step in biological complexing of estrogen action on the target cells. In contrast, the cellular assays include effects from not only ligand-ER binding but also ER-ERE interactions, transcriptional complex effects, translational effects (Fang et al., 2000) and proliferative effects (Soto et al., 1995). The main difference among the reported cellular assays is that the E-Screen assay measures the induction of cell proliferation in an estrogen-sensitive breast cancer cell line (Soto et al., 1995), whereas the other assays measure the expression of a measurable protein such as luciferase and β-galactosidase (Oppen-Berntsen et al., 1994; Routledge and Sumpter, 1996; Legler et al., 1999; Nishihara et al., 2000; Rodgers and Denison, 2002; Villeneuve et al., 2002; Rutishauser et al., 2004; Campbell et al., 2006).

Overlooking differences in analytical methods, the estrogenic activity of a chemical generally depends on its potency and concentration. However, long-term exposure to a less potent compound or weakly estrogenic compound could cause effects if it is present in the environment at a high concentration (Sumpter and Johnson, 2005). Therefore, in order to assess the effects of estrogenic chemicals, exposure duration also needs to be considered.

2.4 Occurrence and concentration of estrogenic endocrine disruptors in surface water and sediment environments in Vietnam

In Vietnam, some data on anthropogenic estrogenic chemicals such as PCBs, OCPs, PAHs and BPA are available. However, for the group of natural estrogenic compounds, there is very little information. Around 27,000 tons of DDT was imported to Vietnam from 1957 to 1990. The usage of DDT was banned in Vietnam in 1995 and other insecticides such as pyrethroid compounds have been used as substitutes (Minh et al., 2008). There is still little information on PCB usage in Vietnam. It is reported that about 27,000 – 30,000 tons of oil contaminated with PCBs were imported from former Soviet Union, China and Rumania. In addition, electrical equipments containing PCBs such as transformers, was imported from Australia until the mid-1980s (Minh et al., 2008).

Most of the different studies on these anthropogenic estrogenic chemicals in Vietnam focus on sediment environments, with only a few studies focusing on surface waters (Table 2.2). The results from these studies show that PCBs and DDTs can be found in surface waters and sediments in different locations in Vietnam (Table 2.2).

In terms of sediments, elevated concentrations of DDTs and PCBs have been found in sediment samples in Ho Chi Minh City (HCMC) (Iwata et al., 1994; Kishida et al., 2007; Minh et al., 2007b), especially in samples taken close to municipal sewage effluents or highly polluted rivers and canals. A study conducted by Hoai et al. (2010) found that among the locations tested in Vietnam, the concentrations of PCBs (mean 104 ng/g) and DDTs (mean 135 ng/g) in sediments were highest in Ha Noi City (HNC), followed by HCMC. Those authors also noted that DDTs and PCBs were the two dominant pollutants in the environment in Vietnam. Moreover, in another study Iwata et al. (2004) reported that DDTs and PCBs were the two dominant organic pollutants in the Mekong Delta and that concentrations of these compounds were higher at sampling sites close to urban areas (Can Tho and Long Xuyen Cities), and decreased downstream in the river.

To evaluate the potential toxicity to the aquatic environments, Hoai et al. (2010) compared the concentrations of DDTs and PCBs in samples taken from urban rivers in HNC against the Interim Sediment Quality Guideline (ISQG) and the Probable Effective Level (PEL) issued by the Canadian Council of Ministers of the Environment (Hoai et al., 2010). They reported that the concentrations of DDE, DDD, and DDT (sum of o,p'- and p,p'-isomers) in all sediment samples exceeded the ISQG values (1.42, 3.54 and 1.19 ng/g, respectively). The concentrations of these compounds also generally exceeded the PEL values (6.75 ng/g for DDE, 8.51 ng/g for DDD and 4.77 ng/g for DDT) but varied depending on the sampling location (Hoai et al., 2010). In contrast, the PCBs concentrations were below the PEL value (340 ng/g for Aroclor 1254), and only half of the samples exceeded the ISQG value (60 ng/g for Aroclor 1254) (Hoai et al., 2010). In other studies, Minh et al. (2007a) and Minh et al. (2007b) also reported that the concentrations at some sampling points in the Mekong River and the Sai Gon-Dong Nai River exceeded the above mentioned ISQG and PEL for DDTs, and the ISQG of 43.1 ng/g and the PEL of 277 ng/g for PCBs. Similar results have been reported for sediments at harbours and industrial areas in Ha Long Bay and Hai Phong Bay in Vietnam (Hong et al., 2008).

PAHs have been detected in the Mekong River and urban drainage canals in Phnom Penh, Can Tho and HCMC (Togo et al., 2004). Major sources seem to be lubricating oil from vehicles and motorbikes. In addition, Kishida et al. (2007) concluded that PAHs pollution in urban and suburban areas is caused mainly by runoff of petrol, whereas in rural areas the combustion of fossil fuels and biomass is the major pollutant source.

The author used data on some chemical groups for which more information exists in Vietnam, e.g. PCBs, DDTs and PAHs, to calculate estrogenic activity. Based on Eq. 1, the author roughly estimated the highest estrogenic activity of these compounds in terms of Estrogenic Equivalents or estrogenic activity (EEQs) by using their highest concentrations reported in different sediment and surface water environments in Vietnam. The results are presented in Table 2.2. However, the author assumed a compound with the highest estrogenic potency within a group to be representative of its group and therefore the values obtained may have been overestimated. Thus, 2',3',4',5'-Tetrachloro-4-biphenylol, o,p-DDE and benz(a)anthracene were selected as the representatives for PCBs, DDTs and PAHs, and their estrogenic potency was estimated to be 1.0×10^{-5}, 2.3×10^{-5} and 5.0×10^{-3}, respectively (Table 2.1).

In order to determine whether the estrogenic activity of PCBs, DDTs and PAHs in sediment samples was influenced by different sampling categories, after converting the data from selected studies into ng EEQs/g, the author arranged them into three major sampling categories (urban, industrial, and rural or suburban areas) (Figure 2.2).

The estrogenic activity obtained for these groups of chemical varied widely, from 9.2×10^{-6} to 38.18 ng/g dry weight sediment (Table 2.2; Figure 2.2), depending on the sampling location and the chemicals analysed in the different studies, i.e. some studies did not analyse all three estrogenic groups (PCBs, DDTs, and PAHs). For example, among the reported samples, only the sediment samples taken in 2002 were analysed for PAHs in addition to PCBs and DDTs, and consequently the estrogenic activity of these samples was markedly higher than that of the others (Figure 2.2). This indicates a considerable contribution of PAHs in the estrogenic activity of sediments. Allowing for the variability in estrogenic activity, median EEQs

obtained in urban areas were higher than in rural or suburban areas in all the studies except one study (sampling year 1995/1996) in which there were only rural samples (Figure 2.2). This indicates that urban discharges are the source of these chemicals. In addition, in two cases industrial samples were analysed in addition to urban and rural samples. The median EEQ of industrial samples taken in 2003/2004 was higher than in the rural samples, whereas it was opposite for those samples taken in 1997 (Figure 2.2). This was because more compounds were analysed in the rural samples than in the industrial samples.

Estrogens such as E1, E2 and EE2 have been reported to predominantly contribute to total estrogenic activity in surface water and sediment environments compared with anthropogenic chemicals. In some cases, their contribution accounts for more than 90% (Furuichi et al., 2004; Pojana et al., 2007; Viganò et al., 2008; Duong et al., 2010). In addition, Duong et al. (2010) found that estrogens are major contributors to the total estrogenic activity of surface water samples taken in Vietnam. Therefore, total estrogenic activity in sediment and surface water samples in Vietnam might be even higher than that reported in Table 2.2 and Figure 2.2, which only originated from anthropogenic chemicals (PCBs, DDTs and PAHs).

Table 2.2: Concentrations of selected estrogenic groups in surface water (pg/L) and sediment (ng/g dry weight) at different locations in Vietnam

Highest EEQ	PCBs	DDTs	PAHs	Sampling location, city	Reference
Concentration in sediment (ng/g dry weight)					
38.18	110	140	6400	Urban river, HCMC	Kishida et al. (2007)
25.65	44	29	4300	Urban canal, HCMC	Kishida et al. (2007)
13.72	21	42	2300	Urban lake, HNC	Kishida et al. (2007)
11.33	7.3	5.5	1900	Suburban, HCMC	Kishida et al. (2007)

Highest EEQ	PCBs	DDTs	PAHs	Sampling location, city	Reference
9.54	21	44	1600	Urban lake, HNC	Kishida et al. (2007)
8.95	5	4.3	1500	Suburban river, HCMC	Kishida et al. (2007)
1.37	0.77	1	230	Rural lagoon, Hue City	Kishida et al. (2007)
1.01	0.67	1.8	170	Rural lagoon, Hue City	Kishida et al. (2007)
0.74	-	-	65-125	Non-urban area, HCMC	Viet (2002)
0.71	0.11	1	120	Rural lagoon, Hue City	Kishida et al. (2007)
0.66	0.27	1.3	110	Rural river, Hue City	Kishida et al. (2007)
0.19	0.19	0.19	33	Rural canal, HCMC	Kishida et al. (2007)
3.6×10^{-2}	-	-	0.02-6	Mekong River	Togo et al. (2004)
2.5×10^{-2}	237-328	82-1100	-	Urban river, HNC	Hoai et al. (2010)
2.1×10^{-2}	630	790	-	Urban canal, HCMC	Iwata et al. (1994)
1.5×10^{-2}	36-139	215-680	-	Urban river, HNC	Hoai et al. (2010)
1.3×10^{-2}	630	360	-	Urban canal, HCMC	Iwata et al. (1994)
1.2×10^{-2}	440	430	-	Urban canal, HCMC	Iwata et al. (1994)
1.0×10^{-2}	9-590.5	1.76-254	-	Urban canal, HCMC	Phuong et al. (1998)
5.5×10^{-3}	20-384	17-109	-	Urban lake,	Hoai et al.

Highest EEQ	PCBs	DDTs	PAHs	Sampling location, city	Reference
				HNC	(2010)
5.5×10^{-3}	0.11-10.1	1.22-274	-	Hai Phong Bay	Hong et al. (2008)
3.6×10^{-3}	140	120	-	Urban canal, HCMC	Iwata et al. (1994)
3.1×10^{-3}	42-122	11-103	-	Urban canal, HNC	Hoai et al. (2010)
2.7×10^{-3}	46-150	12-72	-	Urban canal, HCMC	Minh et al. (2007b)
2.6×10^{-3}	0.45-18.7	1.76-126	-	Hai Phong Bay	Hong et al. (2008)
2.2×10^{-3}	0.04-9.2	0.01-110	-	Mekong River	Minh et al. (2007a)
1.9×10^{-3}	0.97-33.7	7.40-80	-	Canals, HNC	Nhan et al. (2001)
1.8×10^{-3}	1.3-70	6.4-61	-	Urban river, HNC	Hoai et al. (2010)
1.6×10^{-3}	22-153	12-14	-	Urban river, HNC	Hoai et al. (2010)
1.3×10^{-3}	0.18	68	-	Urban area, Hue City	Iwata et al. (1994)
9.9×10^{-4}	7.6	47	-	Rural canal, HCMC	Iwata et al. (1994)
8.8×10^{-4}	37	28	-	Ha Long Bay	Viet et al. (2000)
6.5×10^{-4}	0.33-22	0.21-23	-	Sai Gon-Dong Nai River	Minh et al. (2007b)
5.0×10^{-4}	45	5	-	HNC	Viet et al. (2000)
4.5×10^{-4}	8.6	19	-	Paddy field (PL) & mangroves	Iwata et al. (1994)

Highest EEQ	PCBs	DDTs	PAHs	Sampling location, city	Reference
				(M), HCMC	
3.7×10^{-4}	2.2-10.6	6.9-14.3	-	Rural area, HNC	Nhan et al. (1999)
3.2×10^{-4}	4.8	14	-	PL & M, HCMC	Iwata et al. (1994)
2.9×10^{-4}	8.9	11	-	PL & M, HCMC	Iwata et al. (1994)
2.8×10^{-4}	9.7	10	-	PL & M, Hue City	Iwata et al. (1994)
2.2×10^{-4}	2.28-24.7		-	Tam Giang&Cau Hai Lagoon, Hue City	Frignani et al. (2007)
2.0×10^{-4}	5.1	8.0	-	PL & M, HCMC	Iwata et al. (1994)
1.9×10^{-4}	3.7	8.0	-	PL & M, Hue City	Iwata et al. (1994)
1.7×10^{-4}	1-3.3	3-7.3	-	Along the coast of north Vietnam	Nhan et al. (1999)
1.5×10^{-4}	-	7.8	-	PL, HCMC	Iwata et al. (1994)
1.3×10^{-4}	0.49-2.4	0.15-5.4	-	Sai Gon-Dong Nai River, estuary	Minh et al. (2007b)
1.2×10^{-4}	2.3	5.2	-	Viet Tri City	Viet et al. (2000)
1.2×10^{-4}	0.12-3.7	0.04-4.3	-	Mekong River	Iwata et al. (2004)
8.4×10^{-5}	2.1	3.3	-	PL & M, Hue City	Iwata et al. (1994)
6.2×10^{-5}	2.3	2.1	-	PL & M, HCMC	Iwata et al.

Highest EEQ	PCBs	DDTs	PAHs	Sampling location, city	Reference
					(1994)
3.9×10^{-5}	2.2	1	-	PL & M, Hue City	Iwata et al. (1994)
3.0×10^{-5}	0.04-0.18	0.31-1.46	-	Ba Lat Estuary	Hong et al. (2008)
1.6×10^{-5}	0.65	0.52	-	PL, Hue City	Iwata et al. (1994)
9.2×10^{-6}	0.22	0.37	-	Paddy field, rural area, HCMC	Iwata et al. (1994)
Concentration in surface water (pg/L)					
6.38	-	ND-324000	-	Urban river, HNC	Hung and Thiemann (2002)
3.72	-	ND-189000	-	Urban river, HNC	Hung and Thiemann (2002)
2.6	-	816-132000	-	Irrigation canals, HNC	Hung and Thiemann (2002)
0.56	8000	25000	-	Urban canal, HCMC	Iwata et al. (1994)
0.12	2700	4700	-	PL & M, HCMC	Iwata et al. (1994)
7.0×10^{-2}	-	210-3580	-	Lakes, HNC	Hung and Thiemann (2002)
3.6×10^{-2}	1600	1100	-	Suburban river, Hue City	Iwata et al. (1994)
2.9×10^{-2}	1900	600	-	PL, HCMC	Iwata et al. (1994)

Highest EEQ	PCBs	DDTs	PAHs	Sampling location, city	Reference
2.1×10^{-2}	840	680	-	Urban river, HNC	Iwata et al. (1994)
1.6×10^{-2}	1200	290	-	PL, Hue City	Iwata et al. (1994)
1.6×10^{-2}	570	550	-	PL & M, HCMC	Iwata et al. (1994)
4.5×10^{-3}	-	ND-230	-	Wells, HNC	Hung and Thiemann (2002)

- , no data.

ND, not detectable

Apart from the contribution from estrogens, EEQs originating from PCBs, DDTs and PAHs in some locations in Vietnam are comparable to or even higher than those reported in other regions of the world, e.g. ND to 101 ng EEQ/g in China, 1 to 90 ng EEQ/g in USA, 0.0213 to 0.0299 ng EEQ/g in UK, 2.07 to 12.2 ng EEQ/g in Japan (Zhao et al., 2010) and 4 to 165 ng EEQ/g in Italy (Pojana et al., 2007). So far, there is no information on the exact threshold of adverse effects of estrogenic activity in sediment for aquatic animals. However, Duft et al. (2003) investigated the effects of three individual anthropogenic estrogenic chemicals in sediments (BPA, OP and NP) on the freshwater mud snail (*Potamopyrgus antipodarum*) and found that the Lowest Observed Effect Concentration (LOEC) after eight weeks of exposure was 1 ng/g for BPA and OP, and 10 ng/g for NP. Using the highest values of estrogenic potency of BPA (2.5×10^{-3}), OP (1.3×10^{-3}) and NP (1.1×10^{-3}) listed in Table 2.1, those LOECs are equivalent to 2.98×10^{-3} ng EEQ/g for BPA, 1.36×10^{-3} ng EEQ/g for OP, and 1.7×10^{-2} ng EEQ/g for NP. Considering 1.36×10^{-3} ng EEQ/g as a reference value, the lowest of the three estimated LOECs in terms of ng EEQ/g was exceeded in some sampling locations. This suggests that the estrogenic activity originating from anthropogenic chemicals at these sampling locations posed a potential risk to local aquatic fauna (Figure 2.2).

