# Removal of pesticide residues after simulated water treatment: by-products and acetylcholinesterase inhibition

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**ABSTRACT**: Water is of extreme importance to living creatures. However, due to the actions of humans, water resources have been contaminated by many different compounds, including pesticides. Pesticides in water sources can cause damage to aquatic environments or to those who consume it. On this basis, it is important that water treatment systems can remove these pollutants from water. In this perspective, the objective is to investigate whether conventional water treatment can remove several pesticides, namely, atrazine, ametryn, malathion and chlorpyrifos. According to the results, it was observed that conventional treatment after filtration was not capable of removing these pesticides efficiently, with the organophosphorus pesticides (malathion and chlorpyrifos) removed in a higher percentage than the triazines (atrazine and ametryn). Post-chlorination reduced the pesticide levels, however, malaoxon and ametryn sulphoxide by-products were generated, which caused greater acetylcholinesterase inhibition.

Pesticide removal, by-products generated and acetylcholinesterase inhibitory after conventional water treatment simulation.
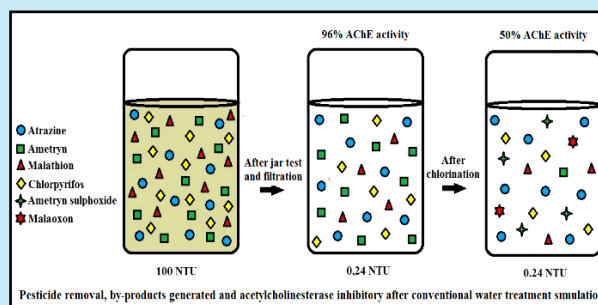
## 1. Introduction

Due to its physical and chemical properties, water was essential for the emergence of the first living organisms, as well as their evolution. It is the most abundant compound in living systems, accounting for ~70% or more of the weight of most organisms, and it plays important roles in their metabolisms. Because it is an essential compound for survival, any contamination can cause damage to humans and the environment[1].

The indiscriminate use of pesticides to accelerate food production, especially in developing countries, has resulted in contamination of natural waters, causing a serious threat to the environment in many parts of the world[2].

Pesticides are chemical compounds used to kill different kinds of pests that cause damage to crop, such as insects, fungi and undesirable plants (weeds)[3], as well as being used to protect food products during processing, storage and transport[4]. Pesticides, due to their nature, are potentially toxic to other organisms, including humans[3].

Acute exposure to pesticides can lead to death or serious illness[5]. Chronic exposure can impair the function of the endocrine, nervous, renal, immune, reproductive, respiratory and cardiovascular systems[6]. From this perspective, there is evidence relating pesticide exposure and the incidence of chronic human diseases, including cancer[7], Parkinson's disease[8], Alzheimer's disease[9] asthma[10], multiple sclerosis[11], diabetes[12],

premature aging[13], reproductive disorders,[14,15] cardiovascular disease[16] and chronic kidney disease[17].

Brazil is the world's largest consumer of pesticides and is responsible for ~20% of the total[18]. In 2014, 317 active ingredients were commercialized in Brazil, consuming ~500 thousand tons of pesticides[19].

Only 0.1% of the pesticides reach the target during application, while the remaining 99.9% has the potential to move to the environment, including surface and groundwater[20]. Thus, there is concern whether conventional water treatment systems can efficiently eliminate these contaminants. Only a few studies have been carried out with organic compounds in treated waters, and most of these studies are with pharmaceuticals and endocrine disrupters[21].

Brazilian conventional water treatment facilities typically use coagulation, flocculation, sedimentation and filtration for the removal of suspended solids and dissolved organic carbon, followed by chlorination for disinfection. This system has been shown to be largely ineffective in removing emerging micropollutants (e.g., endocrine-disrupting compounds, pharmaceuticals, personal care products and pesticides) and the addition of chlorine can result in the reaction and transformation of these compounds[21–23]. Advanced treatment technologies, such as ozonation and advanced oxidation processes, activated carbon adsorption, reverse osmosis and nanofiltration, are effective in removing these compounds[22]. Despite this, due to their high cost, advanced processes in water treatment facilities are still limited, especially in developing countries, like Brazil[24].

