

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170913

## Chemical risks associated with ready-to-eat vegetables: quantitative analysis to estimate formation and/or accumulation of disinfection byproducts during washing

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### Abstract

Fresh produce can become contaminated with disease-causing microorganisms and chemical contaminants at every step of the production and processing chain and in a variety of ways, including through contact with contaminated process water. Water quality is critical to prevent microbial and chemical risks in any of the postharvest and processing operations related to fresh and fresh-cut fruits and vegetables. The wash process requires high volumes of water, which are usually reduced by water reuse. To maintain the microbiological quality of the process water, intervention strategies are needed. Chemical disinfection is the most common method to maintain the microbial quality of process water. However, the use of chemicals leads to the formation/accumulation of disinfection byproducts (DBPs), which can be absorbed by the washed vegetables. This is the case of trihalomethanes (THMs) and chlorates. The presence of high concentrations of DBPs in vegetables has led to an intensive debate on current disinfection practices and how DBPs may enter the food supply chain, becoming a potential health risk for consumers. To assess the risk associated with the formation/accumulation of DBPs in process water, a quantitative analysis was done. Available data have been used to develop mathematical models to predict the formation/accumulation of DBPs (chlorates and THMs) in process water due to the use of chlorine-derived compounds. Preliminary models have been developed, but adjustments are still needed to refine them. The present study contributes more information related to the development of a mathematical model for the accumulation of chlorates and THMs in process water.

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**Keywords:** Modelling, Quantitative Risk Assessment, Chemical Hazard, byproducts, Disinfection

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**Acknowledgements:** This report is funded by EFSA as part of the EU-FORA Program and the project: Inside the project MINECO (Project AGL2016-75878-R) the Center for Produce Safety Grant Agreement (Project 2017-01) and CSIC (Intramural 201670E056).

**Suggested citation:** Gadelha JR, Allende A, López-Gálvez F, Fernández P, Gil MI and Egea JA, 2019. Chemical risks associated with ready-to-eat vegetables: quantitative analysis to estimate formation and/or accumulation of disinfection byproducts during washing. *EFSA Journal* 2019;17(S2):e170913, 12 pp. <https://doi.org/10.2903/j.efsa.2019.e170913>

**ISSN:** 1831-4732

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## Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Chemical sanitisers to maintain the microbiological quality of process wash water.....	4
1.2. Disinfection byproducts.....	4
1.2.1. Chlorates.....	4
1.2.2. Trihalomethanes.....	5
1.3. Predict models.....	5
2. Description of work programme.....	6
2.1. Aims.....	6
2.2. Activities/methods.....	6
3. Conclusions.....	7
References.....	8
Abbreviations.....	9
Annex A – Mechanistic model for chlorates quantitative prediction.....	10
Annex B – Power model to predict THMs concentration.....	12

## 1. Introduction

### 1.1. Chemical sanitisers to maintain the microbiological quality of process wash water

Chemical disinfection, especially using chlorine-derived compounds, is a usual practice to maintain the microbiological quality of process water in the fresh-cut vegetable industry. Chlorine-derived compounds are inexpensive and very efficient reducing microorganisms present in water, when used under the recommended operational standards (Garrido et al., 2019). In general, fresh produce industry uses large volumes of water in the different steps of the postharvest and processing activities, such as washing. The maintenance of an optimal minimum chlorine concentration in the washing tank guarantee the microbiological quality of the process wash water while avoiding the cross-contamination of the different product batches that are washed in the same washing tank (Gil et al., 2016, 2019). However, the use of chlorine-derived compounds has been linked to an increase of chemical risk due to the formation/accumulation of Disinfection byproducts (DBPs). Although other disinfection technologies have been suggested to maintain the microbiological quality of the process water, chlorine-derived compounds are still highly demanded by fresh produce processors and the potential chemical risks should be evaluated (López-Gálvez et al., 2018, 2019).