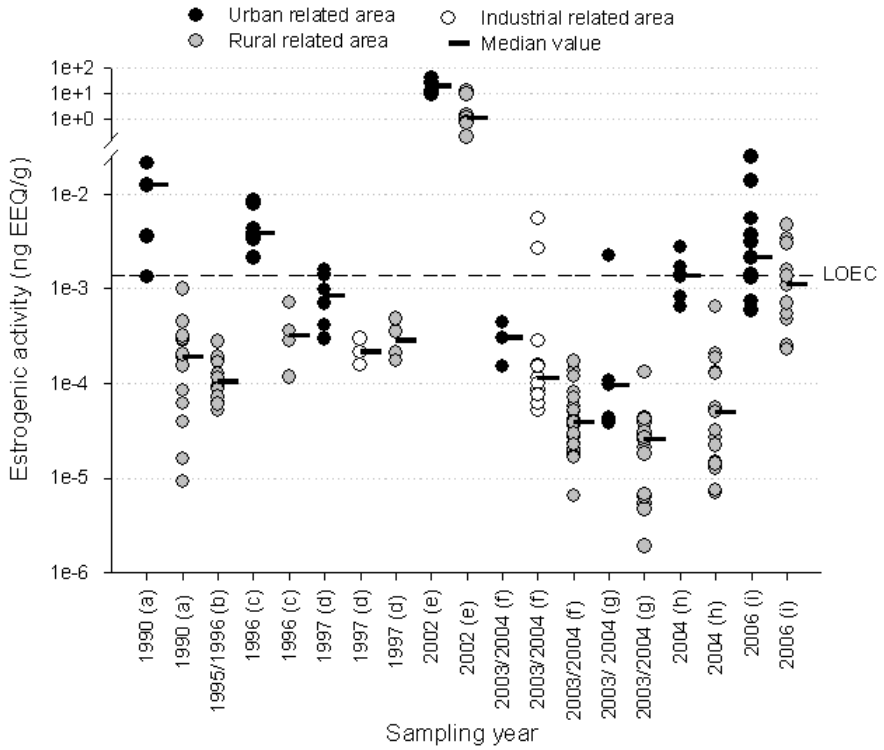

Figure 2.2: Estrogenic activity originating from PCBs, DDTs and PAHs in sediment samples from urban (●), industrial (○) and rural or suburban (◌) areas in Vietnam, (—) median value of each sampling category from different studies: (a) Iwata et al. (1994); (b) Nhan et al. (1999); (c) Phuong et al. (1998); (d) Nhan et al. (2001); (e) Kishida et al. (2007); (f) Hong et al. (2008); (g) Minh et al. (2007a); (h) Minh et al. (2007b); (i) Hoai et al. (2010). The LOEC in terms of estrogenic equivalent for sediment (1.36×10^{-3} ng EEQ/g) was estimated by converting the LOEC of OP (1 ng/g sediment) for the freshwater mud snail after eight weeks of exposure into EEQ ng/g using the estrogenic potency value of 1.3×10^{-3} for OP.

For surface water, there is relatively little information. To the author's knowledge, there are only two studies, focusing on PCBs and DDTs (Table 2.2). Iwata et al. (1994) detected PCBs and DDTs in different water bodies in Vietnam, at concentrations of 0.57 to 8.0 ng/L and 0.29 to 25 ng/L, respectively. In addition, in a survey conducted in 1998/1999, Hung and Thiemann (2002) reported that the mean concentration of DDTs in urban rivers (Red and Duong Rivers) in HNC was 43.7 ± 79.9 ng/L in dry season, and 56.1 ± 65.6 ng/L in rainy season. These studies indicate widespread water contamination by these compounds at considerable concentrations.

Other anthropogenic chemicals (e.g. alkylphenols, phthalates and BPA) have been detected at different locations in the Red River Delta and the Huong River (Viet, 2002). In the Red River Delta, concentrations of alkylphenols (6.7-73 ng/L) were higher than in its estuary (Viet, 2002), suggesting that the phenolic compounds originated upstream and were diluted downstream in the river. The concentrations of BPA in the Red River and its estuary (0.5-1330 ng/L) are comparable to those reported for some developed countries such as Japan, the United States and some western European countries. In addition, the concentration of BPA in some water samples exceeded the ecotoxicological effect value of 1000 ng/L (Predicted No Effect Concentration (PNEC) in water; Viet, 2002). Likewise, water from the Huong River estuary contained elevated concentration of phthalates (DEHP), and in some locations the residues were beyond the environmental risk limit value of 190 ng/L (Viet, 2004).

Concerning estrogens, to the author's knowledge there is only one report analysing different estrogenic chemicals in surface water influenced by municipal discharges, in the Mekong Delta, Vietnam. E1, E2 and EE2 were detected at the highest concentrations of 69.9 ng/L, 11.6 ng/L and 31.7 ng/L, respectively (Duong et al., 2010). According to that study, the total estrogenic activity was 36.9 EEQ/L in 2007 and 46.9 EEQ/L in 2008. The estrogenic concentration obtained for Vietnam was highest for all the countries sampled, which included China, Thailand, Cambodia, Laos, Indonesia, Malaysia. These in turn had higher levels than those reported for European countries, America and Japan (Duong et al., 2010). In another study conducted in the Mekong Delta and focusing on fish, Yamaguchi et al. (2004) reported that the Gonadosomatic Index (GSI) of fish was lower in fish from the urban area of CTC than in fish from rural water bodies, and that fish from the urban area had higher E1 levels than their rural counterparts. This may indicate the occurrence of compounds that disrupt reproductive activities in fish around urban areas in CTC.

To compare estrogenic activity values originating from different e-EDs, despite the limited information available, the author estimated the estrogenic activity of different compounds by using their highest concentrations ever reported in Vietnam (Table 2.3). The results showed that the estrogenic activity of estrogens in surface water was much higher than that of anthropogenic chemicals, and was about one order of

magnitude higher than the PNEC of endocrine disruption (1 ng EEQ/L) (Sumpter and Johnson, 2008). Interestingly, although the estrogenic concentrations of BPA (3.97 ng EEQ/L) and NP (3.15 ng EEQ/L) were about one order of magnitude lower than the estrogens, these concentrations also exceeded the PNEC of 1 ng EEQ/L value, suggesting estrogenic risks of these chemicals for local aquatic animals too. The in vivo studies indicated that at concentrations exceeding 1000 ng NP/L, the impact could be at the same level or even higher than that of E1, E2 (Johnson and Sumpter, 2001). Among the anthropogenic chemicals, OP, DEHP, DDTs and PCBs have weaker estrogenic activity and probably play a minor role in the total estrogenic activity in surface waters (Table 2.3).

Table 2.3: Estrogenic activity and highest concentration of various estrogenic compounds reported in Vietnam

Reported estrogenic compounds	Estimated EEQ	highest conc. reported in Vietnam	Highest EEF from Table 2.1	Reference
In surface water (ng/L)				
EE2	51.93	31.7	1.62	Duong et al. (2010)[a]; Table 2.1 Rutishauser et al. (2004)[b]; Table 2.1
E1	26.76	69.9	0.38	Duong et al. (2010)[a] Rutishauser et al. (2004)[b]; Table 2.1
E2	11.60	11.6	1.0	Duong et al. (2010)[a]
BPA	3.97	1330	2.5×10^{-3}	Viet et al. (2004)[a] Rutishauser et al. (2004)[b]; Table 2.1
NP	3.15	2319	1.1×10^{-3}	Duong et al. (2010)[a] Rutishauser et al. (2004)[b];

				Table 2.1
OP	8.4×10^{-3}	4.9	1.3×10^{-3}	Duong et al. (2010)[a]
				Rutishauser et al. (2004)[b]; Table 2.1
DEHP	2.6×10^{-3}	190	2.0×10^{-5}	Viet et al. (2004)[a]
				Harris and Sumpter (2002)[b]; Table 2.1
Total DDTs	4.9×10^{-4}	25	2.3×10^{-5}	Iwata et al. (1994)[a]
				Legler et al. (2002a)[b]
Total PCBs	7.1×10^{-5}	8	1.0×10^{-4}	Iwata et al. (1994)[a]
				Blair et al. (2000)[b]; Table 2.1
In sediment (ng/g dry weight)				
Total PAHs	38.18	6400	5.0×10^{-3}	Kishida et al. (2007)[a]; Table 2.1
				Villeneuve et al. (2002)[b]
Total DDTs	2.2×10^{-2}	1100	2.3×10^{-5}	Hoai et al. (2010)[a]; Table 2.1
				Legler et al. (2002a)[b]
Total PCBs	5.6×10^{-3}	630	1.0×10^{-4}	Iwata et al. (1994)[a]; Table 2.1
				Blair et al. (2000)[b]; Table 2.1

[a] Reference for the selected concentrations

[b] Reference for the selected EEF of respective estrogenic chemicals

In sediment, information is available for three estrogenic groups. The estrogenic activity of PAHs is remarkably high, about four and five orders of magnitude higher than that of DDTs and PCBs, respectively (Table 2.3). PAHs have been reported to contribute a considerable proportion of the total estrogenic activity in other countries too, e.g. in Tokyo Bay sediment (Kannan et al., 2000) and in European lake sediments (Reyero and Pina, 2005), suggesting that their estrogenic activity in sediment should be considered. Furthermore, sediment has also been reported to be a potential or major sink of estrogens in other countries (Williams et al., 1999; Peck et al., 2004). Data for Vietnam are still lacking.

2.5 Conclusions

There are different estrogenic chemical groups with an estrogenic activity, and their estrogenic potency varies widely. The analytical method used contributes to this variability and for some estrogenic chemical groups, regardless of the target compound; there is a statistically significant difference between methods. In addition, estrogens are generally more estrogenically potent than anthropogenic estrogenic chemicals, regardless of the analytical method used. In Vietnam, although data on estrogens are lacking, there are some data available on anthropogenic estrogenic chemicals. These chemicals ubiquitously occur in water bodies at considerable concentrations, especially in urban areas. In general, the anthropogenic chemicals exhibit relatively low estrogenic activity due to their weak potency. However, in some cases, their estrogenic activity can rise to considerable levels due to their high concentrations in the environment, which may pose an estrogenic risk to local aquatic fauna. The higher concentrations of these estrogenic compounds recorded in urban areas suggest that improperly treated discharges in these areas are the main source. In addition, the high concentrations of estrogens detected in surface water influenced by municipal discharges suggest that human and animal wastes are not sufficiently treated or are not treated at all before being discharged into the environment. Considering the potential risk posed by e-EDs to local animals and humans, estrogenic activity in the environment in Vietnam certainly merits further studies to identify potential sources and to assess estrogenic risk. In any case, municipal discharges, especially human and animal wastes, need to be sufficiently treated in order to reduce estrogenic activity in water bodies in Vietnam.

3. Estrogenic activity in the surface waters of Can Tho City

Abstract

Estrogen contamination in water bodies is a concern due to its potential disruption of the endocrine system of aquatic animals, even at very low concentrations. The Yeast Estrogen Screen (YES) assay was used to monitor estrogenic activity in different surface water categories in Can Tho City (CTC). In general, the water samples either showed estrogenic activity in terms of E2 equivalent (EEQ) or had cytotoxic effects on the yeast cells and thus did not generate estrogenic activity. Although electrical conductivity and/or dissolved oxygen seemed to influence the cytotoxic effect, its underlying causes are still unknown. Estrogenic activity in water samples from urban CTC (range 0.03-33.99 ng EEQ/L, median 0.72 ng EEQ/L) and its industrial areas (range 0.08-11.8 ng EEQ/L, median 1.08 ng EEQ/L) was significantly higher than in samples from suburban areas (range ND-2.99 ng EEQ/L, median 0.256 ng EEQ/L), upstream areas (range 0.015-1.09 ng EEQ/L, median 0.333 ng EEQ/L) and downstream areas (range 0.04-1.57 ng EEQ/L, median 0.485 ng EEQ/L). The Predicted No Effect Concentration (PNEC) value of 1 ng E2/L was exceeded in 56%, 37.5% and 9% of the samples where estrogenic activity was detected for the industrial, urban and suburban areas, respectively, suggesting an estrogenic risk to the local aquatic fauna. The downstream concentrations were higher than upstream in 80% of cases, suggesting introduction of estrogen by CTC into the Hau River (Bassac River) in quantities roughly estimated to be in between 44 to 88 g EEQ/day.

3.1 Introduction

Endocrine Disrupting Chemicals (EDCs) are receiving increasing attention due to their potential negative effects on human and animal health (Sumpter and Johnson, 2005; Leusch et al., 2006). The effects are reported to be include fertility reduction, feminisation in animals (Sumpter, 2005; Campbell et al., 2006), smaller reproductive

organs, and increasing incidence of breast and testicular cancers in humans (Colborn et al., 1993; Aksglaede et al., 2006).

Among a variety of EDCs, the natural estrogens (17β-estradiol (E2), estrone (E1)), and synthetic estrogen (17α-ethynylestradiol (EE2)) are the major contributors to estrogen activity in the environment (Snyder et al., 2001; Furuichi et al., 2004). Other industrial chemicals such as nonylphenol (NP), octylphenol (OP), bisphenol A (BPA), phthalates, polychlorinated biphenyls (PCBs) and certain pesticides have also been reported to exhibit estrogenic activity (Soto et al., 1991; Nishihara et al., 2000; Hurst and Sheahan, 2003; Kojima et al., 2004). However, their contribution to the total estrogen activity is generally low compared with that of natural and synthetic estrogens (Körner et al., 2000; Beck et al., 2006; Ying et al., 2009; Duong et al., 2010).

It has been demonstrated that extremely low concentrations of estrogens such as 1-10 ng E2/L, or as low as 0.1 ng/L in the case of EE2, can cause negative response in fish (Purdom et al., 1994; Young et al., 2002; Johnson and Williams, 2004). Different estrogen guidelines have been proposed to protect aquatic animals (Purdom et al., 1994; Kramer et al., 1998; Metcalfe et al., 2001; Seki et al., 2005; Beck et al., 2006; Caldwell et al., 2008). A tentative long-term Predicted No Effect Concentration (PNEC) for freshwater life of 1 ng E2/L proposed by Young et al. (2002) is regularly used to evaluate the estrogen risk in surface water.

Exposure to a mixture of estrogenic chemicals can cause additive combined effects (Rajapakse et al., 2001; Silva et al., 2002; Jobling et al., 2002b; Sumpter and Johnson, 2005; Filby et al., 2007), even when each chemical is present at a concentration below that which causes an effect (Sumpter and Johnson, 2005). Due to the coexistence of estrogenic chemicals in the environment, total estrogenic activity is often reported in terms of E2 equivalent or estrogenic activity (EEQ). Among various in vitro assays developed to analyse estrogenic activity, the Yeast Estrogen Screen (YES) assay has received particular attention and has been used in many studies as it is a robust, rapid, sensitive and comparatively simple assay in laboratory and field experiments (Beck et al., 2006; Ma et al., 2007).

The occurrence and concentration of EDCs and/or estrogenic activity have been monitored in many developed countries in America, Europe and Asia (Duong et al., 2010), but information is still lacking for developing countries.

The objectives of this chapter were to monitor the occurrence and concentration of estrogenic activities in the surface water samples from urban, industrial and suburban areas of CTC, and to roughly estimate the estrogen discharge from this city (EEQ_{CTC}) into its main water body, Hau River (Bassac River).

3.2 Materials and Methods

3.2.1 Description of the study area and sampling categories

CTC was selected for the study as this city is the most urbanised and industrialised city in the Mekong Delta in Vietnam. The administrative structure of CTC is equivalent to that of a province, and it has urban and rural districts. The city has a total population of 1.2 million and covers an area of around 1400 km^2 (Can Tho DONRE, 2009). It is the largest city in the region, located in the centre of the Mekong Delta in Vietnam, on the south bank of the Hau River (the larger branch of the Mekong River; Figure 3.1). The river water is used as a main resource for agriculture, aquaculture, livestock farming, industry and drinking water. The city is characterised by a dense river and canal system used for irrigation and transportation. As it is located about 100 km from the coast, the surface water in the study areas is influenced daily by the tide. The region is annually affected by flood (inundation period), which usually starts in July and ends in December, while the non-inundation period runs from January to June. The rainy season begins in April and ends in October, while dry season runs from November until March of the following year.

At the time this study was conducted, there was no municipal wastewater treatment plant in CTC. However, there is an unknown number of septic tanks that are not well maintained and their efficiency is not monitored (Yen-Phi et al., 2010).

