รายงานวิจัยฉบับสมบูรณ์

โครงการ การปนเปื้อนยาเคมีบำบัดในน้ำผิวดิน ระบบบำบัดน้ำ และระบบบำบัดน้ำเสียในเขตกรุงเทพมหานคร ประเทศไทย

โดย ดร.นรินทร์ บุญตานนท์

เดือน มิถุนายน ปี ๒๕๕๘

สัญญาเลขที่ RSA5680024

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คณะสิ่งแวดล้อมและทรัพยากรศาสตร์

มหาวิทยาลัยมหิดล

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

และมหาวิทยาลัยมหิดล

กิตติกรรมประกาศ

งานวิจัยฉบับนี้สำเร็จลุล่วงไปได้ด้วยดีจากความช่วยเหลือจากหลายๆ ภาคส่วนที่มีส่วนร่วมและให้การสนับสนุนในการทำวิจัย ตลอดจนคำชี้แนะต่างๆที่เป็นประโยชน์ ขอขอบพระคุณ สถาบันมะเร็งแห่งชาติ ที่ให้การสนับสนุนด้านยารักษามะเร็งเพื่อใช้ในการทดสอบเบื้องต้น และข้อมูลที่เป็นประโยชน์สำหรับงานวิจัย

ขอกราบขอบพระคุณ คณะสิ่งแวดล้อมและทรัพยากรศาสตร์ มหาวิทยาลัยมหิดล และมหาวิทยาลัยมหิดล ในการสนับสนุนงานวิจัย และการประสานงานในการดำเนินงานวิจัยให้สำเร็จลุล่วงด้วยดี

สุดท้ายนี้ขอกราบขอบพระคุณ สำนักงานกองทุนสนับสนุนการวิจัย อย่างสูงที่ได้สนับสนุนเงินทุนวิจัย และเปิดโอกาสให้ผู้วิจัยได้สร้างองค์ความรู้ใหม่ด้านสิ่งแวดล้อมที่เกี่ยวข้องกับสุขภาพที่จะเป็นประโยชน์ต่อสาธารณะต่อไป

ผู้วิจัย

บทคัดย่อ

รหัสโครงการ : RSA5680024

ชื่อโครงการ : การปนเปื้อนยาเคมีบำบัดในน้ำผิวดิน ระบบบำบัดน้ำ และระบบบำบัดน้ำเสีย ในเขตกรุงเทพมหานคร ประเทศไทย

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ยาเคมีบำบัดทำให้เกิดผลข้างเคียงต่อผู้ป่วยที่ได้รับการรักษาโดยผู้ที่ได้รับยาเคมีบำบัดจะผมร่วง หลังจากได้รับยาเนื่องจากยาเคมีบำบัดได้ทำลายเซลล์ขน ซึ่งเป็นเซลล์ปกติในร่างกาย แสดงให้เห็นว่ายาเคมีบำบัดนั้นมีความอันตรายต่อสิ่งมีชีวิตอย่างมีนัยสำคัญ งานวิจัยนี้จึงตระหนักถึงการตกค้างของยาเคมีบำบัดในสิ่งแวดล้อม โดยเฉพาะในส่วนของแหล่งน้ำ เนื่องจากมีการใช้เพื่ออุปโภคบริโภค สุดท้ายแล้วยาเคมีบำบัดอาจเข้าสู่ร่างกายของสิ่งมีชีวิตโดยที่ไม่ได้ตั้งใจได้ ยาเคมีบำบัดที่ศึกษาวิจัยคือ 5- Fluorouracil (5-FU), Cyclophosphamide (CP) และ Hydroxyurea (HU) จากการเก็บข้อมูลจากโรงพยาบาลที่มีการรักษาโรคมะเร็งขนาดใหญ่ในกรุงเทพมหานคร พบว่าเป็นยาเคมีบำบัดที่มีปริมาณการใช้มากที่สุด โดยงานวิจัยนี้มีจุดประสงค์เพื่อที่วิเคราะห์หาปริมาณ 5-FU, CP และ HU ที่ตกค้างในแหล่งน้ำผิวดิน น้ำประปา และน้ำเสียทั้งจากชุมชนและโรงพยาบาล รวมถึงประเมินประสิทธิภาพของระบบปรับปรุงคุณภาพน้ำ และระบบบำบัดน้ำเสียอีกด้วย โดยประยุกต์วิธีการวิเคราะห์จากงานวิจัยที่เกี่ยวข้อง เพื่อให้มีความแม่นยำในการตรวจวัดมากยิ่งขึ้น เริ่มจากการผ่านตัวอย่างน้ำเข้าสู่กระบวนการ solid-phase extraction (SPE) โดยใช้ Oasis® HLB Cartridge และตรวจวัดปริมาณสารด้วย HPLC-MS/MS จากการวิเคราะห์ปริมาณในแม่น้ำเจ้าพระยาตอนล่างในฤดูแล้งช่วง ปี 2013-2014 พบว่าปริมาณ 5-FU, CP และ HU เท่ากับ 1.28, 1.79 และ 1.12 ng/L ตามลำดับ และจากการทำนายปริมาณสารที่ตกค้างในแหล่งน้ำธรรมชาติ ปี 2014 ในสถานการณ์ที่เลวร้ายคาดการณ์ได้ว่าจะพบปริมาณ 5-FU, CP และ HU เท่ากับ 29.53, 12.9 และ 1,711.19 ng/L ตามลำดับ และจากการประเมินประสิทธิภาพในการกำจัด 5-FU, CP และ HU ของระบบปรับปรุงคุณภาพน้ำ และระบบบำบัดน้ำเสียนั้นพบว่าไม่สามารถกำจัดได้ทั้งหมดยังมีตกค้างในน้ำออกบางส่วน จึงได้ทำการประเมินความอันตรายของ 5-FU, CP และ HU จากการอุปโภคบริโภคน้ำในแม่น้ำเจ้าพระยาตอนล่าง และน้ำประปา พบว่าจากปริมาณที่ตรวจวัดได้นั้นไม่มีความเสี่ยงต่อสุขภาพมนุษย์โดยคำนวณจากค่า ADI (Acceptable Daily Intake) โดยทั้งนี้ยังไม่มีมาตรฐานอ้างอิงในสิ่งแวดล้อมที่แน่ชัด

คำหลัก : ยาเคมีบำบัด, ระบบบำบัดน้ำ, Fluorouracil, Cyclophosphamide, Hydroxyurea

Abstract

Project Code : RSA5680024

Project Title : Contamination of Chemotherapy Drugs in Surface Water, Water and Wastewater Treatment Plants in Bangkok Metropolitan, Thailand

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Project Period : 17 June 2013 to 16 June 2015

Chemotherapy drugs are very harmful chemicals which have a lot of side effects on cancer patients such as hair loss. It demonstrates that chemotherapy drugs that are significantly harmful to living things. This research recognizes the residues of chemotherapy in the environment. Especially regarding water resources, because it is used for consumption. Finally, chemotherapy may enter the body of a living organism that is not accidental. Chemotherapy is the interest of this study, 5-Fluorouracil (5-FU), Cyclophosphamide (CP) and Hydroxyurea (HU) from the retention of a large hospital in Bangkok. These are the chemotherapy drugs that have the most usage. The research aims to determine the levels of 5-FU, CP and HU residues in surface water, tap water and wastewater from both the community and hospital and to evaluate the system performance for WTP and WWTPs as well. The analytical processes were performed using solid phase extraction (SPE) with an Oasis® HLB cartridge and measured by HPLC-MS/MS. From the analysis in the Lower Chao Phraya River in the dry season year 2013, the amount of 5-FU, CP and HU were 1.28, 1.79 and 1.12 ng/L, respectively, and predicted a number of contaminants in surface waters, based on the amount of 5-FU, CP and HU consumption in the hospital in 2014, and the amount of 5-FU, CP and HU were 0.42, 0.18 and 24.45 ng/L, respectively. And the assessment of the performance for the removal of 5-FU, CP HU from WTP and WWTPs were unable to eliminate all residuals in the water effluent. Hence, the health risk assessment for 5-FU, CP and HU from the consumption of water in the Lower Chao Phraya River and tap water, is that the amount of 5-FU and CP may not harm the health of the consumer according to ADI (Acceptable Daily Intake). However, there is no reference standard of these substances right now but we should consider about this for prevent these effect in the future.

Keywords : Chemotherapy drug, Water treatment, Fluorouracil, Cyclophosphamide, Hydroxyurea

CONTENTS

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INTRODUCTION

Statement of Problems

Environmental pollutions have an impact on ecosystem functioning, climate regulation and human health. In the current time, potential pollutants would increase continuously because international development in various fields, such as industrial development and medical development, particularly in rapidly developing countries (European Environment Agency, 2015). And the effects of pollutants to living organisms may range from mild discomfort to serious diseases, such as cancer, by the pollutions that caused cancer are called carcinogen, which tend to the increase as well (Union for International Cancer Control, 2011). By seeing database on GLOBOCAN project of International agency for research on cancer (IARC) in 2008, the number of new cancer patients approximately 12.7 million people, while in 2012, the number of new cancer patients, approximately 14.1 million people, and the World Health Organization (WHO) has predicted in the year 2030 there were approximately 27 million of new cancer patients (International Agency for Research on Cancer, 2013), From data of the National Cancer Institute of Thailand, the number of new cancer patients in Thailand has increased as well. In Bangkok in 2001-2003, the number of new cancer patients adjusted 25,209 people and during in 2007-2009 there are a number of new cancer patients adjusted 30,638 people. So, in 8 years the number of new cancer patients in Bangkok is increased by 17 percent (National Cancer Institute of Thailand, 2010; National Cancer Institute of Thailand, 2013). In addition, in Thailand statistic data, the cancer is leading cause of death in the first and morbidity rate of major causes in the second (Bureau of Policy and Strategy, 2014). It can be concluded that the pollutants are a cause of cancer in humans. Cancer treatment can give rise to pollutants as well, which can be explained as follows.

Cancer treatment with Chemotherapy is a popular method which is mostly used in combination with other ways such as treatment with radiation etc. The Method of Chemotherapy followed by intravenous injection, application of a drug to the blood vessel or having the patients taking oral drugs. Chemotherapy drugs for cancer treatment have a mechanism of action by destroying cancer cells or preventing the cancer cell growth. Meanwhile, such drugs also affect to normal cells in the body such as bone marrow cells, hair cells, etc. This is expressed as various kinds of side effects (Brunton et al., 2005). There is also research indicating that chemotherapy drugs have the properties of carcinogen, which

Teratogenic and Mutagenic (Muggia & Ziedler, 1980; Carter, 1984; Buerge et al., 2006; O'Keefe, 2011; Besse et al., 2012). Currently it is found that the therapeutic technology by medications includes a mechanism to destroy cancer cells directly (Target therapy) but this method is still new, expensive, thus not very widespread (National Cancer Institute of USA, 2011). In Thailand, chemotherapy drugs are specially controlled and are the hazardous agent to be prescribed only at the hospitals (Bureau of Drug Control, 2015).

The contamination routes of chemotherapy drugs input and distribute enter to the environment through cancer patients use, carried out from sweat, urine, feces, vomit or disposal. Some of these chemotherapy drugs are not completely removed in wastewater and water treatment systems, therefore, could persist long enough to enter water consumption. The path of contamination with chemotherapy drugs to the environment is shown in Figure 1.1. (Kummerer, 2010). In other countries, contamination with chemotherapy drugs in the environment is likely to arise from the industrial but in Thailand, chemotherapy drugs must be imported only thus there is no contamination from the industrial. However, is arises from a waste in the process of preparing drugs or mixing chemotherapy drugs in the hospitals. (Buerge et al., 2006; Mahnik et al., 2007; Johnson et al., 2008; Garcia-Ac et al., 2008; Jjemba, 2008; Weissbrodt et al., 2009. ; Mullott et al., 2009; Rowney et al., 2009; Kovalova, 2009; Yin et al., 2010; Kümmerer et al., 2010; O'Keefe, 2011; Besse et al., 2012; West and Beaucham, 2014)

Human exposure could then occur from consumption of water (Besse et al., 2012; O'Keefe, 2011; Kummerer, 2010; Rowney et al., 2009; Johnson et al., 2008). It is dangerous to normal human body and cancer risk or mutagenic risk when exposed (Buerge et al., 2006; O'Keefe, 2011; Besse et al., 2012). And, some chemotherapy drugs are not degraded or partially degraded (Buerge et al., 2006; Johnson et al., 2008; Rowney et al., 2009; O'Keefe, 2011; Besse et al., 2012). From the recent studies show that these drugs exposure to human comes from chronic exposure, such as contaminated food (Johnson et al., 2008) and drinking water (Rowney et al., 2009). That drugs are found in environment, such as wastewater treatment, surface water, tap water, drinking water, groundwater, air and sediments (Kiffmeyer et al., 1997; Steger-Hartmann et al., 1997; Buerge et al., 2006; Mahnik et al., 2006; Johnson et al., 2007; Osytek et al., 2007; Rowney et al., 2009; Kovalova, 2009; O'Keefe, 2011; Besse et al., 2012). Some the research found chemotherapy drugs of contaminated in water effect to the health risk of humans and animal life. The pregnant women and infants who are

breastfeeding. Likely to be affected most (Collier, 2007; Johnson et al, 2008; Kovalova, 2009; Rowney et al., 2009; Besse et al., 2012). Currently, no standard chemotherapy drugs used to specially control the discharge into an environment (Zwiener, 2007; Rowney et al., 2009).

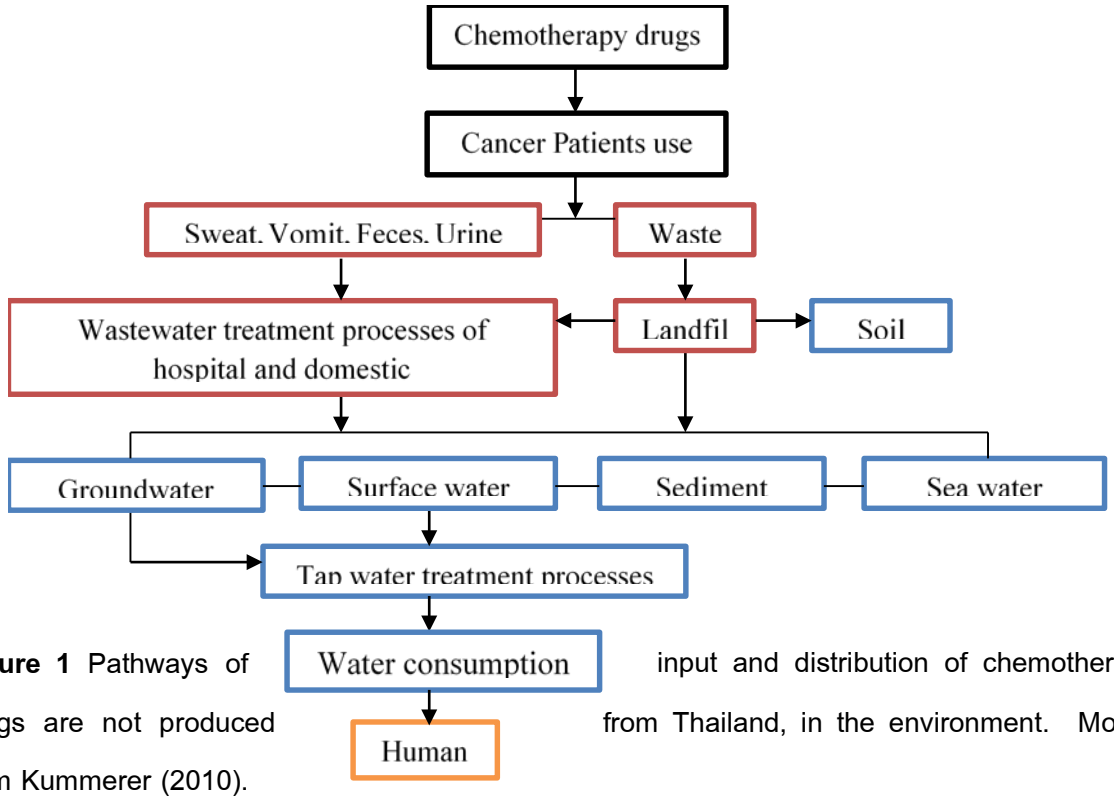


Figure 1 Pathways of input and distribution of chemotherapy drugs are not produced from Thailand, in the environment. Modify from Kummerer (2010).

This research aims to measure the amount of contamination with chemotherapy drugs in the environment, especially in surface water source and water for consumption. The objectives also include measuring the effectiveness for eliminating chemotherapy drugs of the wastewater treatment system and water treatment system and assessing the effects of consuming water from surface water source directly and tap water on people's health. The study area is Bangkok Metropolis, Thailand. Chemotherapy drugs of study are 5-Fluorouracil (5-FU) Cyclophosphamide (CP) and Hydroxyurea (HU), which are commonly used and highly toxic. The expected result of research includes knowledge about the causes of contamination with chemotherapy drugs in the environment, particularly in surface water source and water for consumption. Besides, all sectors become aware of the problem of chemotherapy drugs with the environment, leading to prevention and management of potential effects of chemotherapy drugs.

Objectives of the study

1. To measure the amount of contamination with chemotherapy drugs in surface water, water treatment plants systems and wastewater treatment plants systems.

2. To evaluate the effectiveness of elimination chemotherapy drugs in water treatment plants systems, and wastewater treatment plants systems.

3. To assess the risks to human's health as a result of surface water and consuming water contaminated with chemotherapy drugs in daily life.

Hypothesis of the study

1. The amount of contamination with chemotherapy drugs in water varies in proportion to the number of cancer patients.

2. The water treatment plants system and wastewater treatment plants system using microorganisms tend to be unable to eliminate of chemotherapy drugs entirely.

3. The consumers of water contaminated with chemotherapy drugs in high quantities face high health risks.

Scope of the study

1. Measuring the amount of contamination with chemotherapy drugs, especially 5-Fluorouracil (5-FU), Cyclophosphamide (CP) and Hydroxyurea (HU) in surface water, water treatment plants systems and wastewater treatment plants systems by means of

- Predicted environmental concentrations (PECs): Analysis by PECs model

- Measured environmental concentrations (MECs): Analysis by Solid Phase Extraction method (SPE) coupled with High Performance Liquid Chromatography-tandem Mass Spectrometry (HPLC-MS/MS)

2. The study area is Bangkok, Thailand. The samples and sampling points for research consist of the following.

- Surface water: The samples of water were collected from the lower of Chao Phraya River. The sampling points are based on the Pollution Control Department (PCD).

- Tap water: The samples of water were collected from Samsen water treatment plant

- Hospital wastewater: The samples of water were collected from Siriraj Hospital, Siriraj Piyamaharajkarun Hospital, Ramathibodi Hospital and the National Cancer Institute.

- Domestic wastewater: The samples of water were collected from Din Daeng wastewater treatment plant and Resist Cancer Association of Thailand (convalescent home for cancer patients from the countryside).

Expected results

1. Knowledge about the amount of contamination with chemotherapy drugs in surface water, water treatment plants systems and wastewater treatment plants systems in Bangkok Metropolis.

2. Knowledge about the effectiveness for chemotherapy drug eliminate of water treatment plants systems and wastewater treatment plants systems in Bangkok Metropolis.

3. Awareness of health risks of water consumptions contaminated with chemotherapy drugs in daily life

4. Use as basic information for managing the chemotherapy drug contaminants in surface water, water treatment plants systems and wastewater treatment plants systems in Bangkok Metropolis for raising awareness of the problem, including management and prevention of the problems that may arise from chemotherapy drugs.

Conceptual framework

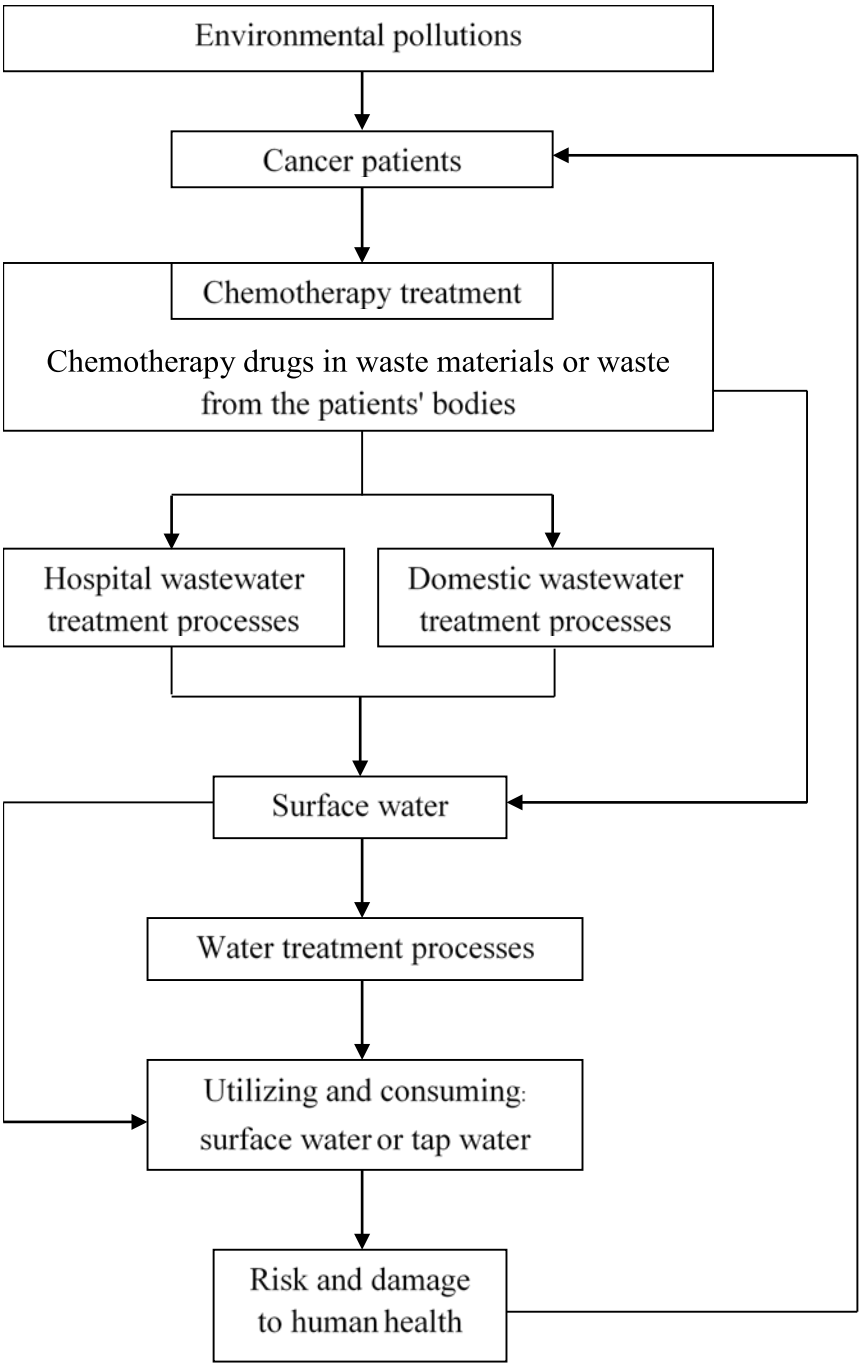


Figure 2 Conceptual framework (followed by Figure 1).

LITERATURE REVIEW

The literature reviews and related researches in this study on contamination of chemotherapy drugs: 5-Fluorouracil (5FU), Cyclophosphamide (CP) and Hydroxyurea (HU) in surface water, water treatment processes and wastewater treatment processes in Bangkok, Thailand are as follows:

Overviews of chemotherapy drugs: 5-FU, CP and HU

Cancer treatment with Chemotherapy mainly uses drugs that act to destroy or prevent the spread of cancer cells. The drugs for cancer treatment can be diversely called, such as an antineoplastic drug, chemotherapy drugs and anticancer drugs, etc. There are many types of drugs for cancer treatment. But this research will discuss 5-FU CP and HU only, which are the commonly used anticancer drugs. General information to be discussed comprises classifications, characteristics, physical and chemical properties, mechanism of action, utilization, environmental fates and toxicity.

Classification

Chemotherapy drugs can be divided into 2 groups, namely Cytotoxic drugs and drugs affecting the immune response (National List of Essential Medicines, 2012). This study focuses on Cytotoxic group only due to being the medications with a mechanism of action affecting DNA of cells. They may cause cell destruction or inability to develop or grow anymore. Various research reports indicated that this group of chemotherapy drugs is toxic to genes with the properties of mutagenic substance and carcinogen too (Carter, 1984; Buerge et al., 2006; O'Keefe, 2011; Besse et al., 2012). Besides, these drugs are commonly used for curing various types of classifications. Table 1 shows classification of Cytotoxic groups.

Table 1 Classification of chemotherapy drugs: Cytotoxic groups (Bureau of Drug Control, 2015)

No.	Cytotoxic groups	Drugs	Note*
1	Alkylating	Busulfan, Chlorambusil, **Cyclophosphamide** and Melphalan	C
		Ifosfamide	D
2	Cytotoxic antibiotics	Bleomycin, Dactinomycin and Doxorubicin hydrochloride	C
		Idarubicin hydrochloride, Mitomycin and Mitoxantrone hydrochloride	D
3	Antimetabolites	Cytarabine, **Fluorouracil**, Mercaptopurine and Methotrexate	C
		Capecitabine, Gemcitabine and Tioguanine	D
4	Vinca alkaloids and etoposide	Etoposide, Vinblastine sulfate and Vincristine sulfate	C
5	Other	Asparaginase, Cisplatin, Carboplatin and **Hydroxyurea**	C
		Paclitaxel and Tretinoin	D
		Docetaxel and Imatinib mesilate	E(2)

Note* C Drugs to used only for the disease specialist or assigned by permitted personal.

D Drugs are multiple function and assigned by permitted personal.

E(2) Drugs special for individual patients and assigned by permitted personal.

Table 1 shows that 5-FU is classified into antimetabolites type, CP is arranged in Alkylating category and HU is in the category of other chemotherapy drugs with the unclear mechanism of action. In Thailand, chemotherapy drugs are specially controlled and are

dangerous medications to be prescribed by specialists in the hospitals only (Bureau of Drug Control, 2015).

Physical and chemical properties of 5-FU CP and HU

5-Fluorouracil or 5-FU or 5-Fluoro-2,4-(1H,3H)-pyrimidinedione is heterocyclic aromatic organic compound. Molecular Formula is $C_4H_3FN_2O_2$. It is a chemotherapy drug in the group of Cytotoxic drugs, type antimetabolite. Its physical structure is similar to pyrimidine DNA and RNA molecules, thus being called pyrimidine antagonist substance. The chemical structure of 5-FU is composed of nitrogen atoms in the ring, hydrogen bond with carbon at position 5 where atoms of fluorine 5-FU are found. The structure is characteristic of crystal and being asymmetric (Zhang et al., 2008; Sigma-aldrich, 2015). The molecular structure, as well as the physical and chemical properties of 5-FU, are shown in Figure 3 and Table 2, respectively.

