O_76

Risk Assessment of Municipal Tap Water Supply for Trihalomethanes, the Chlorination Byproducts: Comparison of Different Boiling Methods

Pinyapat kongngen[1*], Kannikar Chatsantiprapa[2]

Abstract

Introduction: This study was aimed at monitoring and assessing human health risk of trihalomethanes (THMs) in drinking water. Municipal tap water samples were collected in Ban Samran district, Khon Kaen Province, 2 times in November 2014. **Methods:** The water samples were analyzed for baseline THMs and then heated in an open and a closed boiler simultaneously until boiled for 5 min, and re-heated again at 10 and 24 hours. At each boiling interval, water samples were analyzed for THMs by Purge and Trap method with a gas chromatography- electron capture detector. The water samples were also tested for cytotoxicity using MTT assay with Chang liver cells. Health risk of THMs via ingestion model was also estimated. **Results:** The occurrence of THMs levels prior to boiling was chloroform (CF)> bromodichlomethane (BDCM)> chlorodibromomethane (CDBM)> bromoform (BF). The rank of lifetime additional cancer risk was BDCM>CF>CDBM>BF. With increasing boiling time the concentration of THMs were reduced effectively. The test of cell toxicity via MTT assay revealed that boiling of the water samples did not affect the Chang liver cell viability. **Conclusion:** Since the total lifetime additional cancer risk from all THMs in water was marginally acceptable and BDCM presented the highest cancer risk of all THMs studied, compounds providing bromide ions should be avoided in use to reduce the cancer risk for water environment.

Keywords: trihalomethanes, risk assessment, chlorination by–products, drinking water, cytotoxicity

---

[1] M.S., Faculty of pharmaceutical sciences, Khon Kaen University, Thailand

[2] Ph.D., Associate Professor, Faculty of pharmaceutical sciences, Khon Kaen University, Thailand

* **Corresponding author** : Apinya Kongngern, Faculty of pharmaceutical sciences, Khon Kaen University, Thailand

1. Introduction

Chlorination is a common disinfection process for water supply to protect human health against infection by microbiological pathogens. However, the process generates various chlorin ated by-products which affect human health. A group of water chlorination byproducts commonly reported are trihalomethanes (THMs) which consist of chloroform (CF), bromodichloromethane (BDCM), chlorodibromomethane (CDBM) and bromoform (BF) (Carpi and Zufall, 2002). Gen eral properties of these compounds are shown in Table 1. THMs are reported as human carcino gens and cause harm to fetus of pregnant wom an (Do, Ratanapan, Suksaroj, 2011). The US-EPA has published the stage 1 disinfection byproducts (DBPs) rule to regulate total THMs at maximum allowable annual average level of 80 ppb (US-EPA, 1998). The carcinogenicity of CF is generally considered to be a non-genotoxic carcinogen whose mechanism of action involves cytotoxicity and regenerative cell proliferation (IARC, 1999). BDCM, CDBM and BF are gener ally not involved the induction of gene mutation in standard test systems. The rank of cytotoxicity was BF> CDBM> CF> BDCM (IARC, 1999). It is generally assumed that heat and/or sunlight may activate the production of chlorination byproducts from the reactions of organic matters and chlorine dissolved in water. It is a common practice in most households nowadays in Thailand that people boil water in a closed pot (like HottaSharp®, as a common name). It is doubted that the closed pot boiling method will affect the production of chlo rination byproducts in water and hence the health risk of human since volatile compounds are kept in the pot during boiling. Therefore, the purpose of this study was to assess the amount of the 4 compounds of THMs in municipal tap water supply before and after the 2 different methods of boiling. An *open boiler* and a closed boiler were used for comparison, and human health risks were assessed.