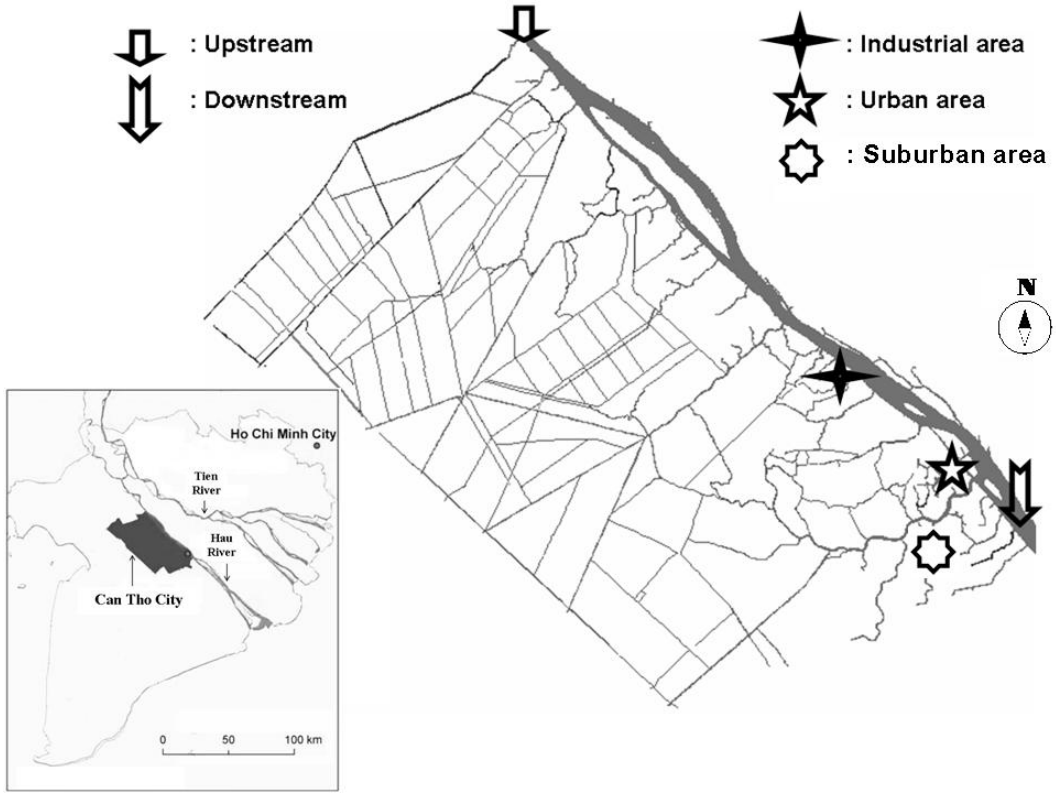

Figure 3.1: Location of CTC in the Mekong Delta (small map) and sampling categories in CTC; grey lines indicate canals and rivers

3.2.2 Urban sampling area

The population of the centre of CTC, where the urban samples were taken, is about 300,000 (Can Tho GSO, 2009). Sampling was repeated every three to four weeks from October 2008 to November 2009. During each sampling event, one sample was taken from each of seven sampling stations in the urban area. However, due to optimisation of the monitoring programme at the beginning of the monitoring period, samples from one or two stations were not taken during some sampling events. During the sampling period, a total of 19 sampling events were conducted, in which 123 samples were taken from different water bodies influenced by domestic wastewater discharge.

3.2.3 Industrial and suburban sampling areas

Sampling in the industrial and suburban areas was performed every three to four weeks from October 2008 to November 2009. Samples were usually taken during the same sampling events, although they were not taken on the same day due to practical reasons.

For the industrial area, water samples were taken at five different sampling stations located in the wastewater drainage trenches of Tra Noc industrial zone, the largest and most industrialised zone in the whole Mekong Delta in Vietnam. Untreated and partially treated wastewater are mixed together in these trenches, and finally discharged into neighbouring water bodies. In total, 15 sampling events were conducted here, in which 71 samples were taken for analysis.

For the suburban area, water samples were taken in the irrigation canals and rivers in Ba Lang Commune to the south of CTC, an area characterised by a mix of paddy rice, vegetables and fruit trees. In total, 16 sampling events were conducted, in which 189 samples were taken from 12 different sampling stations.

3.2.4 Upstream and downstream sampling stations

In the Hau River, which has a width of approx. 1000 to 1500 m in CTC, sampling stations considered to be upstream and downstream of CTC were selected, with the distance between these two sampling stations being about 55 km (Figure 3.1). Samples were taken on the south bank of the Hau River during low tide, and one sample from each upstream and downstream station was taken every three to four weeks from December 2008 to November 2009. In total, 28 samples were taken from these two stations. The upstream station was considered a reference for the sampling stations within CTC (Figure 3.1).

3.2.5 Sample collection and analysis

All water samples were collected as grab samples using 1-litre, pre-cleaned glass bottles with Teflon caps. Samples were taken during the day between 6 am and 8 pm. The samples were then kept on ice, protected from the light, and transported to the laboratory within a few hours, where they were stored at 4^{o}C. Extraction was normally

performed within a week. Estrogenic activity was expressed in terms of EEQ. In addition to analysis of estrogenic activity, water samples were analysed for various water quality parameters in situ, including DO (Dissolved Oxygen), EC (Electrical Conductivity), pH and temperature (T^o).

It was assumed that wastewater would be discharged from the urban areas at a higher rate in certain periods during the day when domestic activities are higher, i.e. from 6 to 8 am; 11 am to 1 pm; and 5 to 7 pm. Samples from the urban category were always taken in these periods, and can thus be considered 'worst case' samples. The analytical procedure was adopted from Routledge and Sumpter (1996), and is thoroughly described in Hong's dissertation (in preparation).

3.2.6 Data analysis

Data sets were analysed and plotted using Excel® (Microsoft Corporation) software packages, SPSS (SPSS Inc.), and Sigma Plot (Systat Software, Inc.). Normality of distribution for each sampling category was checked by the Kolmogorov-Smirnov test. The difference between two sampling categories or two different types of samples was evaluated by the Mann-Whitney U test.

3.3 Results and Discussion

3.3.1 Estrogenic activity in urban sampling area

Estrogenic activity was detected in 65% of urban samples, ranging from 0.03 to 33.99 ng EEQ/L (median 0.72 ng EEQ/L; Figure 3.2; Appendix 1). It was significantly higher than in samples from the suburban area ($p = 0.000$), upstream ($p = 0.007$) and downstream ($p = 0.032$), but not the industrial area ($p = 0.213$). Of the samples that tested positive, 37.5% had a higher concentration than the PNEC of 1 ng/L. All the other samples had cytotoxic effects on the yeast cells. Of the measured water quality parameters, estrogenic activity was only positively and significantly correlated with EC ($p = 0.002$; Figure 3.3). However, this correlation was observed only in the urban area and was weak ($R^2 = 0.1344$).

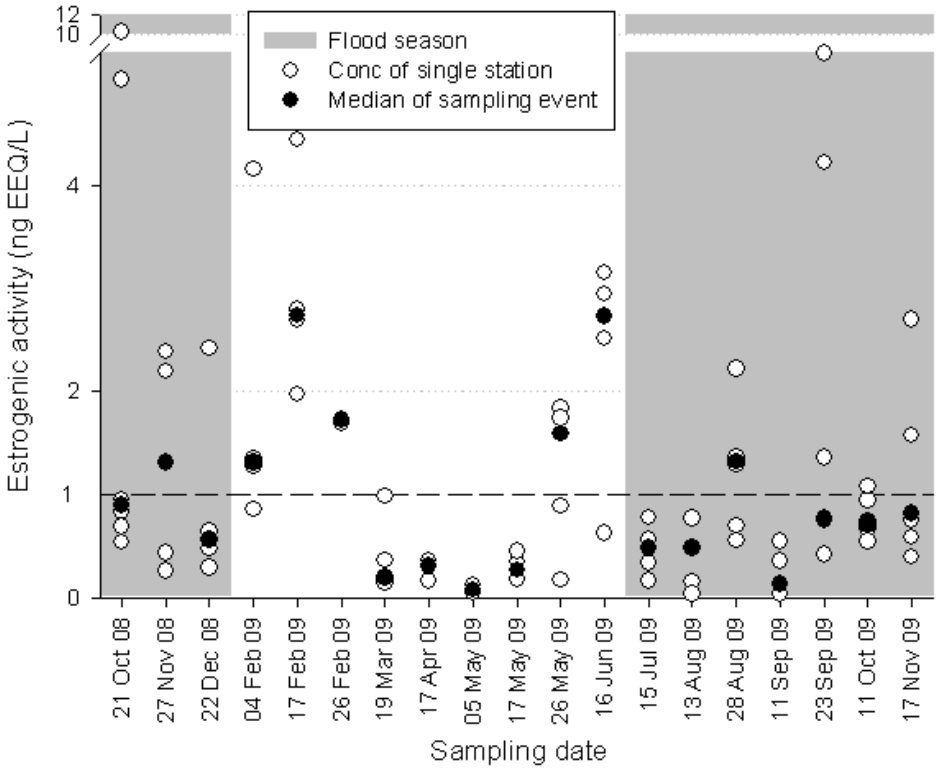

Figure 3.2: Estrogenic activity in urban CTC: Single sampling station (○), median values for each sampling event (●), PNEC = 1 ng E2/L (— — —), one outlier (33.99 ng EEQ/L) measured on 28 Aug 2009 was excluded from the diagram

The variation in each sampling event was most likely because the samples were taken from different sampling stations. During the flood season (inundation period), the variability tended to be higher than in the non-inundation period. It was noted that during the flood season, water sometimes fully penetrated into the drainage system of CTC, which is directly connected to the effluents of septic tanks. As a consequence, water may have penetrated into the septic tanks, where estrogenic chemicals are probably trapped, and flushed them out to the canals and rivers, explaining the higher variability in estrogenic activity during the flood season.

While the median concentration was elevated just before and after the flood season, it was at its lowest between March and May, the driest season of the year (Figure 3.2). Anti-estrogenic compounds are suspected to be the reason for the lower estrogenic activity in the YES assay compared with estimated values (Beck et al., 2006; Sun et al., 2008; Buckley, 2010). The lower concentration observed during March to May in

the present study could be related to the presence of sufficiently high concentrations of anti-estrogenic compounds in the samples to inhibit estrogenic activity.

The concentrations observed in this study are comparable to those reported in other studies of estrogenic activity in surface waters, e.g. 3.2-5.4 ng EEQ/L in South Korea (Duong et al., 2010), average <1 ng EEQ/L in Denmark (Stuer-Lauridsen et al., 2005), 0.1-4.7 ng EEQ/L in China (Ma et al., 2007), 0.7-4.0 ng EEQ/L in Japan (Hashimoto et al., 2005), and in effluents from wastewater treatment plants, e.g. 3 ng EEQ/L in the Netherlands (Murk et al., 2002), <0.8 ng EEQ/L in Germany (Coors et al., 2004), <1 ng EEQ/L in Australia and New Zealand (Leusch et al., 2006), 0.05-0.5 ng EEQ/L in China (Ma et al., 2007) and ND-3.9 EEQ in South Korea (Duong et al., 2010).

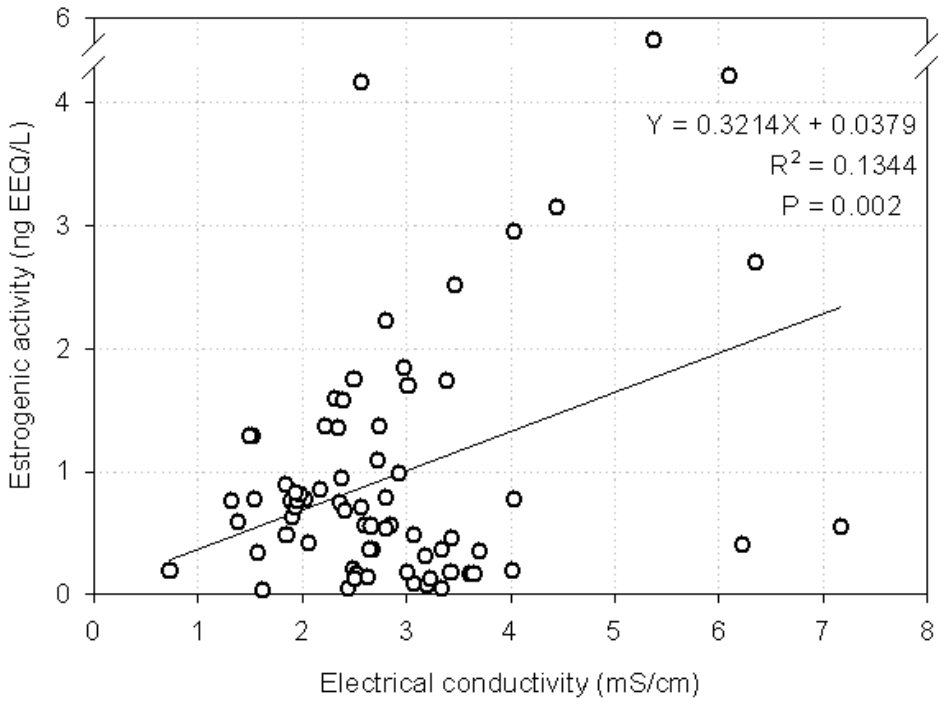

Figure 3.3: Estrogenic activity as a function of electrical conductivity in water samples from urban CTC

Natural steroidal estrogens in the environment are mainly excreted through human and animal faeces and urine. It has been reported that EE2, E2 and E1 account for more than 90% of the total EEQ in wastewater treatment plant effluent and in surface water (Körner et al., 2001; Snyder et al., 2001; Holbrook et al., 2002; Furuichi et al.,

2004; Duong et al., 2010). In a single component analysis of EDC using gas chromatography/mass spectrometry, EE2 and E2 were found to be the main contributors to total EEQ in water samples taken near discharge points of crowded municipal areas in the Mekong Delta, Vietnam (Duong et al., 2010). Those authors reported concentrations of 36.9 ng EEQ/L and 46.9 ng EEQ/L in 2007 and 2008 (one sampling event in each year), respectively, about one order of magnitude higher than the result in this study (median in each sampling event ranged from 0.07 ng EEQ/L to 2.74 ng EEQ/L; Figure 3.2).

The elevated estrogenic activity in urban CTC observed in this study is in line with findings reported by Yamaguchi et al. (2004). They conducted a study in the Mekong Delta in Vietnam and found that fish from the urban areas of CTC had higher E1 levels than fish in the surrounding rural areas, a difference they attributed to the occurrence of compounds that disrupt reproductive activity in fish around CTC. Therefore, the results obtained in this study indicate that estrogenic activity in water may pose an estrogen risk to aquatic fauna inhabiting in urban CTC. However, it must be emphasised that the samples measured in CTC can be considered ‘worst-case’ samples.

3.3.2 Estrogenic activity in industrial and suburban areas

Estrogenic activity values in industrial and suburban areas originating from the same sampling events within months were combined and are presented in Figure 3.4. Estrogenic activity was detected in 58% of industrial samples and 62% of suburban samples. All the remaining industrial samples exhibited cytotoxic effects, while of the suburban samples, 28% were cytotoxic and 10% were under the detection limit (ND = 0.015 ng EEQ/L) (Appendix 2 and 3). The concentration in the industrial samples ranged from 0.08 to 11.8 ng EEQ/L (median 1.08 ng EEQ/L) and was significantly higher ($p = 0.000$) than in the suburban area (range ND-2.99 ng EEQ/L, median 0.256 ng EEQ/L). The estrogenic activity in the industrial samples was also significantly higher than at upstream ($p = 0.003$) and downstream stations ($p = 0.008$), while that in the suburban samples was not ($p = 0.569$ and 0.229, respectively).

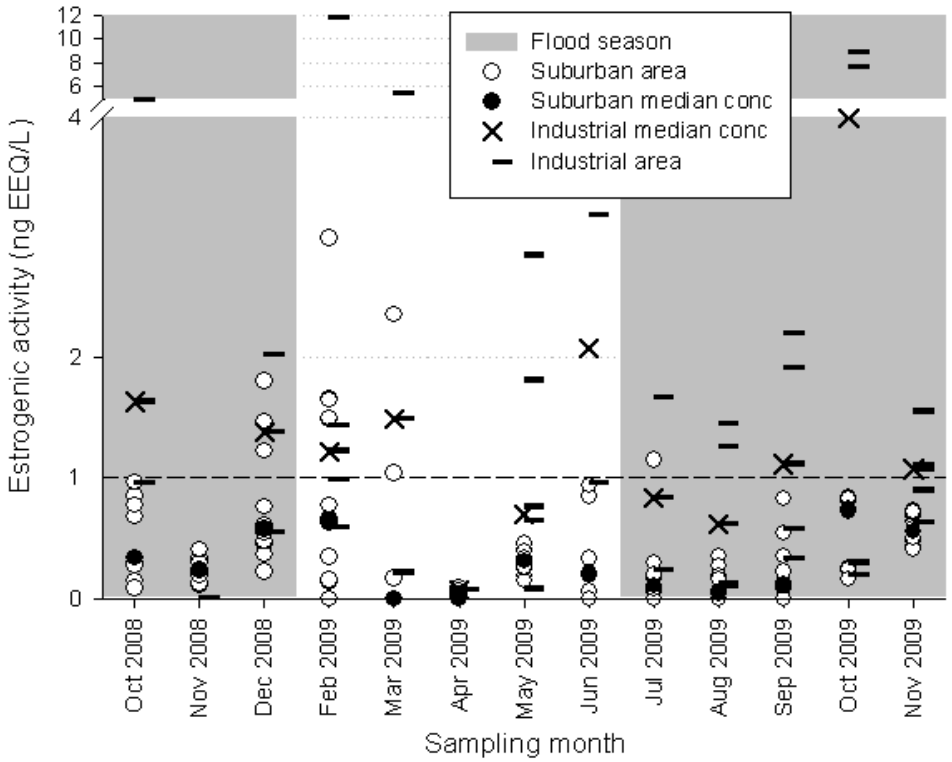

Figure 3.4: Estrogenic activity in the industrial area (—); median value for each industrial sampling event (×); in the suburban area (○); median value for each suburban sampling event (●); PNEC =1 ng/L (— — —)

For the industrial samples, 56% of the samples that tested positive exceeded the PNEC value of 1 ng/L, while the corresponding value for the suburban samples was 9% (Figure 3.4). During the sampling period, it was observed that the samples taken from the industrial area were less influenced by the floods than those from the suburban area, due to the higher topography of the wastewater drainage trenches compared with the canals. The estrogenic activity in the suburban area was at its lowest in April, again suggesting that interference by anti-estrogen compounds during this period could be the reason.