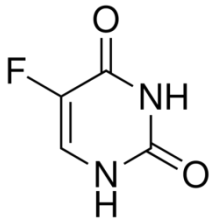


Figure 3 Molecular structure of 5-FU (Zhang et al., 2008).

Cyclophosphamide or CP or (bis(2-chloroethyl) amino)-tetrahydro-2H-1,3,2-oxazaphosphorin-2-oxide monohydrate is organic nitrogen compound or nitrogen mustard. Molecular Formula is $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$. It is a chemotherapy drug in the group of Cytotoxic drugs, type alkylating (Bryniarski, 2011; Sigma-aldrich, 2015). The molecular structure, including the physical and chemical properties of CP, are shown in Figure 4 and Table 2, respectively.

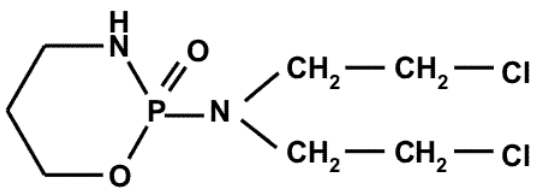


Figure 4 Molecular structure of CP (Bryniarski, 2011).

Hydroxyurea or HU or Hydroxycarbamide is simple organic compound. Molecular Formula is $NH_2CONHOH$. It is hydroxylated derivative of urea molecule with symmetry (Heeney et al., 2004; Sigma-aldrich, 2015). It is a chemotherapy drug in the group of Cytotoxic drugs (Bureau of Drug Control, 2015). The molecular structure, including the physical and chemical properties of HU are shown in Figure 5 and Table 2, respectively.

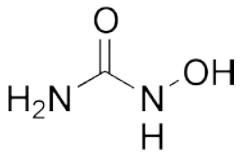


Figure 5 Molecular structure of HU (Heeney et al., 2004).

Table 2 Physical and chemical properties of 5-FU, CP and HU

Properties	Compound name		
	5-FU	**CP**	**HU**
Molecular weight	130.1 [a]	279.1 [a]	76.06 [a]
Vapor Pressure at 25°C (Pa)	2.7 x 10^{-6} [b]	4.5 x 10^{-5} [b]	2.43 x 10^{-3} [b]
solubility in pure water (mg/ml)	<1,000 [c]	10,000-50,000 C[c]	≥100,000 [c]
Log K_{ow}	-0.90 [a]	0.97 [a]	-1.80 [a]
K_H (atm L/mol)	1.66×10^{-10} [b]	7 X10^{-11} [b]	5.4X10^{-11} [b]
pKa	7.6 [d]	6.0 [d]	10.6 [e]
Storage T(°C)	25 [a]	2-8 [a]	2-8 [a]

[a] : Sigma-aldrich (2015); [b] : National Library of Medicine (2015); [c] :

Würsch.(2005) ; [d] : Mahony et al. (2003); [e] : Jong et al. (2003)

From the data presented in Table 2 it can be concluded that both 5-FU, CP and HU dissolve readily in water, but 5-FU is soluble slower than CP and HU.

Mechanism of action, metabolism and elimination from the body of 5-FU, CP and HU

5-FU acts specifically to inhibit the functioning of cells in S phase. The metabolism of 5-FU occurs in tissues. Finally, it inhibits the synthesis of DNA and RNA of cells (Reuters Health Products and Services, 2007). After the patient takes 5-FU into the body, the metabolism occurs at the liver and various tissues and it is eliminated from the body. For the result of metabolism, 5-FU will break down into CO_2, urea and α-fluoro-β alanine. Besides, some of 5-FU does not change. The process of elimination from the body is shown in Figure 6. According to the findings, 5-FU injection of 60-90% into the vein is eliminated from the body within 24 hours and about 20% remains in the form of 5-FU, which is found in the first 6 hours only (Heggie, 1987; Wursch, 2005; Kovalova, 2009; Besse et al., 2012). When taken orally into the body, Capecitabine is metabolized and eliminated from the body in the form of 5-Fluorouracil approximately 0.5% (Judson, 1999; Kovalova, 2009).

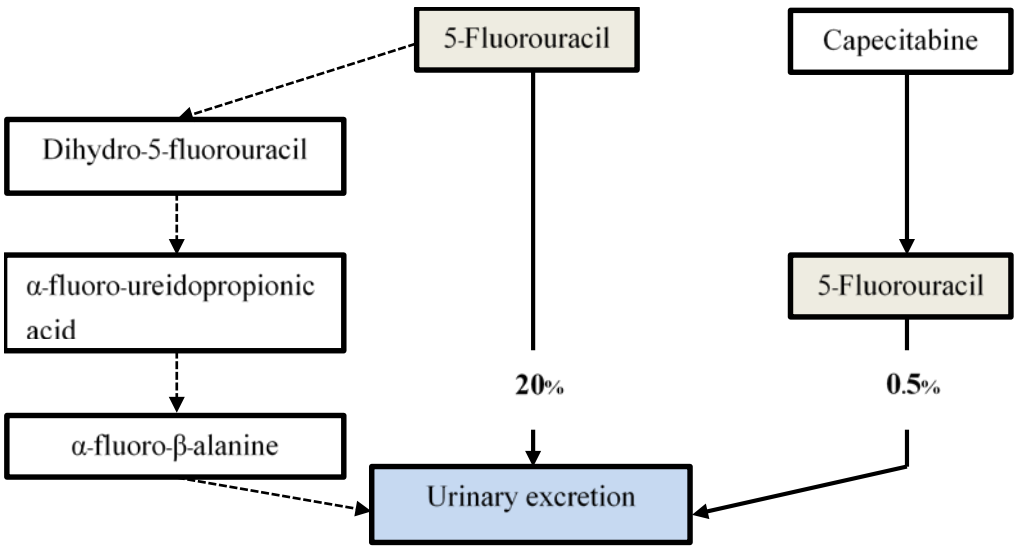


Figure 6 Process of eliminating 5-FU from the body. Modified from Kovalova (2009)

CP is capable of cross-linking with DNA and RNA. This acts to inhibit the protein synthesis of cells and DNA synthesis with no specified stage of reacting with the cells (Sottani et al., 1988). After the patient takes the CP drug into the body, the metabolism occurs at the liver and tissues and it is eliminated from the body. The main results of metabolism are Phosphamide mustard, acrolein, Carboxyphosphamide. Some of CP does not change. The process of elimination from the body is shown in Figure 7. During 3-12 hours after receiving the drug, CP is eliminated from the body immutably approximately 25% (Sottani et al., 1988; Würsch, 2005; Besse et al., 2012).

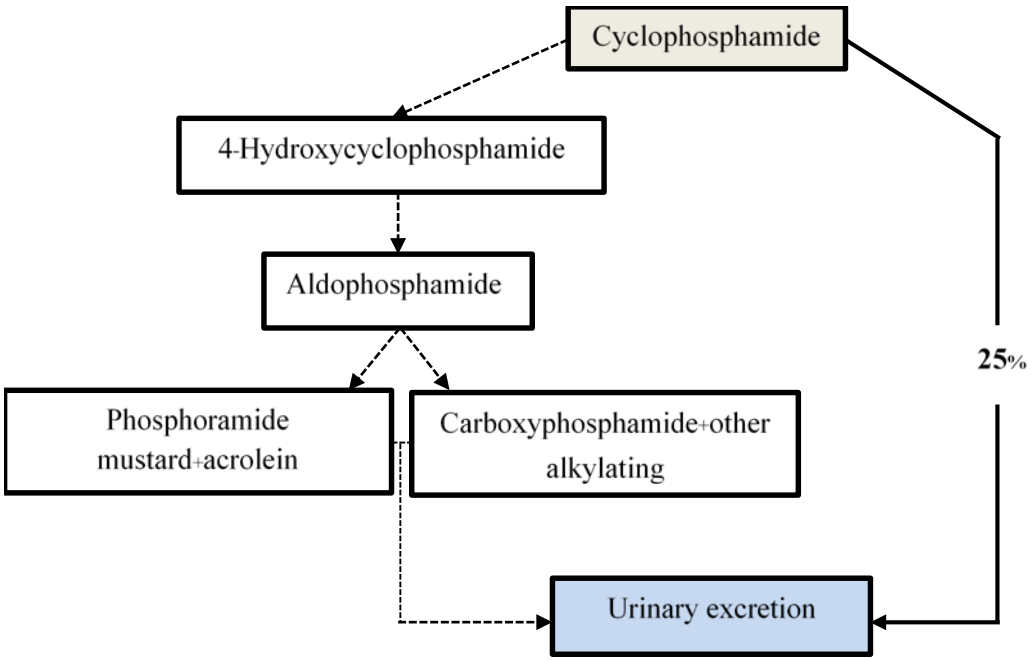


Figure 7 Process of eliminating CP from the body. Modified from Sottani et al. (1988)

HU acts specifically to inhibit the functioning of cells in S phase. No study indicates clearly the method whereby HU can inhibit the synthesis of DNA. But the data state inability to inhibit the synthesis of RNA and protein (Reuters Health Products and Services, 2003). After the patient takes HU drug into the body, the metabolism occurs and it is eliminated from the body. The main results of metabolism are $NOCO_2NH_3$. Some of HU does not change. The process of elimination from the body is shown in Figure 8. After the adult cancer patient

receives HU drug, in 8 hours HU will be eliminated from the body, 50% of elimination does not change (Würsch, 2005; Besse et al., 2012).

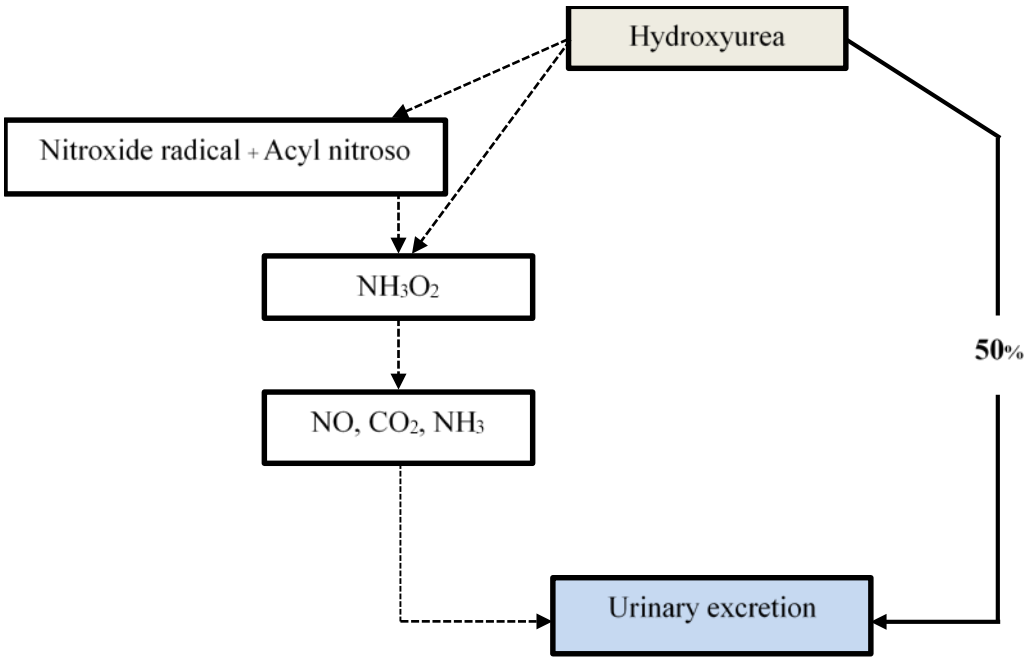


Figure 8 Process of eliminating HU from the body. (Kurose et al., 2006)

Utilization of 5-FU, CP and HU

The cure for cancer with chemotherapy is a treatment method of giving drugs to cancer patients. The drugs act to destroy cancer cells or prevent growth and spread of cancer cells. Each patient must be treated for at least 5 years to be completely cured of the disease. For the use of medications to cure cancer, one type of drug may be given. However, a combination of various drugs is often used for treatment. Each type of cancer uses the different medication. There are many formulas for mixing various drugs. This is left to the discretion of the treating doctor to be most suitable for the disease, stage of disease and patient. Chemotherapy drugs to treat patients include injections for intravenous injection, which are mostly injected by chemotherapy specialists at the licensed hospitals or medical facilities only. The oral drugs or ointment can be orally taken or applied by patients at home (American Cancer Society, 2015).

5-FU is a drug used to cure various cancers: colon cancer (colon), rectum, breast cancer (breast), stomach and pancreas, etc. It can also be used to cure other cancers (Reuters Health Products and Services, 2007).

CP is a drug used to cure various types of cancers: lymphoma, multiple myeloma, leukemia, neuroblastoma, ovarian carcinoma, retinoblastoma, breast cancer etc. It can also be used to cure other diseases apart from cancer such as SLE, etc. Besides, it can be used to cure other cancers (Reuters Health Products and Services, 2007).

HU is a drug used to cure various cancers: melanoma, resistant chronic myelocytic leukemia and ovarian cancer that is recurrent, metastatic or inoperable. It can also be used to cure other cancers (Reuters Health Products and Services, 2007).

Tables 3 and 4 show the amounts of used chemotherapy drugs from 2010 to 2014 of Siriraj hospital, Siriraj Piyamaharajkarun hospital, Ramathibodi hospital and the National Cancer Institute and the amounts of used chemotherapy drugs in 2008 of France, respectively by arrangement in descending order of use. Obviously, the amounts of use are likely to be similar. Thailand included the highest amounts of using Capecitabine, HU, CP and 5-FU arranged in descending order. But when taken into the body, Capecitabine will change to 5-FU and react with cells in the body (Straub, 2010), hence the specific study on 5-FU, CP and HU only.

Table 3 Amounts of used chemotherapy drugs in 2010–2014 in Bangkok

No.	**Drugs**	**Consumption (kg/year)**				
		2010	**2011**	**2012**	**2013**	**2014**
1	**Capecitabine**	205.17	182.53	174.33	205.36	223.68
2	**Hydroxyurea**	-	103.48	107.05	128.65	152.48
3	**Cyclophosphamide**	18.60	19.00	22.05	22.15	28.75
4	**5-fluorouracil**	20.44	20.05	20.48	26.34	41.12
5	**Tegafur**	-	-	5.90	5.50	10.50
6	Cytarabine	1.03	2.58	4.03	5.39	15.61
7	Gemcitabine	-	0.55	1.14	1.27	5.86

(Siriraj hospital, 2015; Siriraj Piyamaharajkarun Hospital, 2015; Ramathibodi hospital, 2015; National cancer institute, 2015)

Table 4 Amounts of used chemotherapy drugs in 2008 in France

No.	Drugs	Consumption (kg/year)
1	**Hydroxycarbamide**	6,838.63
2	**Capecitabine**	5,134.94
3	**Fluorouracil**	1,733.20
4	**Gemcitabine**	379.28
5	**Cyclophosphamide**	305.73
6	Cytarabine	133.59
7	Ifosfamide	103.04
8	Mercaptopurine	94.84

(Besse et al., 2012)

Environmental fate of 5-FU, CP and HU

5-FU is capable of biodegradation as shown in Table 5. The data reviews that the results of various researches are quite different states, so impossibility of clearly concluding it is not persistence substance, but the lighting effects degrade faster than in the dark. In addition to the foregoing, 5-FU also has relatively low bioaccumulation because the bio-concentration factor is approximately 3.6 (Environmental Protection Agency, 2007).

CP is capable of biodegradation as shown in Table 5. The conclusion can be drawn from data that it is not persistence substance, but the lighting effects degrade faster than in the dark, has relatively low bioaccumulation because the bioconcentration factor is about 3 (National Library of Medicine, 2015).

For HU, there is currently no information about various features in the environment.

Table 5 Biodegradation of 5-FU and CP

No.	Drugs	Conc. (ng/L)	Time	Biodegradable	Analysis
1	5-FU dark condition	9 M	28 d	Not degraded	O_2 with oxygen electrode
2	5-FU Zahn-wellens test	175 M	28 d	17%	DOC
3	5-FU OECD test	5 M	2 d	92%	HPLC-DAD
4	5-FU photodegraded	-	16 hr	100%	UV/VIS Spectrophotometer
5	5-FU Aerobic batch	1,000	50 d	50%	GC-MS
6	5-FU Activated sludge	5,000	1 d	>95%	SPE-CE
7	CP photodegraded	-	-	100%	LC-MS/MS
8	CP dark condition	-	80 d	50%	LC-MS/MS
9	CP Activated sludge	-	1 d	Not degraded	LC-MS/MS
10	HU	———	———	No information	———

(2006); 6 : Marnik et al. (2007); 7,8,9 : Buerge et al. (2006).

1, 2 : Kümmerer et al. (1997); 3 : Kiffmeyer et al. (1998); 4 : Tanumihardja (2013); 5 : Yu et al.

Occurrences of chemotherapy drugs: 5-FU, CP and HU in the environment by PECs and MECs

The main routes for human pharmaceuticals to reach the environment are expected to be through the use by patients in hospitals, medical centers or at home, and disposal of unwanted or out-of-date drugs by users. Another way that drugs enter the environment is as waste effluents of the manufacturing processes and from accidental spills during manufacturing or distribution. Following its use, a medical substance was excreted in urine or feces as a mixture of unchanged substance, metabolites or conjugated products. Metabolism partly depends on the type of pharmaceutical compound and the individual patient (Besse et al., 2012; O'Keefe, 2011; Kummerer, 2010; Rowney et al., 2009; Johnson et al., 2008). The substances then enter the sewage system and pass through wastewater treatment before release via sludge, or effluent discharge to surface waters. Sewage treatment plants (STPs) therefore serve as an important pathway of pharmaceutical contaminations. Veterinary products vary considerably from human pharmaceuticals in their pathways to the environment, can follow by Figure 1 (Kummerer, 2010), improve to match Thailand that only import 5-FU, CP and HU from abroad (Bureau of Drug Control, 2015).

In Thailand, 5-FU, CP, and HU were not research on the environment. The relate research in worldwide that detected in the environment as shown in Table 6 and 7 by separate measurement with Measurement Environmental Concentrations (MECs) and Predicted Environmental Concentrations (PECs). HU is no study has been conducted to detect HU in waters or the environment, but some research is discovered contaminated in the air of the chemotherapy office 0.01-0.187 mg/m^3 (Osytek et al., 2007).

Table 6 Related research with Predicted Environmental Concentrations (MECs)

No.	Drug	Country	Source	Analysis	Contamination (ng L)
1	CP	Germany	Hospital WWTP	SPE-GC-MS	143
2	CP	Switzerland	Hospital WWTP	LC-MS/MS	2-10
			Surface water		0.05-017
3	CP	Canada	Domestic WWTP	SPE-LC-MS/MS	3-9
			Surface water		< 3
4	CP	Germany	Landfill effluent	-	97-192
5	CP	Germany	Hospital WWTP	-	< 1-40
			Surface water		0.6-0.7
6	CP	China	Hospital WWTP	UPLC-MS/MS	42
7	5-FU	Switzerland	Hospital WWTP	-	<5-27
8	5-FU	France	Hospital WWTP	GC-MS/MS	0.09-4
9	5-FU	Germany	Domestic WWTP	HPLC-MS/MS	27
10	5-FU	Taiwan	Surface water	LC-MS/MS	100,000-2 M
	CP				25 M 137 M

*effluent of WWTPs

1 : Steger-Hartmann et al. (1997); 2 : Buerge et al. (2006); 3 : Garcia-Ac et al. (2008); 4: Jjemba et al. (2008); 5 : Kümmerer et al. (2010); 6 : Yin et al. (2010); 7 : Weissbrodt el al. (2009); 8 : Mullot et al. (2009); 9 : Kovalova (2009); 10 : Lin et al. (2013).

Table 7 Related research with Measurement Environmental Concentrations (PECs)

No	Drug	Country	Source	Contamination (ng/L)
1.	CP	Switzerland	Hospital WWTP	5.4-100*
			Surface water	ND-0.08*
2	CP	UK	Hospital WWTP	7.02
	5-FU			1.37
3	5-FU	UK	Surface water	5-50*
4	5-FU	Austria	Hospital WWTP	<8,600-1 M
5	5-FU	Germany	Domestic WWTP	1.1
			Surface water	0.03*
			Surface water	0.14
6	5-FU	France	Surface water	39.57
*realistic	CP			6.98 worst case
1 : Buerge et al.	HU			156.13 (2006); 2 :

Rowney et al. (2009); 3 : Johnson et al. (2008); 4 : Mahnik et al. (2007); 5 : Kovalova (2009); 6 : Besse et al. (2012).

Information of the Lower Chao Phraya River, water treatment plants and hospital and domestic wastewater treatment plants

The Lower Chao Phraya River

Chao Phraya River supplies an important metropolitan region. It covers 160,000 km^2, representing 30 percent of the country's total area, and is home to 23 million people. In which, the highly populated areas of Bangkok, a city of more than 11 million people, is located near the Lower Chao Phraya River. Found human communities living along the river too.

The Lower Chao Phraya River (Figure 9) utilize of water resources in the basin extensive both economic activity and consumption in Figure 10. The Lower Chao Phraya River Since the scope of Nonthaburi, Bangkok to Samutprakarn Province total distance of 55 km. Overall, water quality problems, including contamination of coliform bacteria and fecal coliform was high. For domestic wastewater occurs 2,770,121 m^3/day as the amount of BOD loading 221,610 kg/day. For industry, wastewater occurs 267,499 m^3/day as the amount of BOD loading 44,763 kg /day. For agriculture, wastewater occurs 990,022 m^3/day as the quantity of BOD loading 24,520 kg/day. The domestic wastewater that treated can be only partially, because of waterfront community some of the wastewater or waste directly into the river. (Pollution Control Department, 2015)

Figure 9 The Lower Chao Phraya River.

Figure 10 Waterfront communities.

Water treatment plants

Water treatment plants, Places that receive untreated water from natural sources. To improve the quality of raw water that people can safely use. According to, the standards for consumption water. Information Water treatment plants as shown Table 8.

Table 8 Information of Water treatment plants in Bangkok (Metropolitan waterworks authority, 2015)

WTP	**Raw water resources**	**Capacity (m^3/day)**	**Service area**
Samsen	Chao Phraya river (Water supply Canal 31 km)	700,000	Bangkok
Mahasawat	Mae Khlong river (Water supply Canal 107 km)	800,000	Bangkok Nonthaburi
Bangkhen	Chao Phraya river (Water supply Canal 18 km)	3,600,000	Bangkok Nonthaburi
Thonburi	Chao Phraya river (Water supply Canal 27 km)	170,000	Bangkok

The data shows that Samsen Water Treatment Plants. Water treatment plants established as the first located in the heart of Bangkok and local was limited. Water capacity is 700,000 m^3/day by the water supply in Bangkok area. And a point of raw water from the Chao Phraya River situated in the scope of this

Domestic Wastewater treatment plants

Wastewater treatment plants, a place to collected and safe domestic wastewater before discharge into the environment or natural sources. By receiving wastewater from homes in the area, that handles. And efficient removal of contaminants in wastewater. It's based on the

quality of wastewater as well. Information on of Domestic Wastewater treatment plants in Bangkok. Shown in Table 9.

The data shows that Dindaeng wastewater treatment plant, a domestic wastewater treatment plant, supports wastewater for most people in Bangkok was 1.08 million people, as well as, treat wastewater is 350,000 m^3/day. (Department of Drainage and Sewage, 2015)

Table 9 Information on the water quality control plants of large size in Bangkok Metropolis (Department of Drainage and Sewage, 2015)

WWTP	Service Area (km^2)	People	Ability (m^3/day)	Treatment process	Discharge Point
Din Daeng	37	1,080,000	350,000	AS with Nutrients* Removal	Bung Makkasan
Si Phraya	2.7	120,000	30,000	Contact Stabilization AS (CSAS)	Chao Phraya River
Rattanakosin	4.142	76,000	40,000	Two-Stage AS	Khlong Banglumpu
Chong Nonsi	28.5	580,000	200,000	Cyclic AS (CASS)	-
Tungkru	42	177,000	65,000	AS - Vertical Loop Reactor	Khlong Bangjak
Nongkhaem	44	418,000	157,000	AS -Vertical Loop Reactor	Klong Ratburana
Chatuchak	33.40	432,500	150,000	Cyclic AS (CASS)	Klong Bangsue

Hospital Wastewater treatment plants

List of hospitals with cancer chemotherapy in Bangkok (National Cancer Institute of Thailand, 2013).

1. Aekamarin General (Bangkae)

2. Ananta Pattana

3. Bamrasnaradura I.D.

4. Bangkok Adventist

5. Bangkok Cancer Center

6. Bangkok Chest

7. Bangkok General

8. Bangkok Metropolitan Administration General

9. Bangkok Nursing Home

10. Bangkok Pathology-Laboratory

11. Bangmod I

12. Bangmod II

13. Bangmod III

14. Bangna I

15. Bangna II

16. Bangpai

17. Bangpakok I

18. Bangpakok II

19. Bangpakok III

20. Bangpo General

21. Bhumibol Adulyadej

22. Bumrungrad

23. Burachat Chaiyakorn

24. Camilian

25. Cancer Institute, Siriraj

26. Central Chest

27. Central General

28. Chao Phraya

29. Charoenkrung Pracharaks I

30. Chung Jen

31. Deja General

32. Division of Health Statistics Bureau of Health Policy and Plan Office

33. Dr. Panya General

34. Hospital for Tropical Disease

35. Hua Chiew General

36. Institute of Dermatology

37. Institute of Pathology

38. Jetanin

39. Jongjin Foundation

40. Karuna Pitak Cancer

41. Kasemrad Bang Khae

42. Kasemrad Prachachuen

43. King Chulalongkorn

44. Klo ngtun

45. Kluaynamthai

46. Krungdhon I

47. Krungdhon II

48. Ladkrabang

49. Ladprao General

50. Lerdsin

51. Mahachai II

52. Mahaesuk

53. Mayo

54. Metropolitan Electricity

55. Mitraparp Wong Wian Yai

56. Mettapracharak

57. Mongkutwattana

58. Nakorn Thon

59. National Cancer Institute

60. Navaminthra

61. Nopparut Ratchatani

62. Pakkred Vejchakarn

63. Paolo Memorial

64. Petcharavej

65. Piyavate

66. Phaya Thai I

67. Phaya Thai II

68. Phaya Thai III

69. Phra Monkutklao

70. Phranungklao

71. Police General

72. Praram 2

73. Prasat Neurological Institute

74. Priest's

75. Prison of Ministry of Interia

76. Prommitr

77. Queen Sirikit

78. Rajavithi

79. Rajburana

80. Rama 9

81. Ramasuksawat

82. Ramathibodi

83. Ramkhamhaeng

84. Rattarin Hospital

85. Registration Processing

86. Royal Irrigation

87. Saint Louis

88. Salaya

89. Samitivej

90. Samitivej Srinakarin

91. Samrong

92. Siam

93. Sikarin

94. Sin Phaet

95. Somdej Prapinklaow

96. Srisiam

97. Srivichai I

98. Srivichai II

99. Srivichai III

100. St. Carlos Group of Health

101. Sukumvit

102. Surgical Service

103. Taksin

104. Thailand Tobacco

105. Thainakarin

106. Thammasat

107. The Bangkok Christian

108. Theptarin

109. Thon Buri I

110. Thon Buri II

111. Thain Fah

112. Vajira

113. Vejthani

114. Vejsawad

115. Veterans General

116. Vibha – Ram

117. Vibhavadi

118. Vichaiyud

119. Vichanyud

120. Yanhee General

121. Yaowarak

122. Yosae

This research focuses on the large hospitals with available cancer treatment only to collect wastewater samples for analysis. The objectives are to study the amount of chemotherapy drugs 5-FU CP and HU and to investigate the effectiveness of hospital wastewater treatment system too. The said hospitals have a large size according to the number of beds as shown in Table 10. Most wastewater treatment system of public hospitals using Activated Sludge (AS). Meanwhile, a review of relevant research found that this system cannot eliminate 5-FU and CP in effluent at all. Because the part is no photodegradation (Buerge et al., 2006; Marnik et al., 2007).