### 1.2. Disinfection byproducts

Disinfection byproducts are formed by the reaction of chemical disinfectants with byproduct precursors. The use of chlorine-derived compounds leads to the formation/accumulation of different types of DBPs, which can be generated by two pathways: (1) formation of chlorates in chlorinated water as a result of chlorine degradation and (2) formation of halogenated DBPs such as trihalomethanes (THMs) and haloacetic acids (HAAs) due to the reaction of chlorine with the organic matter. In the case of chlorates formation, the main mechanism is the disproportionation of the free chlorine (HClO/CLO), whose balance is determined by the pH. Therefore, to quantitatively predict the accumulation of chlorates in process water, it is important to determine the needed addition of free chlorine to maintain the quality of the water. Regarding THMs and HAAs, the natural organic matter (usually measured as total organic carbon (toc)) and inorganic matter (bromide) are the most significant disinfection byproduct precursors. They react with naturally present fulvic and humic acids, amino acids, and other natural organic matter, as well as iodide and bromide ions, to produce THMs, HAAs, bromate and chlorite. Most of the commonly used chemical disinfectants (e.g. sodium hypochlorite, calcium hypochlorite and chlorine gas) react with organic matter and/or bromide to varying degrees to form different DBPs (Morris, 1966; Adam and Gordon, 1999; Black and Veatch Corporation, 2010) (Table 1).

Chlorine is the most common disinfectant used worldwide, and chlorates, THMs and HAAs are the DBP classes formed at the highest concentrations after chlorination. The DBPs could be originated by organic and inorganic compounds: (1) Halogenated compounds: THMs, HAAs, halonitromethanes, haloaldehydes and haloacetones, haloacetamides, haloacetonitriles and haloalcohols; (2) Non-halogenated compounds: aldehydes and ketones of low molecular weight, other carboxylic acids, keto acids, nitriles and nitrosamines and (3) inorganic byproducts: decyanogen chloride, chlorites, chlorates and bromates.

Additionally, the so-called 'emerging' DBPs such as halonitromethanes, haloacetonitriles, haloamides, halofuranones and iodo-acids such as iodoacetic acid, iodo-THMs (iodotrihalomethanes), nitrosamines and others could also be formed.

#### 1.2.1. Chlorates

Chlorates are substances with high power of oxidation and were widely used as a pesticide in the past, but in European Union, they are banned since 2008. This why, currently, the use of chlorine derived as water disinfectants is by far, the principal source of chlorates in fruits and vegetables. Despite this, the use of chlorine-derived compounds is still widely use in Europe to maintain the quality of process water (Gil et al., 2016).

In the European Union, there is a current debate regarding the maximum residue level (MRL) for chlorate in different fruits and vegetables, because the previous MRL of 0.01 mg kg<sup>-1</sup> is not valid since 2014. In the United States, the regulatory limit for chlorate and chlorite only applies for drinking water and is establish at 700 µg/L for each. Chlorates levels, included in the US Environmental Protection

Agency's monitoring of unregulated contaminants and on the contaminant candidate list, could potentially receive a regulatory determination in the near future. The present report, using available literature along with past and current monitoring data, assesses the presence of chlorate in drinking water and the potential impact of its regulation. Data are still missing regarding the maximum levels that could be recommended in process water in contact with fresh fruits and vegetables (Tables 2 and 3).

There is scarce information regarding the real risk that accumulation of chlorates in fresh fruits and vegetables represents for consumers. Based on available studies conducted to estimate the carcinogenicity and genotoxicity of these compounds in rodents, BPDs seem to be a concern for the human health (SCHER/SCCP/SCENIHR, 2008).

### 1.2.2. Trihalomethanes

Trihalomethanes are a group of four chemicals that are formed along with other DBPs when chlorine-derived compounds are used to maintain the microbiological quality of process water containing high concentrations of organic and inorganic matter. These compounds have been defined as carcinogenic compounds, becoming a relevant concern for the public health. Epidemiological evidence has shown a consistent association between long-term exposure to THMs and the risk of bladder cancer, although the causal nature of the association is not conclusive. Evidence concerning other cancer sites is insufficient or mixed (Villanueva et al., 2015). Numerous studies have evaluated reproductive implications, including sperm quality, time to pregnancy, menstrual cycle and pregnancy outcomes such as fetal loss, fetal growth, preterm delivery and congenital malformation. The body of evidence suggests only minor effects from high exposure during pregnancy on fetal growth indices such as small for gestational age (SGA) at birth. THM formation can be minimised by avoiding the use of pre-chlorination.