The estrogenic activity in the industrial area of CTC was comparable to that reported in China, which was found to range from 0.1 to 13.3 ng EEQ/L but decreased to 0.03-1.6 ng EEQ/L after treatment (Ma et al., 2007). Industrial wastewater has been identified as a source of different EDCs, such as BPA, PCBs, dioxins, pesticides and other anthropogenic estrogenic compounds (Ferguson et al., 2001; Voutsa et al.,

2006). Sun et al. (2008) reported for China that the compounds most frequently identified in wastewater samples with a high ratio of industrial wastewater were long-chain n-alkanes, carboxylic acid, phthalates esters, BPA, NP and OP. Natural and synthetic estrogens such as E2, E1 and EE2 were not detected in the same samples (method detection limit <1 ng/L).

3.3.3 Estrogenic activity contribution from Can Tho City to Hau River

The concentration at the upstream station ranged from 0.015 to 1.09 ng EEQ/L (median 0.33 ng EEQ/L) while at the downstream station it was 0.04-1.57 ng EEQ/L (median 0.49 ng EEQ/L; Figure 3.5; Appendix 4). Although there was no statistical significant difference ($p = 0.616$) between these two stations, the estrogenic activity downstream was higher than that at the upstream station in 80% of cases (Figure 3.5). The median concentrations at the upstream and downstream stations were used to estimate estrogenic discharge from CTC (EEQ_{CTC}) into the Hau River.

The Hau River is 1000 to 1500 m wide in CTC. It was assumed that the water discharge at the upstream and downstream stations was the same, and represented by the average water discharge (Q = 550,000,000 m^3/day) monitored by CTC hydrological monitoring station (Can Tho DONRE, 2009). This hydrological monitoring station is located about 50 km from the upstream station and 5 km from the downstream station. In addition, it was assumed that the estrogen discharge from CTC was either completely mixed (well mixed) in the water body and was the same to that from the north bank of the river or incompletely mixed (non-well mixed). For the non-well mixed scenario, the estrogen discharge from CTC at the south bank was not influenced by that from the north bank of the river (water discharge represented for CTC side was of course smaller than Q).

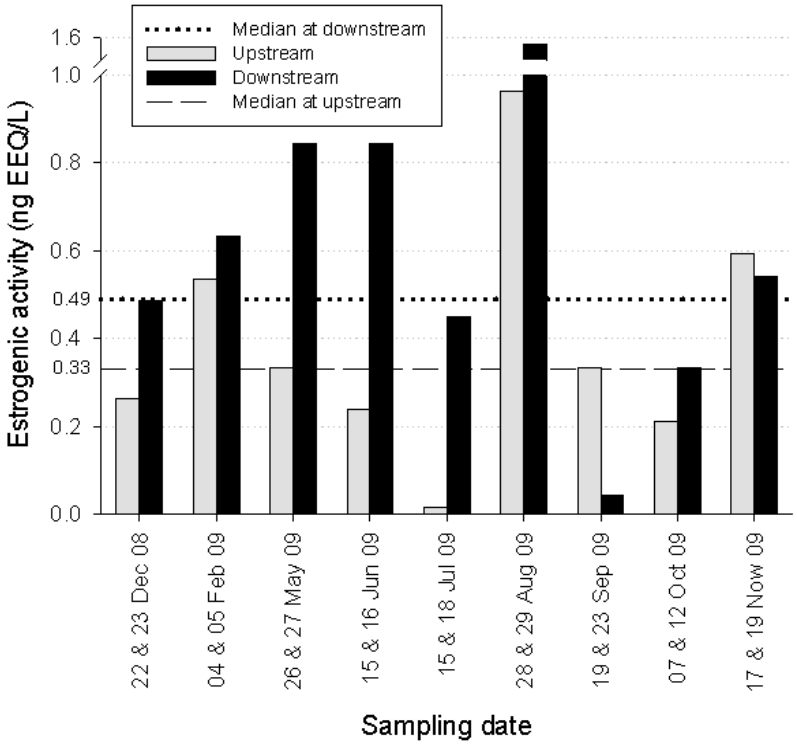

Figure 3.5: Single estrogenic activity value at the upstream station (▭; the day before the sampling event) and the downstream station (▬; the day after the sampling event): median value at upstream station (▪▪▪▪▪▪▪▪); median value at downstream station (— —). Cytotoxic effects occurred at the upstream and downstream stations in some sampling events, and consequently did not generate estrogenic activity.

For the well-mixed scenario, the estrogen discharge from CTC at the south bank was therefore estimated depending on the amount of estrogen discharge from the north bank into the Hau River (Table 3.1). Due to its location between the Tien (Mekong River) and the Hau River (Figure 3.1), the estrogen discharge from the north bank of the Hau River is very likely drained into these two rivers.

Three cases were assumed for the well-mixed scenario: 1. no EEQ discharge from the north bank into the Hau River; 2. discharge of 50% EEQ from the north bank into the Hau River; and 3. discharge of 100% of EEQ from the north bank into the Hau River. The daily estrogen discharge between the upstream and the downstream station for well-mixed scenario was obtained accordingly ((median EEQ/L $_{down}$ –

median EEQ/L_{up}) x Q). The estrogen discharge from CTC was therefore estimated as from 44 to 88 g EEQ/day (Table 3.1).

Table 3.1: Daily estrogen discharge from CTC for different scenarios (g EEQ/day)

Well-mix scenario	Non-well mixed scenario	Estrogen discharge from the north bank into the Hau River (%)
88	< 88	0% discharge = 0 g EEQ/day
66	< 88	50% discharge = 22 g EEQ/day
44	< 88	100% discharge = 44 g EEQ/day

3.3.4 Electrical conductivity, dissolved oxygen and cytotoxic effects

Considering the EC and the DO values the surface waters in the three sampling areas showed relatively low quality (Figure 3.6). The DO values were generally below 4 mg/L, while the EC values varied widely, from one to 80 mS/cm, depending on sampling area (Figure 3.6). Of these, the industrial areas appeared to be the most polluted, followed by the urban and suburban areas. In addition, over-saturation of oxygen was sometimes observed in the urban and the industrial areas (Figure 3.6). The DO values were negatively and significantly correlated with the EC values in the urban and suburban areas ($p = 0.000$), but not in the industrial area ($p = 0.243$). However, from the R^2 values in the graphs, it can be seen that these correlations between the DO and EC were weak (Figure 3.6).

Cytotoxic effects either inhibited yeast cell growth or killed the yeast cells as described in Sohoni and Sumpter (1998). These effects were evaluated by comparison of yeast cell turbidity absorbance at 630 nm between the samples and the solvent blanks (Hong's dissertation in preparation). The cytotoxic effects were observed throughout the monitoring period in all sampling areas, including the upstream and downstream stations, indicating that the chemicals responsible for the cytotoxic effects originated from both CTC itself and upstream provinces (Appendix 1, 2 and 3).

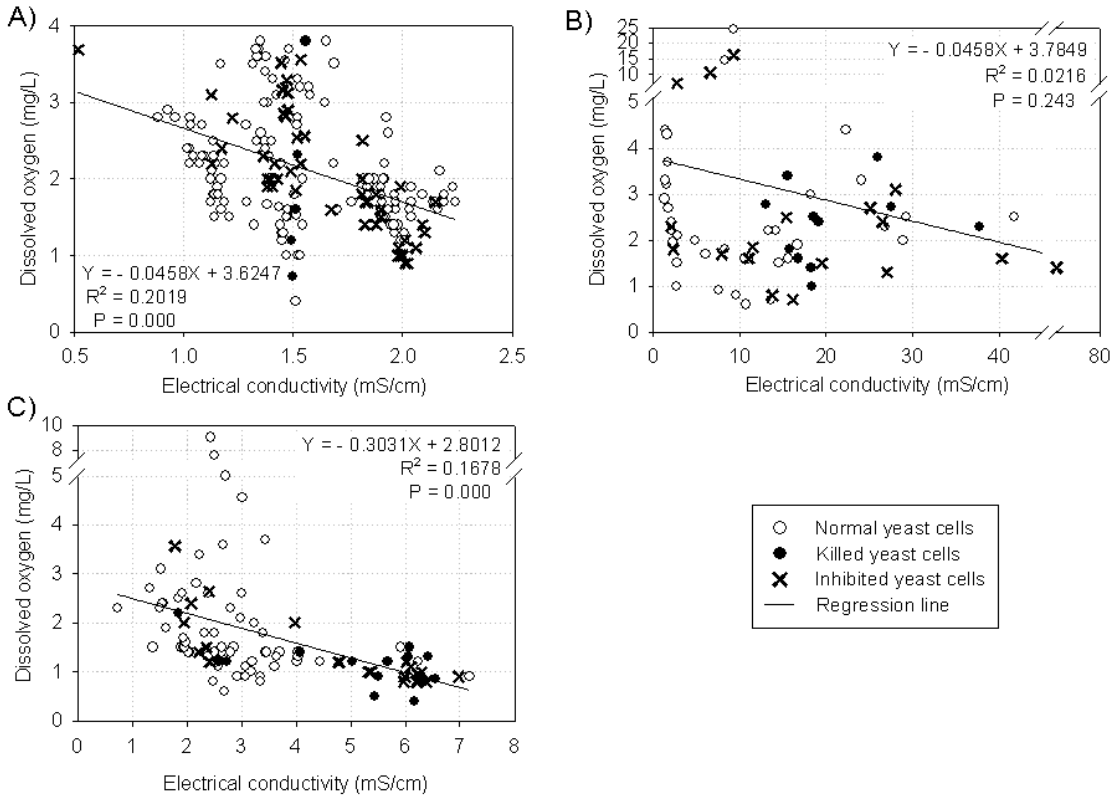

Figure 3.6: Distribution of yeast cell reaction status, EC and DO values for suburban area (A), industrial area (B) and urban area (C): normal yeast cells (○); killed yeast cells (●); inhibited yeast cells (×)

In comparison, the EC and DO values of the normal samples differed from the values in cytotoxic samples in the different sampling areas. In the urban area, the EC (range 1.9-6.9 mS/cm, median 5.5 mS/cm) of the cytotoxic samples was significantly higher ($p = 0.000$) than that of the normal samples (range 0.7-7.2 mS/cm, median 2.6 mS/cm), while DO (range 0.4-3.57 mg/L, median 1.2 mg/L) was significantly lower ($p = 0.000$) than in normal samples (range 0.6-9 mg/L, median 1.5 mg/L). In the industrial area, the EC of the cytotoxic samples (range 2.1-67.6 mS/cm, median 16.9 mS/cm) was significantly higher ($p = 0.002$) than that of the normal samples, but there was no significant difference for the DO ($p = 0.731$) values (range 0.7-16.2 mg/L, median 2.3 mg/L in the cytotoxic samples; range 0.6-24.3 mg/L, median 2.2 mg/L in the normal samples). As in the industrial area, in the suburban area the EC of the cytotoxic samples (range 0.5-2.2 mS/cm, median 1.5 mS/cm) was significantly higher ($p = 0.032$) than in the normal samples (range 0.9-2.2 mS/cm, median 1.5 mS/cm),

whereas there was no significant difference ($p = 0.256$) in DO for the cytotoxic samples (range 0.73-3.8 mg/L, median 1.9 mg/L) and the normal samples (range 0.4-3.8 mg/L, median 2 mg/L). Although the cytotoxic effect was influenced by lower water quality (i.e. either higher EC or lower DO values, or both), the full reason for this effect is unknown.

It is known from other studies that cytotoxic effects on the yeast cells are usually induced by toxic effects of chemicals or heavily polluted water samples (Körner et al., 2000; Hurst and Sheahan, 2003; Tashiro et al., 2004; Nakama et al., 2007). However, the process or substance responsible for the observed cytotoxic effects has not yet been identified. Sewage samples are reported to show some cytotoxic effects (Körner et al., 2000), while Tashiro et al. (2004) reported that water samples from the river passing through the urban area of Osaka strongly inhibited yeast growth in recombinant yeast assays. Although domestic biocides and pesticides have been shown to cause cytotoxic effects on the yeast cells in the YES assay (Hurst and Sheahan, 2003; Nakama et al., 2007), the factors responsible for the cytotoxic effects observed in this study remain to be determined.

3.4 Conclusions

Surface water quality in the city of Can Tho is relatively low. Estrogenic activity was detected in all sampling areas but it was significantly higher in urban and industrial areas, indicating municipal and industrial discharges to be its source. Estrogenic activity seems to be a relevant polluter in the surface water of the Mekong Delta. In the surface water of CTC, the estrogenic activity exceeded the PNEC in many cases, especially in the industrial and urban areas, suggesting an estrogen risk to the local aquatic fauna. An estimated amount to be in between 44 to 88 g EEQ was discharged daily from CTC into the Hau River. The introduction of wastewater treatment plants for both municipal and industrial wastewaters is expected to reduce the estrogenic activity discharged from CTC into the Hau River. Cytotoxic effects were observed in some assays for all sampling areas studied, but especially for urban and industrial areas. Although lower water quality influenced these cytotoxic effects, the underlying reasons are still unknown.

4. Estimation of estrogenic excretion in Can Tho City

Abstract

Endocrine Disruptors (EDs) are pollutants that may cause negative effects on human and animal health at low concentration, and are emerging as a major concern for water quality. Estrogens excreted from humans and animals are considered as the most important source of EDs into the environment. A model was adapted to estimate the total estrogenic activity in terms of 17β-estradiol equivalent (EEQ) excreted by humans and animals from a mid-size city in Vietnam (Can Tho City, CTC) into surface water. The result was about a half of the estimated discharge using analysed data, which was in between 44 to 88 g EEQ/day. Among different estrogens, estrone (E1) and 17β-estradiol (E2) showed to be the major contributors in the total estrogenic activity. Human and animal discharges are the major sources for estrogenic activity, however, there could be other potential sources and their contributions to the total estrogenic activity are not yet identified. A considerable reduction of estrogens into water is expected by the introduction of a wastewater treatment plant. Additionally, if animal excrements would be collected, treated and used as organic fertilizer, the discharge of estrogens in the water body could be decreased.

4.1 Introduction

Endocrine Disruptors (EDs) are a broad class of chemicals including both natural and man-made compounds. They are able to elicit negative effects on the endocrine systems of humans and wildlife. There are different sources of these chemicals to the environment such as municipal and industrial discharges, wastewaters from dairies and aquaculture, livestock feed lots, agricultural runoff (Campbell et al., 2006), leachate from solid waste landfill (Kawagoshi et al., 2003). Among EDs, estrogens such as estrone (E1), 17β-estradiol (E2), estriol (E3) and 17α-ethynylestradiol (EE2), are the major contributors of estrogenic activity in the environment (Hanselman et al., 2003). In addition, these estrogens can induce vitellogeninn synthesis at a very low concentration compared to other chemicals such as nonylphenol (Routledge et al., 1998). They are therefore of particular concern. Estrogens such as E1, E2 and EE2

are the potent estrogenic compounds, while industrial estrogenic chemicals such as nonylphenol, octylphenol, bisphenol A, polychlorinated bisphenyls, organochlorines and pesticides are weaker. Their estrogenic potencies generally range from a few to six orders of magnitude lower than E2 (Soto et al., 1995; Rutishauser et al., 2004). Moreover, estrogens are generally present at low concentrations in the aquatic environment (nanograms per liter), while industrial estrogenic chemicals are present at higher concentrations, ranging from the upper nanogram per liter to tens and even hundreds of micrograms per liter (Sumpter et al., 2006). Therefore, measuring actual concentrations of estrogens in the aquatic environment can be difficult, a time-consuming and expensive work, especially in a highly complex matrix such as wastewater samples (Sumpter et al., 2006; Liu et al., 2009b). It would be helpful for the monitoring of estrogenic activity in environmental samples, e.g. water and sediment samples if the discharge of estrogens can be estimated.

Many estrogenic compounds are not completely removed by existing wastewater treatment systems and they remain with fluctuating concentrations in effluents (Liu et al., 2009a). While the effluent of wastewater treatment system has been reported as a major source of estrogenic activity (Kirk et al., 2002; Campbell et al., 2006), in many developing countries wastewater is not properly treated or even not treated at all before being discharged into the environment. In Vietnam, many water bodies have been polluted with excrements and manure from households and livestock (MONRE, 2005; MONRE, 2006). As a consequence, the discharge of estrogenic chemicals into water bodies may be even higher as compared to developed countries.

The objective of this chapter was to estimate estrogenic activity of human and animal excrements into surface water from a mid-size city in Vietnam (Can Tho City, CTC). The result was compared with an estimate of estrogenic discharge based on monitoring data.

4.2 Materials and Methods

Can Tho City is a mid size city in Vietnam in the Mekong Delta. The region is characterized by a dense river and canal system, where the majority of households are located along these rivers and canals. Human excrements and animal manures

are not commonly used as organic fertilizer in the Mekong Delta (Vu et al., 2007) and they are mainly directly discharged into the water bodies. More information about CTC can be found in the chapter 3.