Table 10 The number of hospital beds

Hospital	Number of Beds
Siriraj	3,000
Siriraj Piyamaharajkarun	2,600
Ramathibodi	1,400
National Cancer Institute	550

(Siriraj hospital, 2015; Siriraj Piyamaharajkarun Hospital, 2015; Ramathibodi hospital, 2015; National cancer institute, 2015)

Analytical method for 5-FU, CP and HU

Predicted Environmental Concentrations

Predicted environmental concentrations or PECs is predicted the concentration of the pollutant residues in the environment by the equation to calculate.

$$PEC_{surface\ water} \quad DOSEai \times Fpen \times Fex \quad WWinhab \times Dilution \qquad (1)$$

where : PEC surface water : Local surface water concentration (mg/L)

DOSEai : Maximum daily dose consume per inhabitant (mg/inhab/day)

Fpen : Percentage of market penetration

Fex : Amount of drug eliminated from the body

WWinhab : Amount of wastewater per inhabitant (L/inhab/day)

Dilution : Dilution factor

If the PEC Surface water value is below 0.01 µg/L (The present action limit is based mainly on acute toxicity data), and no other environmental concerns are apparent, it is assumed that the medicinal product is unlikely to represent a risk for the environment following its prescribed usage in patients (European Medicines Agency, 2006).

Measured Environmental Concentrations

Solid phase extraction (SPE)

The SPE process shown in Figure 11 allows samples which are in solution, free of interfering matrix components, and concentrated enough to permit detected values. The steps are: (1) wash the tube or disk to be in usable condition; (2) add the sample; (3) wash the packing and dry by vacuum; (4) elute compounds of interest.

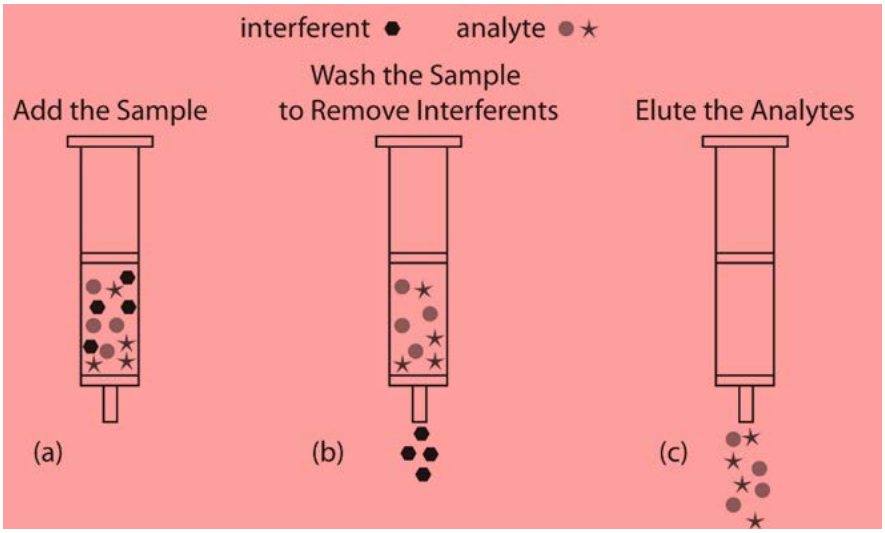


Figure 11 Steps in a typical solid phase extraction. After preconditioning the solid phase cartridge with solvent, (a) the sample is added to the cartridge, (b) the sample is washed to remove interferents, and (c) the analytes eluted (Harvey, 2013).

Liquid Chromatography - Mass Spectrometry (LC/MS)

LC – MS is used in both qualitative and quantitative analysis, and can be applied in several species including substances that are a product of the processes of drugs (metabolite), pesticides, natural products and pollutants. Samples are separated and purified by column. This method is called Liquid Chromatography (LC), and pure substances are measured by Mass Spectrometer. Separation by LC is liquid sample analysis. The sample is pumped into the column which inside is packed with small solid particles acting as the stationary phase. Separation occurs in the column because the individual substances move through the stationary phase at different speeds. Mass Spectrometry (MS) is a technique used to analyze organic and inorganic substances. The samples will be ionized by various methods, such as electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and collision-induced decomposition (CID). Also included are developments involving ionizing with lower energy, such as fast atom bombardment ionization (FAB) and matrix-assisted laser desorption / ionization (MALDI), which can be caused by ions which can be ionizing and the substance does not decompose. It is suitable for substances with large molecules and difficult volatility (Silverstein & Bassler, 1962). Analytical Methods for the analysis of 5-FU, CP and HU as well as phamaceuticals in surface water, water and wastewater are described as follows: Mainly

used Liquid chromatography–mass spectrometry (LC-MS/MS) to determine 5-FU, CP and HU. Table 11-13 shows a summary of analysis method for 5-FU, CP and HU, respectively, in surface water, water and wastewater.

Table 11 Summary of analysis method for 5-FU

Quantification method	Extraction method	Sample	LOD ng/L)	LOQ (ng/L)	Reference
LC-MS/MS	SPE: Oasis HLB	urine	20,000	1,000	(Ndaw et al., 2010)
LC-MS/MS	SPE: Oasis HLB	WW	0.00048	0.0125	(Kovalova, 2009)
LC-MS/MS	SPE: Oasis WCX	Water	1,000	2,500	(Grumbach et al., 2004)
LC-UV	SPE: Amberlyste A-26	Surface water	-	-	(Kiffmeyer et al., 1998)

Table 12

Quantification method	Extraction method	Sample	LOD (ng/L)	LOQ (ng/L)	Reference
LC-MS	SPE: Oasis HLB	WW	-	-	(Gomez-Canela et al., 2012)
LC-MS/MS	SPE: Oasis HLB	WW	0.03	1.1	(Llewel et al., 2011)
LC-MS/MS	SPE: Oasis HLB	urine	270	540	(Marahutta et al., 2011)
LC-MS/MS	-	water	10,000	-	(Wang et al., 2009)
LC-MS/MS	-	WW	0.02	-	(Burge et al., 2006)

Summary of analysis method for CP

Table 13 Summary of analysis method for HU

Quantification method	Extraction method	Sample	LOD (ng/L)	LOQ (ng/L)	Reference
LC-MS/MS	-	blood	500,000	-	(Daniel et al., 2012)
LC-UV	-	air	84.44	-	(Osytek et al., 2007)

Human

Toxicity and Health Risk Assessment

Human Toxicity

Chemotherapy drugs used for cancer treatment have a mechanism of action by destroying cancer cells or preventing cancer cell growth. However, simultaneously they also affect normal cells in the body such as bone marrow cells, hair cells, etc. This is expressed as various side effects (Rahul, 2005). There is the research stating that chemotherapy drugs have the properties of carcinogens that cause Teratogenic and Mutagenic. (Carter, 1984; Buerge et al., 2006; O'Keefe, 2011; Besse et al., 2012).

Toxicity of 5-FU to experimental humans is shown in Table 14. The side effects are shown in Table 15.

Table 14 Toxicity of 5-FU (National Toxicology Program, 2012)

Animals and absorption	Toxicity
LD_{50}(oral,mouse)	115 mg/kg
TD_{Lo}(oral, human)	450 mg/kg/30 days
TD_{Lo}(iv, human)	6 mg/kg/3 days
TD_{Lo}(iv, man)	39 mg/kg/1 day I

LD_{50}: lethal dose 50% kill; TD_{Lo}: lowest published toxic dose; oral: eat; iv: intravenous; I: intermittent

Table 15 Side effects of 5-FU (Chulalongkorn hospital, 2012)

Common Side Effects	Less Common Side Effects
Diarrhea during the first 1-7 days	Nausea
Easy bruising (thrombocytopenia) during the first 7-14 days	Hair loss
Mouth ulcers during the first 5-8 days	Rash
Decreased immunity (Leukopenia) during the first 7-14 days	Sun sensitive skin
Blurred vision, eye irritation	Brittle nails
Darker skin, fingernails, toenails	Palm of hand and foot sole pain
Fatigue	

Toxicity of CP to experimental humans is shown in Table 16. The side effects found in cancer patients receiving CP are shown in Table 17.

Table 16 Toxicity of CP (National Toxicology Program, 2012)

Animals and absorption	Toxicity
TD_{Lo}(oral, human)	45 mg/kg
TD_{Lo}(oral, woman)	20 mg/kg
TD_{Lo}(oral, woman)	16 mg/kg/4 days-I
TD_{Lo}(oral, man)	56 mg/kg/26 days-I
TD_{Lo}(oral, man)	56 mg/kg/4 weeks-I

LD_{50}: lethal dose 50% kill; TD_{Lo}: lowest published toxic dose; oral: eat; I: intermittent

Table 17 Side effects of CP (Chulalongkorn Hospital, 2012)

Common Side Effects	Less Common Side Effects
Decreased immunity (Leukopenia) during the first 7-14 days	Effect on the liver
Anemia (low hemoglobin) during the first 7-14 days	Mouth ulcers
Easy bruising (thrombocytopenia) during the first 7-14 days	Diarrhea
Nausea, vomiting during the first 1-3 days	Dark nails, skin
Poor appetite	Effects on the lungs, heart
Bladder irritation	Some people may feel hot flashes, dizziness, symptoms of nasal congestion, taste perception disorder.
Hair loss	

Toxicity of HU to experimental humans is shown in Table 18. The side effects found in cancer patients receiving HU are shown in Table 19.

Table 18 Toxicity of HU (National Toxicology Program, 2012)

Animals and absorption	Toxicity
TD_{Lo}(oral, human)	80 mg/kg/1 day
TD_{Lo}(iv, woman)	86 mg/kg

~~TD_{Lo}: lowest published toxic dose; oral: eat; iv: intravenous; I: intermittent ; ipr: intraperitone~~al; scu: subcutaneous

Table 19 Side effects of HU (Prince of Songkla University, 2012)

Common Side Effects	Less Common Side Effects
Diarrhea	Mouth ulcers
Drowsiness	Unusual bleeding, blood blotches on skin
Poor appetite	Fingernails and toenails turn black.
Nausea, vomiting	Bloody urine, feces
Bladder irritation	Rash
Cough, hoarseness	Hallucinations, seizures, headache,
Fever	Swollen feet, swollen legs

Health risk Assessment

There is no standard definition for 5-FU, CP and HU in drinking water. Therefore, the health risk assessment of this study based on the principles of US EPA. There are four steps for risk assessment: hazard identification, exposure assessment, dose-response assessment, and risk characterization as shown in Figure 12.

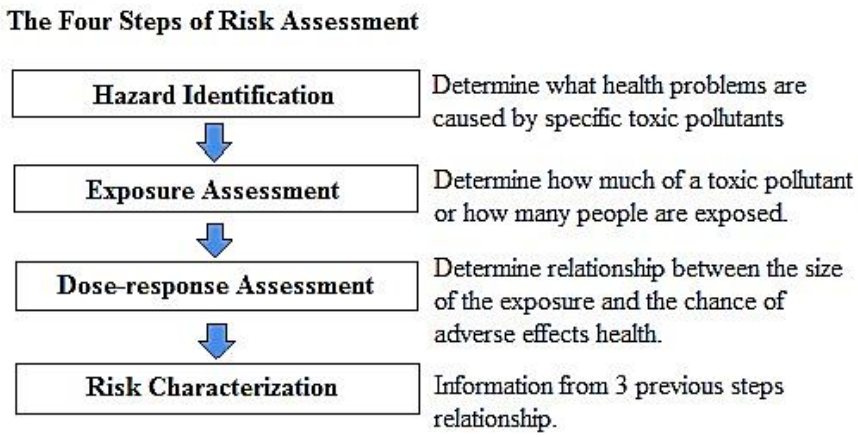


Figure 12 Steps of health risk assessment (US EPA, 2014)

The final step of the quantitative pharmaceuticals risk assessment methodology involves estimation of hazard quotient (HQ) representing ratio of Daily intake (CDI) of pharmaceuticals to acceptable daily intake (ADI) for a particular exposure scenario in equation 2.

$$HQs = DI \; ADI \tag{2}$$

where : HQ Hazard Quotients

DI Daily intake (mg/kg/day)

ADI Accepetable daily intake (mg/kg/day)

If the Hazard Quotient is calculated to be less than 1, then no adverse health effects are expected as a result of exposure. If the Hazard Quotient is greater than 1, then adverse health effects are possible (US EPA, 2014). NOAEL (no-observed-adverse-effect level) of 5-FU, CP and HU as shown in Table 20.

Table 20 NOAEL of 5-FU, CP and HU

Drug	**Study**	**NOAEL mg/kg)**
5-FU	monkey	40 a
CP	human	20 [b]
HU	rat	250 [c]

[a]: (Johnson et al., 2008); [b]: (Schulman et al., 2002); [c]: (Lima et al., 1997)

MATERIALS AND METHODS

Framework of study

This research is the first study in Thailand to evaluate contamination with chemotherapy drugs in aquatic environment. Chemotherapy drugs of this study are 5-fluorouracil (5-FU), cyclophosphamide (CP) and Hydroxyurea (HU). The study is based on analyzing the likely path of contamination from surface water, tap water, domestic water and hospital wastewater in Bangkok Metropolis, Thailand. All water samples were extracted by solid phase extraction (SPE) before analyzed for contamination by High Performance Liquid Chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) and Predicted Environmental Contaminations Model (PECs). The results of 5-FU, CP and HU concentration for surface water and tap water samples were assessed health risk by reference dose of chemotherapy drugs per day. The research was conducted at the water quality laboratory, Faculty of Engineering and the Faculty of Environment and Resource Studies, Mahidol University, Salaya with the overall methodologies are shown in Figure 13.

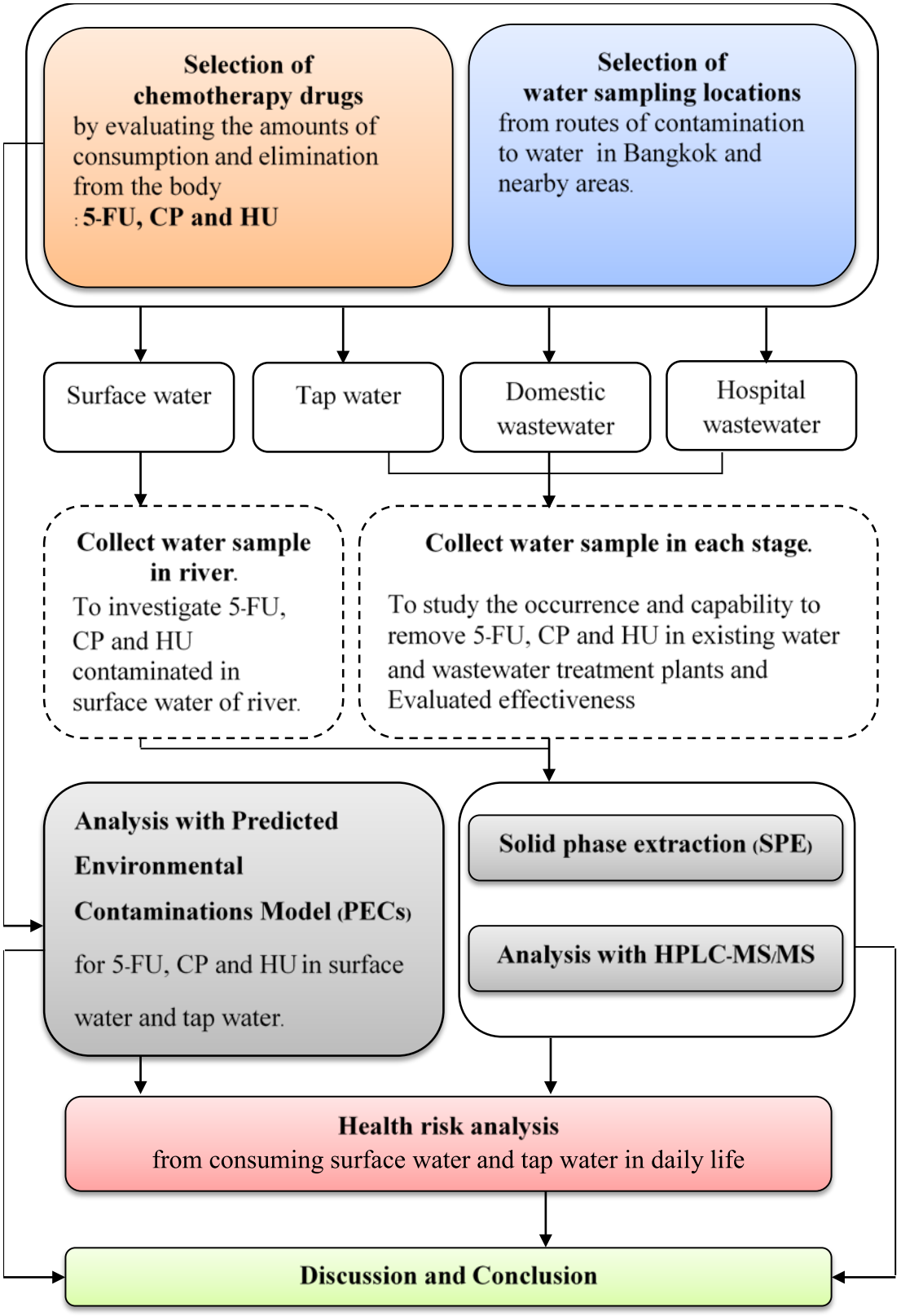


Figure 13 Framework of study.

Chemicals and Equipments

List of all chemicals use

Chemicals use in the extraction processes and HPLC-MS/MS analyses were included the following:

5-Fluorouracil (HPLC grade, ≥99%) from Sigma-Aldrich, USA.

Cyclophosphamide (HPLC grade, ≥98%) from Sigma-Aldrich, USA.

Hydroxyurea (HPLC grade, ≥98%) from Sigma-Aldrich, USA.

Formic acid (HPLC grade, 98-100%) from EMD Millipore Company, Germany.

Methanol (HPLC grade >99.99%) purity from EMD Millipore Company, Germany.

Acetonitrile (HPLC grade >99.8%) purity from EMD Millipore Company, Germany.

Ammonium acetate (99.9999%) purity from Merck KGaA, Germany.

Methanol, (ACS grade >97%) purity from EMD Millipore Company, Germany.

List of equipment use

The details of equipment use in this study are given below:

Liquid chromatography tandem mass spectrometry (LC-MS/MS), Agilent 1200SL HPLC and Agilent 6400 MS/MS, Agilent Technologies, Japan.

Accelerated Solvent Extraction, Dionex ASE 200 model.

Solid phase extraction (SPE)

Nitrogen gas purge

Vacuum manifold

Vortex mixer

PET bottle 1.5 Liters

1 mm G/B glass microfiber filter

Standards solutions preparation

A stock mixed standard solution of 5-FU, CP and HU were prepared at a concentration of 5 mg/L by dissolving 0.125 mg of the chemicals standard in 25 mL methanol. The mixed standard solution of 5-FU, CP and HU were conducted to prepare the calibration standard. Calibration standard was prepared at a concentration range of 1 to 50 µg/L by dilution of the mixed standard solutions with methanol. All standards and fortification solutions were stored in polypropylene bottle and kept in refrigerator at 4 ºC.

Sampling location and Sample Collection

Surface water

Water samples from surface water source were collected from the Lower Chao Phraya River. The sampling points are based on the Pollution Control Department. The points of surface water sampling are shown in Table 21 and Figure 14.

Sampling was kept 2 times in the rainy and dry seasons. The analysis of other parameters of water sample quality includes pH, Temperature (ºC), Turbidity, DO and TOC. Samples were collected by direct grab-sampling from the river. 3 liters PET bottles covered with foil paper were used as sampling containers. The PET bottles were washed with methanol and dried before use. Containers were rinsed 3 times with the sample before collection. After sampling, the samples were kept at 4 °C and brought back to the laboratory and filtrated by using a 1 mm GF/B glass microfiber filter.

Table 21 The sampling points in surface water

Lower Chao Phraya River	Sampling point	
	Latitude	Longitude
RamaVI Bridge, Nontaburi (CH1)	13º48·38"N	100º31·03"E
Bangkok Noi Canal, Bangkok (CH2)	13º45·39"N	100º29·11"E
Mon Canal, Bangkok (CH3)	13º44·52"N	100º29·13"E
Bangkok Yai Canal, Bangkok (CH4)	13º44·28"N	100º29·26"E
Bhudthayodpha Bridge, Bangkok (CH5)	13º44·20"N	100º29·50"E
Bangkok Bridge, Bangkok (CH6)	13º42·03"N	100º29·29"E
Daokanong Canal, Bangkok (CH7)	13º41·41"N	100º29·16"E
Ladluang Canal, Bangkok (CH8)	13º40·02"N	100º32·21"E
Prakhanong Canal, Bangkok (CH9)	13º42·09"N	100º34·50"E
Bangkok Harbour, Bangkok (CH10)	13º41·55"N	100º34·55"E
Sumrong Canal, Samutprakarn (CH11)	13º39·31"N	100º34·17"E
Phrapradaeng, Samutprakarn (CH12)	13º39·28"N	100º32·04"E
Bangplakod Canal, Samutprakarn (CH13)	13º36·48"N	100º33·19"E
Prasamutjadee, Samutprakarn (CH14)	13º36·02"N	100º35·19"E

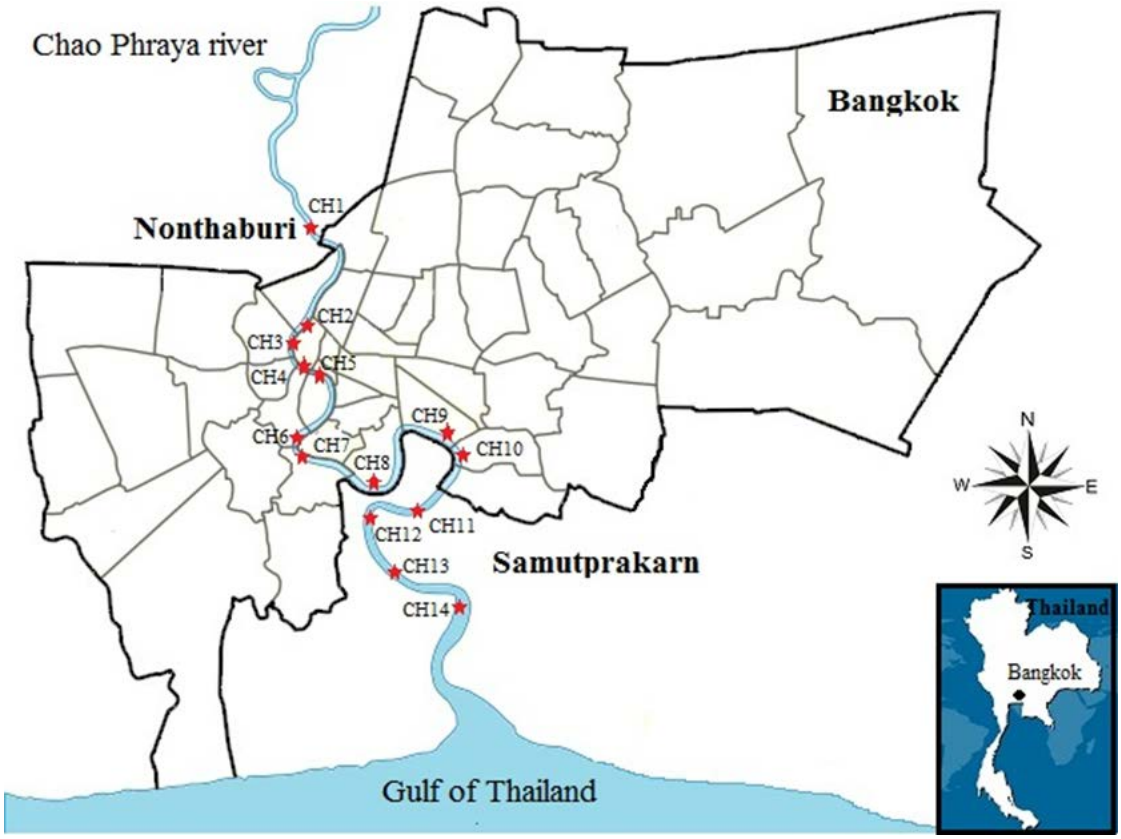


Figure 14 Points of surface water sampling in the lower Chao Phraya River : Sampling points.

Tap water

Water samples were collected from the conventional treatment plant in Bangkok province. Samsen Water Treatment Plant (WTP) was selected because it is the first plant of the Metropolitan Waterworks Authority (MWA) and situated in the central city and very small footprint with a source of raw water from the Chao Phraya River. The production max capacity is 700,000 cubic meters per day, which supply tap water in Bangkok province (Metropolitan waterworks authority, 2015). The treatment process and sampling point in this study shown in Figure 15 and 16.