Table 1: General properties of trihalomethanes (THMs)

THMs	Chemical structures	Boiling point (°C)	Melting point (°C)
Chloroform (CF)	$CHCl_3$	61.3	-63.2
Bromodichloromethane (BDCM)	$CHBrCl_2$	90	-57.1
Chlorodibromomethane (CDBM)	$CHBr_2Cl$	119	-
Bromoform (BF)	$CHBr_3$	149 – 150	8.3

(WHO, 1998)

2. Material and Methods

Sample collection and analysis

Municipal tap water samples were collected in Ban Samran district (9 Km from the chlorination station) in Khon Kaen Province, two times (on November 1 and 2, 2014) in clean, ice-bathed, tightly screw-capped bottles (filled until over-full). The water samples were stored at 4 oC prior to experiments. Before boiling, 40 ml of sample was transferred to a clean glass vial at full vial for leaving no headspace, for THMs analysis. Then the water sample was separated into an open boiler and a closed boiler for boiling. The water samples were heated simultaneously to 100^{0}C for 5 minutes, then 40 ml of each sample was collected immediately in a clean glass vial (to full vial). The water samples were re-heated again at 10 and 24 hours and collected in the same manner. Each water sample was analyzed for THMs and cytotoxicity.

Chemical analysis

THMs calibration mix in methanol (Supelco) with percent purity of BDCM 99.4%, BF 96.5%, CF 99.0%, CDBM 99.9% was diluted and used as reference chemicals.

GC-ECD-Purge and Trap was used for analysis: Purge & Trap sample concentrator (Tekmar 3100), Gas chromatography-electron capture detector system (Agilent 4890 D). Column: SPB-608 fused silica capillary column (30 m length x 0.53 mm inner diameter x 0.5 μm film thickness, J&W Scientific).

GC conditions: experiment time 15 min, injector temperature 150^{o}C, oven temperature 50^{o}C for 15 min, carrier gas 2ml/min, detector temperature 250 ^{o}C. Purge and Trap conditions see Table 2.

Table 2: The purge and trap conditions

The purge and trap parameter	Condition
Valve oven temp (^{o}C)	150
Transfer line temp (^{o}C)	150
Sample mount Temp (^{o}C)	40
Purge ready temp (^{o}C)	40
Purge temp (^{o}C)	40
Purge ready time (min)	5
Desorb preheat temp (^{o}C)	225
Desorb time (min)	1
Desorb temp (^{o}C)	225
Bake time (min)	5
Bake time (^{o}C)	250

Cytotoxicity assay

Chang liver cell line (CLS), passage 30th, were cultured in full growth media consisting of DMEM with 4.5 g/l glucose, 2 mM L-glutamine, 1% non-essential amino acid, 10% fetal bovine serum and 1% antibiotic (penicillin, streptomycin). The cells dilution used was approximately 80,000 cells/ml. MTT assay was performed as described by Mosmann (1983). Full growth medium was used as negative control and 10% dimethyl sulfoxide (10% DMSO) was used as positive control.

Health risk assessment

An exposure assessment of THMs via ingestion model of water was carried out using chronic daily intake (CDI) estimation as follows.

CDI = (C)(IR)(EF)(ED)/(BW)(AT) (LaGrega, *et al.,* 2001, cited by Panyakapo and Paopuree, 2007) (input parameters see Table 3)

C = chemical concentration of each compound detected (see Table 5)

Table 3 Input parameters and values for lifetime exposure assessment used in calculation for Thai people.

Notation	Parameter	Value	Unit	References
IR	Average ingestion rate	2	l/day	USEPA (1997)
EF	Exposure frequency	365	day/year	Intranont (2005), cited by Panyakapo and Paopuree (2007)
ED	Exposure duration (Lifetime, Thai)	64	year	Intranont (2005), cited by Panyakapo and Paopuree (2007)
BW	Average body weight (Thai people)	55	kg	Intranont (2005), cited by Panyakapo and Paopuree (2007)
AT	Average time (Lifetime, Thai)	64× 365	day	Intranont (2005), cited by Panyakapo and Paopuree (2007)

The lifetime additional cancer risk assessment or cancer risk assessment of eachcompound was calculated from: Cancer risk = CDI x Slope factor.

The non-cancer risk assessment or Margin of safety (MOS) of each compound was calculated from: MOS = Tolerable Daily Intake or TDI/CDI.

For slope factor and TDI of each compound of THMs see Table 4.

