# Evaluation of chlorination and chloramination to degrade the Endocrine Disruptor Compounds in ground water

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## Abstract

Emerging public health concerns relating to the epigenetic effects of Endocrine Disruptor Compounds (EDCs), along with the reconceptualization of dose response curves, provides a compelling rationale for addressing estrogenically active contaminants in drinking water. These environmental health concerns are now known to have long lasting impacts, especially on fetal development. For this ground water research, the estrogenic EDCs are identified and the treatment processes, Chlorination and Chloramination are done. The quantification of EDCs present in sample/treated sample were done by Liquid Chromatography/Mass Spectrometry in tandem mass spectrometry. The EDC found in the sample was Ethinylestradiol (EE2). By comparing the reduction in EE2 present in the treated sample implied that the effective treatment process is Chlorination. Thus, the conclusion made from this research is that Chlorination is the process that is more capable of reducing EE2 present in the sample that the Chloramination process.

## 1. Introduction

Endocrine disruptors are introduced to the environment through many venues. Once these contaminants are in the environment, they are difficult to detect and often impossible to avoid. Since endocrine disruptors are active at very low levels, the amounts found in the environment are often deemed harmless yet have potential implications to the functioning of the body's endocrine system [1].

The endocrine system is very complex and has many pathways that can be disrupted internally (endogenously) and externally (exogenously). Complex systems are characterized by such interactions that lead to the emergence of new relationships at different levels of chemical organization [2].

The endocrine system, as a complex system, is composed of ductless glands that secret various hormones into the vascular system which are then transported to distant target tissues, where the hormones produce specific effects [3]. Chemical regulators are also released into the vascular system by the nervous system (neuro hormones) to regulate the release of hormones. This activity typically takes place within the liver, heart, kidneys and adipose tissue [4].

Endocrine Disrupting Compounds (EDCs) can also interrupt other pathways in the body and are not limited to the endocrine system. Human exposure to these chemicals in food, water and the environment is a growing critical concern with unknown long-term and multi-generational impacts. Some EDCs have been linked to birth defects, infertility, immune system suppression, deformities to the reproductive organs, and various other health problems [5]. Exposure to EDCs, such as estrogenic EDCs, has been implicated in the observed decrease in sperm counts in
human males. More significantly, the developing fetus is exceptionally sensitive to both the natural hormone signals used to guide its development, and the exogenous chemical signals that reach it from the environment [6]. These natural signals and exogenous "morphogens" guide the fetus through its developmental path and help set the sensitivity to subsequent hormonal signals. This involvement of setting sensitivity can have life-long and multi-generational consequences. The primary objective of this study is to identify and quantify the Endocrine disruptors present in drinking water. The main objective is to find effective disinfection method that degrades higher quantity of Endocrine disrupting.

## 2.0Materials and methods

The samples were collected from Classic Clinical Lab at Vellaripatti near Melur, Tamil Nadu, India. The samples collected were ground water samples which were contaminated by the untreated wastewater released from this clinical lab where the drugs are being manufactured including the tablets that are made endocrine disrupting chemicals.

The some of the tablets manufactured in this laboratory are Novelon, Femilon that has only Ethinylestradiol not any other estrogenic EDCs like Diethylstilbestrol as per recommendation regulated by Central Drugs Standard Control Organization (CDSCO).

Due to the chemical diversity of endocrine disrupting compounds, the range of instrumental techniques available for their analysis is very large [7]. Within modern analytical techniques applicable to trace analysis of endocrine disrupting compounds (EDCs), gas chromatography (GC) and liquid chromatography (LC) in tandem with mass spectroscopy (MS) or with tandem mass spectroscopy (MS-MS), play an important role in providing sufficient selectivity and inherent sensitivity in the analysis of complex environmental matrices [8].

Most of the analytical procedures developed for environmental determination of

EDCs and emerging contaminants have been designed for analysis of specific classes of compounds [9]. Analysis of each byproduct of individual EDCs will assist in developing multi-residue methods in which different compound classes can be identified in a single analysis.

## 3.0 Result and discussion

The first sample can be examined for any endocrine disrupting compounds and can be quantified even the presence of EDCs in milligram per litre (mg/l) or picogram per litre (pg/l) range by the Liquid Chromatography in tandem Mass Spectrometry.

The result obtained from sample sent into the Liquid Chromatography in tandem with Mass spectrometry describes that the EDC present in the sample is Ethinylestrdiol and the quantity of Ethinylestradiol (EE2) present in the taken sample is 0.85mg/l.