Regulations in developed countries governing DBPs have established varying thresholds for the THM presence in drinking water. The maximum contaminant levels (MCL) of total THMs (the sum of chloroform, bromodichloromethane, dibromochloromethane and bromoform) have been set at 80 µg/L in the United States and 100 µg/L in the European Union (EU) (EPA, 2011). While no parametric values have been set for DPBs other than THMs and bromate, there is a requirement under Regulation 13 of the Drinking Water Regulations that 'any contamination from DPBs is kept as low as possible without compromising the disinfection, in accordance with any such directions as the supervisory authority may give' (EPA, 2011).

### 1.3. Prediction Model

Mathematical models are abstract representations of physical or chemical systems able to predict the system's response to some conditions without need of performing new experiments. During the model-building process, one of the key steps is the calibration of the proposed model through experimental data. We can distinguish two main groups of mathematical models: (i) Deterministic models, which are based in mass and energy balances as well as in physical laws and kinetics, and (ii) Empirical models, which usually fit experimental data to certain mathematical functions. In the first case, the mechanism of the process is known, and such models are able to make predictions in different conditions from those used to calibrate the model. In the case of empirical models, they are useful when the process mechanisms are not known. Its application to different conditions from the calibration one must be cautious.

Mathematical modelling is a mathematical and statistical method of studying events and predicting outcomes in different scenarios without the need of retrieving new experimental data.

Time series data of free chlorine (FC), Chemical Oxygen Demand (COD), pH and ultraviolet (UV) absorbance of process water as well as the chlorates and THMs concentrations have been used to construct mathematical models able to predict the DBP's concentration along experiments of wash water disinfection of different processing lines of leafy greens. The results of the study would help to improve the confidence on a disinfection system predicting the DBPs formation and reducing the risks to human health.

In the present study, available data have been used to develop mathematical models able to predict the formation/accumulation of DBPs (chlorates and THMs) in process water due to the use of chlorine-derived compounds. A mechanistic model was developed in the case of chlorates whereas an empirical model based on multiple linear regression was built for THMs quantitative prediction.

## 2. Description of work programme

### 2.1. Aims

The goals of the current project were to collect the data generated in the last 2 years in the CEBAS-CSIC research group (the host institution) and use them to develop mathematical models that can explain the formation/accumulation of chlorates and THMs in process wash water obtained from different processing lines of fresh produce (Gil et al., 2016, 2019; López-Gálvez et al., 2018, 2019). Four different process, wash water have been evaluated including: iceberg lettuce (*Lactuca sativa* L. var. *capitata*), a mix of three types of baby leaves (rocket; *Eruca sativa* Mill., red oak leaf; *Lactuca sativa* L. cv. oak leaf and red swiss chard; *Beta vulgaris* L. var. *cycla*), red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) and diced onion (*Allium cepa* L.). Based on the available data, the aim was to determine the potential risks associated with the accumulation of chlorates and THMs in different types of process water. Different scenarios were evaluated taking into account the different types of products, physico-chemical parameters of process water, format of the fresh produce, the washing time as well as the concentration of DBPs generated in the process wash water. Therefore, the aim of the project was to develop mathematical models able to predict the formation/accumulation of DBPs (chlorates and THMs) in process water due to the use of chlorine-derived compounds.

### 2.2. Activities/methods

The experimental data used for the development of the model were obtained in lab scale experiments simulating the industrial fresh produce wash systems (Tudela et al., 2019). In these experiments, the disinfectant concentrations needed to avoid cross-contamination in the washing tank between different product lots was established as well as the potential formation and/or accumulation of DBPs formed during the washing. The selected DBPs were chlorates (inorganic DPBs) and THMs (halogenated DPBs). Both are chlorine DBPs and relevant for human health. With the use of probabilistic distributions, a model was developed to describe how different factors may or may not contribute to the risk. Model predictions provided an estimation of the disinfectant concentrations necessary to eliminate the microorganisms but assuring the chemical safety to human being.