Table 4 Slope factor and Tolerable Daily Intake (TDI) of each compound of trihalomethanes

Trihalomethanes	Slope factor (kg·day/mg) (LaGrega, *et al.,* 2001, cited by Panyaka-po and Paopuree, 2007)	TDI (mg/kg/day) (Rookett, *et al.,* 2010)
Chloroform (CF)	0.0061	0.015
Bromodichloromethane (BDCM)	0.062	-
Chlorodibromomethane (CDBM)	0.084	0.0214
Bromoform (BF)	0.0079	0.0179

Statistical analysis

Data were analyzed using statistical software statistix10 (Analytical Software, USA) with one-way analysis of variance (ANOVA) followed by Tukey HSD multiple-comparison test. A p value less than 0.05 was considered as statistically significant.

3. Results

Chemical analysis

THMs from municipal tap water supply prior to boiling are shown in Table 5. Of all the 4 compounds, CF gave the highest concentration. After boiling in 2 different methods for 5 min, then at 10 h, 24 h (Table 6), CF still gave the highest concentration of all, followed by BDCM, DBCM, and BF, respectively. Every compound was lower in concentration than the level detected prior to boiling, started from the first interval of boiling, except for BF. BF was higher in concentration after 5 min boiling from both boilers (Table 6) than what found prior to boiling (Table 5). However, after 3 boiling intervals, all the compounds were reduced to insignificant levels (Table 6 and Figures 1-4).

Comparing between the 2 boiling methods, in the first boiling interval, although CF, BDCM, and CDBM were slightly higher in a closed boiler than an open boiler (Table 6 and Figure 1-3), they were not significantly different. However, in the second (10 h) and third (24 h) boiling intervals, the open boiler gave higher CF, BDCM, and CDBM than the closed boiler.

Table 5 Trihalomethanes (THMs) detected in Khon Kaen municipal tap water supply prior to boiling.

	THMs μg/l (mean ±SD)			
Source	CF	BDCM	CDBM	BF
Tap water	85.62 ± 0.93	31.81 ± 0.20	4.94 ± 0.12	0.01 ± 0.01

CF=Chloroform, BDCM=Bromodichloromethane, CDBM=Chlorodibromomethane, BF=Bromoform

Table 6 Trihalomethanes (THMs) detected in an open boiler and a closed boiler at 3 boiling intervals.

	THMs µg/l (mean ±SD)			
Source	CF	BDCM	CDBM	BF
Open boiler at 5 min	63.43± 2.35	26.79 ± 0.54	3.36 ± 0.31	0.09 ± 0.01
Open boiler at 10 h	42.99 ± 1.45	13.87 ± 1.48	0.94 ± 0.03	ND
Open boiler at 24 h	3.92 ± 0.53	0.37 ± 0.05	0.34 ± 0.01	ND
Closed boiler at 5 min	65.68 ± 2.39	28.48 ± 0.82	4.23 ± 0.36	0.09 ± 0.01
Closed boiler at 10 h	2.92 ± 2.07	0.42 ± 0.04	0.35 ± 0.01	ND
Closed boiler at 24 h	1.64 ± 0.12	0.33 ± 0.02	0.34 ± 0.00	ND

CF=Chloroform, BDCM=Bromodichloromethane, CDBM=Chlorodibromomethane, BF=Bromoform

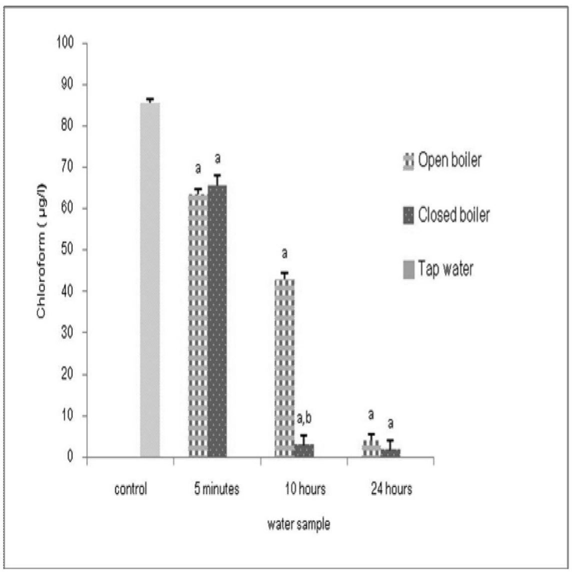

Figure 1 Chloroform (CF) concentrations in µg/l detected from an open boiler and a closed boiler in 3 boiling intervals, 5 min, 10 h and 24 h, with water from municipal tap before boiling being control. After boiling, CF concentration continued to decrease significantly ($p<0.05$) compared to control in every water sample as denoted with [a], and the closed boiler gave significantly lower concentration ($p<0.05$) than the open boiler at 10 h boiling as denoted with [b].