Sampling was kept in dry seasons. The analysis of other parameters of water sample quality includes pH, Temperature (ºC), Turbidity, DO and TOC. Samples were collected by direct grab-sampling from the process. 3 liters PET bottles covered with foil paper were used as sampling containers. The PET bottles were washed with methanol and dried before use. Containers were rinsed 3 times with the sample before collection. After sampling, the samples

were kept at 4 °C and brought back to the laboratory and filtrated by using a 1 mm GF/B glass microfiber filter.

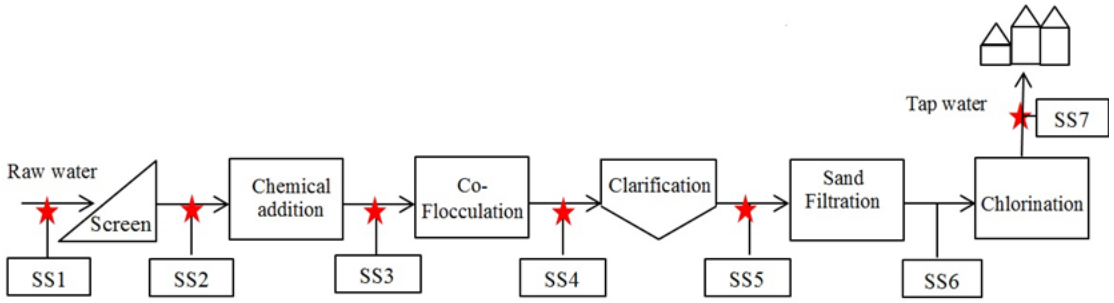


Figure 15 The treatment process and sampling point of Samsen WTP; Sampling points.

Figure 16 Location of Samsen WTP location; Sampling point.

Domestic wastewater

Wastewater samples were collected from 2 locations, namely Resist Cancer Association of Thailand that for patients from the countryside to recuperate and represented household wastewater and Municipal Wastewater Treatment Plant (WWTP) in Bangkok was selected for

this study: Din Daeng Water Treatment Plant with capacity for wastewater treatment of 350,000 cubic meters per day. It is responsible for treating the maximum wastewater when compared with other wastewater treatment plants in Bangkok. The treatment process is activated sludge with nutrients removal (AS) (Department of Drainage and Sewage, 2015). The treatment process and sampling point in this study shown in Figure 17 – 19.

The analysis of other parameters of water sample quality includes pH, Temperature (°C), Turbidity, DO and TOC. Samples were collected by direct grab-sampling from the process. 1.5 liters PET bottles covered with foil paper were used as sampling containers. The PET bottles were washed with methanol and dried before use. Containers were rinsed 3 times with the sample before collection. After sampling, the samples were kept at 4 °C and brought back to the laboratory and filtrated by using a 1 mm GF/B glass microfiber filter.

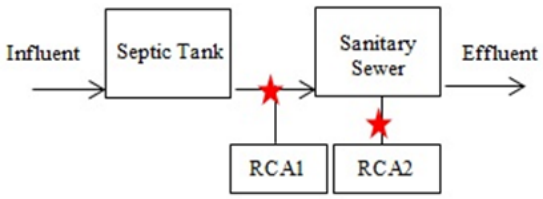


Figure 17 The treatment process and sampling point of Resist Cancer Association; ★ Sampling point.

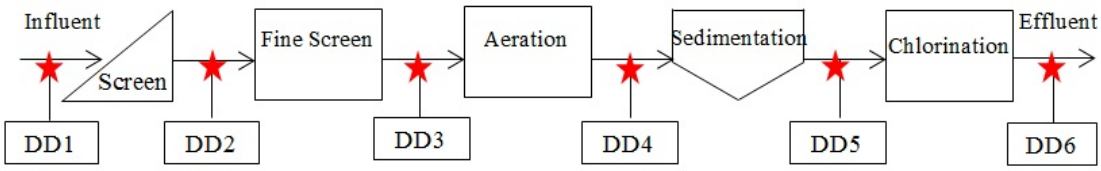


Figure 18 The treatment process and sampling point of DinDaeng WWTP; Sampling points.

★

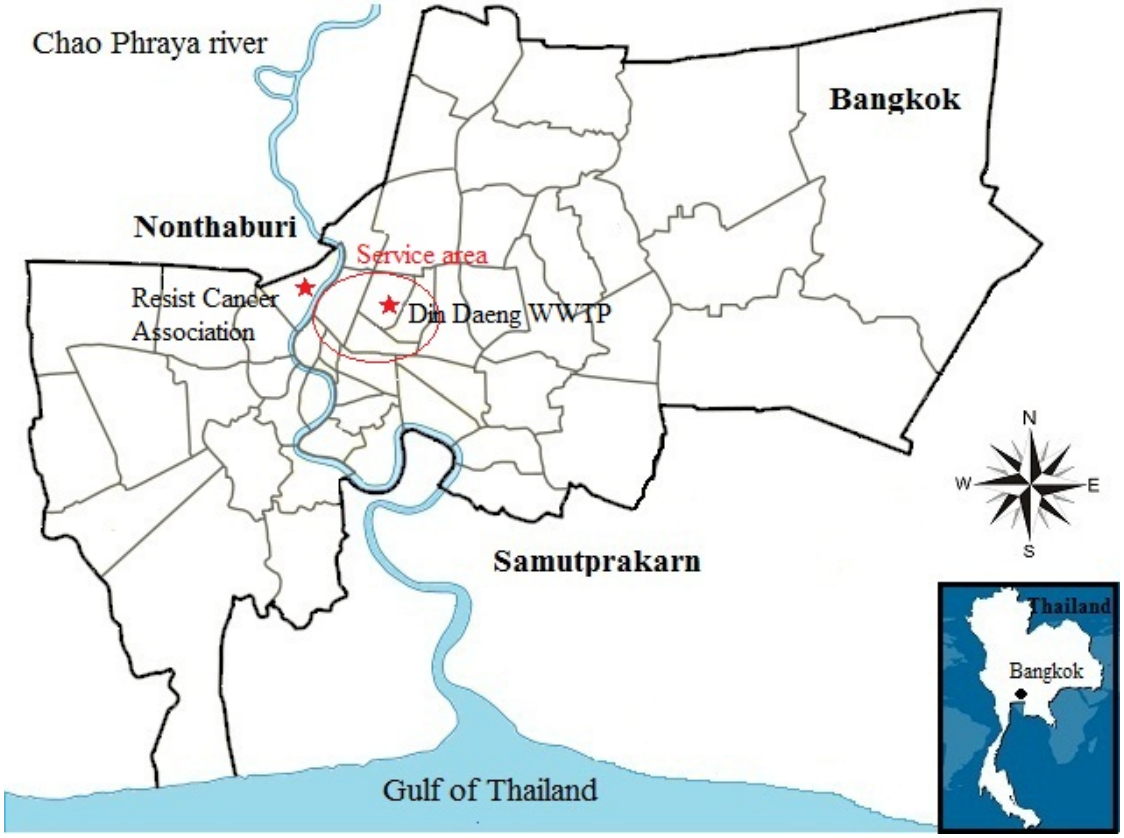


Figure 19 Locations of Domestic wastewater sampling locations; ★ Sampling points.

Hospital wastewater

Hospital wastewater samples were collected from 4 large hospitals with available cancer treatment in Bangkok, including Siriraj Hospital, Siriraj Piyamaharajkarun Hospital, Ramathibodi Hospital and the National Cancer Institute. The wastewater treatment process is typical of activated sludge (AS). The treatment process and sampling point in this study shown in Figure 20 - 24.

The analysis of other parameters of water sample quality includes pH, Temperature (°C), Turbidity, DO and TOC. Samples were collected by direct grab-sampling from the process. 1.5 liters PET bottles covered with foil paper were used as sampling containers. The PET bottles were washed with methanol and dried before use. Containers were rinsed 3 times with the sample before collection. After sampling, the samples were kept at 4 °C and brought back to the laboratory and filtrated by using a 1 mm GF/B glass microfiber filter.

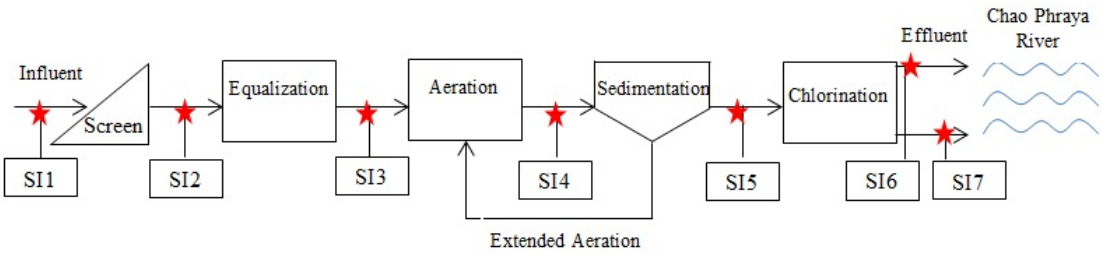


Figure 20 The treatment process and sampling point of Siriraj Hospital; ★ Sampling points.

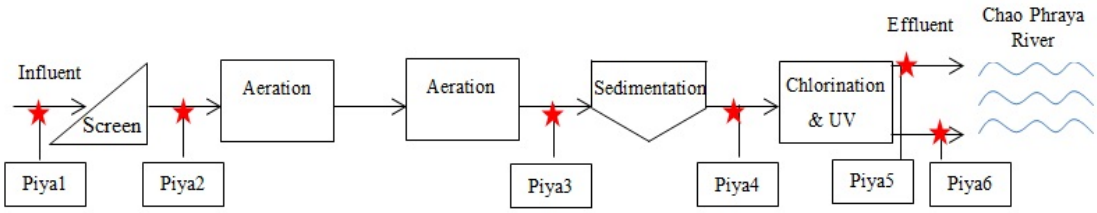


Figure 21 The treatment process and sampling point of Siriraj Piyamaharajkarun Hospital; ★ Sampling points.

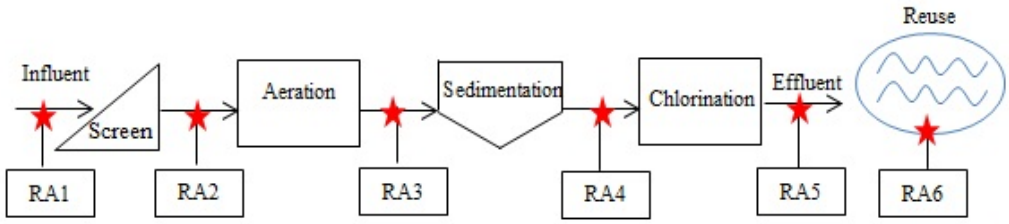


Figure 22 The treatment process and sampling point of Ramathibodi Hospital; ★ Sampling points.

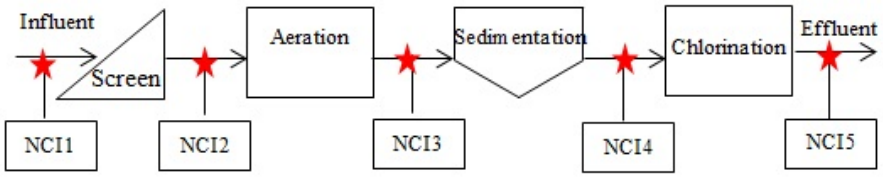


Figure 23 The treatment process and sampling point of National Cancer Institute; ★ Sampling points.

Figure 24 Locations of hospital wastewater sampling locations;★ Sampling points.

Approach to finding the suitable method of analysis

Development of analysis by High Performance Liquid Chromatography coupled with tandem Mass -Spectrometry (HPLC / MS-MS)

This research applies and is based on the method: High Performance Liquid Chromatography coupled with tandem Mass Spectrometry (HPLC / MS-MS) from related research to get suitable conditions for 5-FU, CP and HU.

Development of Solid Phase Extraction (SPE) method

This research applies and is based on the method: Solid phase extraction (SPE) from related research to get Conditions suitable for 5-FU, CP and HU.

Method Validation

Linearity refers to the ability of method to analyze, then get results that are proportional to the concentration of substances analyzed in the given concentration range. The standard solution was added to have at least 5 lowest to highest levels of concentrations. Concentrations were analyzed repeating 3 times for each concentration level. The values obtained were written as line graph and calculated the correlation coefficient, r^2. For acceptance criteria, generally the value of r^2 must be between 0.995 - 1.000 (Irish National Accreditation Board, 2012).

Precision refers to the precision of analysis repeated several times. The difference of results from this repeated analysis is often expressed as SD or Coefficient of Variation, CV shown as %RSD.

$$\%RSD = (SDx100)/mean \quad (3)$$

Accuracy refers to the accuracy of the measurement method. The measured values closest to the actual values show that such analysis has high accuracy. But the measured values far from the actual values indicate that the test has low accuracy.

$$\%recovery = \frac{(Value\ of\ sample\ with\ added\ standard\ solution)-(Value\ of\ sample\ with\ no\ addition)\ x100}{(Value\ of\ concentration\ of\ standard\ solution\ added)} \quad (4)$$

LOD and LOQ (limit of detection and limit of quantization) LOD (limit of detection) means the lowest concentration analyzed in sample which can be measured. LOQ (limit of quantization) refers to the lowest concentration analyzed in sample, which can detect the quantity or report with acceptable accuracy and precision. LOD and LOQ can be determined by measuring sample blank repeatedly, which are used to calculate the mean and SD.

LOD = mean of sample blank + 3 SD.

LOQ = mean of sample blank +10 SD

If the sample blank cannot be determined, the standard solution with lower concentration must be added in sample blank by repeating to find SD.

LOD = 3 SD, LOQ = 10 SD

Analysis of samples

The overall methodological analysis to obtain the results is shown in Figure 25. Water samples were filtered through a glass fiber filter to remove suspended solids. Filtered water was loaded into an Oasis HLB cartridge using a concentration system (SepPak Concentrator). Cartridges were dried under light vacuum and eluted by 1 mL 0.1% formic acid in methanol (HPLC grade), followed by 2 mL 5% ammonium hydroxide solution into the vial. High purity nitrogen gas was used to dry solvent inside the vial at a temperature of 40°C. After the sample in the vial dried completely, methanol was added to 0.5 mL reconstitute before being transferred to HPLC-MS/MS vials. This analysis was performed using an Agilent 1200 SL high performance liquid chromatography interfaced to an Agilent 6400 triple quadrupole mass spectrometer

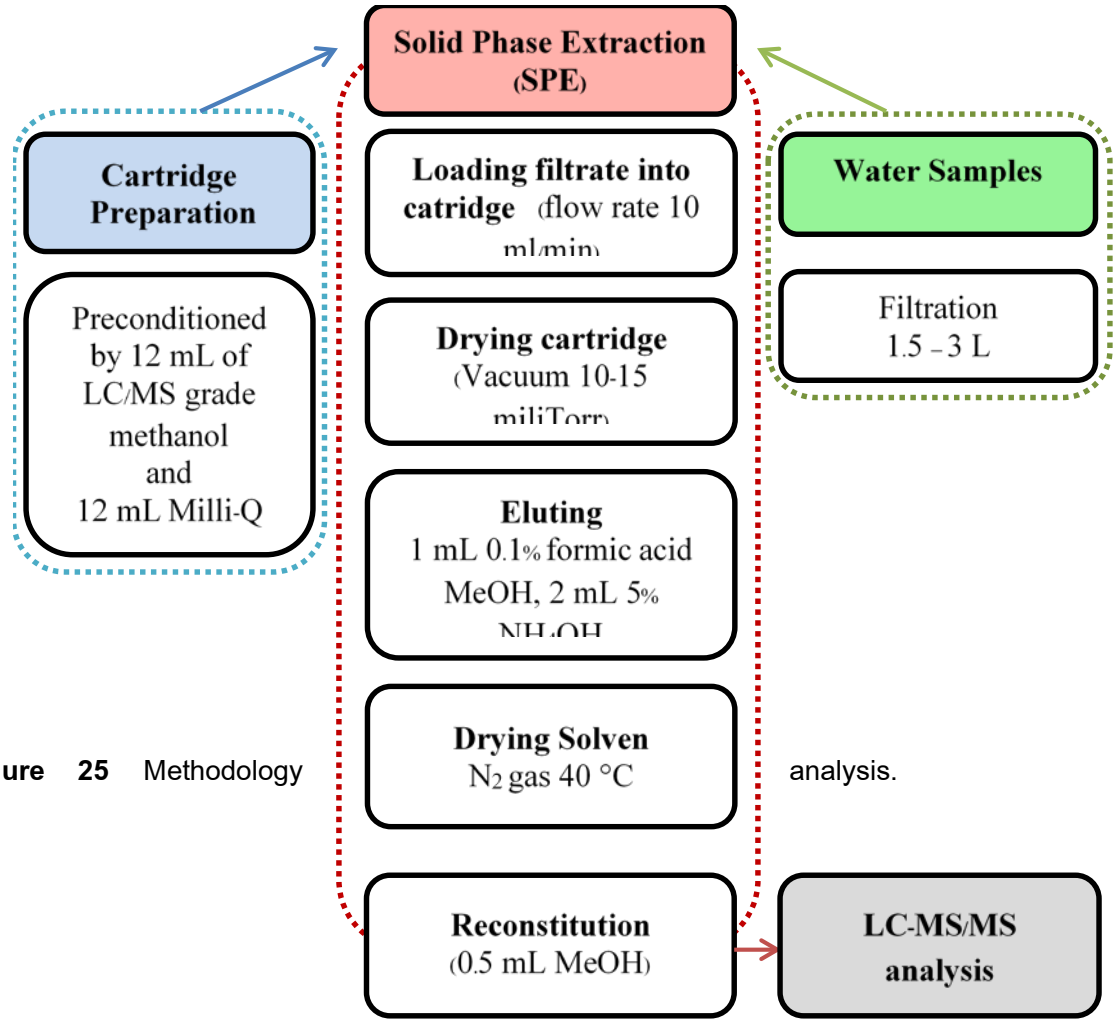


Figure 25 Methodology analysis.

Solid phase extraction (SPE)

The filtered water samples were extracted through an optimized Solid Phase Extraction (SPE) technique to prepare for analysis on LC-MS/MS. The process of SPE is regular extraction methods used to provide the data for 5-FU, CP and HU migrated into liquid. Filtered water were loaded into an Oasis HLB cartridge at a flow rate of 10 mL/min using a concentration system (SepPak Concentrator). Before use, the cartridge was conditioned by passing 12 mL of HPLC grade methanol at a flow rate of 10 ml/min immediately followed by 12 mL Milli-Q water, and the concentrator were cleaned by methanol for 3 minutes and followed by Milli-Q water for 10 minutes respectively. After loading is finished, the cartridges were dried under light vacuum and eluted by 1 mL 0.1% formic acid methanol (HPLC grade) followed by 2 mL 5% ammonium hydroxide (NH_4OH) into the vial. High purity nitrogen gas was used to dry the solvent inside the vial at a temperature of 40°C for 1-2 hours as shown in Figure 26. After the sample in the vial has completely dried, MeOH was added to 0.5 mL reconstitute before transfer to HPLC-MS/MS vials.

Figure 26 Drying solvent.

Analytical procedure

This analysis was performed using Agilent 1200 SL high performance liquid chromatography (HPLC) interfaced to an Agilent 6400 triple quadrupole mass spectrometer (MS/MS). The mobile phase A was composed of 0.1% formic acid in ultrapure water and the mobile phase B was 0.1% formic HPLC grade MeOH with a flow rate of 0.25 mL/min. The substances that were investigated in this study included 5-FU, CP and HU.

Predicted environmental concentrations (PECs)

PECs Model or predicted environmental concentrations is the assessment of contamination with pollutants in the environment in the past, at present to the future by the principles, which is probable and close to reality. The objective is to predict the likely contamination with substances in the future and to find ways to prevent contamination before affecting the health of living things. Calculation is based on the equation (European Medicines Agency, 2006).

The penetration factor (Fpen) represents the proportion of the population being treated daily with a specific drug substance. The default penetration factor was derived from a wide range of individual market penetration factors (European Medicines Agency, 2006), which were calculated as follows:

$$\textbf{Fpen} = \textbf{(Consumption x 100) / (inhab x 365)} \quad (5)$$

where : Fpen = 95 percentile of 0.954 % was calculated as the default penetration factor (Fpen). It is proposed to use an Fpen of 0.01 (1%) in the risk assessment. (%)

Consumption = Consumption of 5-FU, CP and HU (mg/year)

Inhab = Bangkok population = 10,172,000 (non-registered population include) (National Statistical Office, 2014)

365 = Day.

PEC surface water = (DOSEai x Fpen x Fex) / (WWinhab x Dilution) (6)

where : PEC surface water = Local surface water concentration. (mg/L)

DOSEai = Maximum daily dose consume per inhabitant. (mg/inhab/day) (assume: Acceptable dose intake; ADI)

Fpen = Percentage of market penetration.

Fex = Amount of drug eliminated from the body

WWinhab = Amount of wastewater per inhabitant. (300 L/inhab/day) (Pollution Control Department, 2015)

Dilution = Dilution factor (10)

Evaluating of the Removal efficiency of treatment processes

The effectiveness for eliminating chemotherapy drugs of different water treatment systems was evaluated, including hospital wastewater treatment system, sewage treatment system and water treatment system. This can be calculated by the applied equation of Kantachote et al. (2009) as follows.

$$\% \text{ Removal efficiency} = \frac{\text{Concentration of Influent [I]} - \text{Concentration of Effluent [E]} \times 100}{\text{Concentration of Influent [I]}}$$

(7)

* Note: In constant Flow condition

Health Risk Assessment

Exposure assessment

This step is to assess the risk of 5-FU, CP and HU residue exposure via ingestion of drinking water calculated using the Daily intake (DI).

$$DI = \frac{IR \times CW \times EF \times ED}{BW \times AT} \quad (8)$$

where : DI = Daily intake (mg/kg/day).

CW = Concentration of 5-FU, CP and HU (mg/L).

IR = Amount of water for drinking per day (2 L/day).

EF = Exposure frequency (365 days/year).

ED = Exposure duration (70 years).

BW = Body weight (average 70 kg).

AT = Averaging time (for non-carcinogenic; AT = ED x 365 days)

Risk characterization

$$ADI = \frac{NOAEL}{BW \times UF} \quad (9)$$

where : ADI = Acceptable Daily intake (mg/kg/day).

NOEAL = No-observed-adverse-effect level (mg/kg/day)

UF = Uncertainty factor, consists of (1) inter-species variability (2) intra-117 species variability (3) extrapolation from a Low-observable-adverse-effect-level (LOAEL) (4) duration of exposure in toxicological studies (5) quality of data

$$\mathbf{HQs = DI / ADI} \quad (10)$$

This step is an assessment of the toxicity effects of 5-FU, CP and HU ingestion to human health. It was shown as a hazard quotient (HQ), calculated using. If a hazard quotient value is equal or less than 1, the risk is not considered significant to human health.

RESULTS AND DISCUSSTION

This part presents the test results and discussion according to the research purposes, including measurement of the amount of contamination with 5-FU, CP and HU in the water by using HPLC-MS/MS, and evaluating effectiveness for eliminating 5-FU, CP and HU of the WTP and WWTPs process in various agencies and the results of evaluating the risks to human health from consuming tap water or surface water in daily life, the results of the study are as follows:

Analytical Method Performance

For this research, HPLC-MS/MS method was used to analyze the contaminated samples (5-FU, CP and HU) in surface water, WTP and WWTPs in Bangkok to determine the amount of residues and the risks to human health.

From the review of literature found no previous studies that analyze only 5-FU, CP and HU contaminants in the environment. Thus, this research must find a way to analyze and conditions suitable for Solid phase extraction (SPE) and HPLC-MS/MS. Thus, this research

includes the development of method for analyzing 5-FU, CP and HU to be convenient and precise and to give the results more quickly as detailed below.

Development of analysis by HPLC-MS/MS

The important thing to consider for method development is the type of sample to be analyzed, HPLC- MS/MS analysis condition and Standardization. This research applied and developed the method of related research to determine the conditions of HPLC-MS/MS suitable for 5-FU, CP and HU and to develop the effective analysis of the amount of substances to study in water samples.

Also, less time was used to analyze when compared with the previous researches. With exposure to light or heat, the substances of study, 5-FU, CP and HU are degradable. Thus, if the results can be quickly analyzed, the analysis outcomes will be more accurate and precise. The suitable conditions are summarized and shown in Table 22. The analysis was performed using an Agilent 1200 SL high performance liquid chromatography (HPLC, Agilent Technologies) interfaced with an Agilent 6410 triple quadrupole mass spectrometer (MS/MS, Agilent Technologies). Analyst ions were monitored by using multiple reactions monitoring (MRM) mode. The mobile phase was used (A) 0.1% formic acid in Milli-Q water and (B) 0.1% formic acid in methanol HPLC grade (MeOH).

Table 22 Summarizes the suitable conditions of LC-MS/MS for analyzing 5-FU, CP and HU

HPLC		MS	
Instrument	Agilent 1200 SL HPLC	Instrument	Agilent 6410 triple quadrupole mass spectrometer
Column	Guard column Agilent Zorbax Eclipse XDB C_{18} (I.D. 4.6x50 mm, 1.8 µm)	Ionization	Electrospray ionization
	Analytical column Agilent Zorbax Eclipse Plus XDB C_{18} (I.D. 2.1x100 mm, 1.8 µm)	Polarity-Mode	Negative for 5-FU Positive for CP, HU
Mobile Phase	A: 0.1%formic acid Milli-Q water	Nebulizer	N_2 (50 psi
		Gas flow	N_2(10 mL/min)
	B: 0.1% formic acid MeOH		
Gradient application	2 min, A 50%; B = 50%	Gas Temp.	300 ºC
	5 min, A = 0%; B = 100%	Capillary-Voltage	3,500 V
	5.5 min, A = 20%; B = 80%		
	9 min, A = 20%; B = 80%		
	11 min, A = 5 0%; B = 50%	MRM mode	
	15 min, A = 50%; B = 50%	5-FU:	129.0 > 42.0(m/z)
Flow rate	0.25 mL/min	CP:	261.0 > 140.0(m/z)
Column-Temp	40 ºC	HU:	77.1 > 44.0 (m/z)
Injection-Volume	10 µL		

Figure 27 shows Chromatograms of 5-FU, CP and HU from developing conditions of HPLC-MS/MS. Apparently, the analysis of 1 sample can be completed within 12 minutes when compared with the previous research that analyzed one by one and took longer time as shown in Table 23. For details of developing Conditions of CP and HU, electrospray ionization (ESI) was used as + and - for 5-FU. The important part is that retention times of 5-FU and HU overlapping made it impossible to analyze. Thus, the problem could be solved by switching ESI

modes and injecting the sample 2 times. For the first sample injection, the device could analyze HU and CP in ESI + mode, respectively.

After that, the device would switch mode to ESI -. For the second sample injection, the device could analyze the amount of 5-FU because retention times did not overlap anymore.