Factors which influenced DBP formation include:

- Type of disinfectant used\*;
- ✓ Concentration of disinfectant used\*;
- ✓ Concentrations of organic matter and other DBP precursors in water to be disinfected;
- ✓ Water temperature (seasonality)\*;
- ✓ pH\*;
- ✓ Contact time;
- ✓ Length of the distribution network;
- ✓ Vegetable type
- ✓ Bromide ion concentration (THMs case)

\*controlled parameter

Briefly, the experimental set-up aimed to simulate a real agro-industry disinfection system (Tudela et al., 2019). First, an aqueous solution with a high concentration of free chlorine was elaborated. This concentrated chlorinated solution was used to maintain a constant free chlorine concentration in the washing tank. Four different concentrations of free chlorine were selected (0, 10, 20 and 30 ppm). A washing tank of approximately 15 L capacity was used to simulate a washing tank of the fresh produce industry. The washing tank was filled with about 6 L of pipe water (with undetectable concentrations of chlorates and THMs). Following a dynamic system, a concentrated aqueous solution of organic matter (about 2,000 mg/L, COD) obtained after washing approximately 10 kg of produce in a small volume of water was constantly added to the washing tank to simulate the continuous entrance of produce in the washing tank. At the same time, the selected concentration of free chlorine (0, 10, 20 and 30 ppm) was constantly maintained by adding chlorine from the concentrated chlorinated solution. The system was running for 80 min for each chlorine concentration. In order to measure and assure the controlled conditions, physico-chemical parameters, such as free chlorine, COD and pH, were measured each 5 minutes. Water samples were collected every 20 minutes to measure the DPB concentrations. Phosphoric acid was also added to the solution, in order to keep the pH constant at ~~6.5~~ 5.6 in order to guarantee a high concentration of hypochlorous acid in the washing

solution, which is guarantee of the efficacy of chlorine. Microbiological parameters were also evaluated to determine the antimicrobial capacity of the chlorine solution (Tudela et al., 2019).

The working plan of the fellow included: (1) to learn microbiological techniques to get familiar with the microbiological parameters involved in a disinfection process, (2) to understand the microbial data and to determine the best methods to analyse microbiological data, (3) to develop mathematical models able to predict the formation/accumulation of DPBs in process water used to wash fresh produce, (4) to get familiar with different tools used to elaborate statistical analyses of data including Bioinactivation (Garre et al., 2018) and R programming language (R Core Team, 2018). To this, the fellow has performed a Statistical course related to use the R software on March 11–15 (hosting by CIIMAR, Porto-Portugal) to improve her knowledge in the topic and finally to apply the concepts 'learning-by-doing' using the previous data produced to design a mathematical model.

This research project aims to give a tool to the industry that allow the prediction of the formation/accumulation of DPBs in process water. This can lead to the establishment of good handling practices in order to avoid the presence of these compounds in the final product, reducing the exposure of the consumers to these DBPs.

### 3. Conclusions

This work is still in draft form and the main conclusions are not yet formulated since the analysis is ongoing. The information presented here is therefore to be considered provisional. Indeed, our system has a dosing scheme to replenish the possible losses of free chlorine or to maintain its level within a desired range.

We identify the sources of production or depletion of chlorates in process water obtained during the washing of different leafy greens. Each type of vegetable generates a different type of process water, which might affect the formation of DBPs in the water and consequently to have a different impact on the chemical risk for consumer.

The present study constitutes only one step of all the steps needed to establish a Chemical Risk Assessment regarding the risks posed by DPBs present in process water which can be absorbed by fresh fruits and vegetables in contact with the water. However, this is a relevant step in order to estimate the formation and accumulation of DPBs in process water. The final objective will be to determine risks linked to the consumption of fresh fruits and vegetables.