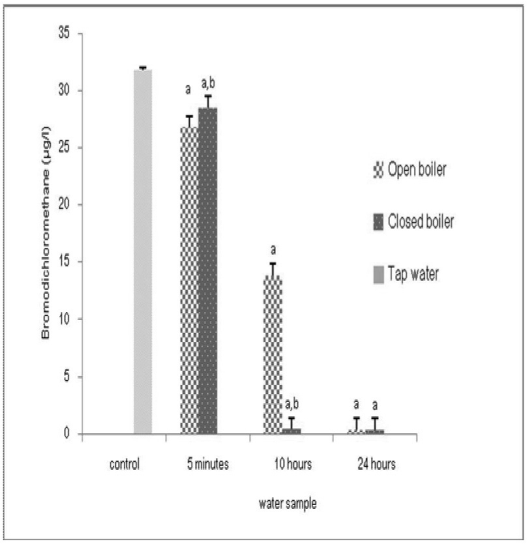

Figure 2 Bromodichloromethane (BDCM) concentrations in µg/l detected from an open boiler and a closed boiler in 3 boiling intervals, 5 min, 10 h and 24 h, with water from municipal tap before boiling being control. After boiling, BDCM concentration continued to decrease significantly ($p<0.05$) compared to control in every water sample as denoted with [a], and the closed boiler gave significantly different concentration ($p<0.05$) from the open boiler at 5min and 10 h boiling as denoted with [b].

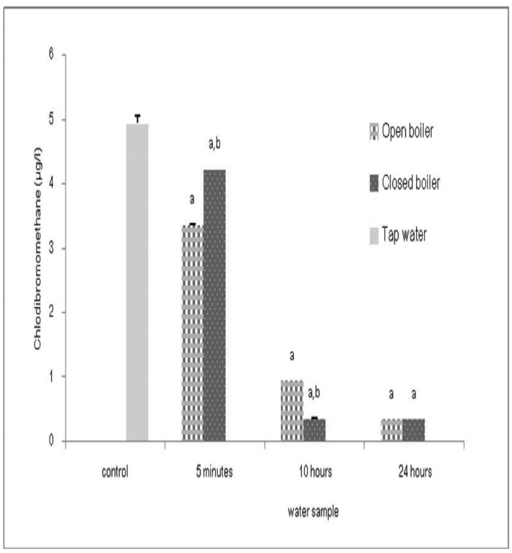

Figure 3: Chlorodibromomethane (CDBM) concentrations in µg/l detected from an open boiler and a closed boiler in 3 boiling intervals, 5 min, 10 h and 24 h, with water from municipal tap before boiling being control. After boiling, CDBM concentration continued to decrease significantly ($p<0.05$) compared to control in every water sample as denoted with [a], and the closed boiler gave significantly different concentration ($p<0.05$) from the open boiler at 5 min and 10 h boiling as denoted with [b].

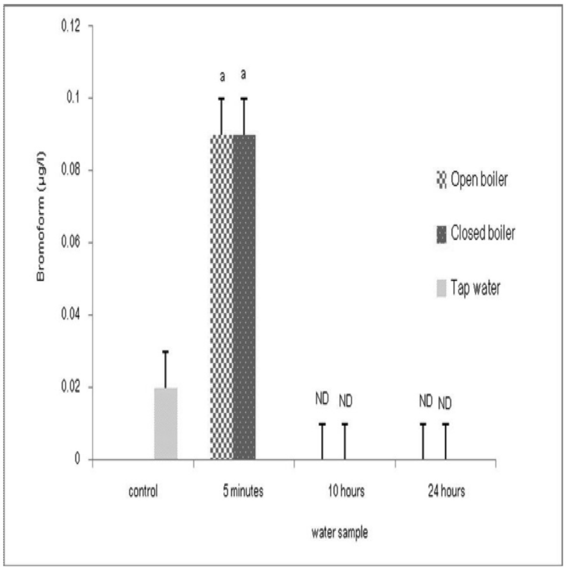

Figure 4: Bromoform (BF) concentrations in µg/l detected from an open boiler and a closed boiler in 3 boiling intervals, 5 min, 10 h and 24 h, with water from municipal tap before boiling being control. After boiling, BF was found significantly higher ($p<0.05$) than control at 5 min boiling interval as denoted with [a]. After that, BF was not detected (ND=not detected).