Table 23 Compare the time spent on 5-FU, CP and HU analysis by HPLC-MS/MS

Related researches	Compound	Retention time (min)
This research	5-FU, CP and HU	12
Mahnik et al., 2006	5-FU	4
Kovalova, 2009	5-FU	5.91
Burge et al., 2006	CP	6.3
Llewellyn et al., 2011	CP	5.8

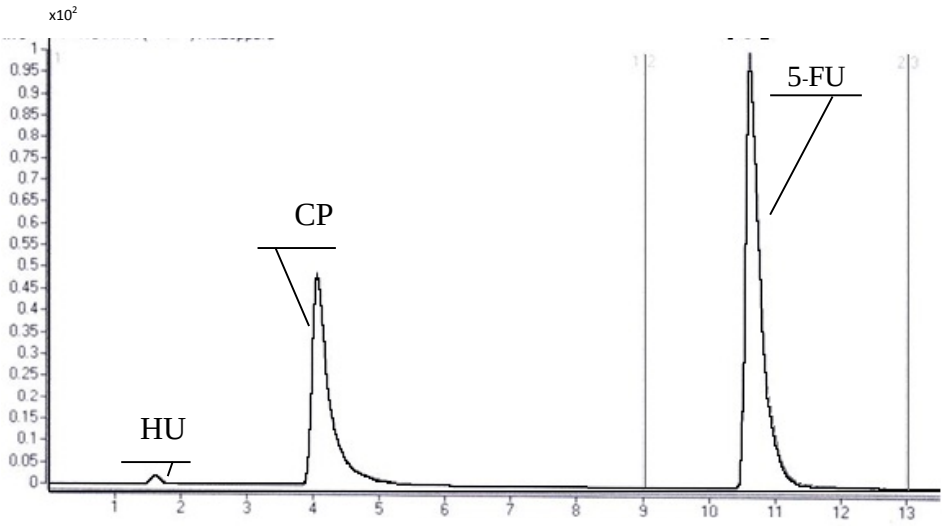


Figure 27 Chromatograms of 5-FU, CP and HU Analysis by LC- MS/MS.

Development of Solid Phase Extraction (SPE)

Preparation of water samples by the Solid Phase Extraction (SPE) method to be suitable before analysis with HPLC-MS/MS. The procedure was developed from other related researches. The objective is to select the method based on the analysis effectiveness from the procedure of previous research, which was used to further develop the approach. The development of procedure for preparing the samples contaminated with 5-FU, CP and HU easily and quickly. The effectiveness of analysis method was tested and found to be acceptable. With exposure to light or heat, the substances of study, i.e. 5-FU, CP and HU are degradable. So if the results can be quickly analyzed, the outcomes will be more accurate and precise.

Spike STD. 5-FU, CP and HU concentrations of 25 and 50 ng/L in Milli-Q water, repeating 2 times for each samples. % Recovery analyzed is equal to 95-118% (see. Appendix D). This is consistent with the standard due to being in the range 70-120%, which is acceptable (Irish National Accreditation Board, 2012).

SPE method appropriate for this research was available. Cartridge Oasis® HLB (Hydrophilic-Lipophilic-Balanced) was used. Cartridge was prepared with 12 mL of HPLC-MS/MS grade methanol and 12 mL Milli-Q water, Eluted with 1 mL 0.1% formic in LC/MS grade methanol follow 2 mL 5% ammonium hydroxide and Reconstituted with 0.5 mL LC/MS grade methanol.

Method Validation

Due to development, adjustment from reference methods, so the method of study must be validated for analyzing the samples to be reliable, acceptable. This also helps to know the features, conditions or limitations of that analysis method (Irish National Accreditation Board, 2012).

Figure 28 shows Linearity graph by analyzing the standard substances: 5-FU, CP and HU in concentrations of 1, 5, 10, 25 and 50 ng/L to determine the relationship with Peak Area from analysis by HPLC/MS-MS. The findings indicate that Linearity graphs of 5-FU, CP and HU have correlation coefficient or R^2 in the range of 0.997 - 0.999. This meets the standard between 0.995 - 1.000 (Irish National Accreditation Board, 2012), which is acceptable.

The determination of Precision of this method is based on analyzing the samples repeatedly 5 times in the same conditions (Repeatability). The standard substances: 5-FU, CP

and HU were analyzed at concentrations of 25 ng/L and 50 ng/L. The results of analyzing precision of this method are shown as %RSD in Table 24. Obviously, the precision of this method is very high because the value of %RSD is in the range of 0.1 - 1.3. According to the standard, %RSD must not exceed 10. (Irish National Accreditation Board, 2012)

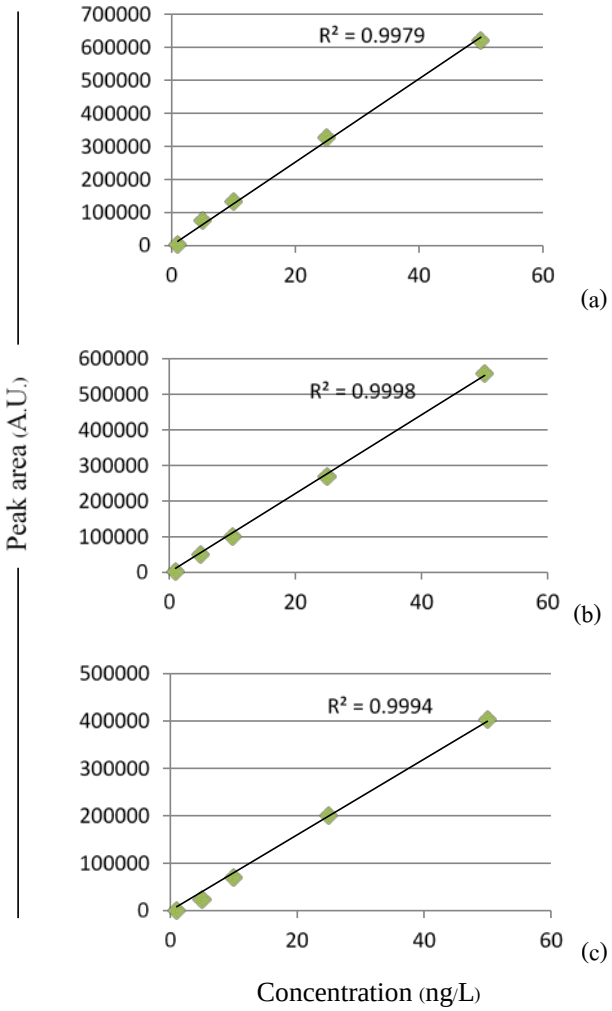


Figure 28 The calibration curves of (a) 5-FU, (b) CP and (c) HU.

Table 24 Precision of the method from %RSD

Drug	**Low concentration (25 ng L)**		**High concentration (50 ng L)**	
n=5	**Peak area (Mean)**	**%RSD**	**Peak area (Mean)**	**%RSD**
5-FU	325,142.44	1.1	692,502.71	1.3
CP	262,055.18	0.2	600,121.58	0.1
HU	239,107.60	0.4	396,582.30	0.6

The accuracy of this analysis method is determined by finding the accuracy of spike standard substances: 5-FU, CP and HU in samples of surface water Milli-Q water and wastewater at concentrations of 25 and 50 ng/L. The results of analyzing the accuracy of this method are shown as %Recovery in Table 25. Obviously, this method has the acceptable accuracy due to %Recovery in the range of 77-113%, which meets the acceptable standard, i.e. 70-120% (Irish National Accreditation Board, 2012).

Table 25 Accuracy of the method from %Recovery

Drug	**% Recovery**					
n=5	**Surface water**		**Wastewater**		**Milli-Q water**	
	25 ng/L	**50 ng/L**	**25 ng/L**	**50 ng/L**	**25ng/L**	**50 ng/L**
5-FU	79±4	87±7	77±6	103±4	92±4	112±6
CP	96±6	97±7	108±4	92±5	98±6	86±6
HU	104±5	103±5	83±4	95±11	113±3	75±2

The determination of LOD and LOQ (Limit of detection and Limit of quantization) of this method is based on calculating S/N (Signal to noise) (Irish National Accreditation Board, 2012) with the results as shown in Table 26. Obviously, 5-FU could be analyzed with LOD and LOQ equaling 0.179 and 0.597 ng/L, respectively. CP has LOD and LOQ equaling 0.442 and 1.475 ng/L, respectively and HU has LOD and LOQ equaling 0.270 and 0.901 ng/L, respectively. When compared with other researches, obviously contamination with substances can be

analyzed at low concentrations in the range found in the environment such as surface water, etc.

Table 26 Shows the values of LOD and LOQ of the analysis method.

Drug	LOD (ng/L)	LOQ (ng/L)
5-FU	0.179	0.597
CP	0.442	1.475
HU	0.270	0.901

Contamination of 5-FU, CP and HU in Surface water

Natural water source is important in Bangkok: Chao Phraya River is the source of water for consumption. Communities are living along the river massively some households excreted of waste dumping directly to the river. Meanwhile, it has a water activity of children at riverbank as well and the boat. Through traffic around the lower Chao Phraya River the whole day. Water quality of the Lower Chao Phraya river is considered low. Determination of contamination 5-Fu CP and HU in the lower Chao Phraya River the first samples were collected during the summer of 2013 and again during the rainy season of 2014, sampling points from a total of 14 points. Samples were collected along the route of the lower Chao Phraya River according to the sampling point of Pollution Control Department (PCD). The measurement of the amount of contamination of the 5-FU CP and HU as follow:

Table 27 5-FU, CP and HU concentrations in the Lower Chao Phraya River

No.	Name	Site	Dis-tance (km)	Dry season n=2			Wet season n=2		
				5-FU (ng/L)	CP (ng/L)	HU (ng/L)	5-FU (ng/L)	CP (ng/L)	HU (ng/L)
1	CH1	Nontaburi	0.00	0.49	1.29	0.81	0.39	1.27	0.78
2	CH2	Bangkok	7.50	0.79	1.21	0.74			
3	CH3	Bangkok	8.90	1.35	1.24	1.09			
4	CH4	Bangkok	9.65	1.29	1.49	1.20			
5	CH5	Bangkok	10.35	2.08	2.30	1.19	1.94	2.37	1.09
6	CH6	Bangkok	16.60	2.05	3.03	1.08	1.68	2.24	0.92
7	CH7	Bangkok	17.24	1.53	2.48	1.25			
8	CH8	Bangkok	23.60	1.48	2.76	1.28			
9	CH9	Bangkok	31.20	1.14	1.90	1.48			
10	CH10	Bangkok	32.77	1.27	1.60	1.32	1.02	1.59	1.14
11	CH11	Samutprakarn	36.80	1.09	1.34	1.12			
12	CH12	Samutprakarn	40.74	1.20	1.59	1.20	0.98	1.47	0.99
13	CH13	Samutprakarn	46.20	1.12	1.63	0.90			
14	CH14	Samutprakarn	51.80	1.07	1.26	1.06	<LOD	1.41	0.46
			Average	1.28	1.79	1.12	1.20	1.72	0.90

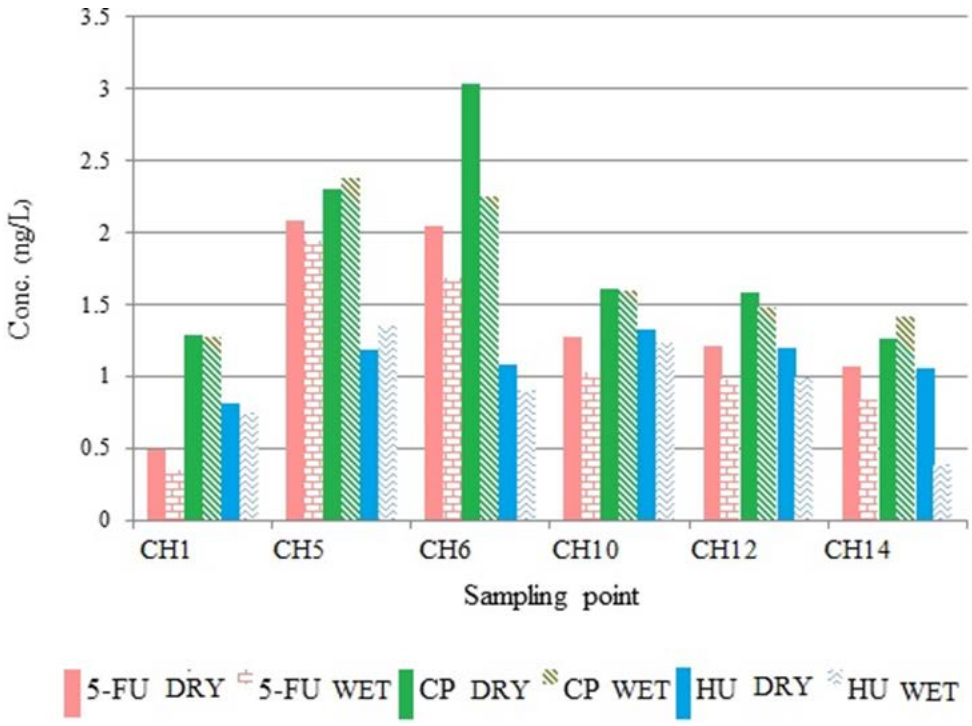


Figure 29 Contamination of 5-FU, CP and HU in Chao Phraya River during the dry season compared with the wet season.

Table 27 has shown the amount of contamination of 5-FU, CP and HU in the Lower Chao Phraya River is the first sampling in the dry season found that 5-FU is highest at Bhudthayodpha bridge, and Bangkok bridge was 2.08 and 2.05 ng/L, respectively, and the least in Nonthaburi was 0.49 ng/L and during the dry season the amount of 5-FU in the lower Chao Phraya River average of 1.28 ng/L. For CP is highest at Bangkok bridge was 3.03 ng/L, and the least in Bangkoknoi canal was 1.21 ng/L and during the dry season the amount of CP in the lower Chao Phraya River average of 1.79 ng/L. For HU is highest at Prakhanong canal was 1.48 ng/L, and the least in Bangkoknoi canal was 0.74 ng/L and during the dry season the amount of HU in the lower Chao Phraya River average of 1.12 ng/L.

In the second measurement is sampling during the rainy season. Due to limitations in the sampling was to keep samples at the river only and found 5-FU is highest around that same point earlier was 1.94 ng/L and the least in Nonthaburi at the same point as well was 0.39 ng/L during the rainy season the amount of 5-FU in the lower Chao Phraya River average of 1.20 ng/L. For CP is highest at Bhudthayodpha bridge was 2.37 ng/L and the least in Nonthaburi was 1.27 ng/L during the rainy season the amount of CP in the lower Chao Phraya River average of 1.72 ng/L. For HU is highest at Bangkok harbor was 1.14 ng/L and the least in Samutprakarn was 0.46 ng/L during the rainy season the amount of HU in the lower Chao Phraya River average of 0.90 ng/L.

From Figure 29 is a graph comparing of 5-FU, CP and HU in the Lower Chao Phraya River in the dry season with the rainy season, which will see the volume determined during the dry season, slightly higher than during the rainy season due to the rainy season the water content may dilute more during the dry season. It shows that seasonal effects to the water quantity in the surface water have increased as a result of the dilution of 5-FU, CP and HU in the environment significantly.

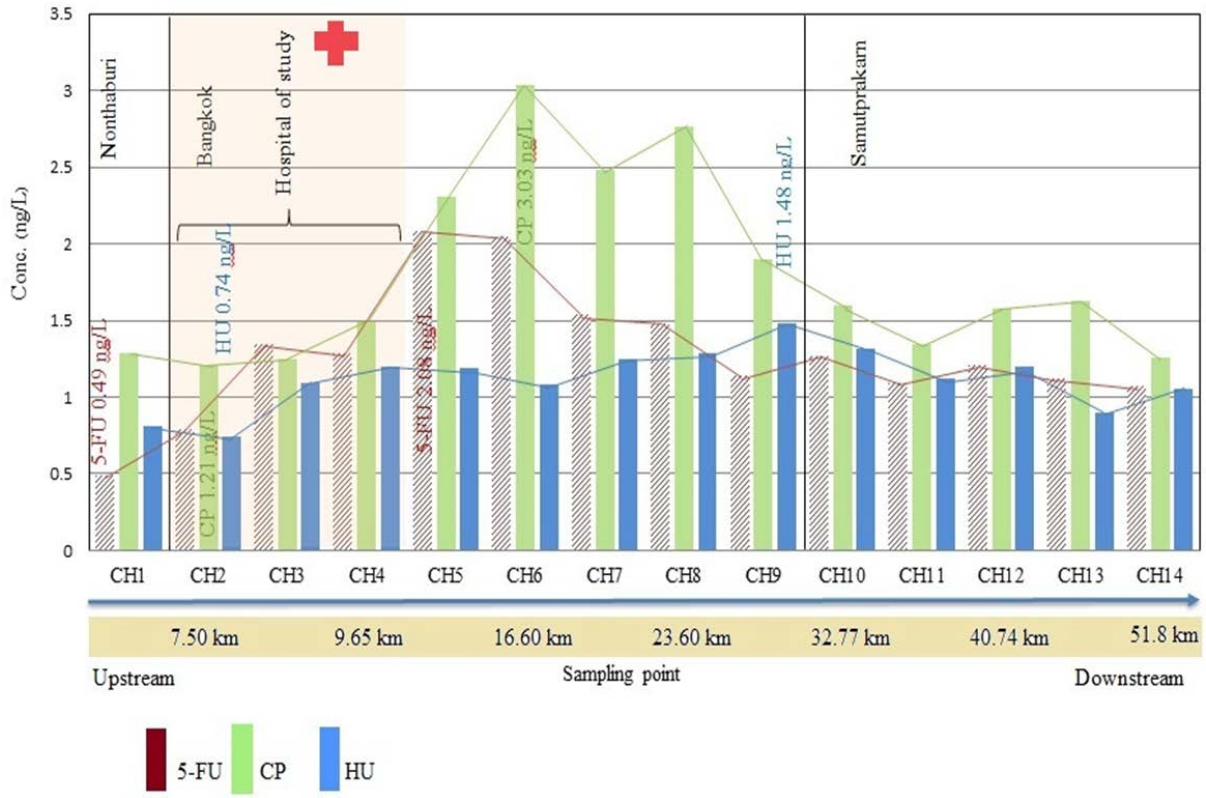


Figure 30 Contamination of 5-FU, CP and HU in Chao Phraya River during the dry season with location.

Figure 30 and 31 shows the amount of contamination of 5-FU, CP and HU in the Lower Chao Phraya River, a distance of 51.8 km measured in the dry season. By comparing the measured with distance to the sampling point. The overall graph, apparently contamination of CP and 5-FU is found in the middle area of the Lower Chao Phraya River as Bhudthayodpha bridge to Daokanong canal. While the area has a large hospital is detected in decreasing amounts because contaminants may have been blowing out by the pollutant source of approximately 6 km to accumulate in the field of the detector. Due to the chemical properties of 5-FU, CP and HU is relatively stable in water but sensitive to the sun. Meanwhile according to the general appearance of the river is sunny but the water in the river is quite turbid and much sediment, hence it may be possible to detect 5-FU, CP and HU. While the lower Chao Phraya River in Nonthaburi, the amount of contamination of 5-FU, CP and HU in a few because it is outside the city so not densely populated, as well as a large hospital for cancer treatment is not found in this area. In the area of Samut Prakan End of the Chao Phraya River, the amount of contamination of 5-FU CP and HU tends to decrease from the middle of the river, probably due

to the width of the river is wider and diluted with water. There is no large hospital cancer treatment as well.

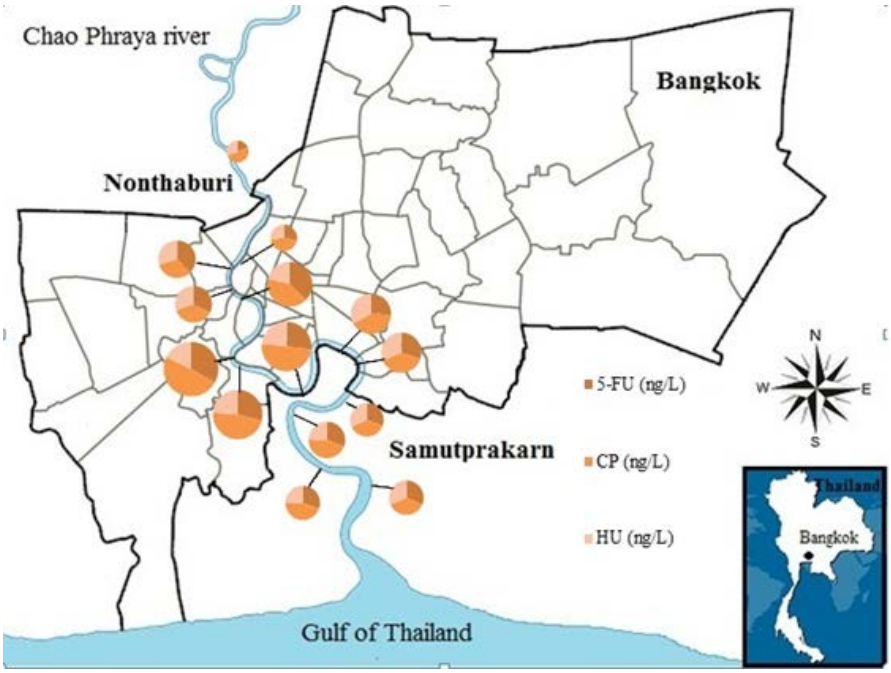


Figure 31 Contamination of 5-FU, CP and HU in Chao Phraya River during the dry season with Bangkok map.

Table 28 5-FU concentrations in surface water by PECs

Year	5-FU					
	Consumption (mg/year)	Fpen %	ADI (mg/p/day)	Fex	PEC (mg/L)	PEC (ng/L)
2010	20,440,000	0.550530869	0.39998	0.2	0.000015	14.68
2011	20,050,000	0.540026611			0.000014	14.40
2012	20,480,000	0.551608229			0.000015	14.71
2013	26,340,000	0.709441443			0.000019	18.92
2014	41,120,000	1.107525897			0.000030	29.53

Table 29 CP concentrations in surface water by PECs

Table 30 HU concentrations in surface water by PECs

Year	HU					
	Consumption (mg/year)	Fpen %	ADI (mg/p/day)	Fex	PEC (mg/L)	PEC (ng/L)
2010	-	-			-	-
2011	103,480,000	2.787129860	2.49998	0.50	0.001161	1,161.29
2012	107,050,000	2.883284224			0.001201	1,201.36
2013	128,650,000	3.465058528			0.001444	1,443.76
2014	152,480,000	4.106895642			0.001711	1,711.19
	(mg/year)					
2010	18,660,000	0.500972317	0.19999	0.25	0.000008	8.35
2011	19,000,000	0.511745915			0.000009	8.53
2012	22,050,000	0.593894602			0.000010	9.90
2013	22,150,000	0.596588001			0.000010	9.94
2014	28,750,000	0.774352372			0.000013	12.9

Note: Fpen = (Consumptionx100)/(inhabx365); inhab = populations in Bangkok (10,172,000 people) PEC surface water = (ADIxFpenxFex)/(WWinhabxDilution); WWinhab = 300 L/people/day; Dilution = 10; People = 70 kg.

From Table 28-30 showed PECs of 5-FU CP and HU in the years of 2010-2014 in natural waters are adjusted by the amount of 5-FU CP and HU consumption per capita per year in Bangkok. And the percentage of the excreted from the body and ADI information. Calculated results found that the trend of contamination and 5-FU, CP and HU in natural water resources will increase each year varies according to consumption shown as Figure 32. Corresponds to the number of cancer patients is increasing steadily each year. It was found that in 2010-2014 the amount of contamination and 5-FU, CP and HU increased annually by the year 2014 showed the stain of 29.53 ng/L, 12.9 ng/L and 1,711.19 ng/L, respectively. As is evident that compared with real volume measurement see that the predicted values are much higher. The calculations using data in worst case scenario other the case one can see that from the measurements showed that HU has concentrated a little more substance in the three compared

with the results from the calculation finds. HU is the most valuable because of high consumption. There are constants to eliminate from the body up to 50% is calculated to be worth that are overdone beyond that. Therefore, might need to be revised, the calculation for the study of HU. HU residues in the environment, the current research not found that monitoring the residues in the environment, because it is less toxic than 5-FU and CP.

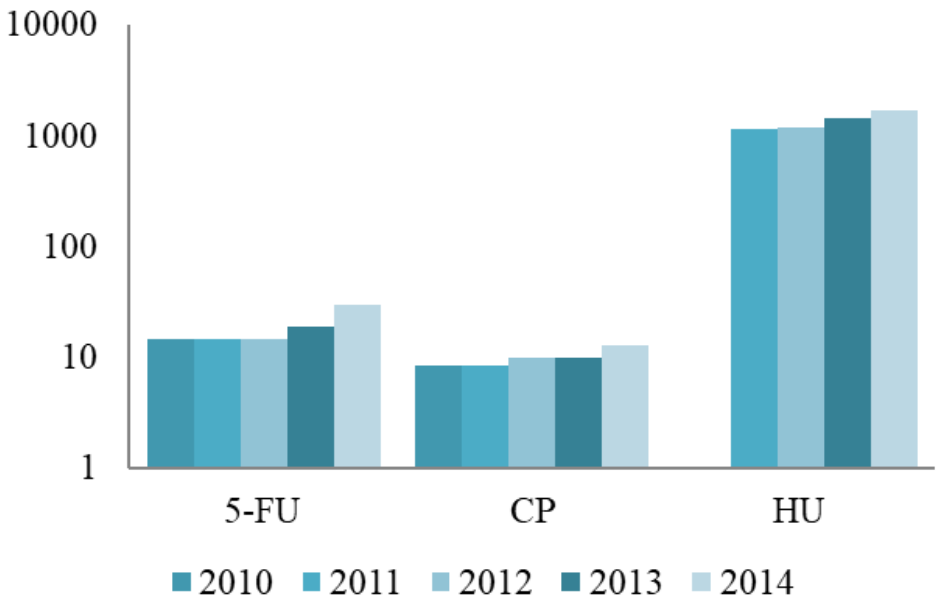


Figure 32 Concentrations of 5-FU, CP and HU in surface water by PECs.