**Table 1:** Disinfectant and the respective byproducts generated by the disinfection process (Adapted from USEPA Drinking Water Guidance on Disinfection byproducts)

Disinfectant	Disinfectant byproducts	Disinfectant byproducts
Chlorine (e.g. gas, sodium hypochlorite, tablets, OSEC)	Trihalomethanes, Haloacetic Acids, Chloramines1, Chlorinated Acetic Acids, Halogenated Acetonitriles, Chloral Hydrate, Chlorophenols, MX2, bromate3, chloropicrin, halofurans, bromohydrins.	Trihalomethanes, Haloacetic Acids, Chloramines1, Chlorinated Acetic Acids, Halogenated Acetonitriles, Chloral Hydrate, Chlorophenols, MX2, bromate3, chloropicrin, halofurans, bromohydrins.
Chlorine Dioxide	Chlorite, Chlorate and Chloride	Chlorite, Chlorate and Chloride
Ozone	Bromate, Formaldehyde, Aldehydes, Hydrogen Peroxides, Bromomethanes.	Bromate, Formaldehyde, Aldehydes, Hydrogen Peroxides, Bromomethanes.
Chloramines	Dichloramines, Trichloramines, Cyanogen Chloride, Chloral Hydrate.	Dichloramines, Trichloramines, Cyanogen Chloride, Chloral Hydrate.

**Table 2:** Components of DBPs in drinking water, their effects and regulatory limits (Adapted from Chowdhury et al., 2009)

Main Group	Compounds	Acronym	Main disinfectant*	Effects	Toxicity to Human	Regulations (µg/L)						
					Human	RFD	SF	HC (2007)	USEPA (2006)	WHO (2004)	Aus-NZ (2004)	UK (2000)
Trihalomethanes (THMs)	Chloroform	TCM	Chlorine	Animal Liver tumors	B-2	0.01	0.01			300		
	Bromodichloromethane	BDCM	Chlorine	Kidney tumors	B-2	0.02	0.062	16		60		
	Bromochloromethane	BCM	Chlorine	Colon tumor	B-2	0.02	0.0079			100		
TTHMs	Dibromochloromethane	TBM	Ozone, Chlorine	Liver tumors	C	0.02	0.0084			100		
								100	80		250	100
Haloacetic acids (HAAs)	Bromochloroacetic acid	BCAA	Chlorine	Liver tumors								
	Bromodichloroacetic acid	BDCAA	Chlorine	Liver tumors								
	Chlorodibromoacetic acid	CDBAA	Chlorine	Liver tumors								
	Dibromoacetic acid	DBAA	Chlorine	Liver tumors								
	Dichloroacetic acid	DCAA	Chlorine		B-2	0.004	0.05			50	100	
	Monobromoacetic acid	MBAA	Chlorine									
	Monochloroacetic acid	MCAA	Chlorine									
HAA5	Tribromoacetic acid	TBAA	Chlorine								150	
	Trichloroacetic acid	TCAA	Chlorine	Liver tumors	C					100	100	
								60				
Haloacetonitriles (HANs)	Bromochloroacetonitrile	BCAN	Chlorine	Embryo-death								
	Dibromoacetonitrile	DBAN	Chlorine	Skin tumor						70		
	Dichloroacetonitrile	DCAN	Chlorine	Embryo-death						20		
	Trichloroacetonitrile	TCAN	Chlorine	Embryo-death	C							
Haloketones (HKs)	1,1-dichloropropanone	DCP	Chlorine									
	1,1,1-trichloropropanone	TCP	Chlorine									
Aldehydes	Formaldehyde		Ozone, Chlorine							900	500	
	Acetaldehyde		Ozone, Chlorine									
	Glyoxal		Ozone, Chlorine									
Carboxylic acids	Methyl glyoxal		Ozone, Chlorine									
	Formate		Ozone									
	Acetate		Ozone									
Nitrosamines	Oxalate		Ozone									
	Nitrosodimethylamine		Chloramine		B-2		51					
Cyanogen halides	Chloramine		Chloramine							70	80	
	Cyanogen bromide		Chloramine									
Chloral hydrate		CH	Chlorine							10	20	
Bromate			Ozone		B-2	0.004	0.7	10	10	10	20	10
Chlorate			Chlorine Dioxide							700		
Chlorite			Chlorine Dioxide		D	0.03	-	1000	700	300		