Cytotoxicity tests

The MTT assay revealed that water samples from both open and closed boilers at all boiling intervals showed non-significantly different cell viability to the negative control (full growth media) after 24 h incubation period (Figure 5). However, after 48 h incubation period, the closed boiler water at 5 min boiling, and the open boiler water at 10 h and 24 h boiling showed significantly higher cell viability than the negative control (Figure 6). There was no different cell viability found between waters from an open or closed boiler.

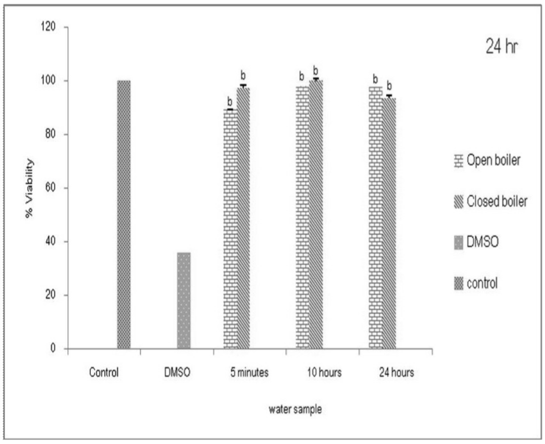

Figure 5: Percentage of cell viability tested on Chang liver cell line, passage 30th, with 10% water samples collected after boiling in an open boiler and a closed boiler at 5 min, 10 h, 24 h, using full growth media as negative control and 10%DMSO as positive control for cytotoxicity tests for 24 h incubation period. After boiling, cell viability was not significantly different from negative control and when comparing between the 2 boiling methods. Cell viability was significantly different ($p<0.05$) to positive control (DMSO) as denoted with [b].

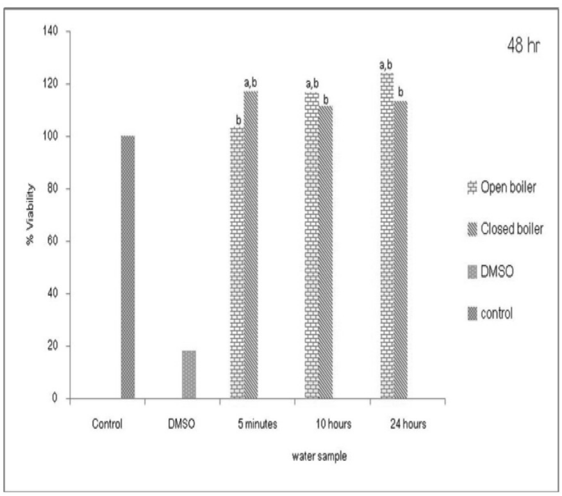

Figure 6: Percentage of cell viability tested on Chang liver cell line, passage 30th, with 10% water samples collected after boiling in an open boiler and a closed boiler at 5 min, 10 h, 24 h, using full growth media as negative control and 10%DMSO as positive control for cytoto xicity tests for 48 h incubation period. After boiling, cell viability in a closed boiler at 5 min and in an open boiler at 10h, 24 h boiling was significantly different ($p<0.05$) from negative control as denoted with [a]. Cell viability in all water samples after boiling was significantly higher ($p<0.05$) than positive control (DMSO) as denoted with [b]. Cell viability was not significantly different between the 2 boiling methods.

Health risk assessment

Lifetime additional cancer risk from each compound of THMs was calculated and shown in Table 7. Only THMs detected from tap water samples before boiling were used in the calculation for human health risk since after boiling, all THMs compounds continually decreased until insignificant amount just after 3 boiling intervals. Hence, the amount of THMs prior to boiling represents the maximum health risks of THMs in the municipal water supply. It was found that the total lifetime additional cancer risk from the 4 compounds of THMs, 1.05×10^{-4} (Table 7) were in a marginal safe range since US-EPA suggested the acceptable lifetime additional cancer risk from management is 10^{-7} - 10^{-4} (US-EPA, 1989), The margin of safety (MOS) of all compounds were more than 1 with CF the lowest MOS of all the 4 compounds due to the highest concentration detected in water supply. However, CF did not present the highest additional cancer risk. BDCM without TDI provided to calculate for MOS, gave the highest lifetime additional cancer risk (68% of total additional cancer risk, Figure 7) despite a lower concentration than CF was found.