**Table 3.1: EDC present in the sample before Disinfection process**

| S.No | Endocrine Disrupting Compound present in the taken sample detected by Liquid Chromatography | The quantity of Endocrine Disrupting Compound present in the taken sample analyzed by Mass Spectrometry (mg/l) |
|---|---|---|
| 1 | Ethinylestradiol (EE2) | 0.85 milligram/litre |

Two separate samples collected from the contaminated site are disinfected by Chlorination disinfection process and Chloramination disinfection process. Here both of these disinfection methods are implemented in two separate samples in order to find out the most effective disinfection method by comparing the quantity of Ethinylestradiol percentage reduction after each disinfection methods.

The first disinfection method carried out was Chlorination. The chlorination was done by introducing 6.0mg/l of Calcium hypochlorite ($Ca(OCl)_2$) i.e Bleaching powder for minimum 5hrs.

$$Ca(OCl)_2 + H_2O \leftrightarrow HOCl + Cl^- + H^+$$

(Hypochlorous acid)

The primary disinfecting agent in the chlorination process is HOCl, while $OCl^-$ is a less effective disinfectant.

$$HOCl + Cl^- + H^+ \leftrightarrow ClO^- + H^+$$

(Hypochlorite ion)

The primary advantage of using chlorination is that after the initial reaction, the treated water can retain a chlorine residual, which can help protect the quality of drinking water throughout the distribution system [11].

According to the Safe Drinking Water Act, regulated by the EPA, the maximum allowable level of chlorine residual as $Cl_2$ is 4.0 mg/L. With high levels of $Cl_2$ residual, there is potential for irritation to eyes and nose, as well as stomach discomfort following long term exposure.

Endocrine Disruptor + HOCl + $H^+ \leftrightarrow$ byproducts

Endocrine Disruptor + HOCl $\leftrightarrow$ byproducts

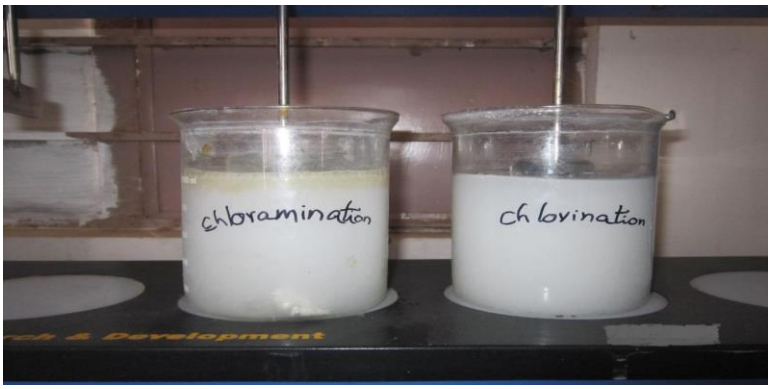


**Figure 3.1: Chloramination and Chlorination during mixing.**

Since chlorination is the most widely used disinfection process, there have been numerous studies on the treatment byproducts resulting from reactions of free chlorine with EDCs