**Table 3:** Basic information and attributes of disinfectants (Adapted from Chowdhury et al., 2009)

Issue		Chlorine	Chloramine	Chlorine dioxide	Ozone	Ultraviolet radiation	Reference
Application		Most common	Common	Occasional	Common	Emerging use	USEPA (2006)
Cost		Lowest	Moderate (>chlorine)	High	High	Extremely High	Clark et al. (1994)
Disinfection efficiency	Bacteria ( <i>V. cholerae</i> , <i>Coliform</i> , <i>E. coli</i> , etc)	Excellent	Good	Excellent	Excellent	Good	MWH (2005), Sadiq and Rodríguez (2004)
	Viruses (Polio virus, Rota virus, MS2 coliphase, etc)	Excellent	Fair	Excellent	Excellent	Fair	
	Protozoa ( <i>G. lamblia</i> , <i>C. parvum</i> , <i>E. intestinalis</i> , etc)	Fair to poor	Poor	Good	Good	Excellent	
	Endospore	Good to poor	Poor	Fair	Excellent	Fair	
Organisms regrowth		Unlikely	Unlikely	Likely	More likely	More likely	MWH (2005)
Limits on free residuals		4 mg/L	4 mg/L	0.8 mg/L	-	-	USEPA (2006)
Byproducts	Regulated	4 THMs, HAAs	Traces of THMs and HAAs	Chlorite	Bromate	None	USEPA (2006)
	Unregulated	Many	Many: cyanogen halides, NDMA	Many: chlorate	Biodegradables organics	None know	Richardson (2005)
Oxidation		Strong	Weak	Selective	Strongest	None	Chlorine Chemistry Council (2003)
Odor and taste removal		Excellent	Good	Excellent	Good to poor	None	
Stability		Stable	Stable	Unstable	Unstable	Unstable	

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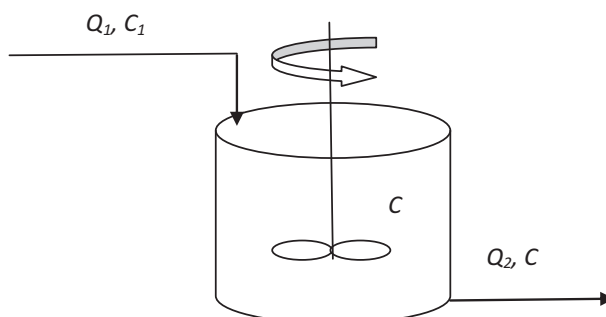
## Abbreviations

DBPs	Disinfection byproducts
CEBAS	Centro de Edafología y Biología Aplicada del Segura
CIIMAR	Interdisciplinary Centre of marine and Environmental Research
COD	Chemical Oxygen Demand
CRA	Chemical Risk Assessment
CSIC	Consejo Superior de Investigaciones Científicas
EU	European Union
FC	Free Chlorine
HAAs	Haloacetic Acids
MCL	Maximum Contaminant Level
MRL	Maximum Residue Level
SGA	Small for Gestational Age
THMs	Trihalomethanes
toc	total organic carbon
USEPA	United States Environmental Protection Agency
UV	Ultraviolet radiation

## Annex A – Mechanistic model for chlorates quantitative prediction

### Evolution of chlorates

The following scheme represents the system used in this study: A Stirred continuous tank.



Where  $Q_1$  and  $Q_2$  represent, respectively, the inlet and outlet flow rate. We assume that the liquid volume ( $V$ ) remains constant inside the tank, thus  $Q_1 = Q_2$  (we call them  $Q$ ).  $C_1$  is the chlorates concentration of the inlet stream (which we assume it is zero or very close) whereas  $C$  is the chlorates concentration  $[ClO_3^-]$  of the outlet stream, which varies with time and equals the concentration inside the tank if it is perfectly stirred.

We have to identify the sources of production or depletion of chlorates in the solution.