Figure 7 presents the percentage of lifetime additional cancer risk shared by the 4 compounds of THMs when maximum risk was calculated. The rank of additional cancer risk was BDCM>CF>CDBM>BF.

Table 7 Health risk assessment for maximum THMs detected in water samples before boiling.

THMs	Concentration	CDI (mg/kg body weight. day)	Slope factor (kg.d/ mg)	Lifetime additional cancer risk	Margin of safety
CF	85.62	3.11×10^{-3}	0.0061	1.89×10^{-5}	4.82
BDCM	31.81	1.16×10^{-3}	0.0620	7.17×10^{-5}	-
CDBM	4.94	1.80×10^{-4}	0.0840	1.51×10^{-5}	119.04
BF	0.01	3.90×10^{-7}	0.0079	3.04×10^{-9}	46371.27
Total risk				1.05×10^{-4}	

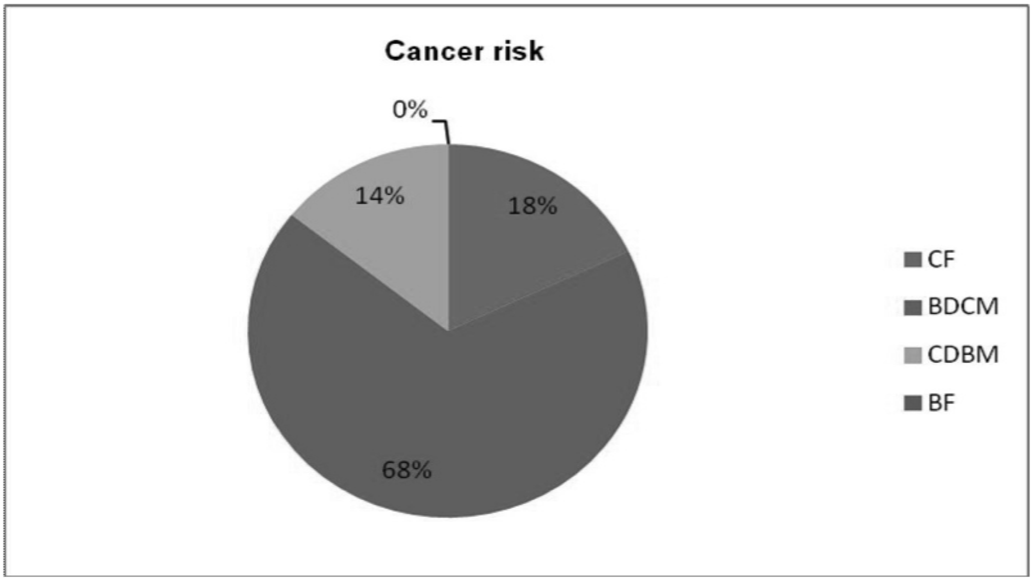

Figure 7: Percentage of additional cancer risk shared by the 4 compounds of THMs when maximum risk was calculated

4. Discussion

From chemical analysis, it could be concluded that boiling is an effective method in reducing THMs in the water supply and either one of the methods, open boiling or closed boiling, could be used to reduce THMs. The longer boiling time results in lesser THMs remained in the water. This is due to the volatility nature of THMs. Therefore, more heat input was, lesser residues remained. Although BF at 5 min boiling interval increased in concentration compared to control due to its higher boiling point (149-150^{0}C) than water, repeated boiling also reduced its concen tration to undetectable level.

The finding that THMs compounds were higher from the open boiler than the closed boiler in longer boiling intervals (10 h and 24 h), could be due to the different properties of the boilers. In a closed boiler, to collect water sample for the analysis, water ran by a pump. The pressure performed by dispensing pump in addition to the boiling temperature could increase the evaporation of volatile substances like THMs. Thus, this caused lower concentration of compounds detected in a closed boiler than an open boiler.