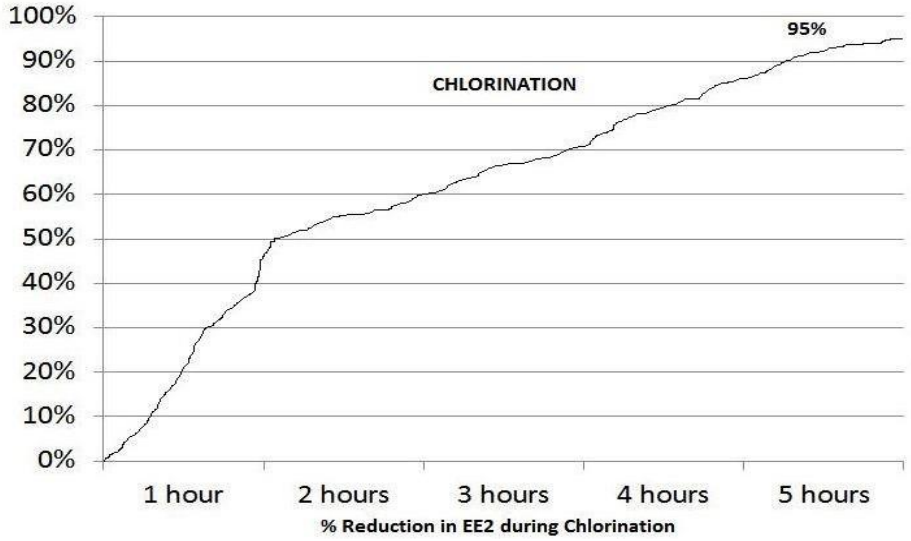


**Figure 3.2: % Reduction in EE2 during Chlorination.**

## 3.1Chloramination

The second disinfection method carried out in this project was Chloramination. To increase the lifetime of the residual chlorine disinfectant, some drinking water utilities add ammonia to finished water, forming chloramines; monochloramine ($NH_2Cl$), dichloramine ($NHCl_2$), and trichloramine ($NCl_3$) [12]. There are four types of chloramination, which differ based on the order of ammonia and free chlorine introduction: pre-ammoniation, pre-chlorination, simultaneous addition and preformed chloramines.

Chloramine does not change the pH of water, is safe to use for watering plants and the beneficial soil bacteria will not be harmed, will not affect swimming pools, will not be found in cow's milk, when they would drink chloraminated water, does not cause asthma, is not associated with heart failure, is not a carcinogen, in drinking water is safe for babies and pregnant women, does not bio-accumulate (either in fish, animals or humans) [13]. Only problem with the chloramination is that chloramines cannot be removed by reverse osmosis or boiling.

$2H_2O + Cl_2 \leftrightarrow 2HOCl + 2H^+$

(Hypochlorous acid)

Instead of adding Ammonia to chlorine to get the desired chloramination reaction, the desired chloramination effect was attained by adding 6.0mg/l of ($Ca(OCl)_2$) with 1.5mg/l of Ammonium Chloride($NH_4Cl$) (i.e in 4:1 ratio).(for minimum 5hrs).

$HOCl + NH^+_4 \leftrightarrow NH_2Cl + H_3O^+$
(Monochloramine)

$NH_2Cl + HOCl \leftrightarrow NHCl_2 + H_2O$
(Dichloramine)

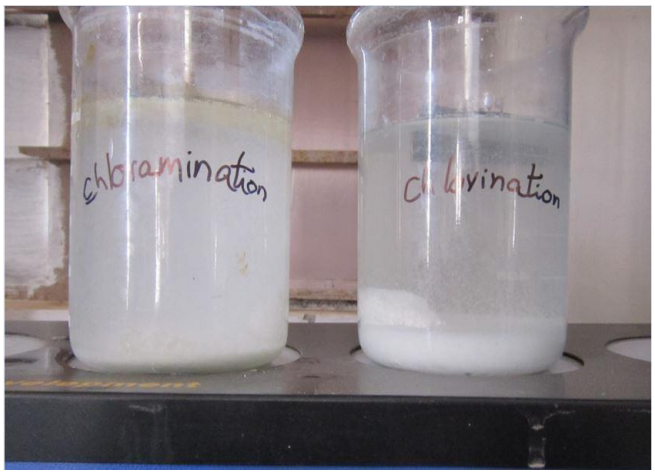


**Figure 3.3: Chloramination and Chlorination after mixing.**

Chloramines have a much lower reaction rate compared to the chlorination reaction rates with organic contaminants and are typically used as a secondary disinfectant. The primary purpose for chloramine addition is to control microbes [14]. There are potential long-term chloramine exposure effects, such as anemia, eye and nose irritation and stomach discomfort. According to the SDWA, the MRDL for chloramines as $Cl_2$ is 4.0 mg/L.

$NHCl_2 + HOCl \leftrightarrow NCl_3 + H_2O$

(Trichloramine)

If the chloramine formation rate is slower than the free chlorine reaction rate with the estrogenic compounds, then the same daughter products will be produced as discussed in the free chlorine reaction. Since the published research on chloramination reactions with estrogenic compounds is limited, daughter products formed from the reactions have not been identified for any estrogenic EDCs.

Conversely, if the chloramine formation rate is faster than the free chlorine reaction rate with the contaminant, then new daughter products may be formed.

Since the reaction rates and products for chlorine and ammonia are pH dependent, the daughter products produced will consequently depend on the pH of the system.

Chloramination is widely used, however, as a secondary disinfectant and could result in byproduct formation within a distribution system. The reaction rates with chloramines are relatively slow compared to other types of disinfectants.