- 1) Dilution of chlorates by continuous inlet stream free of chlorates.
- 2) Increase of chlorates for reaction of hypochlorite to produce chlorates. If we consider a first order kinetics:

$$\frac{d[ClO_3^-]}{dt} = K_{obs}[FC] \quad (1)$$

Where  $[FC]$  is the free chlorine concentration which is controlled in our system by addition of a concentrated NaOCl solution. In this study, the value of  $[FC]$  has been considered as a constant value calculated as the mean value of the measured free chlorine concentration along the experiment. The constant ( $K_{obs}$ ) is estimated using the experimental data to minimise the differences between the measured and the predicted values of chlorates concentration.

- 3) Increase of chlorates by addition of mother solution of NaClO which contains a certain concentration of chlorates,  $C_M$ .

Indeed, our system has a dosing scheme to replenish the possible losses of free chlorine or to maintain its level within a desired range. The 'mother' solution of  $Na_2ClO$  contains a certain concentration of chlorates,  $C_M$ , due to chlorine disproportionation. We applied a dosing strategy with a fixed period  $\tau = 5min$  that consist of adding an amount of mother solution with a given rate every 5 min during a very short time,  $\tau_0 \approx 1s$ . Then, the increase of chlorates concentration in the tank due to this fact can be expressed as:

$$\frac{d[ClO_3^-]}{dt} = \frac{1}{V} \sum_{k=1}^N (Q_{M,k} \cdot C_M) \chi[k\tau, k\tau + \tau_0] \quad (2)$$

Where  $V$  is the volume of the tank,  $Q_{M,k}$  is the mother solution flow rate added each time  $k$ ,  $C_M$  is the chlorates concentration in the mother solution and  $\chi[k\tau, k\tau + \tau_0]$  is an indicator function, taking the value 1 on time interval  $[k\tau, k\tau + \tau_0]$  for some small time increment  $\tau_0$  and zero elsewhere,  $N$  is the number of doses added.

The times when we measured the THMs were on 0, 20, 40, 60 and 80 minutes, where the chlorine addition was made each 5 minutes.

Summarising all the considered terms, the change of chlorates concentration with time in our system can be represented as:

$$\frac{d[\text{ClO}_3^-]}{dt} = -\frac{Q}{V}[\text{ClO}_3^-] + K_{obs}[\text{FC}] + \frac{1}{V} \sum_{k=1}^N (Q_{M,k} \cdot C_M) \chi[k\tau, k\tau + \tau_0], \quad (3)$$

which corresponds with the points 1, 2 and 3 considered above.

The following plots show the experimental data (dots) and the model prediction (solid line) for the lettuce case in the three controlled scenarios (10, 20 and 30 ppm of free chlorine in the wash water).

## Annex B – Power model to predict THMs concentration

According to the experimental data retrieved in the laboratory, we propose a power model to predict THMs concentration as a function of the identified relevant variables: COD, UV absorbance, theoretical FC concentration and time (Abbreviation list). The proposed power model has the following form.

$$THM = \beta_0 \cdot DQO^{\beta_1} \cdot UV^{\beta_2} \cdot FC^{\beta_3} \cdot (time + 10)^{\beta_4}. \quad (1)$$

Similar to some of the models presented in the review by Chowdhury et al. (2009).

Note: We add a constant term equal to 10 to avoid numerical problems in later logarithmic transformations.

Taking logarithms from Equation 1 we obtain:

$$\log(THM) = \log(\beta_0) + \beta_1 \cdot \log(DQO) + \beta_2 \cdot \log(UV) + \beta_3 \cdot \log(FC) + \beta_4 \cdot \log(time + 10) \quad (2)$$

This formulation allows a multiple linear regression analysis. Preliminary results with lettuce indicate that there are highly correlated explanatory variables. In particular, DQO, UV and time are highly correlated. After performing a model selection procedure, the identified most accurate model contains de variables UV and time. For lettuce, the fitted model is:

$$\log(THM) = 3.45 + 0.125 \cdot \log(UV) + 0.303 \cdot \log(FC). \quad (3)$$

The coefficient of determination is  $R^2 = 0.79$ . All the coefficients are statistically significant ( $p < 0.001$ ) and the residual analysis indicates that the normality and heteroscedasticity assumptions are met.

Similar models will be developed for the other substrates (Lettuce, baby leaves, onion and col) and a model comparison procedure will be carried out to check whether these different substrates have an influence in the THMs concentration.