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It was assumed that estrogenic excretion in CTC is mainly originated by humans and animals as reviewed in the chapter 1. Therefore, a model that has proved to be reasonably accurate (Johnson and Williams, 2004) was adapted to estimate estrogenic discharge in CTC. Additionally, pig and cattle were selected as representative livestock as a source of estrogens. Poultry (about 1.6 millions duck and 0.3 million chicken; GSO, 2009) are also typical in the region, however, their estrogen excretion rates are unknown. They were omitted from the calculation.

In literature, reported (Estrogenic Equivalent Factor or estrogenic potency) EEFs vary depending on how they were analysed. As this study used the Yeast Estrogen Screen (YES) assay to analyse the estrogenic activity, only EEFs reported from the YES assay were used: EEFs of all estimated compounds (E1, EE2 and E3) were reported in the study of Rutishauser et al., (2004). The EEFs of E1, EE2 and E3 were 0.38, 1.19 and 0.0024, respectively (Rutishauser et al., 2004).

EEQt is calculated as:

$$EEQt = EEQ^{h} + EEQ^{a} \quad (1)$$

EEQt: total daily estrogenic equivalent excretion in CTC

EEQ^{h}: total daily estrogenic equivalent excretion from the human population

EEQ^{a}: total daily estrogenic equivalent excretion from the animal population

$$EEQ^{h} = 1/0.74(0.38S^{h}_{E1} + S^{h}_{E2} + 0.0024S^{h}_{E3}) + 1.19S^{h}_{EE2} \quad (2)$$

The factor of 1/0.74 is due to the author's assumption that E1, E2 and E3 contribute 74% into human estrogenic excretion rate (described below).

$$EEQ^{a} = 0.38S^{a}_{E1} + S^{a}_{E2} \quad (3)$$

S: is the total of an estrogen in a specific form (E1, E2, EE2 and E3) estimated by the model developed by Johnson and Sumpter (2004) as Eq. (4) with some adjustments described below.

$$S_T = (1-k_T) \sum f_i(U_i + F_i) + S_s \qquad (4)$$

S_T: total of an estrogen in all forms arriving at the sewage treatment work

k_T: overall fraction of steroid lost in transit through the sewerage network

U_i: amount estrogen excreted in urine in all forms by the i^{th} fraction (f_i) of the population

F_i: amount estrogen excreted in feces by the i^{th} fraction (f_i) of the population

S_s: internal generation of the estrogen from other estrogens

Full details of the theory behind the model is thoroughly described in Johnson et al. (2000) and Johnson and Williams, (2004).

4.2.1 Estimation of human estrogenic excretion

For human population, there are four different population fractions (i = 1 to 4): 1. pregnant females (19,200); 2. menstrual females were assumed to be in between the ages of 15 and 49, which represent 55.7% of the female population (320,000 persons); 3. menopausal females were taken as being above 49 years of age, which represent 19.4% of the female population (118,000 persons); and 4. males (591,600) (estimated from GSO, 2007 and GSO, 2009). The group of females taking Hormone Replacement Therapies (HRT) was omitted because of the unavailable data of this part of the population. Thus, the estrogenic excretion of menopausal females will be underestimated. HRT either contains natural estrogens (E1, E2) or synthetic estrogen (EE2), although products containing EE2 have been largely replaced by products containing natural estrogens (Johnson and Sumpter, 2004). Orally treated HRT menopausal women, the preferential method in the United States, would excrete higher levels of estrogens than untreated HRT menopausal women, e.g. E1 and E2 excretion rates were 28400 ng/day and 56100 ng/day in menopausal women using

HRT compared to 1800 ng/day and 1000 ng/day in untreated ones, respectively (Johnson and Sumpter, 2004).

In this study, it was assumed that there is no estrogen degradation in a wastewater treatment (K_T = 0). A portion of human excrements is preliminary treated by household not well maintained septic tanks (Yen-Phi et al., 2010), which accounted for about 23.6% of the population (CTC-GSO, 2007). The remaining (76.4%) excreta are directly discharged into water bodies nearby since there was no wastewater treatment plant in CTC at the time this study was conducting. The short passage from the house to the canal makes steroid transformation unlikely and in contrast to developed countries, where the sewer transit may take a few hours. Therefore, steroid estrogen transformation in the sewer was considered as zero (S_s = 0).

Hanselman et al. (2003) reported that steroid estrogens are mainly excreted in the urine of humans and mammalians, with a small proportion in the faeces. In addition, E1, E2 and E3 contribute 66 to 82% of the total EEQ excretion rate in human urine (Liu et al., 2009b). Therefore it was assumed that these three compounds contribute 74% to the total human EEQ excretion rate. The remaining 26% derived from the other natural estrogens.

D'Ascenzo et al. (2003) reported that natural estrogens in urine were proven to mainly exist in conjugated forms, which are negligible estrogenic potency and easily changed to their more potent forms (free estrogens) for the case of glucuronide estrogens (Johnson et al., 2006; Liu et al., 2009b). In faeces, most of these compounds exist as free estrogens (Liu et al., 2009b). Therefore, similar to the assumption made by Johnson and Sumpter (2004), all glucuronide conjugates, whether excreted in the urine or in the faeces, were assumed to be in the free forms for the model in this study. Finally, human estrogenic excretion in this study is estimated by Eq (2). In this model, E3 excretion rates were used according to the study of Johnson et al. (2000).

4.2.2 Estimation of animal estrogenic excretion

For animal population, there are two groups of considered animals (i = 2). They are: 1. cattle (population 7,100); 2. pig (population 113,000) (GSO, 2009).

Estrogenic excretion is only estimated for E1 and E2 due to the unavailability of data for other estrogens in literature. Johnson et al. (2006) reported that almost all estrogens in faeces and urine of cattle and pig are in the form of free and conjugated estrogens, respectively. Glucuronide conjugates, which are presumed to be rapidly deconjugated to their free forms, are therefore considered. Sulfate conjugates are excluded from the model because they are more persistent, and may result in an underestimation (Johnson et al., 2006) of the overall estrogen discharge. Since livestock wastes in CTC are mainly directly discharged into water bodies, it is assumed that S_s and K_T are zero.

For E1 and E2 excretion rates of cattle and pig, the author used values reported in Johnson et al. (2006), where urinal and fecal excretion rates of cattle and pig are 838 and 384 µg/cattle/day and 201 and 104 µg/pig/day for E1 and E2, respectively.

4.3 Results and Discussion

An overall amount of 37 g EEQ/day is released into the surface water in CTC. Human excretion contributed to 60%, the remaining 40% derived from animal excretion (Figure 4.1, Appendix 5) although the number of pigs and cattle were relatively small, just about 10% of the human population. Among the different estrogenic contributors, the pregnant group was the smallest in numbers (1.6% of the human population) but showed the highest estrogen release with 44% of the total estrogenic activity (16.28 g EEQ/day). The second biggest contributor is pig livestock, followed by cattle. The other groups contributed considerably less, especially the group of menopausal women (Figure 4.1).

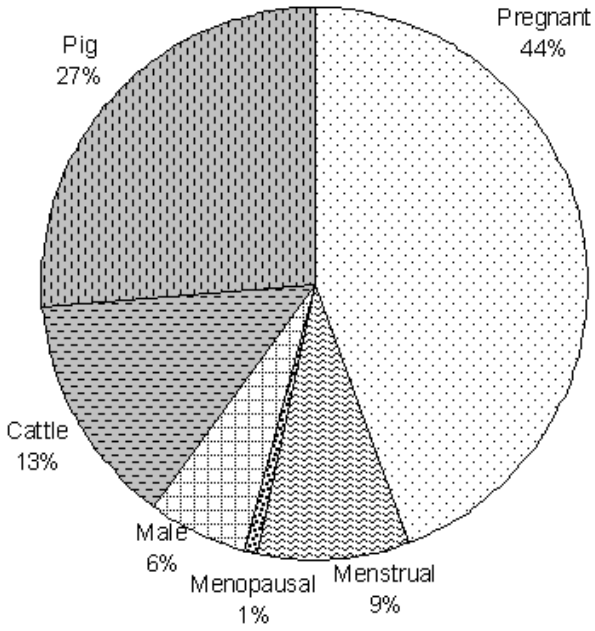

Figure 4.1: Estrogenic contributions from different sections of human (white background) and animal (grey background) populations to the total estrogenic excretion (37 g EEQ/day) from CTC

E1 and E2 showed to be the major estrogens, their respective contributions were 46% and 36.7% to the total estrogenic activity excreted from CTC (Figure 4.2, Appendix 5). The contribution of E3 and EE2 was minor and accounted for about 2% of the total estrogenic activity, only. In a single component analysis, Duong et al. (2010) found that EE2 was the main contributor to the EEQ of water samples taken from near discharge points of crowed municipal areas in Long Xuyen City, a neighboring city of CTC. Although the other compounds such as E1, E2, EE2, nonylphenol, octylphenol, bisphenol A and genistein were also detected, their contribution to the EEQ was lower than EE2 (Duong et al., 2010), suggesting that in Vietnam EE2 might be used in a larger extent as compared to the estimation in this study.

In a study conducted in the year of 2009, the author monitored the estrogenic activity in surface water of CTC by the YES assay and estimated the daily discharge to be in between 44 to 88 g EEQ (chapter 3). Although the difference is not too big between modeled and measured values, it is reasonable that result from the monitoring activity is higher. The YES assay is also sensitive to other EDs such as nonylphenol, octylphenol, bisphenol A and some pesticides (Rutishauser et al., 2004; Hurst and

Sheahan 2003). These chemicals may have contributed to the monitoring result. Additionally, there could be other possible sources in the city, which were not included in the author's estimation.

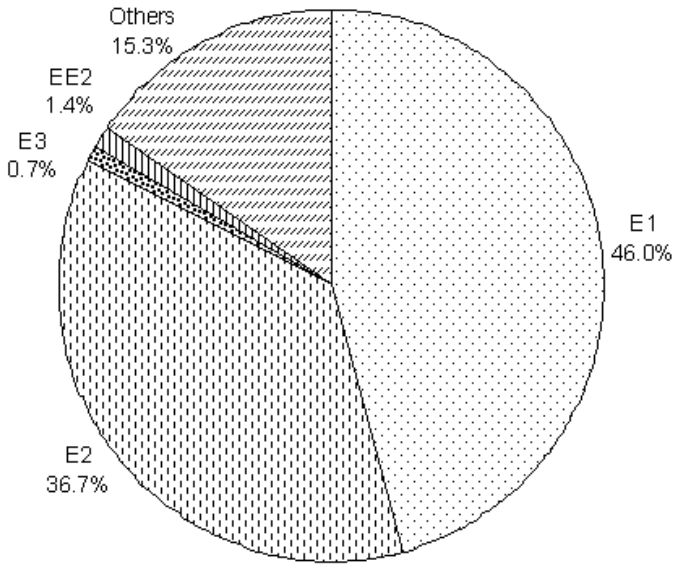

Figure 4.2: Estrogenic contributions from different estrogens to the total estrogenic excretion (37 g EEQ/day) from CTC

For example, open dumping sites have been reported as a potential source of several estrogenic compounds (Aoki, 2001; Tanabe and Minh, 2010). In CTC, it is assumed that about 40% out of 800 ton of fresh municipal solid waste is dumped into water bodies daily. The majority of the waste (60%) is mixed with other solid waste fractions and is dumped into the open dumping site (Can Tho DONRE, 2007; Loan, 2010). Industrial wastewater in CTC could be another source of estrogenic activity. In total, CTC daily releases about 45,000 m^3 of industrial wastewater, and most of that are not treated properly before discharging into the environment (Loan, 2010). Loan, 2010 reported from the biggest industrial zone in CTC, that only 28 of 130 factories have installed wastewater treatment systems. The other 102 factories discharge their wastewater into the water bodies directly.

Another source of estrogenic activity is fishpond discharge (Kolodziej et al., 2004). About 70,000,000 m^3/day of untreated water from fishponds in CTC is discharged into

surface water (Loan, 2010). The estrogenic activity may originate partly from commercial fish feed that may contain estrogenic activity ranging from 0.2 to 6.2 ng EEQ/g (Matsumoto et al., 2004). Agricultural runoff or discharge from irrigation water may contribute to estrogenic activity in surface waters because pesticides have been reported to have estrogenic activities (Hurst and Sheahan, 2003; Kojima et al., 2004). As pesticides are heavily used in the Mekong Delta (Berg, 2001; Khanh et al., 2006), it is likely that some of the estrogenic activity in surface water is contributed from pesticides. However, the estrogenic potency of pesticides is rather low.

4.4 Conclusions

The model predicts the discharge of estrogenic activity into water bodies well. In CTC, human and animal discharges are the major sources for estrogenic activity. If ongoing plans and construction activities are realized to install a sewage system and a wastewater treatment plant in CTC, then the discharge of estrogenic activity will be reduced as estrogens are degraded to a certain extent in the wastewater treatment process. If animal excrements would be collected, treated and used as organic fertilizer, then the complete contribution of estrogenic activity from animals could be avoided. However, this would require a change in the animal manure management system.

5. Estrogenic activity in the surface waters of agro-ecosystems of Dong Thap Province and Can Tho City

Abstract

The concentration and occurrence of estrogenic activity in surface water was monitored in different agro-ecosystems in the Mekong Delta, Vietnam. In the period from October 2008 to November 2009, water samples were taken from different categories including agricultural fields, fishponds, and irrigation canals in agricultural and suburban in Dong Thap Province (DTP) and Can Tho City (CTC), respectively. The estrogenic activity was analyzed by the Yeast Estrogen Screen (YES) assay, and expressed in terms of estrogenic activity or 17β-estradiol equivalent (EEQ). Ranging from under the detection limit (ND = 0.015 ng EEQ/L) to 3.62 ng EEQ/L (median 0.3 ng EEQ/L), the concentrations in CTC were significantly higher than those in DTP, ranging from ND to 4.97 ng EEQ/L (median 0.19 ng EEQ/L). In each sampling area, more than 10% of the detected samples exceeded the Predicted No Effect Concentration (PNEC) of 1 ng/L, indicating a potential estrogenic risk to the local aquatic fauna in both CTC and DTP. Domestic discharge is likely to be the reason for the observed estrogenic activities. Fishponds had slightly elevated the estrogenic activities in their surface waters. The estrogenic activity in the agricultural fields in DTP was lower as compared to the fields in CTC.

5.1 Introduction

The Mekong River is the longest river in southeastern Asia, which flows a distance of almost 4800 km originating in China, and runs through Myanmar, Thailand, Laos, Cambodia, and Vietnam before emptying its water into the sea. Water discharge averages are 14000 m^3/s, and the drainage basin of the Mekong River covers 795000 km^2 (Cenci and Martin, 2004). Cover an area of 39000 km^2, with a population of about 20 million people, the Mekong Delta in Vietnam is one of the most highly productive agricultural areas in the world (Minh et al., 2007a). Agriculture and aquaculture are the major agronomic activities in the Mekong Delta in Vietnam. Population growth and

economic development in this area in recent years has increasingly polluted its surface waters by agrochemicals (Berg, 2001; Phuong and Gopalakrishnan, 2003; Dasgupta et al., 2007), discharges from fishponds, and human and livestock wastes (Loan, 2010).

Among a variety of estrogenic chemicals, the natural estrogens (17β-estradiol (E2), estrone (E1)), and synthetic estrogen (17α-ethynylestradiol (EE2)) are the major contributors of estrogenic activity in the environment (Snyder et al., 2001; David Holbrook et al., 2002; Furuichi et al., 2004). While estrogens are mainly excreted by humans and livestock (Ying et al., 2002a; Hanselman et al., 2003; Campbell et al., 2006), industrial estrogenic chemicals derive from industry and domestic usages (Staples et al., 1998; Campbell et al., 2006; Sun et al., 2008). Although wastewater treatments remove these chemicals to a certain extent (Desbrow et al., 1998; Körner et al., 2000; Drewes et al., 2005; Chimchirian et al., 2007), the effluent concentrations may cause an estrogenic response in the aquatic animals (Folmar et al., 1996; Jobling et al., 1998; Gross-Sorokin et al., 2006), and have been therefore considered as the major source of estrogenic activity in surface water (Legler et al., 2002b; Kirk et al., 2002; Coors et al., 2004; Campbell et al., 2006).

Runoff from cultivated fields is also considered as a potential source as it may contain natural estrogenic chemicals (Stuer-Lauridsen et al., 2005; Campbell et al., 2006; Lavado et al., 2009), industrial estrogenic chemicals such as pesticides (Hurst and Sheahan, 2003; Xie et al., 2005; Lavado et al., 2009) and other estrogenic chemicals (e.g. nonylphenol ethoxylates; Campbell et al., 2006). In addition to humans and livestock, fish has also been implicated to be responsible for the elevated estrogenic activity in surface water (Stuer-Lauridsen et al., 2005; Barel-Cohen et al., 2006) although relatively little information is available.

EDs have been suspected to cause the negative effects on human and animal health (Colborn et al., 1993; Safe, 2000; Waring and Harris, 2005; Aksglaede et al., 2006; Campell et al., 2006). It is reported that at extremely low concentration the estrogens (E2, EE2) could generate the abnormal response in the fish (Purdom et al., 1994; Young et al., 2002; Johnson and Williams, 2004). Young et al. (2002) proposed the Predicted No Effect Concentration (PNEC) of 1 ng E2/L to protect the aquatic animals.