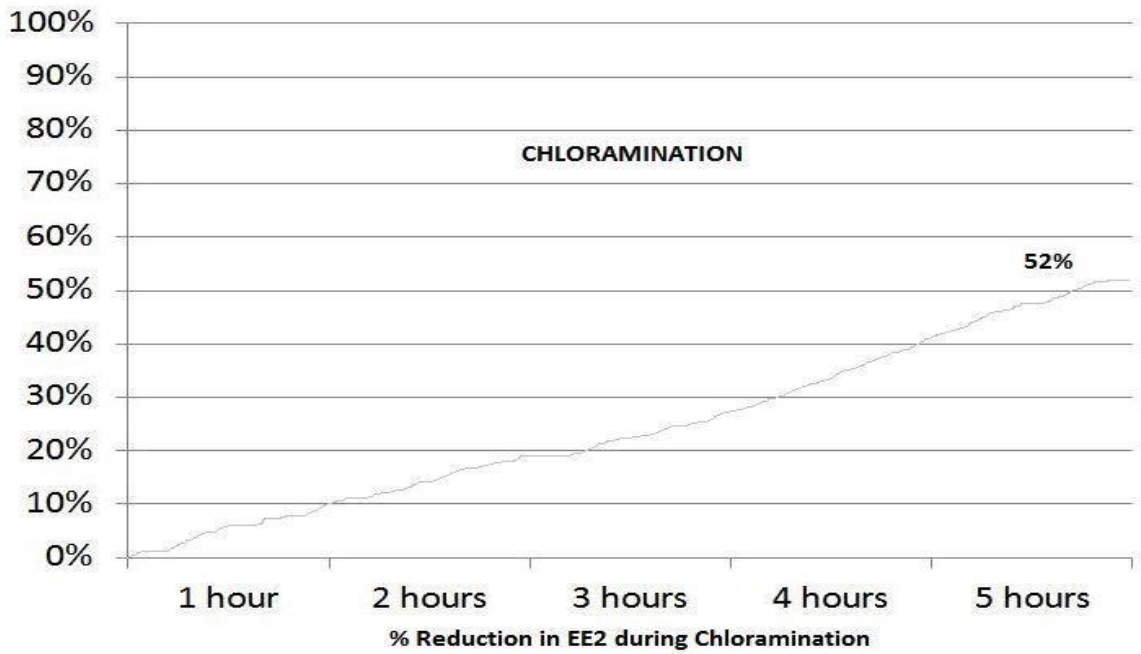


**Figure 3.5: % Reduction in EE2 during Chloramination.**

## 3.2LIQUID CHROMATOGRAPHY/ MASS SPECTROMETRY

**Liquid chromatography** generally utilizes very small particles packed and operating at relatively high pressure, and is referred to as high performance liquid chromatography (HPLC); modern LC-MS methods use HPLC instrumentation, essentially exclusively, for sample introduction. In HPLC, the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase generally composed of irregularly or spherically shaped particles chosen or derivatized to accomplish particular types of separations. HPLC methods are historically divided into two different sub-classes based on stationary phases and the corresponding required polarity of the mobile phase.

Sample components are separated using an HPLC column where the analytes are differentially partition between the mobile phase (eluent) and the stationary phase (coated onto a support material and packed into the column).

For HPLC analysis the analyte must be soluble in the mobile phase. HPLC samples are prepared in a solvent system that has the same or less organic solvent than the mobile phase and injection volumes of 1 to 50 μl are common (1-10μg of analyte per 1g packing material)

**Mass spectrometry** (MS) is an analytical technique that measures the mass- to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule, and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. MS works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios.

The Mass Spectrometry technique has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is thus useful to study physical, chemical, or biological properties of a great variety of endocrine disrupting compounds.

The quantification and identification of target endocrine compound Ethinylestradiol (EE2) present in the two samples after undergoing two different disinfection treatment process done through the Liquid Chromatography in tandem with Mass Spectrometry is as follows,

The Chlorinated sample is taken as sample 1 and it is sent through Liquid Chromatography/ Mass Spectrometry. The LC/MS targeting Ethinylestradiol (EE2) measures the quantity of EE2 as 0.04 mg/l. Thus the quantity of Ethinylestradiol reduced by Chlorination process is 0.81mg/l which means EE2 was reduced by 95%.