Table 31 Comparative analysis of the amount 5-FU, CP and HU in surface water with other countries research

Research	MECs (ng/L)			PECs (ng/L)		
	5-FU	CP	HU	5FU	CP	HU
This study, 2014	1.28	1.79	1.12	29.53	12.9	1711.9
Taiwan, 2013	2,000,000	13,700,0000				
France, 2012				39.57	6.98	156.13
Germany, 2010	0.7			0.14		
UK, 2008				50		
Canada, 2008	3					
Switzerland, 2006	0.17			0.08		

(refs: Lin et al., 2013; Besse et al., 2012; Kummerer et al., 2010; Johnson et al., 2008; Garcia-Ac et al., 2008; Buerge et al., 2006)

Table 31 shows a comparison between this research and the other research of international. In Taiwan contamination of 5-FU in larger quantities than other countries up to a million. Opposed to Switzerland, CP contaminants found in water in tiny amounts of 0.05-0.17 ng/L may be because of developed countries, and a wastewater treatment system is effective when compared with countries in the asia.

For the prediction of contamination by PECs of drug residues in the environment rather popular. Due to the measuring quickly and a variety of substances. On the other hand, an internationally accepted standards. Switzerland and Germany, the predictive value was found to be low. Probably because of these two countries have good environmental management and HU have not found research that studied the residues in the environment yet.

Contamination of 5-FU, CP and HU in Water Treatment Plant

Water is important to people. All people need to drink or use water. Raw water to produce Tap water in Bangkok from surface water sources, such as Chao Phraya River. And detect contamination 5-FU CP and HU in the Chao Phraya River, so the assumption that 5-FU, CP and HU is contaminating to tap water too. This section, therefore, aims to evaluate the efficiency of the water treatment process to the removal 5-FU, CP and HU. By measured water samples from Samsen Water Treatment Plant, situated in the city and the limited space and the

first water treatment plants, that the systems are not modern. By getting raw water from the Chao Phraya River submitted by canal water, which is an open system, total length of 31 km.

Table 32 5-FU, CP and HU concentrations in Water Treatment Process

No.	Sam-ple	Process	WTP (ng/L)				
			5-FU		CP		HU
			Conc.	Removal (%)	Conc.	Removal (%)	Conc.
1	SS1	Raw water influent	0.95	-	0.62	-	<LOD
2	SS2	Screen	1.03	-8.42	0.92	-48.39	<LOD
3	SS3	Chemical addition	0.86	16.5	1.13	-22.83	<LOD
4	SS4	Co-Flocculation	0.57	33.72	0.48	57.52	<LOD
5	SS5	Clarification	0.53	7.02	0.59	-22.92	<LOD
6	SS6	Filtration	0.28	47.17	0.43	27.12	<LOD
7	SS7	Chlorination or tap water	0.21	25	0.44	-2.33	<LOD
8	SS1-SS8		-	77.8	-	29.03	-

The measuring of contamination of 5-FU, CP and HU in the Samsen Water Treatment Plant shown Table 32. Trend of the contamination of the system is reduced, the amount of contaminants in the raw water from the Chao Phraya River that meet the 5-FU and CP was 0.95 ng/L and 0.62 ng/L respectively. While the tap water, after disinfection with Cl_2 found contamination of 5-FU and CP was 0.21 ng/L and 0.44 ng/L,

respectively, which representing 5-FU reduced 77.8% and CP reduced 29.03% for HU, the amount of contamination low over LOD detection.

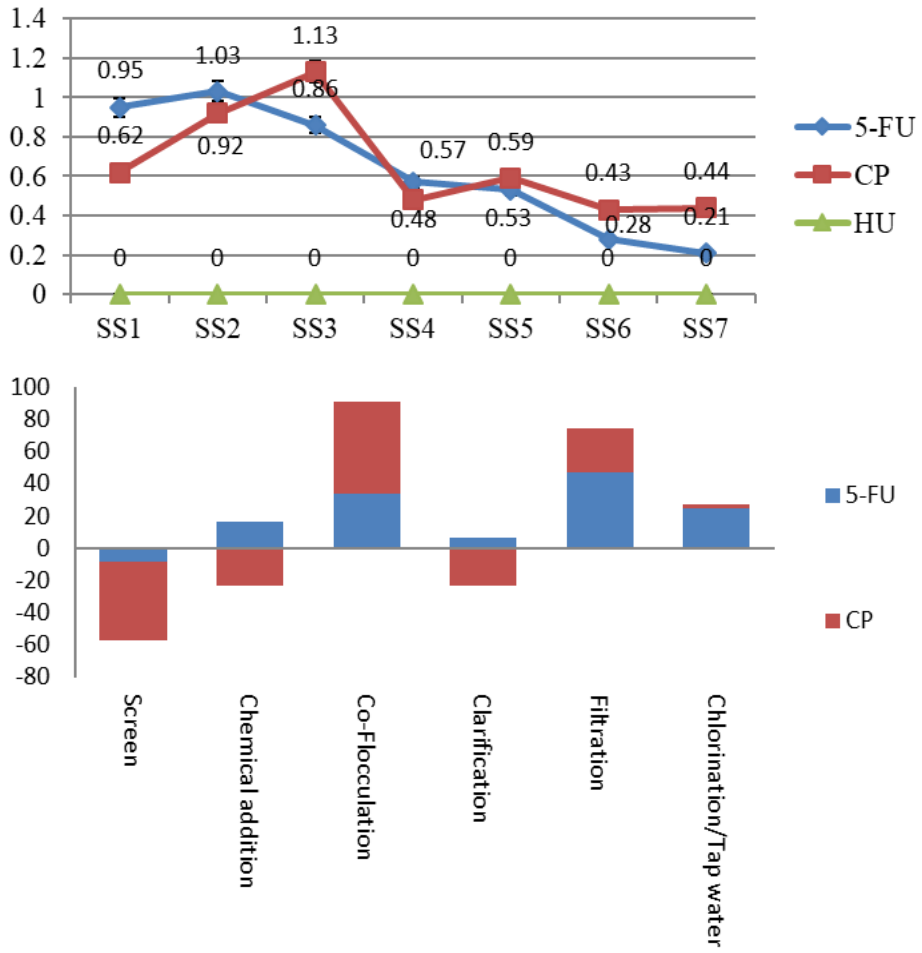


Figure 33 5-FU, CP and HU concentrations in Water treatment process and removal efficiency.

When evaluating the efficiency of 5-FU and CP elimination showed that the procedure of screen has found that the amount of contamination has increased, thus cannot eliminate 5-FU and CP was in the process of chemical addition to precipitate the amount of CP increased, but was 5-FU reduced. In the process of co-flocculation that continually decreased and in step Clarification the amount of CP increased but 5-FU continued to decrease and in step Filtration found contaminants both types is reduced in steps. Finally, the addition of Cl_2 to kill believes that CP has increased slightly, but 5-FU reduced shown as Figure 33 and the data analysis found that some of the steps in the process, the amount of 5-FU and the CP may increase because of the water samples collected during the same period. The assessment of the

contamination of the system thus, not continuous. And during the period the amount of contamination that may increase due to the accumulation of contaminants in the system. Overseas research showed that there is no research.

Contamination of 5-FU, CP and HU in Domestic Wastewater Treatment Plants

Wastewater treatment plants that collected sewage water from commercial, industrial and accommodation sources into various treatment processes for remove pollutants in wastewater to provide better quality and do not cause damage to the natural resources. The wastewater will be treated and then discharged into the river or parts can be reused in the field of agriculture and other industries also reduces a load of water to clean itself naturally and help prevent contaminants from entering the water contamination in the water supply.

The measuring of contamination of 5-FU, CP and HU in the Din Daeng Wastewater Treatment Plant shown Table 33. Trend of the contamination of the system is reduced, the amount of contaminants in the wastewater from domestic that meet the 5-FU, CP and HU were 17.73 ng/L, 28.61 ng/L and 5.49 ng/L respectively. While the water effluent, after disinfection with Cl_2 found contamination of 5-FU, CP and HU were 2.95 ng/L, 1.94 ng/L and <LOD respectively, which representing 5-FU reduced 83.3%, CP reduced 93.2% and HU reduced >65.3%

When measuring the performance for the removal of 5-FU, CP and HU from Din Daeng wastewater treatment process was found in Screen process 5-FU was eliminated slightly, CP meet increased and HU fell moderately. In the Fine Screen. both 5-FU, CP and HU greatly reduced. In steps Aeration found that 5-FU has increased slightly, but CP and HU has declined steadily. In step Sedimentation found that 5-FU and CP at the most reduced. While HU dropped to less than LOD. In the final stage after disinfection with Cl_2 5-FU and CP were to be removed shown as Figure 34. The analysis of data from Din Daeng wastewater treatment plant is an activated sludge which will use bacteria to eliminate contaminants can eliminate 5-FU, CP and HU.

Table 33 5-FU, CP and HU concentrations in Din Daeng Domestic Wastewater Treatment Processes

NO.	Sample	Process	Din Daeng WWTP (ng/L)					
			5-FU		CP		HU	
			Conc.	Removal (%)	Conc.	Removal (%)	Conc.	Removal (%)
1	DD1	Influent	17.73	0	28.61	0	5.49	0
2	DD2	Screen	16.98	4.23	28.71	-0.35	4.32	21.31
3	DD3	Fine Screen	11.21	33.98	22.28	22.40	2.94	31.94
4	DD4	Aeration	12.60	-12.40	19.11	14.23	1.90	35.37
5	DD5	Sediment-ation	5.79	54.05	3.93	79.43	<LOD	-
6	DD6	Chlorina-tion, effluent	2.95	49.05	1.94	50.64	<LOD	-
7	DD1-DD6			83.36		93.21		>65.39

Table 34 5-FU, CP and HU concentrations in Household Wastewater Treatment Processes (Resist Cancer Association).

NO.	Sample	Process	Resist Cancer Association WWTP (ng/L)					
			5-FU		CP		HU	
			Conc.	Removal (%)	Conc.	Removal (%)	Conc.	Removal (%)
1	RCA1	Septic tank	3.14	0	2.40	0	<LOD	-
2	RCA2	Sanitary sewer	2.48	21.02	1.21	49.58	<LOD	-

The measuring of contamination of 5-FU, CP and HU in Resist Cancer Association shown Table 34. Trend of the contamination of the system is reduced, the amount of contaminants in the wastewater from domestic that meet the 5-FU, CP and HU were 3.14 ng/L, 2.40 ng/L and <LOD ng/L respectively. While the water effluent, after out from sanitary sewer found contamination of 5-FU and CP were 2.48 ng/L and 1.21 ng/L, respectively which representing 5-FU reduced 21.02% and CP reduced 49.58% shown as Figure 35.

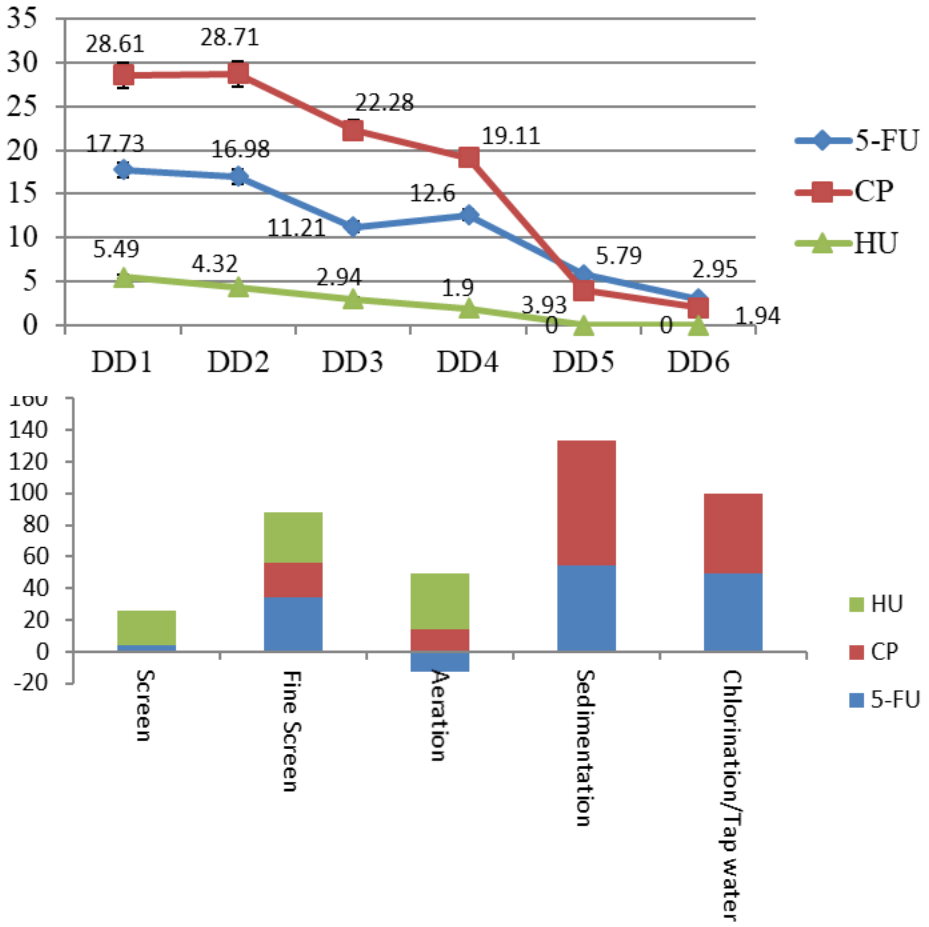


Figure 34 5-FU, CP and HU concentrations in Din Daeng Domestic Water Treatment Process with removal efficiency.

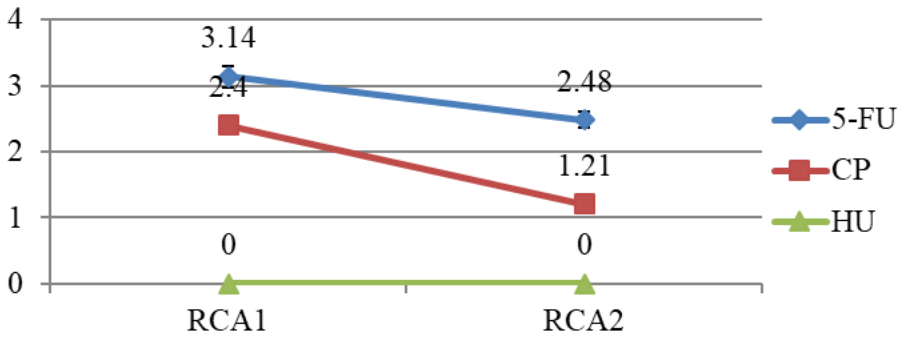


Figure 35 5-FU, CP and HU concentrations in Household Wastewater Treatment Processes (Resist Cancer Association).

Although it is a small wastewater treatment. A household septic 5-FU, CP and HU can be removed partially. After the wastewater into the sewer. It is diluted with rainwater and into wastewater treatment systems by the next. The comparison with the research that has measurements for 5-FU from the wastewater treatment plant of Germany, it is found that is to

27 ng/L and Canada found CP as 3-9 ng/L (Buerge et al., 2006; Kovalova, 2009), which was significantly higher than that of this research. But since the wastewater in Bangkok can be just treated 40% of all that occur. Thus, the measured values may not be all.

Contamination of 5-FU, CP and HU in Hospital Wastewater Treatment Plants

Hospitals wastewater treatment, each with an efficiency of treatment. different out hospitals are more likely to have detectable amounts of 5-FU, CP and HU high. Because cancer patients have been admitted to hospitals. Cancer patients some require a hospital stay of several nights. It must also excrete waste. Nurse administrators or mixed chemotherapy, have to use the water to wash hands or equipment contaminated with chemotherapy as well. Therefore, study wastewater treatment process of the hospital same to study the source of contamination before 5-FU, CP and HU residues in the environment.

The measuring of contamination of 5-FU, CP and HU in Siriraj hospital shown Table 35. Trend of the contamination of the system is reduced, the amount of contaminants in the wastewater from hospital that meet the 5-FU, CP and HU were 65.46 ng/L, 32.55 ng/L and 40.71 ng/L, respectively. While the water effluent after out from Chlorination found contamination of 5-FU, CP and HU were 30.68 ng/L, 6.64 ng/L and 4.94 ng/L, respectively which representing 5-FU reduced 53.13%, CP reduced 79.60% and HU reduced 90.59% shown as Figure 36.

When measuring the performance for the removal of 5-FU, CP and HU from Siriraj wastewater treatment process was found in Screen process 5-FU, CP and HU were eliminated slightly. In the Equalization. Both 5-FU, CP and HU significantly reduced. In steps Aeration found that 5-FU has increased slightly, but CP and HU have declined steadily. In step, Sedimentation found that 5-FU, CP and HU at the most reduced. In the final stage after disinfection with Cl_2 5-FU, CP and HU were to be removed shown in Figure 36. While a number of contaminants in water effluent is also with the 5-FU. Effluent of the system discharge to surface water directly to the river.

Table 35 5-FU, CP and HU concentrations in Siriraj Hospital Wastewater Treatment Processes

NO	Sample	Process	Siriraj WWTP (ng/L)					
			5-FU		CP		HU	
			Conc.	Removal (%)	Conc.	Removal (%)	Conc	Removal (%)
1	SI1	Influent	65.46	0	32.55	0	40.71	0
2	SI2	Screen	63.12	3.57	28.71	11.80	36.80	9.60
3	SI3	Equaliza-tion	60.51	4.13	20.12	29.92	33.17	9.86
4	SI4	Aeration	68.26	-12.81	14.67	27.09	18.28	44.89
5	SI5	Sedimenta-tion	33.79	50.50	8.28	43.56	7.14	60.94
6	SI6(1)	Chlorina-tion, Effluent 1	30.68	9.20	6.64	19.81	3.83	46.36
7	SI6(2)	Chlorina-tion, Effluent 2	27.91	9.03	7.26	-9.34	4.94	-28.98
8	SI1-SI6			53.13		79.60		90.59

The measuring of contamination of 5-FU, CP and HU in the Siriraj Piyamaharajkarun hospital wastewater treatment plant shown Table 36. Trend of the contamination of the system is reduced, the amount of contaminants in the wastewater from hospital that meet the 5-FU, CP and HU were 16.4 ng/L, 11.17 ng/L and 4.32 ng/L respectively. While the water effluent, after disinfection with Cl_2 and UV found contamination of 5-FU, CP and HU were 0.96 ng/L, 0.87 ng/L and <LOD respectively, which representing 5-FU reduced 94.15%, CP reduced 92.21% and HU reduced >40.5%.

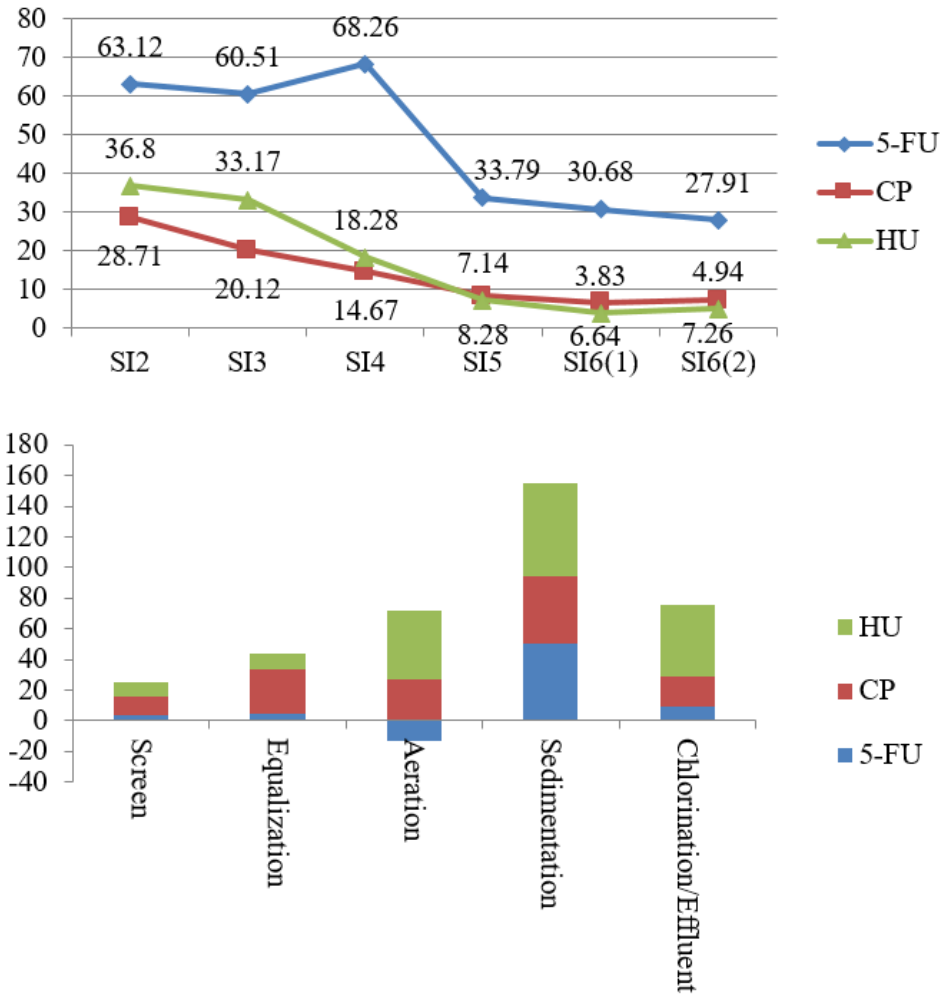
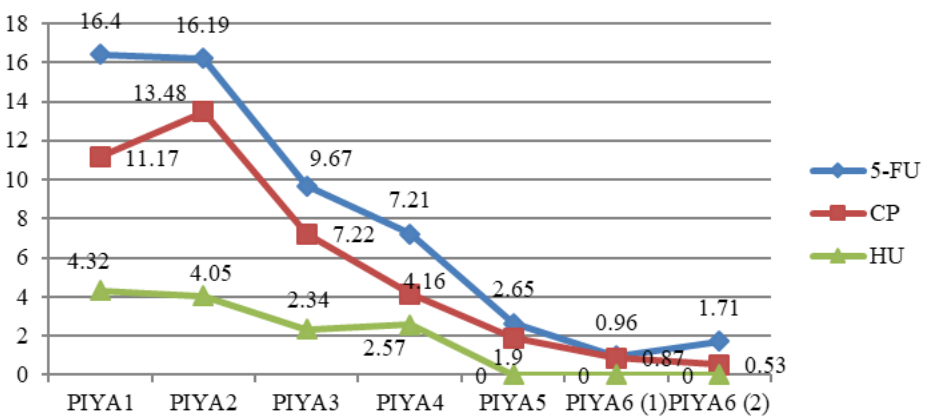


Figure 36 5-FU, CP and HU concentrations in Hospital Wastewater Treatment Processes (Siriraj hospital) with removal efficiency.

When measuring the performance for the removal of 5-FU, CP and HU from Siriraj Piyamaharajkarun hospital wastewater treatment process was found in Screen process 5-FU and HU were eliminated slightly, but CP increased. In the Aeration (1). Both 5-FU, CP and HU significantly reduced. In steps Aeration (2) found that Both 5-FU, CP and HU have declined steadily. In step, Sedimentation found that 5-FU, CP and HU at the most reduced. In the final stage after disinfection with Cl_2 5-FU, CP and HU were to be removed shown in Figure 37.

Table 36 5-FU, CP and HU concentrations in Siriraj Piyamaharajkarun Hospital Wastewater Treatment Processes

NO.	Sample	Process	Siriraj Piyamaharajkarun WWTP (ng/L)					
			5-FU		CP		HU	
			Conc.	Removal (%)	Conc.	Removal (%)	Conc	Removal (%)
1	PIYA1	Influent	16.40	0	11.17	0	4.32	0
2	PIYA2	Screen	16.19	1.28	13.48	-20.68	4.05	6.25
3	PIYA3	Aeration (1)	9.67	40.27	7.22	46.44	2.34	42.22
4	PIYA4	Aeration (2)	7.21	25.44	4.16	42.38	2.57	-9.83
5	PIYA5	Sedimenta-tion	2.65	63.25	1.90	54.33	<LOD	-
6	PIYA6 (1)	Chlorina-tion +UV, Effluent (1)	0.96	63.77	0.87	54.21	<LOD	-
7	PIYA6 (2)	Chlorina-tion+UV, Effluent (2)	1.71	-78.13	0.53	39.08	<LOD	-
8	PIYA1-PIYA6			94.15		92.21		>40.50



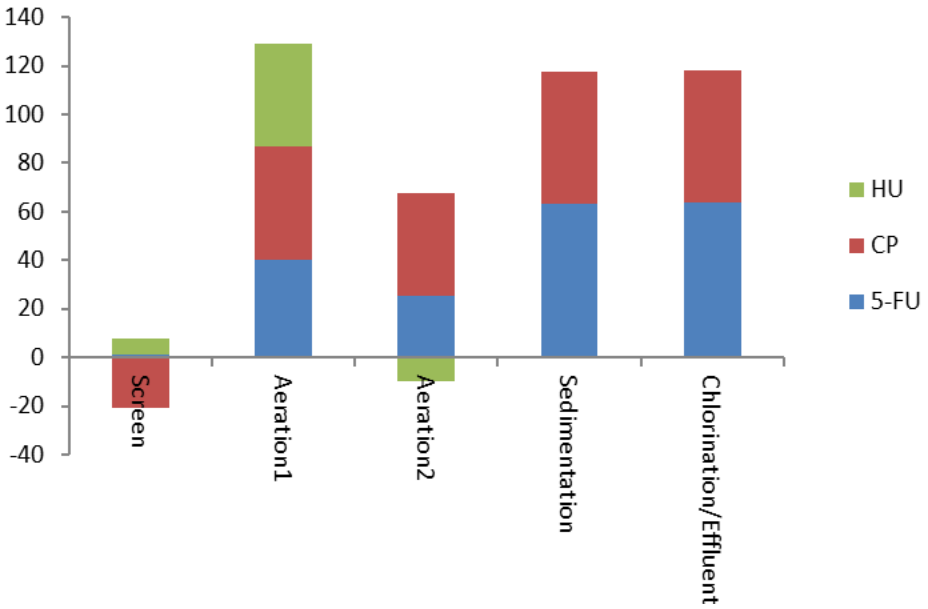


Figure 37 5-FU, CP and HU concentrations in Siriraj Piyamaharajkarun Hospital Wastewater Treatment Processes with removal efficiency.