High contamination of estrogenic activity in the surface waters of the Mekong Delta in Vietnam may be expected due to its high population density and the intensive agronomic activities. However, to the best of the author's knowledge, there is no information relating estrogenic activity in the agro-ecosystems in the Mekong Delta in Vietnam. The objective of this chapter was to monitor the occurrence and concentration of estrogenic activity in surface waters of some agro-ecosystems including agricultural runoff, fishponds and irrigation canals in two representative areas in the Mekong Delta, Vietnam.

5.2 Materials and Methods

Tam Nong district in Dong Thap Province (DTP) and Cai Rang district in Can Tho City (CTC) were selected as a representative for an agricultural and suburban area in the Mekong Delta, respectively (Figure 5.1). Tam Nong district is located in the north-western part upstream of the Mekong Delta in Vietnam, on the north bank of Tien River (the smaller branch of the Mekong River). It is also referred to as "Plain of Reeds". This study area is located upstream of CTC where water from Tien River partially flows into Hau River (Bassac River) after passing Tam Nong district. The local households are mainly located along some irrigation canals and separated from the rice fields, which represents an agricultural pattern with two intensive paddy rice crops per year. The region is annually and heavily affected by flood in the rainy season (Table 5.1).

CTC located in the center of the Mekong Delta, just on the south bank of Hau river (the bigger branch of the Mekong river), is the most urbanized and industrialized city in the region (Figure 5.1). The administrative structure of this city is equivalent to those of a province, and it has urban as well as rural districts. Cai Rang district is the second study site located in a sub-urban and agricultural area of CTC. In this area, the majority of households are located along the canals, however, there are a number of households located near or directly on the fields, where a mixture of paddy rice, vegetables and fruits is grown.

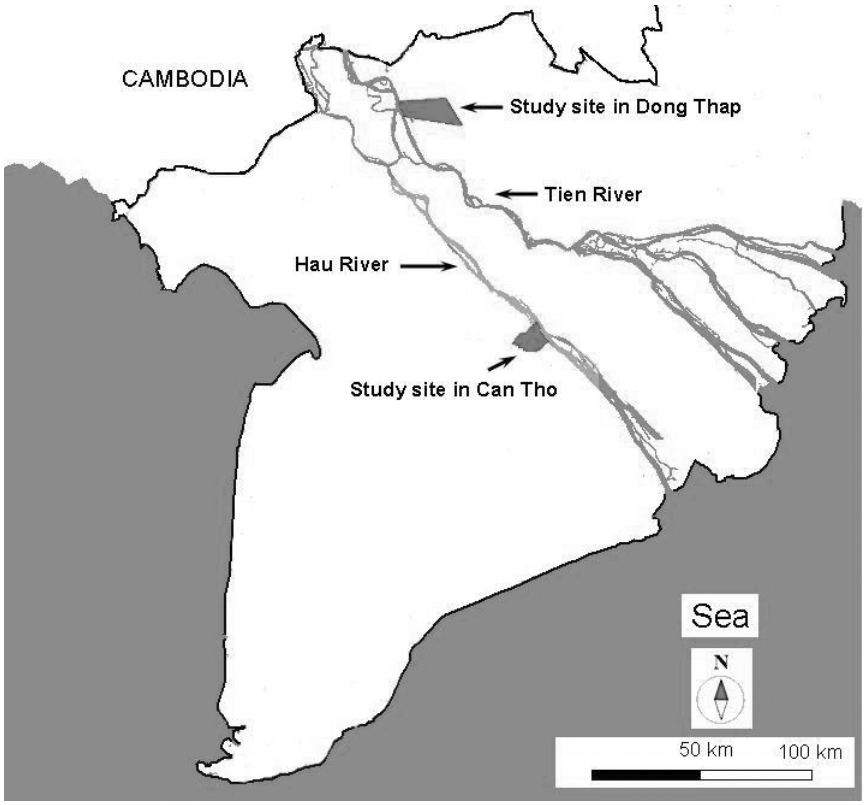

Figure 5.1: Locations of the study sites in the Mekong Delta, Vietnam

Table 5.1: Climatic patterns in the Mekong Delta, and rice seasonal calendar in Dong Thap Province (adapted from Wassmann et al., 2004)

Month	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Rice season in DTP												
Flood season												
Rainy season												

The Mekong Delta is characterized by a dense system of rivers and canals, and water from the Mekong River is used as the main water resource for agriculture, aquaculture, livestock farming, industry and drinking water in the region. The water levels in the rivers and canals are daily influenced by the tide (about 200 km and 100 km from the coast for Tam Nong and Cai Rang respectively). In both areas, the rainy season is from April to November, while the dry one is from December to March of the following year (Table 5.1).

5.2.1 Sampling in Dong Thap Province

Different water categories in DTP were sampled including a reference sampling station upstream of the study area, canals, agricultural fields, fishponds, and protected national wetland park (Tram Chim National Park). Sampling was conducted in every six weeks. In total, 10 sampling events were conducted from November 2008 to November 2009, in which 174 samples were taken and analyzed from 25 sampling stations. In detail, they were: 76 samples from ten stations in the canals; 47 samples from nine station in the rice fields; 22 samples from three stations in the national park; 19 samples from two stations in the fishponds; ten samples from one station at the reference upstream of the study area. Due to practical reasons, not all sampling point could be considered during each sampling event.

5.2.2 Sampling in Can Tho City

Sampling categories in CTC included a reference station, canals, agricultural fields and fishponds. Sampling was conducted every three weeks. In total, 16 sampling events were conducted from October 2008 to November 2009, in which 364 samples were taken and analyzed from 26 sampling stations. In detail, they were: 189 samples from 12 stations in the canals; 114 samples from 10 stations in the agricultural fields; 30 samples from two stations in the fishponds and 15 samples from one station for fishponds reference; 16 samples from one station at the reference upstream of the study area. Similar to DTP, not all sampling points could be considered during each sampling event due to practical reasons.

5.2.3 Sample collection and analytical procedure

Water samples were collected as grab samples using 1-litre, pre-cleaned glass bottles with Teflon caps. Samples were taken during the day between 6 am and 8 pm. The samples were then kept on ice, protected from the light, and transported to the laboratory within a maximum 36 hours, where they were stored at 4°C. Extraction was normally performed within a week. In addition to estrogenic activity analysis, water samples were analysed for various water quality parameters on in situ, including DO (Dissolved Oxygen), EC (Electrical Conductivity), pH and temperature (T°). The analytical procedure was adopted from Roultedge and Sumpter (1996), and thoroughly described in Hong's disseration (in preparation).

5.2.4 Data analysis

Data sets were analyzed and plotted using Excel® (Microsoft Corporation) software packages, SPSS (SPSS Inc.), and Sigma Plot (Systat Software, Inc.) respectively. Normality of distribution was checked by the Kolmogorov-Smirnov test. The difference between two sampling categories was evaluated the Mann-Whitney U test.

5.3 Results and Discussion

5.3.1 Estrogenic activity in Dong Thap Province

Estrogenic activity occurred in all the sampling events. In 59.2% of the total samples, estrogenic activity was detected ranging from under the detection limit (ND = 0.015 ng/L) to 4.97 ng EEQ/L (median 0.19 ng EEQ/L). The remaining 40.8% were not detected (33.3% cytotoxic, 7.5% under the detection limit). Although water discharge is higher during the flood season (inundation period), the variation in this period was comparable to that during the non-inundation period (Figure 5.2). This may be related to the fact that domestic waste in this region is directly discharged into rivers, canals and ponds. During the rainy season, estrogenic compounds from domestic waste are discharged into the rivers and canals directly. During the non-inundation season, they are trapped in the ponds located behind the small sewers and may be flushed out of

the ponds to the rivers and canals during the flood season, suggesting the estrogenic variation in this period.

Among those samples showing estrogenic activity, 13% of the samples exceeded the PNEC value of 1 ng/L. These samples were either taken in the national park or the canals (Figure 5.3A). Interestingly, in March and April (the driest month of the year) most of the samples were cytotoxic to the yeast cells, and consequently did not generate estrogenic activity. The other samples with estrogenic activity had a low EEQ.

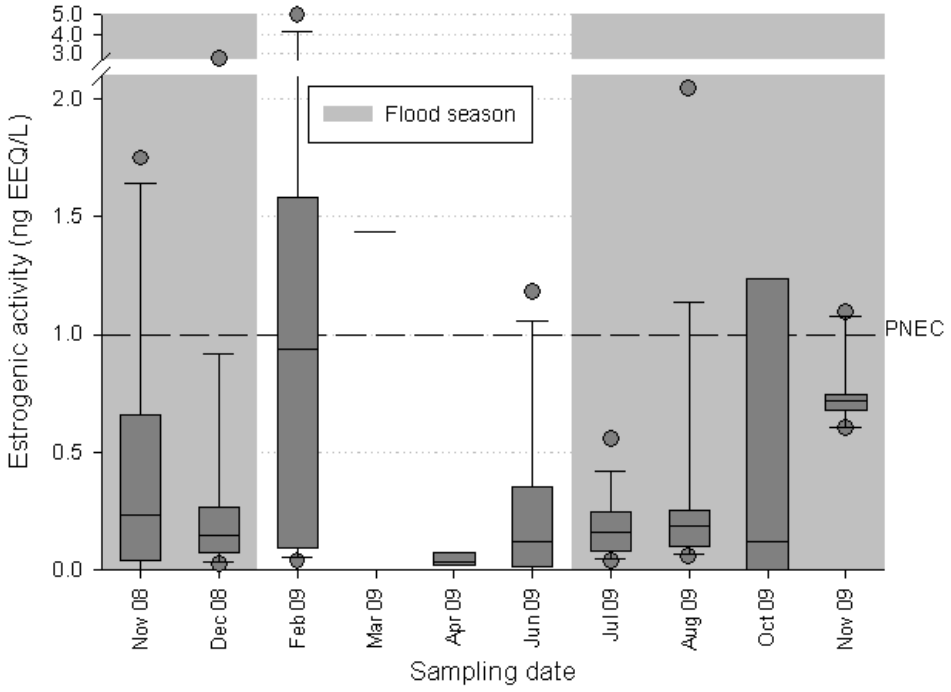

Figure 5.2: Estrogenic activity in the sampling area in DTP: the boundary of the box closest and farthest to zero indicate the 25th, and 75th percentile respectively; a line within the box marked the median; the error bars above and below the box indicated the 90th, and 10th percentile respectively; each outliner (○); PNEC (– – –)

In detail for each category (Appendix 6, 7 and 8), the estrogenic activities in the canals ranged from ND to 2 ng EEQ/L (median 0.19 ng EEQ/L), and tended to be higher than the reference upstream station (range ND to 0.45 ng EEQ/L, median 0.01

ng EEQ/L, p = 0.08 in the Mann-Whitney U test). The concentration in the park ranged from 0.07 to 4.97 ng EEQ/L (median 0.99 ng EEQ/L) and was higher as compared to the canals (p = 0.000 in the Mann-Whitney U test). In the fields the concentration ranged from 0.02 to 0.58 ng EEQ/L (median 0.16 ng EEQ/L) tended to be lower than that in the canals (p = 0.585 in the Mann-Whitney U test). For the fishpond, the concentrations (range ND to 0.75 ng EEQ/L, median 0.14 ng EEQ/L) tended to be higher as compared to its influent station (range ND to 1.6 ng EEQ/L, median 0.08 ng EEQ/L, p = 0.525 in the Mann-Whitney U test). Among all sampling categories, the highest median concentration was found in the national park, followed by the canals, the fishpond and the rice fields.

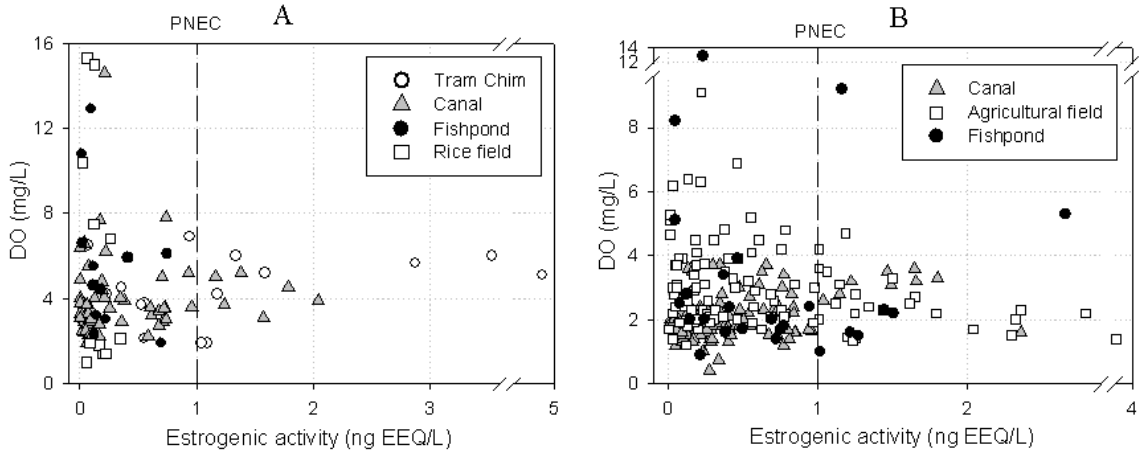

Figure 5.3: Estrogenic activity and its respective DO value in DTP (A) and CTC (B): Tram Chim (○), canal (△), fishpond (●), rice field or agricultural field (□), PNEC (— — —)

Water inside the park is less exchanged compared to that in the canals, because the whole park is protected by a complete dyke system. In addition, the national park is connected to a residential area locating in the park by a semi-dyke system; as a consequence, there is an exchange of potentially polluted water from the residential area and the water in the park. Both, the rather stagnant water and the connection to a potential source of estrogenic activity may be the reason for the high estrogenic activity in the park as compared to the other categories (Figure 5.3A). Although untreated domestic waste is also directly discharged into the canals, it is probably more diluted due to a higher exchange of water as compared to the park. This may be

the reason of the lower estrogenic activity in the canals as compared to the park. Consider all these facts, it can be said that untreated domestic discharge is most likely the reason for the observed estrogenic activity since there was no industrial factories, and sewage treatment system in this area.

The data analysis did not show any correlation between the estrogenic activity and the other measured quality parameters. However, the estrogenic activities tended to increase with decreasing oxygen concentration (Figure 5.3A).

5.3.2 Estrogenic activity in Can Tho City

Estrogenic activity occurred in all sampling events. In 68% of all samples, estrogenic activity was detected and ranged from ND to 3.62 ng EEQ/L (median 0.3 ng EEQ/L; Figure 5.4). From those, 17% of the samples had a higher estrogenic activity than the PNEC value. The other 32% of the samples were either cytotoxic (24.7%) or under the detection limit (7.3%). Similar to DTP, the variation during the inundation period was comparable to that during the non-inundation period (Figure 5.4). Nevertheless, the estrogenic activity in CTC was significantly higher ($p = 0.008$ in the Mann Whitney U test) than in DTP. Interestingly, while in the sampling event on the fourth of May all the samples either showed a cytotoxic effect or the estrogenic activity was below the detection limit. During the end of February and April (the driest season of the year), estrogenic activity in general was low (Figure 5.4). Anti-estrogenic compound has been suspected to be the reason for lowering estrogenic activity in the YES assay compared to prognosed values (Beck et al., 2006; Sun et al., 2008; Buckley, 2010). The lower concentration of estrogenic activity during the driest season of the year might be related to the effect of higher concentration of anti-estrogenic compounds in the samples. Thus in the presence of these compounds at sufficient high concentration, the estrogenic activity in the samples may be inhibited.

There was no significant difference between the canals (range ND to 2.99 ng EEQ/L, median 0.256 ng EEQ/L) and the reference station (range 0.07 to 0.8 ng EEQ/L, median 0.28 ng EEQ/L, $p = 0.7$ in the Mann Whitney U test). For the agricultural fields the estrogenic activity tended to be higher as compared to the canals (range ND to 3.6 ng EEQ/L, median 0.3 ng EEQ/L, $p = 0.066$ in the Mann Whitney U test, Figure

5.3B, Appendix 9). This contradiction result with DTP may be due to the direct discharge of wastewater of the households into the agricultural fields.

Similar to DTP, the estrogenic activity in the fishponds (range 0.05 to 2.66 ng EEQ/L, median 0.69 ng EEQ/L) tended to be higher as compared to that in the influent station (range 0.29 to 1.35 ng EEQ/L, median 0.56 ng EEQ/L, p = 0.98 in the Man-Whitney U test) (Appendix 10).

Except for the canal category (p = 0.22), the estrogenic activities in the fields and the fishponds in CTC were significantly higher than those in their respective categories in DTP (p = 0.003 and 0.001 in the Man-Whitney U test, respectively).