From MTT assay, it could be concluded that water samples after boiling did not reduce the cell viability of Chang liver cells and the 2 boiling methods were not significantly different in cell viability. Although after 48 h incubation period, the closed boiler water at 5 min boiling, and the open boiler water at 10 h and 24 h boiling showed significantly higher cell viability than the negative control, this may be due to the nature of Chang liver cell growth and undetected matters remained in the water after boiling. This finding may need further investigation to explain.

The total lifetime additional cancer risk from the 4 compounds of THMs were found in a marginal safe range with BDCM sharing 68% of total additional cancer risk. This should be concerned since a little increase of this brominated compound could result in unacceptable cancer risk. Therefore, reduction or avoidance of brominated compounds in use should be promoted to keep the water environment safer.

5. Conclusions

This study monitored the occurrence of THMs in municipal water supply in Khon Kaen Province in order to obtain baseline data and compared the amount of THMs remained after 2 different boiling methods. This is due to the boiling practice in modern households has been changed to a closed boiling pot not the conven tional open boiler as in the old days. This was aimed to test for different health impacts for further decision making. The study found that after boiling in either ways, THMs were reduced effectively and the longer boiling time resulted in the more reduction of THMs. The test of cell toxicity via MTT assay found that the 2 boiling methods were not significantly different in cell viability and both methods did not affect cell viability of Chang liver cells. Since the total lifetime additional cancer risk from THMs is marginally acceptable and BDCM, the brominated compound, presented the highest cancer risk of all THMs studied, brominated compounds should be avoided in use to keep the water environment safer.

6. Acknowledgments

The study was supported by Khon Kaen University research grant, fiscal year 2013, and graduate research grant of Faculty of Pharma ceutical Sciences, Khon Kaen University, The authors would like to express their gratitude to the staff of Khon Kaen municipal water supply and local people in Baan Samran district for their kind support during the sample collection.

References

Carpi, M., Zufall, C. (2003). GC analysis of trihalomethanes in drinking water - A rapid and direct quantitative method. Lc Gc N. Am., 88-91.

Do M, Ratanapan C, Suksaroj T. (2011). Health risk from exposure to trihalomethane in swimming pools. Journal of pubic health and development. Dev. 9(2):208-22.

LaGrega, M.D., Buckingham, P.L., Evans, J.C., (2001). *Hazardous waste Management.* McGraw-Hill. 2nd Edition. Washing ton, D.C., cited by Mallika Panyakapo and Pongsi Paopuree. (2007). Occurrence of Trihalomethanes in chlorinated drinking water: a case study of the water supply system of Nakhon Pathom municipality. Department of environmental science, Faculty of Science, Silapakorn University.

Intranont, K. (2005). *Ergonomics book. Chulalongkorn University Press.* Chulalongkorn University, Bangkok, cited by Mallika Panyakapo and Pongsi Paopuree. (2007). Occurrence of Trihalomethanes in chlorinated drinking water: a case study of the water supply system of Nakhon Pathom municipality. Department of environmental science, Faculty of Science, Silapakorn University.

Mosmann, T. (1983). Rapid colorimetric for cellular growth and survival:Application to roliferation and cytotoxicity assay. *J Immuno Methods* 65: 55-63.

IARC. (1999). Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances, Vol.73, International Agency for Research on Cancer, Lyon, France.

Rockett L., Ewence A., Gardner M., Young W. and Rumsby P. A., (2010). Drinking water inspectorate. A review of the current knowledge on dermal and inhalation toxicity of regulated THMs. WRC Swindon, Frankland Road, Blagrove, Swidon, Wiltshire.

US-EPA. (1989). Risk assessment guidance for superfund, Vol 1, Human health evaluation manual EPA/540/1-89/002, Washington DC.

US-EPA. (1997). Exposure factors handbook. General Factors I, US EPA, Washington.

US-EPA. (1998). Disinfectants and disinfection by – products. Rule : Final Rule, “Fed Reg. 63.241, Dec16.

World Health Organization. (1998). Trihalomethanes in drinking water guidelines for drinking water quality, 2nd ed. Addendum to Vol. 2. Health criteria and other supporting information. Geneva.