The measuring of contamination of 5-FU, CP and HU in Ramathibodi hospital wastewater treatment processes as shown Table 37. Trend of the contamination of the system is reduced, the amount of contaminants in the wastewater from hospital that meet the 5-FU, CP and HU were 29.71 ng/L, 21.65 ng/L and 16.32 ng/L respectively. While the water effluent after disinfection with Cl_2 found contamination of 5-FU, CP and HU were 14.42 ng/L, 14.17 ng/L and 3.07 respectively, which representing 5-FU reduced 79.33%, CP reduced 56.53% and HU reduced 93.19%

Table 37 5-FU, CP and HU concentrations in Ramathibodi Hospital Wastewater Treatment Processes

NO.	Sample	Process	Ramathibodi WWTP (ng L)					
			5-FU		CP		HU	
			Conc.	Removal (%)	Conc.	Removal (%)	Conc	Removal (%)
1	RA1	Influent	29.71	0	21.65	0	16.32	0
2	RA2	Screen	31.02	-4.41	22.16	-2.36	15.04	7.84
3	RA3	Aeration	28.37	8.54	19.49	12.05	15.19	-1.00
4	RA4	Sediment a-tion	25.43	10.36	15.31	21.45	3.44	77.35
5	RA5	Chlorina-tion, Effuent	14.42	43.30	14.17	7.45	3.07	10.76
6	RA6	Effuent reuse	6.14	57.42	9.41	33.59	1.11	63.84
7	RA1-RA6			79.33		56.53		93.19

When measuring the performance for the removal of 5-FU, CP and HU from Ramathibodi hospital wastewater treatment processes was found in Screen process HU were eliminated slightly, but 5-FU and CP increased. In the aeration both 5-FU and CP reduced, but HU increased. In steps, Sedimentation found that 5-FU, CP and HU at the most reduced. In the final stage after disinfection with Cl_2 5-FU, CP and HU were to be removed shown in Figure 38.

For here, wastewater has to be treated, reuse benefits, such as fish or plants or wipe the floor cleaning etc. The results showed that the 5-FU, CP and HU drop of wastewater from the treatment system is highly % removal were 57.42%, 33.59% and 63.84%, respectively probably because of the water that comes out treatment system that utilization on outdoor ponds drug 5-FU, CP and HU will decompose faster.

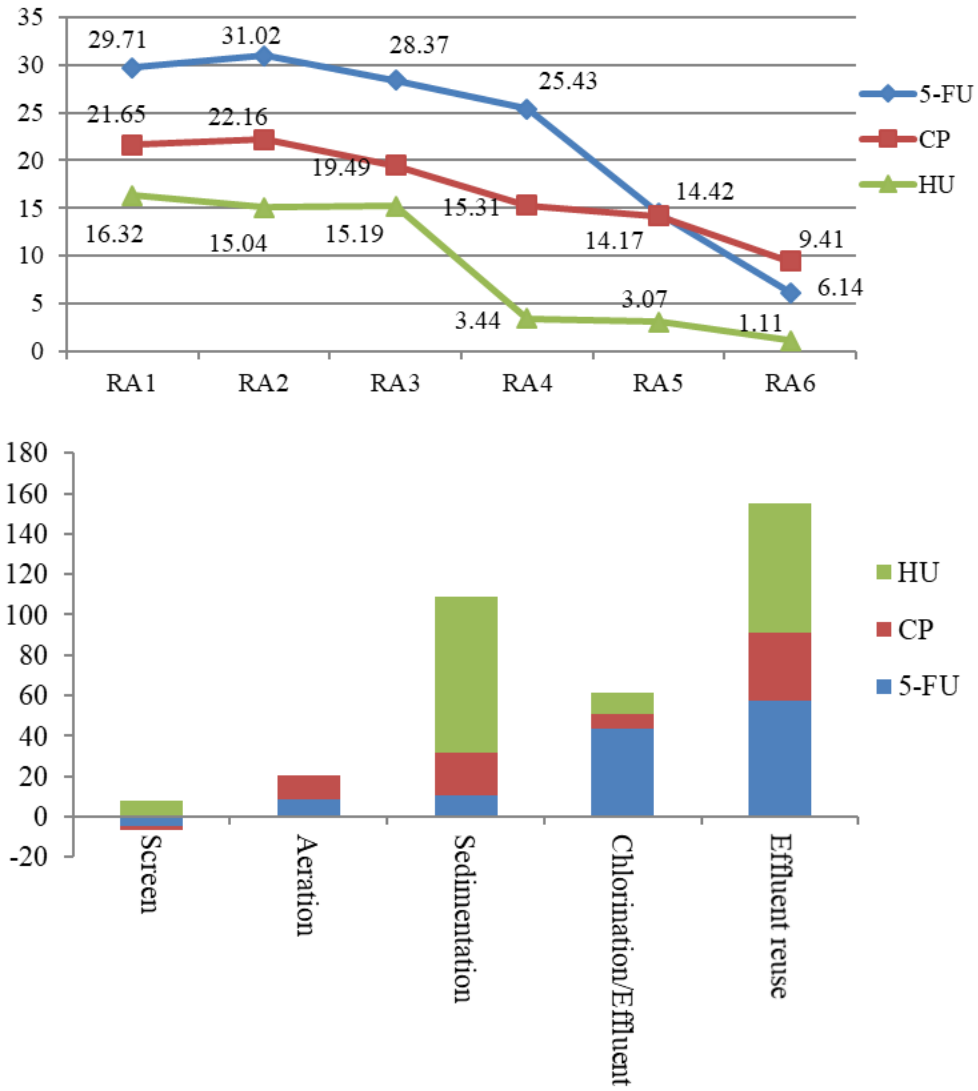


Figure 38 5-FU, CP and HU concentrations in Ramathibodi Hospital Wastewater Treatment Processes with removal efficiency.

For this hospital have restrictions on the size of the treatment plant, two shared with hospital wastewater treatment plant located in the city, and get treatment for cancer patients in particular. The measuring of contamination of 5-FU, CP and HU in National Cancer Institute hospital wastewater treatment processes as shown Table 38. Trend of the contamination of the system is reduced, the amount of contaminants in the wastewater from hospital that meet the 5-FU, CP and HU were 54.95 ng/L, 32.86 ng/L and 40.41 ng/L, respectively. While the water effluent after disinfection with Cl_2 found contamination of 5-FU, CP and HU were 19.25 ng/L,

14.13 ng/L and 2.56, respectively, which representing 5-FU reduced 64.97%, CP reduced 56.99% and HU reduced 93.66%

Table 38 5-FU, CP and HU concentrations in National Cancer Institute Hospital Wastewater Treatment Processes

NO.	Sample	Process	National Cancer Institute WWTP (ng L)					
			5-FU		CP		HU	
			Conc.	Removal (%)	Conc.	Removal (%)	Conc	Removal (%)
1	NCI1	Influent	54.95	0	32.86	0	40.41	0
2	NCI2	Screen	45.94	16.40	31.11	5.33	39.49	2.28
3	NCI3	Aeration	42.33	7.86	34.78	-11.80	38.19	3.29
4	NCI4	Sedimenta-tion	37.16	12.21	28.91	16.88	12.70	66.75
5	NCI5	Chlorina-tion, Effuent	19.25	48.20	14.13	51.12	2.56	79.84
6	NCI1-NCI5			64.97		56.99		93.66

When measuring the performance for the removal of 5-FU, CP and HU from National Cancer Institute hospital wastewater treatment processes was found in Screen process 5-FU, CP and HU were eliminated slightly. In the aeration, both 5-FU and HU reduced, but CP increased. In steps, sedimentation found that 5-FU, CP and HU at the most reduced. In the final stage after disinfection with Cl_2 5-FU, CP and HU were to be removed shown in Figure 39.

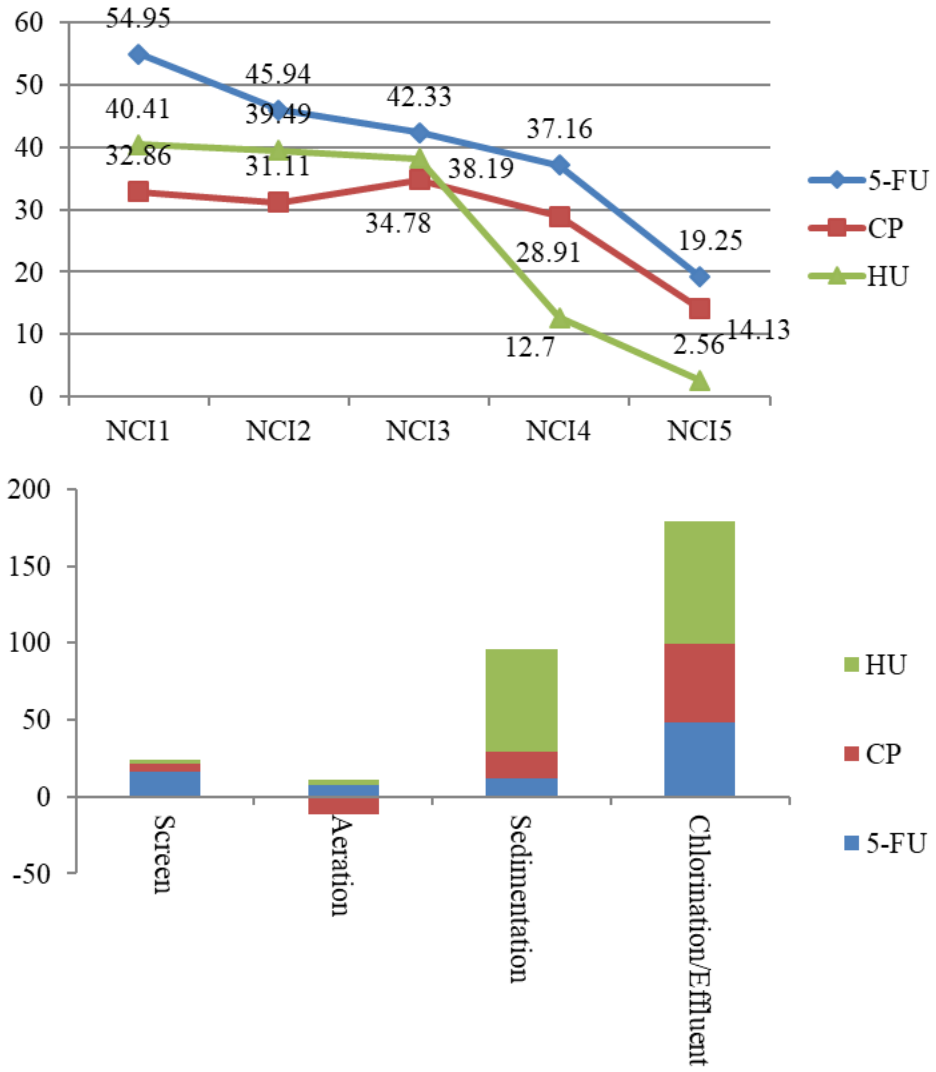


Figure 39 5-FU, CP and HU concentrations in National Cancer Institute Hospital Wastewater Treatment Processes with removal efficiency.

Health risk assessment

From the calculation in Table 19, the risk of hazards substances, 5-FU and CP were the ratio exceeds <1, therefore is that there are not risks to human. Due to contamination in tap water and surface water in quantities that may cause toxicity to human health. However, in fact, we usually do not drink water from the tap water. and the direct surface water. However, due to the risk than one might be concerned and the aware of the problem is the same if you eat or drink water contaminated in the long term is likely to be carcinogenic, teratogenic and mutagenic as well (Muggia & Ziedler, 1980; Carter, 1984; Buerge et al., 2006; O'Keefe, 2011; Besse et al., 2012). Research over the past 5-FU and CP said that despite the small amount

found in the environment and the drug for cure however in the meantime, it is a dangerous drug. Some the research found chemotherapy drugs of contaminated in water effect to the health risk of humans and animal life. The pregnant women and infants who are breastfeeding. Likely to be affected most (Collier, 2007; Johnson et al, 2008; Kovalova, 2009; Rowney et al., 2009; Besse et al., 2012). Currently, no standard chemotherapy drugs used to specially control the discharge into an environment (Zwiener, 2007; Rowney et al., 2009).

Table 39 The result of risk assessment for 5-FU, CP and HU in tap water and surface water by hazard quotient (HQ)

Water samples	**5-FU**			**CP**			**HU**		
	Conc. (ng/L)	Daily intake (mg/kg/d)	HQ	Conc. (ng/L)	Daily intake (mg/kg/d)	HQ	Conc. (ng/L)	Daily intake (mg/kg/d)	HQ
Tap water	0.21	$6x10^{-9}$	$1x10^{-12}$	0.44	$1.2x10^{-6}$	$4x10^{-10}$	<LOD	-	-
Surface water (dry season)	1.28	$3.6x10^{-8}$	$6x10^{-12}$	1.79	$5.1x10^{-8}$	$1x10^{-11}$	1.12	$3.2x10^{-8}$	$8x10^{-7}$
Surface water (wet season)	1.20	$3.4x10^{-8}$	$6x10^{-12}$	1.72	$4.9x10^{-8}$	$1x10^{-11}$	0.90	$2.5x10^{-8}$	$7x10^{-7}$

Note: Daily intake (DI) = (Concentration x IR) / BW; IR = water drinking a day (2 l day^{-1}); BW = average weight (70 kg)

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Chemotherapy drugs are very harmful chemicals that are used for cancer patients. They have a lot of side effects on cancer patients or anyone else who consumes them, such as hair loss. It demonstrates that chemotherapy drugs that are significantly harmful to living things. This research recognizes the residues of chemotherapy in the environment. Especially regarding water resources, because it is used for consumption. Finally, chemotherapy may enter the body of a living organism that is not accidental. Chemotherapy is the interest of this study, 5-Fluorouracil (5-FU), Cyclophosphamide (CP) and Hydroxyurea (HU) from the retention of a large hospital in Bangkok. These are the chemotherapy drugs that have the most usage. The research aims to determine the levels of 5-FU, CP and HU residues in surface water, tap water and wastewater from both the community and hospital and to evaluate the system performance for WTP and WWTPs as well. The analytical processes were performed using solid phase extraction (SPE) with an Oasis® HLB cartridge and measured by High performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS). A separation system consisting of a guard column Agilent@ Zorbax Eclipse XDB C18 (I.D. 4.6 x 50 mm, 1.8 μm particle size) and analytical column Agilent@ Zorbax Eclipse Plus C18 (I.D. 2.1 x 100 mm, 1.8 μm particle size) using a gradient mixture of MeOH + 0.1% formic acid and Milli-Q water + 0.1% formic acid as the mobile phase with a multiple injection mode. Simultaneous anticancer drugs were detected by MS/MS using electrospray ionization and multiple reaction monitoring (MRM) for both positive (CP and HU) and negative (5-FU) charges. The method validations included acceptable, accuracy, precision and specificity for the detection of 5-FU, CP and HU, showed that linearity was R^2>0.99 and the calculated limit of detection (LOD) for 5-FU, CP and HU were 0.179, 0.442 and 0.270 ng/L, respectively. From the analysis in the Lower Chao Phraya River in the dry season year 2013, the amount of 5-FU, CP and HU were 1.28, 1.79 and 1.12 ng/L, respectively, and predicted a number of contaminants in surface waters, based on the amount of 5-FU, CP and HU consumption in the hospital in 2014, and the amount of 5-FU, CP and HU were 0.42, 0.18 and 24.45 ng/L, respectively. And the assessment of the performance for the removal of 5-FU, CP HU from WTP and WWTPs were unable to eliminate all residuals in the water effluent. Hence, the Health risk assessment for 5-FU, CP and HU from the consumption of water in the Lower Chao Phraya River and tap water, is that the amount of 5-FU and CP may harm the health of the consumer. However, there is no reference standard and these substances can be dissolved using light acceleration.

Recommendations

This study involved only a certain period. Therefore, studies conducted in other times, such as other seasons, may give varied results. Studies using a wider period are essential for further studies. In addition, the flow rate of the water in a water treatment process is also important since the amount of time for the water to flow through the individual treatment stages is different. Therefore, the flow rate and individual amount of water flowing time should be taken into account if there is any additional study in the future.

This study, the results showed that the use of chemotherapy in order to predict the volume of contaminated substances, there are not cover the total quantity because there is no agency to collect any amount of drugs from all hospitals in Bangkok.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

Usawanuwat J., Boontanon N. and Boontanon S.K. Analysis of Three Anticancer Drugs (5-Fluorouracil, Cyclophosphamide and Hydroxyurea) in Water Samples by HPLC-MS/MS. *International Journal of Advances in Agriculture and Environmental Engineering*, **2014**, 1(1), 72-76. (published)

2. การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงวิชาการ โดย คณะสิ่งแวดล้อมและทรัพยากรศาสตร์ มหาวิทยาลัยมหิดล

มีการนำไปใช้ในการนำเสนอผลงานการวิจัยในหัวข้อ Analysis of Three Anticancer Drugs (5-Fluorouracil, Cyclophosphamide and Hydroxyurea) in Water Samples by HPLC-MS/MS ในงานประชุมวิชาการนานาชาติ International Conference on Biological, Chemical and Environmental Sciences (BCES-2014) Jan. 21-22, 2014 Patong Beach, Phuket (Thailand)

และมีการสร้างนักวิจัยใหม่ในระดับบัณฑิตศึกษา จำนวน 1 คน

3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

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ภาคผนวก

Analysis of Three Anticancer Drugs (5-Fluorouracil, Cyclophosphamide and Hydroxyurea) in Water Samples by HPLC-MS/MS

Jareerat Usawanuwat, Narin Boontanon, and Suwanna Kitpati Boontanon

***Abstract*—**Anticancer drugs are very harmful chemical which use for cancer patient. It has a lot of side effect to cancer patient or anyone who consume contaminated body intake. A rapid, reliable and highly selective performance analysis method was developed for commonly used anticancer drugs (5-Fluorouracil, Cyclophosphamide and Hydroxyurea) residual in water samples. The analytical processes were performed using solid-phase extraction (Oasis@ HLB cartridge) and measured by High performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS). Separation system consist with guard column Agilent@ Zorbax Eclipse XDB C18 (I.D. 4.6 x 50 mm, 1.8 µm particle size) and analytical column Agilent@ Zorbax Eclipse Plus C18 (I.D. 2.1 x 100 mm, 1.8 µm particle size) using gradient mixture of methanol + 0.1% formic acid and water + 0.1% formic acid as mobile phase with multiple injection mode. Simultaneous anticancer drugs were detected by MS/MS using electrospray ionization and multiple reaction monitoring (MRM) for both positive (Cyclophosphamide and Hydroxyurea) and negative (5-Fluorouracil) charges. The method validations were included acceptable, accuracy, precision and specificity for detection of 5-Fluorouracil, Cyclophosphamide and Hydroxyurea shown linearity was achieved from 1 to 50 µg/L, R2>0.99 and the calculated limit of detected for 5-Fluorouracil 0.013 µg/L, for Cyclophosphamide was 0.006 µg/L, for Hydroxyurea was 0.050 µg/L. The results from various water sample type were compared to predicted environmental concentrations (PECs) for environmental and human health risk assessment.

***Keywords*—**5-Fluorouracil, Cyclophosphamide, Hydroxyurea, HPLC-MS/MS, water sample.

I. Introduction

ANTICANCER or antineoplastic drugs refers to any drugs used in chemotherapy of oncological patients. These drugs act by interfering directly of tumour cells and growth cells but acting non-selective and healthy cells may also be damaged which, cause side effects several organisms [1-2].

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Some antineoplastic drugs have already been classified by International Agency for Research on Cancer (IARC) as carcinogens in humans: group 1 such as, cyclophosphamide [3]. Most commonly used of anticancer drugs for the cancer treatment are 5-fluorouracil (5-FU), cyclophosphamide (CP) and hydroxyurea (HU) which, are an antimetabolite, alkylating and other of antineoplastic agents respectively [4] (Fig.1). The contamination routes of these drugs reach to water in the environment by excreted of urine or feces and released via the hospital or domestic wastewater and wastewater treatment plants (WWTPs) [5-7]. Contamination level of pharmaceuticals and personal care products as pollutants (PPCPs) in water samples are importance for environmental and human risk assessment meanwhile, recently considered as emerging environmental contaminants [7]. The aquatic environmental and human health impact of anticancer drugs were imprecised although, there are highly cytotoxic, carcinogenic, embryotoxic, mutagenic and teratogenic [1], [8-9].

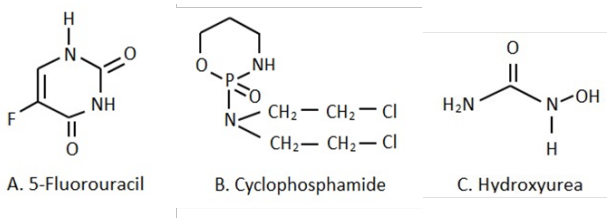

Fig. 1 Structures of most commonly use anticancer drugs

Currently, has not been report for analysis of HU in water samples and other researches were limit or difficulty for analytical method of 5-FU and CP in water samples. Many researchers had been reported by asynchronous drugs and its derivatives while the treatment process possibly use of those three drugs simultaneously [5], [10-20]. Thus, the excretion from cancer patients may contain the residual of those three anticancer drugs with different portion. HPLC-MS/MS as the highly solution performance for qualitative and quantitative analysis, this instrument has been developed and used widely for extensive clinical and environmental studies. Hence, HPLC-MS/MS technique could be developed for

measurements of 5-FU, CP and HU in agents environmental sample, since the simultaneous measurement of 5-FU, CP and HU has not been yet establishes.

The aims of this studier are modifier and developer HPLC-MS/MS method for the simultaneously quantification of 5-FU, CP and HU in water samples. This validated method will be used for measurement the residual of those drugs in various sources of water samples from surface water and domestic wastewater effluent. The contamination in various water samples will be compared to predicted environmental concentrations (PECs) by calculating [21-22], based on consumption data in Thailand.

II. Materials and Method

A. Analytical Method Modification

Analytical method modifications will be done by modified from the single drug analysis of previous researches [10-20]. Advantage and disadvantage of each one will be considered and applying for the simultaneous measurement of those three drugs, such as the mobile phase, gradient and column. Furthermore, those three anticancer drugs have both positive and negative charges for detection which is most difficulty for setting up the analytical procedure with the single run.

B. Chemicals and Reagents

5-Fluorouracil (HPLC-Grade, ≥99%), cyclophosphamide (HPLC-Grade, ≥98%) and hydroxyurea (HPLC-Grade, ≥98%) were purchased from Sigma-Aldrich (USA). Methanol (HPLC-grade, >99.99%) and formic acid (HPLC-Grade, 98-100%) were purchased from Merck (Germany) and ammonia (30%) were purchased from Panreac (Spain).

A stock mixed standard solution of 5-FU, CP and HU were prepared at a concentration of 5 mg/L by dissolving 0.125 mg of the chemicals standard in 25 mL methanol. The mixed standard solution of 5-FU, CP and HU were conducted to prepare the calibration standard. Calibration standard was prepared at a concentration range of 1 to 50 µg/L by dilution of the mixed standard solutions with methanol. All standards and fortification solutions were stored in polypropylene bottle and kept in refrigerator at 4 ºC.

C. Instrumentation

All qualification and quantification were performed using an Agilent 1200 SL HPLC coupling with Agilent 6410 triple quadrupole mass spectrometer.

D. Sample Collection and Preparation

Samples of surface water were collected from Chao Phraya River and domestic wastewater effluent from accommodation for cancer patient by grab samples. All the samples were collected in 9-10 and 30 November 2013, respectively and were analyzed within 48 hours. Water samples were collected in polyethylene terephthalate (PET) bottle previously washed with Milli-Q water and methanol before using and rinse by water sample. The collected sample were avoid to sunlight and sent to laboratory, stored in refrigerator at 4°C until analysis.

The methods for analyses the concentration of 5-FU, CP and HU in water samples consisted by using the solid phase extraction (SPE) method coupling with HPLC-MS/MS for quantification [7], [10]. The analysis procedure were shown in Fig. 2

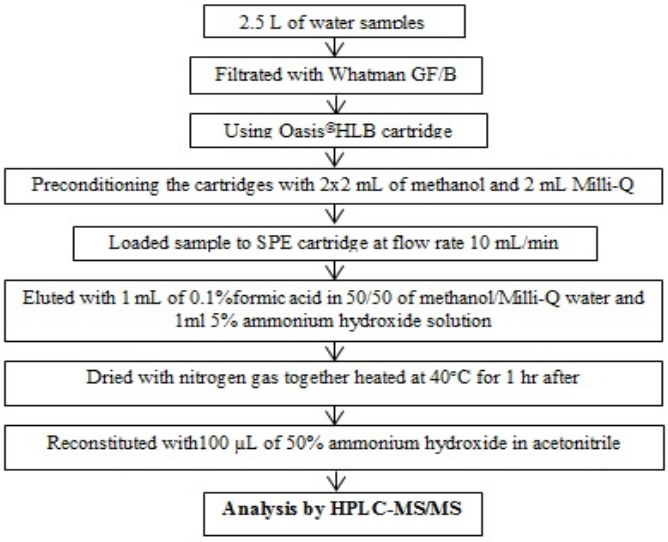

Fig. 2 Water sample analytical procedure

E. Validation of the HPLC-MS/MS Method

The linearity of the experiment will be obtained by using concentration levels of 1, 5, 10, 25 and 50 µg/L with 5 replicates. Acceptance criteria were acceptable accuracy and precision data. The reproducibility and recovery will be obtained by using low and high concentrations relative to calibration range which prepared by using standard of 25 and 50 µg/L spiking 50 mL to 2.5 L of surface water samples with 5 replicates per sample. Calculation of percent recovery as of response in extracted samples compared to control samples and calculation of respective value of relative standard deviation)RSD(. Acceptance criteria were acceptable sensitivity and reproducible recovery.

Estimated of limit of detection)LOD(and limit of Quantitation)LOQ(will be calculated by signal to noise ratio)S/N(. LOD and LOQ were expressed as S/N equal 3 and 10, respectively. The model for calculation of LOD = 3 x S/N and LOQ = 10 x S/N [23].

F. Predicted Environmental Concentrations (PECs)

The preliminary exposure assessment of 5-FU, CP and HU contaminant in surface water will be implemented by calculating PECs [21-22] using the following this parameters model and compared to measurement environmental concentrations in surface water at Chao Phraya river, Bangkok.

$$\text{PECs (mg/L)} = \frac{\text{amount of consumption (mg/year) x excretion fraction of agent x emission of agent to surface water}}{\text{wastewater/person/day(L) x number of people x 365 day x Dilution to surface water}} \quad (1)$$

where consumption is the quantity of an active molecule consumed by patients and data were collected in 2012 at hospital in Bangkok. The total amounts of 5-FU, CP and HU calculated from usage drugs data in 22 hospitals in Bangkok which about 55% of total hospitals for cancer treatment (Table I). The excretion fraction of 5-FU, CP and HU are 0.20, 0.25 and 0.50 respectively. The fraction of emission of the drug from WWTPs directed to surface water (=1). The volume of wastewater per person per day (default value = 250). In Bangkok have 7 million people. 365 is the number of days per year (day/year). The dilution factor from wastewater treatment plant (WWTP) effluents to surface waters (default value set at 140).