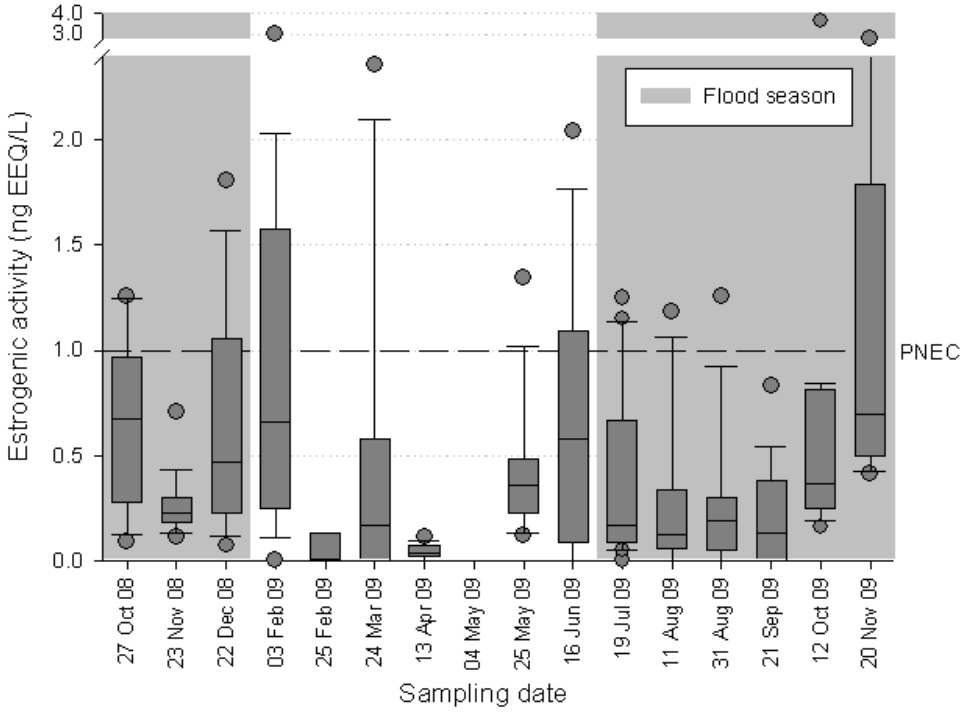

Figure 5.4: Estrogenic activity in the sampling area in CTC: the boundary of the box closest and farthest to zero indicate the 25th, and 75th percentile respectively; a line within the box marked the median; the error bars above and below the box indicated the 90th, and 10th percentile respectively; each outliner (○); PNEC (— — —)

Similar to DTP, there was no correlation between the estrogenic activity and other measured quality parameters, and the estrogenic activity tended to be higher at low DO (Figure 5.3B). DOs in CTC were lower as compared to those in DTP (Figure 5.3).

The EEQs in this study (ND to 4.97 ng EEQ/L) are in line with studies from other countries such as ND to 8.64 ng EEQ/L in Taiwan, 2.2 to 12.1 ng EEQ/L in China, ND to 7.4 ng EEQ/L in Korea, 0.3 to 4.5 ng EEQ/L in France (Shue et al., 2010), 1.32 to 11.79 ng EEQ/L in Australia (Ying et al., 2009), 0.7 to 4.0 ng EEQ/L in Japan (Hashimoto et al., 2005), 0.05 to 2.7 ng EEQ/L (median 0.4 ng EEQ/L) in Denmark (Stuer-Lauridsen et al., 2005). A comparison of estrogenic activity is limited because the concentration depends on the water discharge in the rivers. The sites in this study were hydrologically connected to the Mekong River that has a rather high discharge (average discharge of about 14000 m^3/s; Cenci and Martin, 2004).

Agricultural runoff has been reported as one source of estrogenic activity (Burnison et al., 2003; Xie et al., 2005; Campbell et al., 2006; Lavado et al., 2009). The concentration in the drainage from fields amended with manure was from 0.05 to 1.1 ng EEQ/L (median 0.7 ng EEQ/L) or from 0.05 to 36.1 ng EEQ/L (median 2.3 ng EEQ/L) when sewage sludge was applied (Stuer-Lauridsen et al., 2005). The estrogenic activity in the agricultural areas of California was from ND to 242 ng EEQ/L (Lavado et al., 2009). In comparison to these studies, the estrogenic activity obtained in the agricultural sampling fields in this study was low. This result might be related to the fact that organic fertilizers are not commonly applied in the Mekong Delta in Vietnam (Vu et al., 2007). This is particularly true for the sampling areas in this study. In addition, Toan et al. (2010), who analyzed pesticide concentrations in the same rice field and period that the author took the samples, showed that surface water in this sampling area has been contaminated by recently used pesticides. Moreover, the pesticide contamination in surface water in the Mekong Delta has also been reported in other studies (Berg, 2001; Huan et al., 2005; Khanh et al., 2006). On the one hand, although some pesticides have been shown to exhibit estrogenic activity (Xie et al., 2005; Campbell et al., 2006; Lavado et al., 2009), their potency is generally low compared to E2 (a few to six orders of magnitude lower than E2; Hurst and Sheahan 2003; McCarthy et al., 2006) and may not significantly influence to the total estrogenic activity. On the other hand, other reports showed that some pesticides were anti-

estrogenic (Tran et al., 1996; Alvarez et al., 2008) that could be able to mask the estrogenic activities of estrogenic compounds in the agricultural runoff.

Kolodziej et al. (2004) reported that in the raceways and effluents of the fish hatcheries, the endogenous steroid estrone was detected at concentrations near 1 ng/L. The authors also highlighted that aquaculture effluents, and even spawning fish can lead to detectable concentrations of steroid hormones in surface water. In another study, the estrogenic activity in receiving water downstream was slightly elevated compared to its upstream of discharging points from fish farms during fall season. The estrogenic activity was 2 ng EEQ/L and 3.5 ng EEQ/L in the upstream and downstream, respectively (Stuer-Lauridsen et al., 2005). In addition, commercial fish feed was also reported containing 0.2 to 6.2 ng EEQ/g (Matsumoto et al., 2004). It was noted that in this study fish in the sampling ponds was mainly fed by commercial fish feed as well. Although the estrogenic activity obtained in the fishponds was slightly elevated compared to the influent stations, the concentrations analyzed in this study were lower compared to those mentioned in literature. This could be related to the fact that the sampling fishponds in this study were neither hatcheries nor spawnings which may have higher estrogenic activity due to the steroidal hormones are increasingly excreted by fish before and during the production periods (Kolodziej et al., 2004).

5.3.3 Cytotoxic effects in Dong Thap Province

In this study, cytotoxic effects were observed throughout the sampling period. These effects either occurred in the form of yeast cells growth inhibition (inhibited yeast cells) or in the form of yeast cells death (killed yeast cells). The cytotoxic effects were evaluated upon the comparison of the yeast cells turbidity absorbance at 630 nm between samples and the solvent blanks (Hong's disseration in preparation).

In DTP, cytotoxic effects mainly occurred in the rice fields and in the canals, and rarely in other sampling categories (Appendix 6 and 7). In the canals, 76 samples were analyzed. From those 30.3% (14.5% killed, and 15.8% inhibited) showed cytotoxic effects. Moreover, cytotoxic effects in the canals occurred not only during the rice season but also during the food season, e.g. November 2008 and October 2009

(Figure 5.5B). At that time, cytotoxic effects were also observed at the reference station in the Tien River, suggesting that chemicals responsible for the cytotoxic effects (cytotoxic chemicals) originated from upstream.

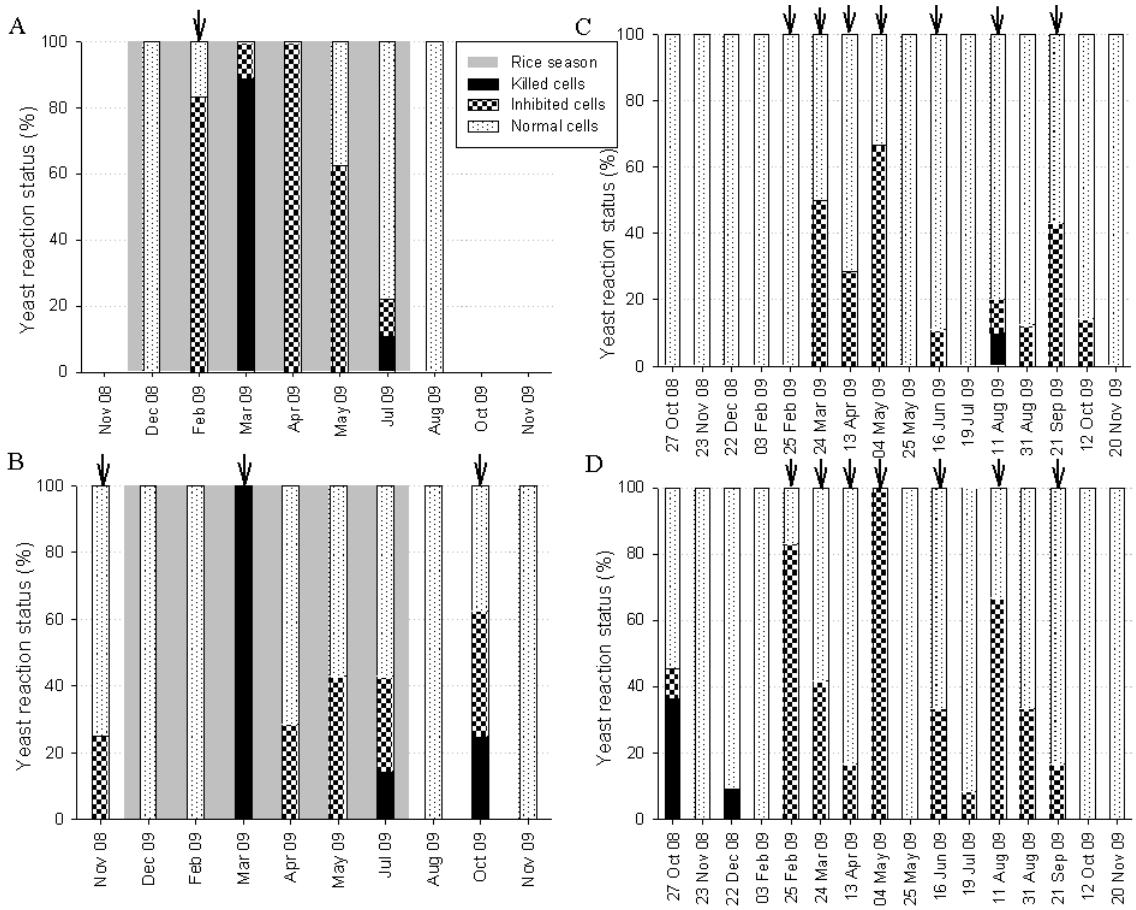

Figure 5.5: Distribution of the yeast reaction status in the samples taken in the rice fields (A) and the canals (B) in DTP, the agricultural fields (C) and the canals (D) in CTC: killed yeast cells (■), inhibited yeast cells (▩), normal yeast cells (▯), rice season (▭), cytotoxic effect at upstream station (↓)

In the rice field, 47 samples were analyzed. From those 59.6% (19.2% killed, 40.4% inhibited) of the total samples showed cytotoxic effects, which occurred only in the period from February to July 2009 (during the rice season, Figure 5.5A). In December and August, when the rice was just seeded and about 20 days after harvesting, respectively, there were no cytotoxic effects to the yeast cells (Figure 5.5A). It is noted that pesticides were less or even not applied at all during the seeding time and about

two weeks before harvesting, suggesting non-cyctotoxic effect in these sampling events.

Cytotoxic effects occurred during the rice season in both the rice fields and the canals. However, in February this effect was observed only in the rice fields but not in the canals (Figure 5.5A and 5.5B), suggesting that chemicals responsible for the observed cytotoxic effect originated from the rice fields. Since there was no rain in February, these chemicals could be trapped in the fields. They were flushed out into the canals during the sampling in March, which witnessed a heavy rainfall event, and consequently caused the cytotoxic effects to the samples either taken in the canals or the rice fields (Figure 5.5A and 5.5B).

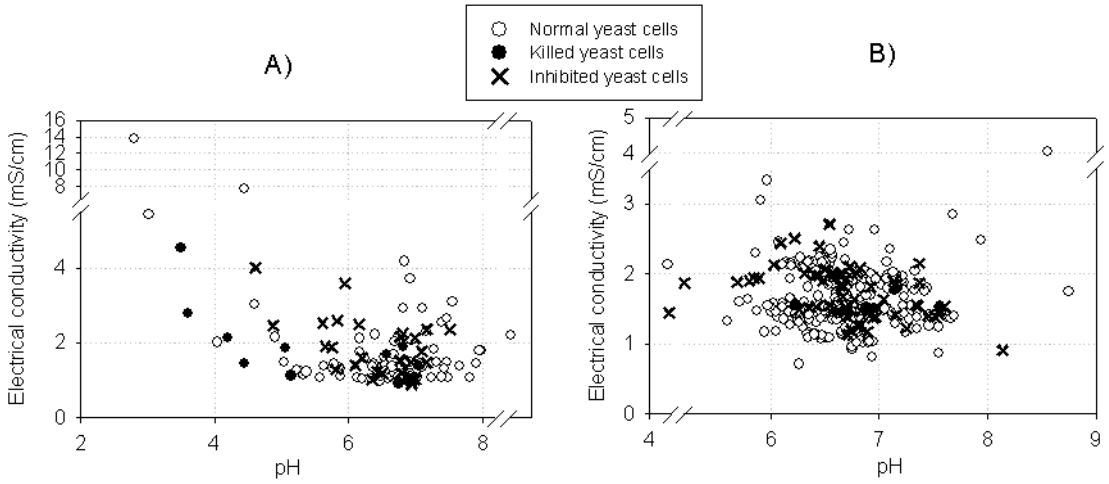

Figure 5.6: EC, pH and cytotoxic distribution in the samples in DTP (A) and in CTC (B)

While pesticides have been shown to be cytotoxic to the YES assay (Hurst and Sheahan, 2003), the sampling surface water in DTP was shown to be contaminated by recently used pesticides (Toan et al., 2010), suggesting that pesticides were likely responsible for the observed cytotoxic effects although sometimes these effects occurred at the upstream station for unknown reason. Moreover, in the same rice fields and sampling period that the author took the samples Toan et al. (2010) reported that average pesticide concentration ranged from 0.01 to 3.96 µg/L. The fungicide isoprothiolane and the insecticide buprofezin occurred with the highest concentration (up to 20.77 and 16.53 µg/L, respectively). Additionally, one of the most

common fungicides used (propiconazole) in the Mekong Delta (Berg, 2001) that has been shown to be cytotoxic to the YES assay (Hurst and Sheahan 2003) was also detected.

There was no significant difference between the measured water quality parameters of the cytotoxic samples and those of the normal samples. In addition, the EC tended to decrease with increasing pH values (Figure 5.6A).

5.3.4 Cytotoxic effects in Can Tho City

In total, 114 and 189 samples were taken and measured in the agricultural fields and canals, respectively. In the fields 16% (1% killed, and 15% inhibited) of the samples were cytotoxic while it was 29% (3% killed, and 26% inhibited) for the samples taken in the canals (Figure 5.5C and 5.5D).

In most of the sampling events with cytotoxic effects, these effects occurred at the reference sampling station (Figure 5.5C and 5.5D), indicating that the reason of cytotoxic effects may originate from upstream. Whereas in the other sampling events the cytotoxic effects occurred only in the agricultural fields (12 Oct 2009), in the canals (27 Oct 2008, 22 Dec 2008, 19 July 2009) or both in the field and canals (31 August 2009) but not at the reference sampling station (Figure 5.5C and 5.5D), suggesting that the reason of cytotoxic effects may originate from study area itself. However, it is unclear what were responsible for the cytotoxic effects in CTC. It is known that yeast cells are rather sensitive to toxic effects of chemicals or in heavily polluted surface waters (Körner et al., 2000; Hurst and Sheahan, 2003; Tashiro et al., 2004; Nakama et al., 2007). Domestic biocides may cause cytotoxic effects on the yeast cells in the YES assay at the lowest concentration of 2.7 µg/L (Nakama et al., 2007). Also surface waters polluted with municipal wastewater may inhibit the yeast cell growth (Tashiro et al., 2004). Such an unspecific pollution may be the reason for the cytotoxic effects in this study, because the water in the canals passed CTC and other cities before entering the canals.

Among the measured water quality parameters, the EC values of the cytotoxic samples (median 1.6 mS/cm) were significantly higher ($p = 0.032$ in the Man-Whitney

U test) than those of the normal samples (median 1.5 mS/cm). Additionally EC values tended to be higher when their respective pH values were decreasing (Figure 5.6B).

5.4 Conclusions

Estrogenic activity can be found all over the Mekong Delta in Vietnam. The concentration in surface water of the suburban area in CTC is significantly higher than in the agricultural area in DTP. The PNEC value of 1 ng/L is exceeded in both the surface waters in DTP and in CTC, indicating an estrogenically potential risk to the local aquatic animals. Domestic discharge is likely the main estrogenic source in these two areas. Additionally, although the estrogenic activity in the fishponds seemed to be elevated as compared to the influent stations, no significant difference was recorded. In DTP, estrogenic activity in the fields is low, whereas it is elevated in the fields in CTC. Pesticides may have caused the cytotoxic effects to the yeast cells in DTP. In CTC, the reason for these effects is unknown.