The Chloraminated sample is taken as sample 2 and it is also sent through Liquid Chromatography/ Mass Spectrometry. The LC/MS targeting Ethinylestradiol (EE2) records the quantity of EE2 as 0.41 mg/l. Thus the quantity of Ethinylestradiol reduced by Chloramination process is 0.45mg/l which means EE2 was reduced by 52%.

**Table 3.2: Ethinylestradiol (EE2) present in samples after disinfection process.**

| S.No | Disinfection Method | EE2 before Disinfection (mg/l) | EE2 after Disinfection (mg/l) | EE2 reduction % |
|---|---|---|---|---|
| 1 | Chlorination | 0.85 | 0.04 | 95 |
| 2 | Chloramination | 0.85 | 0.41 | 52 |

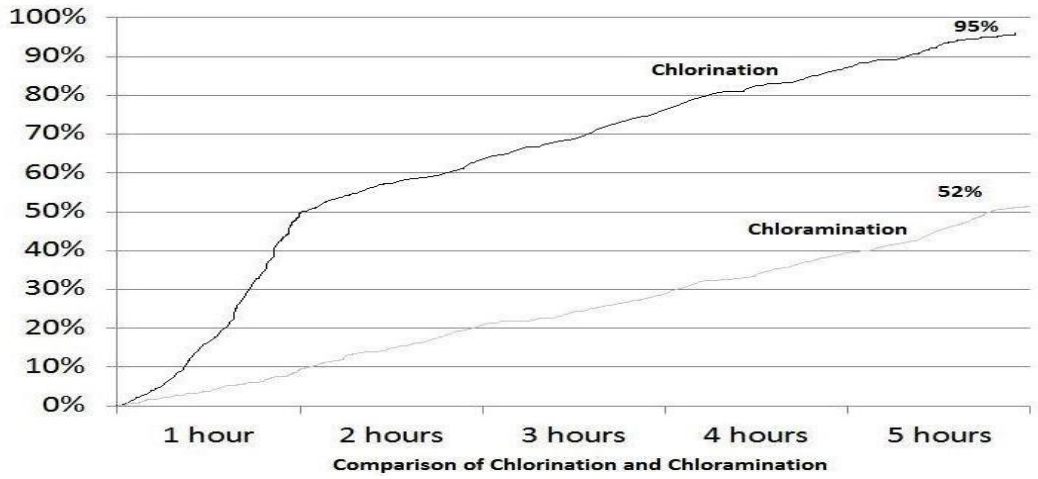


**Figure 3.6: Comparison of Chlorination and Chloramination.**

## 4.0 Conclusion

The samples collected from the ground water contaminated site are examined with Liquid Chromatography in tandem with Mass Spectrometry (LC-MS) in order to find the EDCs and quantity of EDCs present in the sample.

The LC-MS-MS analysis of estrogenic compounds is much faster and less affected by error. Because the Gas Chromatography utilizes the gas phases during analysis, a derivitization step must be added during sample preparation to condition analytes from the liquid to gas phase change. In LC/MS, the Liquid Chromatography section is which helps to check the presence of target chemicals in the sample whereas the Mass Spectrometry (in tandem) section uses analytical technique which helps to find even to the detection limit of milligram per litre (mg/l) or picogram per litre range (pg/l).

Then the conventional disinfection methods Chlorination and Chloramination are carried out in separate samples. The primary disinfecting agent in the chlorination process is HOCl, while $OCl^-$ is a less effective disinfectant. Whereas Chloramines have a much lower reaction rate compared to the chlorination reaction rates with organic contaminants and are typically used as a secondary disinfectant. The primary purpose for chloramine addition is to control microbes. Monochloramine has several advantages over chlorine as a secondary disinfectant. Monochloramine is more chemically stable than chlorine.

Now once again LC/MS technique is used to examine both of the disinfected samples in order to find the quantity of Ethinylestradiol (EE2). The percentage decrease in EE2 by Chlorination is 95% and the percentage decrease in EE2 by Chloramination is 52%. By interpreting the results obtained from LC/MS, the effective disinfection method for treating EDCs was found. Chlorination disinfection method was found to be effective than Chloramination disinfection method.

The consequences of having low quantities of EDCs in water are not completely known, it is imperative that changes to wastewater and drinking water treatment techniques be considered in order to optimize degradation, while keeping the original integrity of the treatment system. Without placing adequate attention on EDCs in the environment, we may reach a tipping point where increased hormonal activity could do irreparable damage to our ecosystems and future generations.

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