TABLE I
CONSUMPTION DATA OF 5-FU, CP AND HU IN BANGKOK

Anticancer drug	Total amounts (µg)
5-FU	259,068
CP	118,124
HU	158,026

TABLE II
HPLC-MSMS INSTRUMENTATIONS AND THEIR OPTIMIZED CONDITIONS DEVELOPMENT FOR QUANTIFICATION OF 5-FU, CP AND HU

HPLC		**MS**	
Instrument	Agilent 1200 SL HPLC	Instrument	Agilent 6410 triple quadrupole mass spectrometer
Column	Guard column Agilent@ Zorbax Eclipse XDB C_{18} (I.D. 4.6x50 mm, 1.8 µm) Analytical column Agilent@ Zorbax Eclipse Plus XDB C_{18} (I.D. 2.1x100 mm, 1.8 µm)	Ionization	Electrospray ionization
		Polarity mode	Negative for 5-FU Positive for CP, HU
Mobile Phase	A: Milli-Q water+0.1% formic acid B: methanol+0.1% formic acid	Nebulizer	N_2 (50 psi)
		Gas flow	N_2 (10 mL/min)
Gradient-application	2 min, A = 50%; B = 50% 5 min, A = 0%; B = 100% 5.5 min, A = 20%; B = 80% 9 min, A = 20%; B = 80% 11 min, A = 50%; B = 50% 15 min, A = 50%; B = 50%	Gas temperature	300 ºC
		Capillary voltage	3500 V
		MRM mode	5-FU : 129.0>42.0 (m/z) CP : 261.0>140.0 (m/z) HU : 77.1> 44.0 (m/z)
Flow rate	0.25 mL/min		
Column temperature	40 ºC		
Injection volume	10 µL		

III. RESULT AND DISCUSSION

A. Analytical Method Modification

The simultaneous quantification of 5-FU, CP and HU were performed by applying of mobile phase, gradient and column as shown in TABLE II. The chromatographic conditions were optimized to obtain the better resolution within a shorter analytical time. Two mobile phase systems Milli-Q water + 0.1% formic acid and methanol + 0.1% formic acid were tested result in the best separation of the investigated compounds. For quantitative determination was used electrospray ionization (ESI) for both positive (Cyclophosphamide and Hydroxyurea) and negative (5-Fluorouracil) charges with capillary voltage 3500V. Analyst ions was monitored by using multiple reactions monitoring (MRM) mode. The representative chromatograms were shown in Fig. 3.

Usually, MS/MS can be analyzed both negative and positive charges with short changing time, however, in this case, those 5-FU and HU retention times was overlap, which cannot be analyzed by the usual method. Therefore, we solved this problem by switching detection charges together with multiple injection mode. That mean, during quantification, the first sample injection was detecting positive charge and after CP was detected, the second injection and charge changing were done for negative charge. The procedure mentioned that allows the simultaneous analysis of 5-FU, CP and HU at once.

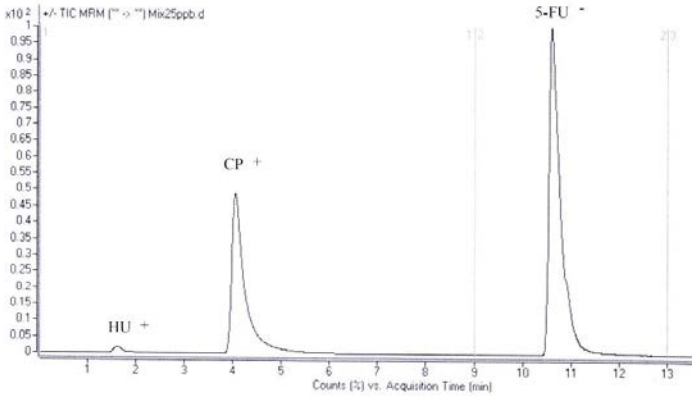

Fig. 3 Representative high performance liquid chromatography (HPLC/MS/MS) chromatograms 5-FU, CP and HU

B. Validation

The linearity was studied in the range from 1 to 50 µg/L of standard 5-FU, CP and HU. Five concentration range were assayed in duplicate. 5-FU, CP and HU standards mixture showed very good linearity. The correlation coefficient (R^2) was always greater than 0.997. Therefore, from results those concentrations with the peak area data measured by this analytical method were correlated and appropriated as shown in Fig 4.

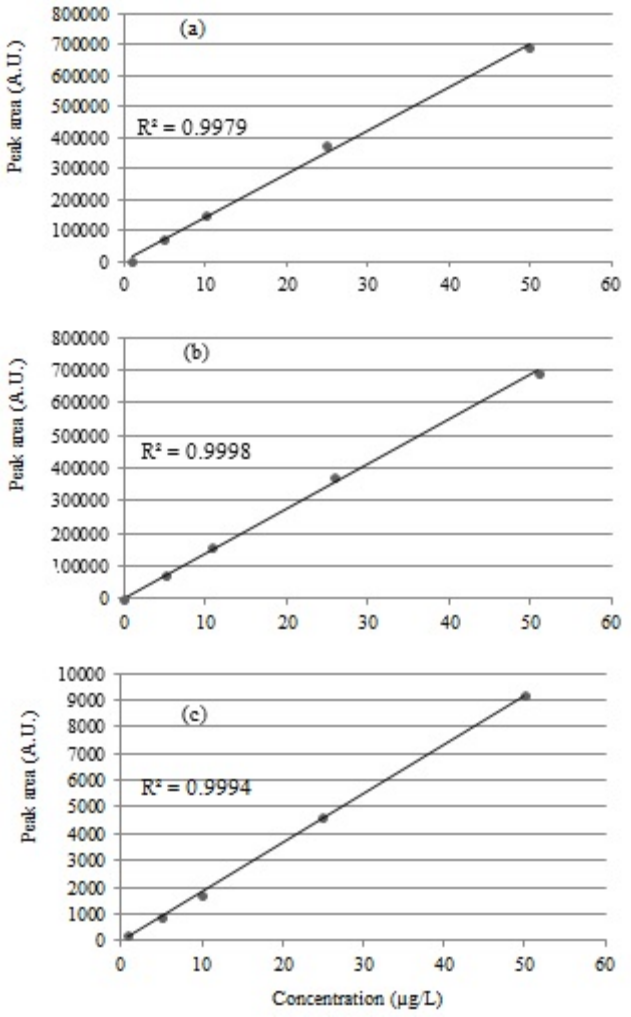

Fig. 4 Linearity of (a) 5-FU, (b) CP and (c) HU

Reproducibility was calculated by five replications of low and high concentration levels. The relative standard deviation variations of 5FU, CP and HU were 1.350 %, 0.163 % and 0.622 %, respectively. The result shows a good reproducibility and high precision for the quantification of 5-FU, CP and HU (TABLE III) under an agreement between experimental and theoretical values.

Considering on the results of 5-FU, CP and HU were found that the recovery were within the range 77-108%, while the mean recovery at each fortification level and for each sample matrix should be in the range of 70-120% [24]. The recoveries were fall within the acceptable range which indicated that the developed method was reliable and accurate (TABLE IV).

LOD and LOQ of 5-FU, CP and HU with acceptable precision and accuracy, in the present study, were calculated from signal to noise and the data were shown in TABLE V.

C. Predicted environmental concentrations (PECs)

The results from surface water samples were compared to calculated predicted environmental concentrations (PECs) -(1) and the results show that PECs were higher than the actual measured values (Table VI).

TABLE III
REPRODUCIBILITY

Anticancer drugs (*n=5*)	Low concentration Peak area (Mean)	%RSD	High concentration Peak are (Mean)	%RSD
5-FU	78561.55	1.150	221894.17	1.350
CP	69590.67	0.163	177650.02	0.135
HU	65807.73	0.451	126894.67	0.622

TABLE IV
RECOVERY

Anticancer drugs	% Recovery	
	Domestic wastewater	Surface water
5-FU	77	79
CP	108	96
HU	83	104

TABLE V
EVALUATION OF LOD AND LOQ

Anticancer drugs	LOD (µg/L)	LOQ (µg/L)
5-FU	0.013	0.043
CP	0.006	0.020
HU	0.050	0.166

Because the predictive assessment of the situation has limited such as the assumed lower excretion value, wastewater treatment is not available or may disappear in environmental due to the dilution by the natural environment such as rainwater or infiltration. Such phenomenon, the analyzed values may, possibility, found under the estimated. The calculated predicted environmental concentrations of 5-FU, CP and HU in this study were shown similarly to the reported in France and England [21-22]. However, the contamination of 5-FU, CP and HU in surface water might cause the health risk when consumed those water.

TABLE VI
PREDICTED AND MEASUREMENT ENVIRONMENTAL CONCENTRATIONS

Anticancer drugs	Predicted environmental concentrations (µg/L)	Measurement environmental concentrations (µg/L)
5-FU	7.890	0.578
CP	5.750	1.907
HU	3.564	0.788

IV. CONCLUSION

The study of contamination of 5-FU,CP and HU in water samples can be concluded as follows: Modification and development HPLC-MS/MS method for the simultaneously quantification of 5-FU, CP and HU in water samples were acceptable with high accuracy, precision and specificity for the detection. The linearity was achieved R^2 higher than 0.99 and the calculated LOD for was 5-FU 0.013 µg/L, for CP was 0.006 µg/L and for HU was 0.050 µg/L.

ACKNOWLEDGMENT

This research was supported by the Center of Excellence on Environmental Health and Toxicology, Science & Technology Postgraduate Education and Research Development Office (PERDO), Ministry of Education, National Cancer Institute of Thailand (NCI) and Thailand Research Fund (TRF).

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Some antineoplastic drugs have already been classified by International Agency for Research on Cancer (IARC) as carcinogens in humans: group 1 such as, cyclophosphamide [3]. Most commonly used of anticancer drugs for the cancer treatment are 5-fluorouracil (5-FU), cyclophosphamide (CP) and hydroxyurea (HU) which, are an antimetabolite, alkylating and other of antineoplastic agents respectively [4] (Fig.1). The contamination routes of these drugs reach to water in the environment by excreted of urine or feces and released via the hospital or domestic wastewater and wastewater treatment plants (WWTPs) [5-7]. Contamination level of pharmaceuticals and personal care products as pollutants (PPCPs) in water samples are importance for environmental and human risk assessment meanwhile, recently considered as emerging environmental contaminants [7]. The aquatic environmental and human health impact of anticancer drugs were imprecised although, there are highly cytotoxic, carcinogenic, embryotoxic, mutagenic and teratogenic [1], [8-9].

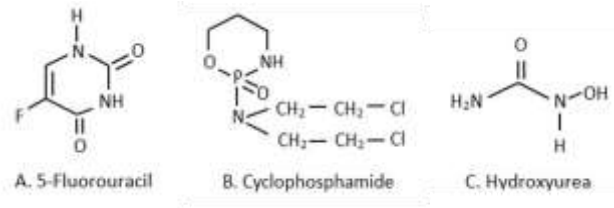

Fig. 1 Structures of most commonly use anticancer drugs

Currently, has not been report for analysis of HU in water samples and other researches were limit or difficulty for analytical method of 5-FU and CP in water samples. Many researchers had been reported by asynchronous drugs and its derivatives while the treatment process possibly use of those three drugs simultaneously [5], [10-20]. Thus, the excretion from cancer patients may contain the residual of those three anticancer drugs with different portion. HPLC-MS/MS as the highly solution performance for qualitative and quantitative analysis, this instrument has been developed and used widely for extensive clinical and environmental studies. Hence, HPLC-MS/MS technique could be developed for

measurements of 5-FU, CP and HU in agents environmental sample, since the simultaneous measurement of 5-FU, CP and HU has not been yet establishes.

The aims of this studier are modifier and developer HPLC-MS/MS method for the simultaneously quantification of 5-FU, CP and HU in water samples. This validated method will be used for measurement the residual of those drugs in various sources of water samples from surface water and domestic wastewater effluent. The contamination in various water samples will be compared to predicted environmental concentrations (PECs) by calculating [21-22], based on consumption data in Thailand.

II. Materials and Method

A. Analytical Method Modification

Analytical method modifications will be done by modified from the single drug analysis of previous researches [10-20]. Advantage and disadvantage of each one will be considered and applying for the simultaneous measurement of those three drugs, such as the mobile phase, gradient and column. Furthermore, those three anticancer drugs have both positive and negative charges for detection which is most difficulty for setting up the analytical procedure with the single run.

B. Chemicals and Reagents

5-Fluorouracil (HPLC-Grade, ≥99%), cyclophosphamide (HPLC-Grade, ≥98%) and hydroxyurea (HPLC-Grade, ≥98%) were purchased from Sigma-Aldrich (USA). Methanol (HPLC-grade, >99.99%) and formic acid (HPLC-Grade, 98-100%) were purchased from Merck (Germany) and ammonia (30%) were purchased from Panreac (Spain).

A stock mixed standard solution of 5-FU, CP and HU were prepared at a concentration of 5 mg/L by dissolving 0.125 mg of the chemicals standard in 25 mL methanol. The mixed standard solution of 5-FU, CP and HU were conducted to prepare the calibration standard. Calibration standard was prepared at a concentration range of 1 to 50 µg/L by dilution of the mixed standard solutions with methanol. All standards and fortification solutions were stored in polypropylene bottle and kept in refrigerator at 4 ºC.

C. Instrumentation

All qualification and quantification were performed using an Agilent 1200 SL HPLC coupling with Agilent 6410 triple quadrupole mass spectrometer.

D. Sample Collection and Preparation

Samples of surface water were collected from Chao Phraya River and domestic wastewater effluent from accommodation for cancer patient by grab samples. All the samples were collected in 9-10 and 30 November 2013, respectively and were analyzed within 48 hours. Water samples were collected in polyethylene terephthalate (PET) bottle previously washed with Milli-Q water and methanol before using and rinse by water sample. The collected sample were avoid to sunlight and sent to laboratory, stored in refrigerator at 4°C until analysis.

The methods for analyses the concentration of 5-FU, CP and HU in water samples consisted by using the solid phase extraction (SPE) method coupling with HPLC-MS/MS for quantification [7], [10]. The analysis procedure were shown in Fig. 2

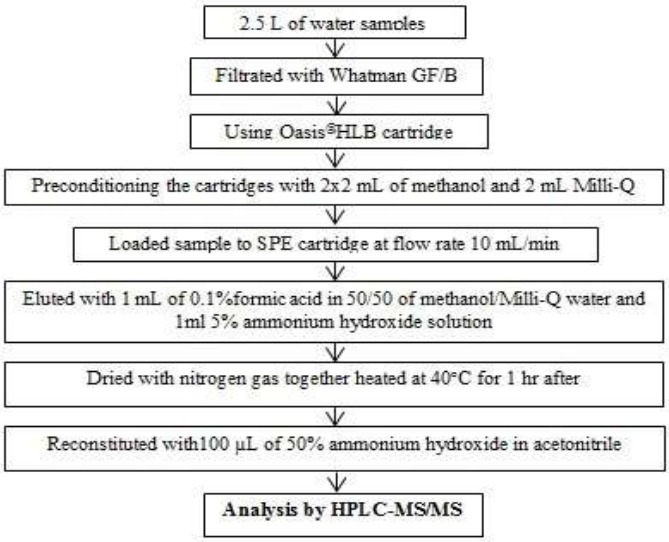

Fig. 2 Water sample analytical procedure

E. Validation of the HPLC-MS/MS Method

The linearity of the experiment will be obtained by using concentration levels of 1, 5, 10, 25 and 50 µg/L with 5 replicates. Acceptance criteria were acceptable accuracy and precision data. The reproducibility and recovery will be obtained by using low and high concentrations relative to calibration range which prepared by using standard of 25 and 50 µg/L spiking 50 mL to 2.5 L of surface water samples with 5 replicates per sample. Calculation of percent recovery as of response in extracted samples compared to control samples and calculation of respective value of relative standard deviation)RSD(. Acceptance criteria were acceptable sensitivity and reproducible recovery.

Estimated of limit of detection)LOD(and limit of Quantitation)LOQ(will be calculated by signal to noise ratio)S/N(. LOD and LOQ were expressed as S/N equal 3 and 10, respectively. The model for calculation of LOD = 3 x S/N and LOQ = 10 x S/N [23].

F. Predicted Environmental Concentrations (PECs)

The preliminary exposure assessment of 5-FU, CP and HU contaminant in surface water will be implemented by calculating PECs [21-22] using the following this parameters model and compared to measurement environmental concentrations in surface water at Chao Phraya river, Bangkok.

$$PECs\ (mg/L) = \frac{\text{amount of consumption (mg/year) x excretion fraction of agent x emission of agent to surface water}}{\text{wastewater/person/day(L) x number of people x 365 day x Dilution to surface water}} \quad (1)$$

where consumption is the quantity of an active molecule consumed by patients and data were collected in 2012 at hospital in Bangkok. The total amounts of 5-FU, CP and HU calculated from usage drugs data in 22 hospitals in Bangkok which about 55% of total hospitals for cancer treatment (Table I). The excretion fraction of 5-FU, CP and HU are 0.20, 0.25 and 0.50 respectively. The fraction of emission of the drug from WWTPs directed to surface water (=1). The volume of wastewater per person per day (default value = 250). In Bangkok have 7 million people. 365 is the number of days per year (day/year). The dilution factor from wastewater treatment plant (WWTP) effluents to surface waters (default value set at 140).

TABLE I
CONSUMPTION DATA OF 5-FU, CP AND HU IN BANGKOK

Anticancer drug	Total amounts (µg)
5-FU	259,068
CP	118,124
HU	158,026

TABLE II
HPLC-MSMS INSTRUMENTATIONS AND THEIR OPTIMIZED CONDITIONS DEVELOPMENT FOR QUANTIFICATION OF 5-FU, CP AND HU

HPLC		**MS**	
Instrument	Agilent 1200 SL HPLC	Instrument	Agilent 6410 triple quadrupole mass spectrometer
Column	Guard column Agilent@ Zorbax Eclipse XDB C_{18} (I.D. 4.6x50 mm, 1.8 µm) Analytical column Agilent@ Zorbax Eclipse Plus XDB C_{18} (I.D. 2.1x100 mm, 1.8 µm)	Ionization	Electrospray ionization
		Polarity mode	Negative for 5-FU Positive for CP, HU
Mobile Phase	A: Milli-Q water+0.1% formic acid B: methanol+0.1% formic acid	Nebulizer	N_2 (50 psi)
		Gas flow	N_2 (10 mL/min)
Gradient-application	2 min, A = 50%; B = 50% 5 min, A = 0%; B = 100% 5.5 min, A = 20%; B = 80% 9 min, A = 20%; B = 80% 11 min, A = 50%; B = 50% 15 min, A = 50%; B = 50%	Gas temperature	300 ºC
		Capillary voltage	3500 V
		MRM mode	5-FU : 129.0>42.0 (m/z) CP : 261.0>140.0 (m/z) HU : 77.1> 44.0 (m/z)
Flow rate	0.25 mL/min		
Column temperature	40 ºC		
Injection volume	10 µL		

III. RESULT AND DISCUSSION

A. Analytical Method Modification

The simultaneous quantification of 5-FU, CP and HU were performed by applying of mobile phase, gradient and column as shown in TABLE II. The chromatographic conditions were optimized to obtain the better resolution within a shorter analytical time. Two mobile phase systems Milli-Q water + 0.1% formic acid and methanol + 0.1% formic acid were tested result in the best separation of the investigated compounds. For quantitative determination was used electrospray ionization (ESI) for both positive (Cyclophosphamide and Hydroxyurea) and negative (5-Fluorouracil) charges with capillary voltage 3500V. Analyst ions was monitored by using multiple reactions monitoring (MRM) mode. The representative chromatograms were shown in Fig. 3.

Usually, MS/MS can be analyzed both negative and positive charges with short changing time, however, in this case, those 5-FU and HU retention times was overlap, which cannot be analyzed by the usual method. Therefore, we solved this problem by switching detection charges together with multiple injection mode. That mean, during quantification, the first sample injection was detecting positive charge and after CP was detected, the second injection and charge changing were done for negative charge. The procedure mentioned that allows the simultaneous analysis of 5-FU, CP and HU at once.

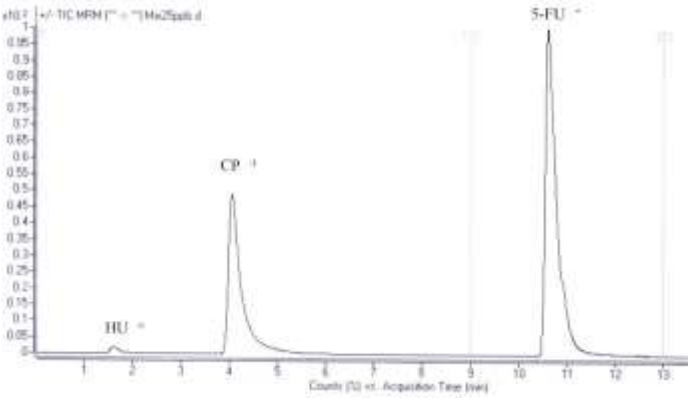

Fig. 3 Representative high performance liquid chromatography (HPLC/MS/MS) chromatograms 5-FU, CP and HU

B. Validation

The linearity was studied in the range from 1 to 50 µg/L of standard 5-FU, CP and HU. Five concentration range were assayed in duplicate. 5-FU, CP and HU standards mixture showed very good linearity. The correlation coefficient (R^2) was always greater than 0.997. Therefore, from results those concentrations with the peak area data measured by this analytical method were correlated and appropriated as shown in Fig 4.

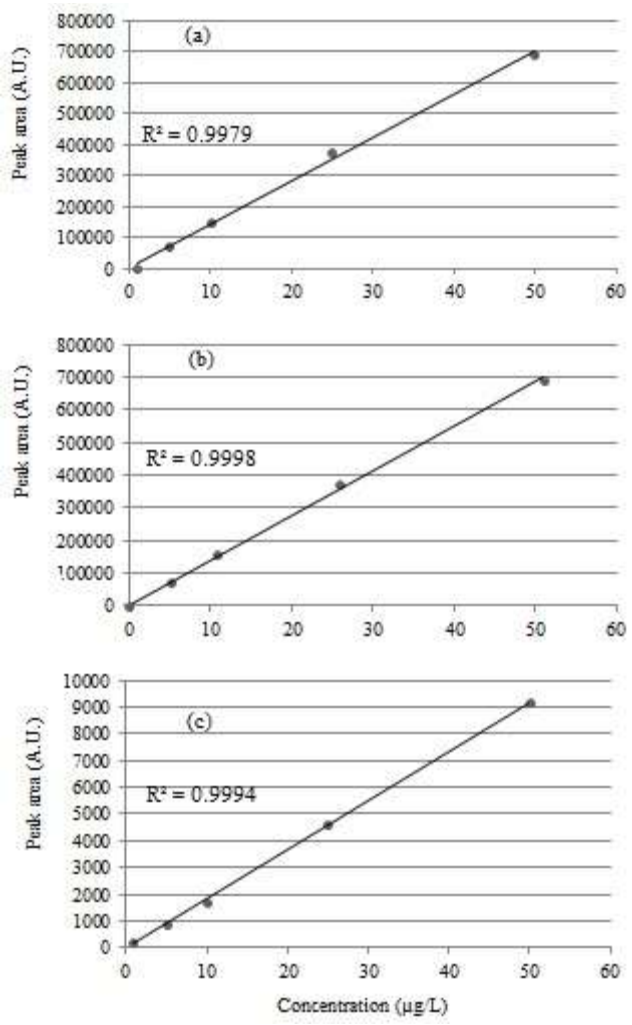

Fig. 4 Linearity of (a) 5-FU, (b) CP and (c) HU

Reproducibility was calculated by five replications of low and high concentration levels. The relative standard deviation variations of 5FU, CP and HU were 1.350 %, 0.163 % and 0.622 %, respectively. The result shows a good reproducibility and high precision for the quantification of 5-FU, CP and HU (TABLE III) under an agreement between experimental and theoretical values.

Considering on the results of 5-FU, CP and HU were found that the recovery were within the range 77-108%, while the mean recovery at each fortification level and for each sample matrix should be in the range of 70-120% [24]. The recoveries were fall within the acceptable range which indicated that the developed method was reliable and accurate (TABLE IV).

LOD and LOQ of 5-FU, CP and HU with acceptable precision and accuracy, in the present study, were calculated from signal to noise and the data were shown in TABLE V.

C. Predicted environmental concentrations (PECs)

The results from surface water samples were compared to calculated predicted environmental concentrations (PECs) -(1) and the results show that PECs were higher than the actual measured values (Table VI).

TABLE III
REPRODUCIBILITY

Anticancer drugs (*n=5*)	Low concentration Peak area (Mean)	%RSD	High concentration Peak are (Mean)	%RSD
5-FU	78561.55	1.150	221894.17	1.350
CP	69590.67	0.163	177650.02	0.135
HU	65807.73	0.451	126894.67	0.622

TABLE IV
RECOVERY

Anticancer drugs	% Recovery	
	Domestic wastewater	Surface water
5-FU	77	79
CP	108	96
HU	83	104

TABLE V
EVALUATION OF LOD AND LOQ

Anticancer drugs	LOD (µg/L)	LOQ (µg/L)
5-FU	0.013	0.043
CP	0.006	0.020
HU	0.050	0.166

Because the predictive assessment of the situation has limited such as the assumed lower excretion value, wastewater treatment is not available or may disappear in environmental due to the dilution by the natural environment such as rainwater or infiltration. Such phenomenon, the analyzed values may, possibility, found under the estimated. The calculated predicted environmental concentrations of 5-FU, CP and HU in this study were shown similarly to the reported in France and England [21-22]. However, the contamination of 5-FU, CP and HU in surface water might cause the health risk when consumed those water.

TABLE VI
PREDICTED AND MEASUREMENT ENVIRONMENTAL CONCENTRATIONS

Anticancer drugs	Predicted environmental concentrations (µg/L)	Measurement environmental concentrations (µg/L)
5-FU	7.890	0.578
CP	5.750	1.907
HU	3.564	0.788

IV. CONCLUSION

The study of contamination of 5-FU,CP and HU in water samples can be concluded as follows: Modification and development HPLC-MS/MS method for the simultaneously quantification of 5-FU, CP and HU in water samples were acceptable with high accuracy, precision and specificity for the detection. The linearity was achieved R^2 higher than 0.99 and the calculated LOD for was 5-FU 0.013 µg/L, for CP was 0.006 µg/L and for HU was 0.050 µg/L.

ACKNOWLEDGMENT

This research was supported by the Center of Excellence on Environmental Health and Toxicology, Science & Technology Postgraduate Education and Research Development Office (PERDO), Ministry of Education, National Cancer Institute of Thailand (NCI) and Thailand Research Fund (TRF).

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