6. General conclusions and recommendations

In the Mekong Delta, Vietnam, in despite of the rather high water discharge estrogenic activity ubiquitously occurred in its surface waters, with the detection frequency of 100% in the urban and industrial areas of CTC, and about 90% in the suburban and rural areas of CTC and DPT, respectively. The estrogenic activity occurred in all sampling water categories, with significantly higher concentration in the urban and industrial areas of CTC. Although in the suburban area the concentration was significantly lower than that in the urban and industrial areas of CTC, it was significantly higher than that in the more agricultural area of DTP, an upstream area of CTC. In general, the concentration of estrogenic activity varied widely depending on the sampling location, and in many cases it exceeded the PNEC value of 1 ng/L and may cause an estrogenic risk to local aquatic fauna, especially in the urban and the industrial areas of CTC. In addition, the risk of estrogenic activity might be extended to the suburban and agricultural areas as well since in these areas the concentration sometimes exceeded the PNEC value.

Most likely, the ubiquitous occurrence and considerable concentration of estrogenic activity in the surface water of the Mekong Delta, Vietnam is related to the direct discharge of improperly treated or even not treated at all wastewater into water bodies. This situation warrants effects of estrogenic chemicals on aquatic fauna and probably human health. Human and animal wastes seemed to be the major sources for estrogenic activity. Therefore, sufficiently treatment of wastes, especially human and animal wastes are necessary to reduce estrogenic activity and its risk on aquatic fauna and human health, especially in highly populated urban areas. It is expected that the introduction of wastewater treatment plants for both municipal and industrial wastewaters, then the discharge of estrogenic activity could be reduced. In addition, the reduction of estrogenic activity could be obtained if animal waste would be collected, treated and used as organic fertilizer.

There are different groups of chemicals with an estrogenic activity, and their estrogenic potencies vary widely. The analytical methods could contribute to the variability of their potencies. However, regardless of the analytical methods estrogens

are generally more potent than anthropogenic estrogenic chemicals whose potencies are from a few to eight orders of magnitude lower than E2.

In Vietnam, there is lacking of studies on estrogenic chemicals in the environment, especially for the group of estrogens. There are some studies on anthropogenic estrogenic compounds such as PCBs, DDTs, and these persistent EDs ubiquitously occur in the environment at considerable concentrations, especially in urban areas. Due to their weak potency, these chemicals generally exhibit relatively low estrogenic activity, and contribute a minor portion to the total estrogenic activity of environmental samples. However, in some cases even without the contribution of estrogens, the estrogenic activity of these anthropogenic estrogenic chemicals occurs at considerable levels due to their high concentrations in the environment, which may cause an estrogenic risk for local aquatic fauna.

Considering the potential effects of estrogenic chemicals, it is clear that more efforts should be made to deal with the environmental problems caused by these chemicals since the scientific community is still in the initial steps to mitigate the concentrations and the effects of these estrogenic compounds. In Vietnam, this topic certainly deserves further studies. To further identify the sources, to elucidate the composition of total estrogenic activity, and to understand the risk of these chemicals, studies with integrated methods using the combination of chemical and biological analyses would be highly recommended. While in surface water E1, E2, EE2, nonylphenols, BPA should be considered in the chemical analysis, in sediment in addition to those mentioned compounds, the following persistent anthropogenic chemicals, PCBs, DDTs, and PAHs should be added. In addition, studies using bioindicators such as vitellogenin, an egg-yolk protein, which is normally only produced by adult females, can also be considered to evaluate the effects of estrogenic activity.

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Appendices

Appendix 1: Sampling date and estrogenic activity (ng EEQ/L) in the urban are of Can Tho City

21 Oct 08	27 Nov 08	22 Dec 08	04 Feb 09	17 Feb 09	26 Feb 09	19 Mar 08	17 Apr 08	05 May 09	17 May 09	26 May 09	16 Jun 09	15 Jul 09	13 Aug 09	28 Aug 09	11 Sep 09	23 Sep 09	11 Oct 09	17 Nov 09
0.953		0.289	4.161	2.69	1.738	0.981	0.161	0.07	0.191	1.84	3.152	0.774	0.48	0.552	0.356	0.414	0.748	0.752
0.836	2.194	0.477	1.35	4.452		0.363	0.363	0.048	0.35	1.745	2.946	0.161	0.777	1.362	0.129	0.771	0.673	0.81
-	0.431	-	-	1.972	-	0.2	0.31	0.078	0.447	1.59	2.517	0.559	0.16	0.7	0.12	0.752	0.943	0.817
0.538	2.391	-	0.853	2.799	1.694	0.15		0.123	0.178	0.17			0.035	2.221	0.55	1.359	1.084	1.572
0.688	0.258	0.64	1.281			0.192				0.888	0.626	0.335	0.77	1.285	0.035	0.755	0.696	0.588
10.18		2.422										0.48		34		5.284		0.398
5.032	-	-	-	-	-											4.221	0.547	2.701

	Sample killed the yeast cells
	Sample inhibited the yeast cells
-	Not available

Appendix 2: Sampling date and estrogenic activity (ng EEQ/L) in the industrial area of Can Tho City

22 Oct 08	23 Dec 08	05 Feb 09	26 Feb 09	19 Mar 09	15 Apr 09	05 May 09	27 May 09	15 Jun 09	18 Jul 09	07 Aug 09	29 Aug 09	19 Sep 09	07 Oct 09	18 Nov 09
0.96	1.383	1.439	0.985	0.217		0.079	0.761	0.962	0.232	0.126	1.257	0.33	0.191	0.897
4.904	0.55	0.59					1.812	3.186	1.672	0.104		1.117	0.297	1.553
1.634	2.024			5.39			2.851		0.837			0.576	7.679	1.076
-	-	11.8		1.488	0.077						1.452	2.204		1.113
-	-	1.224				0.085	0.643			0.623		1.911	8.904	0.632

	Sample killed the yeast cells
	Sample inhibited the yeast cells
-	Not available

Appendix 3: Sampling date and estrogenic activity (ng EEQ/L) in the suburban rivers and canals of Can Tho City

	27 Oct 08	23 Nov 08	22 Dec 08	03 Feb 09	25 Feb 09	24 Mar 09	13 Apr 09	04 May 09	25 May 09	16 Jun 09	19 Jul 09	11 Aug 09	31 Aug 09	21 Sep 09	12 Oct 09	20 Nov 09
Ref canal	0.069	0.177	0.188	0.654					0.231		0.108		0.48	0.33	0.804	0.502
canal		0.232	0.444	0.135			0.018		0.29		0.05	0.352	0.068	0.133	0.838	0.56
canal	0.852	0.111	1.469	ND			0.069		0.409		0.095			0.083	0.84	0.475
canal	0.676	0.299	0.611	1.659		1.039	0.024		0.39		0.103		0.267	ND	0.728	0.425
canal	0.148	0.332		1.492		0.17	ND		0.243	0.056	0.165	0.172	ND	0.095	0.814	0.557
canal		0.219	1.226	1.648	0.164	2.36	0.079		0.454		0.296		ND	ND	0.801	0.639
canal	0.965	0.232	1.805	1.499	ND		ND		0.391	0.329	1.151			0.214	0.236	0.484
canal	0.774	0.13	-	0.658		ND	0.089		0.394	0.849				0.539	0.26	0.411
canal	0.287	0.297	0.363	0.63		ND	0.038		0.329	0.942	0.083			0.223	0.252	0.502
canal	0.088	0.19	0.224	2.99		ND			0.187	ND	0.197		0.184	0.833	0.246	0.681
canal	0.279	0.404	0.475	0.151			0.062		0.282	0.208	ND		0.042	ND	0.159	0.728
canal	0.34	0.239	0.545	0.347					0.144	0.184	0.108	ND	0.163	ND	0.827	0.705
canal	-	-	0.763	0.772		ND	0.017		0.26	ND	0.077	ND	0.035	0.349	0.743	0.72

	Sample killed the yeast cells
	Sample inhibited the yeast cells
-	Not available
ND	Under the detection limit

Appendix 4: Sampling date and estrogenic activity (ng EEQ/L) in the upstream and downstream stations out of Can Tho City

Sampling station	23 Dec 08	05 Feb 09	27 Feb 09	23 Mar 09	15 Apr 09	05 May 09	27 May 09	15 Jun 09	18 Jul 09	09 Aug 09	29 Aug 09	20 Sep 09	09 Oct 09	19 Nov 09
Upstream	0.260	0.536		1.091			0.334	0.238	ND		0.964	0.333	0.211	0.594
Downstream	0.486	0.632					0.842	0.296	0.451		1.569	0.041	0.334	0.542

	Sample killed the yeast cells
	Sample inhibited the yeast cells
-	Not available
ND	Under the detection limit

Appendix 5: Estimation of estrogenic discharge from human and animal populations of Can Tho City

Compound	Sex	Population fraction	F (ng/day)	U + F (ng/day)	f, (head)	EEQ (g)
Human contribution						
E1	Female				608000	5.56
		Pregnant	100000	550000	19200	4.04
		Menstrual	300	11700	320000	1.43
		Menopausal	70	1800	118000	0.08
	Male		400	2600	591600	0.59
EEQ_{E1}						**6.15**
E2	Female				608000	8.69
		Pregnant	202000	393000	19200	7.54
		Menstrual	200	3200	320000	1.02
		Menopausal	90	1000	118000	0.12
	Male		630	1800	591600	1.06
EEQ_{E2}						**9.75**
EE2(**EEQ_{EE2}**)	Female		6000	10500	41400	**0.52**
E3	Female			6000000	608000	0.264
		Pregnant		4800	19200	0.261
		Menstrual		1000	320000	0.003
		Menopausal		1500	118000	0.000
	Male				591600	0.002
EEQ_{E3}						**0.267**
$EEQ^{H} = ((EEQ_{E1} + EEQ_{E2} + EEQ_{E3})/0.74) + EEQ_{EE2}$						**22.36**
Animal contribution						
E1		Cattle		838000	7100	2.28
		Pig		201000	113000	8.69
SE1						**10.97**
E2		Cattle		384000	7100	2.73
		Pig		10400	113000	1.17
SE2						**3.9**
$EEQ^{A} = EEQ_{E1} + EEQ_{E2}$						**14.87**
Total human and animal contribution						**37.23**

Appendix 6: Sampling date and estrogenic activity (ng EEQ/L) in the Tram Chim National Park in Dong Thap Province

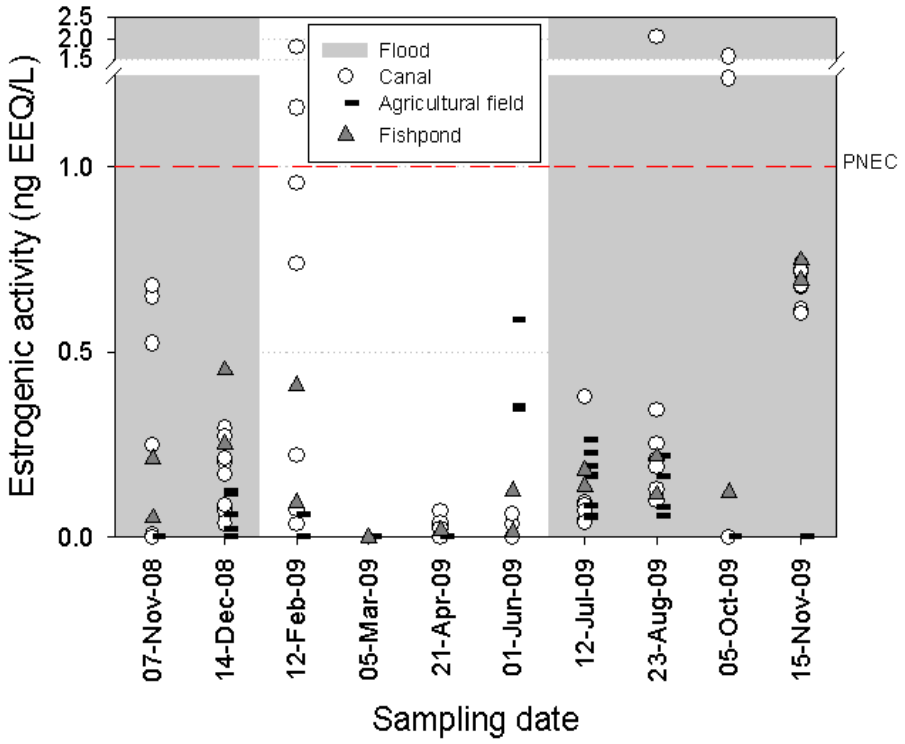
06 Nov 08	13 Dec 08	11 Feb 09	04 Mar 09	20 Apr 09	31 May 09	11 Jul 09	22 Aug 09	04 Oct 09	14 Nov 09
0.113	2.77	4.971	2.871	0.226	1.179	0.356	0.528	0.548	1.096
1.748	0.143	3.533	-		0.118	0.556	0.074		1.042
-	-	1.335	-	-	-	-	-	-	-
-	-	1.584	-	-	-	-	-	-	-
-	-	0.939	-	-	-	-	-	-	-

	Sample killed the yeast cells
	Sample inhibited the yeast cells
-	Not available
ND	Under the detection limit

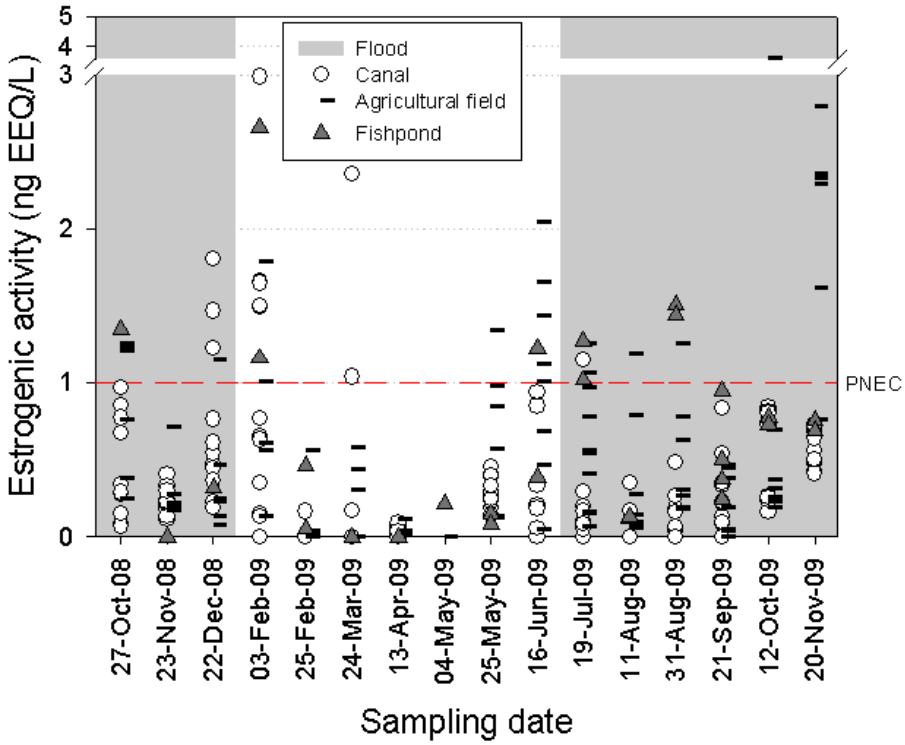
Appendix 7: Sampling date and estrogenic activity (ng EEQ/L) in the fishponds and reference stations in Dong Thap Province

Sampling station	07 Nov 08	14 Dec 08	12 Feb 09	05 Mar 09	21 Apr 09	01 Jun 09	12 Jul 09	23 Aug 09	05 Oct 09	15 Nov 09
Reference for canal		ND	ND		ND	0.381	0.368	0.025		0.45
Reference for fishpond	-	0.078	0.074			ND	0.085	0.209	1.239	0.676
Fishpond	0.213	0.452	0.41	ND		0.125	0.138	0.218		0.694
Fishpond	0.052	0.253	0.094	-	0.02	0.016	0.181	0.116	0.121	0.749

	Sample killed the yeast cells
	Sample inhibited the yeast cells
-	Not available
ND	Under the detection limit



Appendix 8: Estrogenic activity (ng EEQ/L) and sampling date from three different water categories in Dong Thap Province



Appendix 9: Estrogenic activity (ng EEQ/L) and sampling date from three different water categories in Can Tho City

Appendix 10: Sampling date and estrogenic activity (ng EEQ/L) in the fishponds of Can Tho City

Sampling station	22 Oct 08	23 Dec 08	05 Feb 09	23 Feb 09	19 Mar 09	15 Apr 09	06 May 09	27 May 09	15 Jun 09	18 Jul 09	09 Aug 09	30 Aug 09	20 Sep 09	24 Sep 09	08 Oct 09	17 Nov 09
Ref Fishpond	1.346	0.486	0.632					0.842	0.296	0.451		0.878	0.295	-	0.467	0.733
Fishpond	1.349	0.32	2.657	0.467			0.218	0.15	1.221	1.274	0.119	1.509	0.245	0.502	0.773	0.759
Fishpond	-	-	1.163	0.051				0.08	0.386	1.019	0.13	1.447	0.373	0.949	0.725	0.694

	Sample killed the yeast cells
	Sample inhibited the yeast cells
-	Not available