Therefore, the main objective of this work is to verify, by means of simulations, whether a conventional Brazilian water treatment system could remove some selected pesticide, namely, atrazine and ametryn, which belong to the class of triazines, and the organophosphorus pesticides, malathion and chlorpyrifos (Figure 1). These pesticides were chosen because they have recently been reported in the literature due to their high concentration in Brazilian surface waters, above the tolerable limit for aquatic life[18].
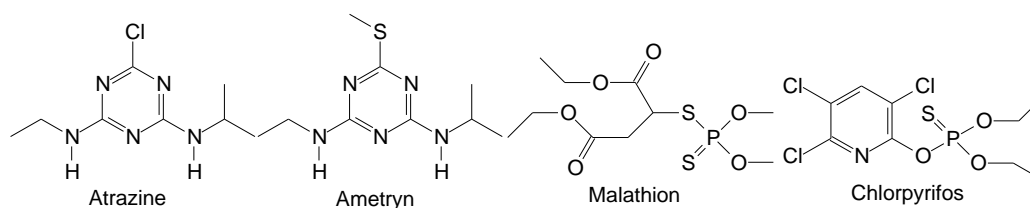


**Figure 1.** Pesticides used in this research.

## 2. Experimental

### 2.1. Materials and reagents

Atrazine (98.8% purity), ametryn (98.5% purity), chlorpyrifos (99.2% purity) and Mmalathion (99.1% purity) were purchased from Sigma-Aldrich. Aluminium sulfate ($Al_2(SO_4)_3.18H_2O$, analytical grade) and calcium hydroxide ($Ca(OH)_2$, analytical grade) were obtained from VETEC Química Fina Ltda. Kaolin was purchased from Prominérios Comércio de Minérios Ltda. and commercial sodium hypochlorite was obtained from Indústrias Anhembi Ltda. The concentration of the sodium hypochlorite solution was confirmed by a standard sodium thiosulfate titration[25].

Stock solutions of 800 mg $L^{-1}$ of the individual pesticides were prepared in pure acetone. All the stock solutions were stored in amber glass bottles at 4 ºC.

The experimental artificial sample was prepared by adding a kaolin suspension with $100 \pm 5$ NTU turbidity (TB100p MS Tecnopon) and 450 uH apparent color (Alfakit equipment)[21]. Organic and inorganic compounds were not added to artificial water because the aim was to isolate the variables and verify the treatment without interferents. The effect of a real matrix will be investigated in the next step of this work.

### 2.2. Experimental procedures

#### 2.2.1 Analytical method and recuperation tests

The method of analysis used to quantify the pesticides and to identify the by-products was based on the extraction/concentration of these

substances by means of solid phase extraction. Samples of 50 mL were submitted to solid phase extraction employing OPT 3 mL × 60 mg. Agilent cartridges were conditioned with 6.0 mL of methanol and 6.0 mL of ultrapure water in a 12-port vacuum manifold system. The analytes were then eluted using 7.5 mL of ethyl acetate with subsequent quantification by GC-MS.

*Parent compound quantification*

The extracts were analyzed by GC-MS (Shimadzu GC - 17A and MS - QP 5050) using selected ion monitoring mode. In the experiments, the injection volume was 1 µL and a VF-5ms capillary column (Varian, 30 m, I.D. 0.25 mm, 0.25 µm) was used. A flow rate of 1 mL min$^{-1}$ of helium gas, with a constant pressure of 203 kPa and a 1:10 split ratio was used. The oven temperature started from 100 °C then increased at 25 °C min$^{-1}$ to 250 °C and finally increased at 15 °C min$^{-1}$ to 270 °C. Temperatures were set at 240 °C in the injector and 230 °C in the interface to the detector. The ions monitored for atrazine detection were: m/z 215, 200 and 173; for ametryn: m/z 227, 212 and 170; for malathion: 173, 125 and 93; for chlorpyrifos: 314, 197 and 97. Quantification was performed by external standardization, with the area obtained compared with the appropriate analytical curve (Table 1)[26].

*By-product identification*

The by-products of the pesticides were also analyzed by GC-MS using similar conditions as described for the parent compounds. A split ratio of 1:10, an injection volume of 1 µL and scan mode were used for this analysis. Compounds were identified using fragmentation analysis, and isotope clustering patterns were found with the aid of the NIST library.

Preliminary tests were carried out to evaluate the recovery of the pesticides in the aqueous matrixes used in the experiments to determine the efficiency of the extraction method. The pesticide recovery ratio was determined (in triplicate) by comparing peak areas from water samples spiked with a known amount of non-extracted pure standards. The linearity of the method, calibration equation, limit of detection (LOD) and limit of quantification (LOQ) were also determined (Table 1). The detection and quantification limits were calculated using the standard deviation of the blank (synthetic water extract after simulated water treatment without pesticides) from the following equations[27]:

*LOD* = 3.3 x (blank standard deviation)/slope (Equation 1).

*LOQ* = 10 x (blank standard deviation)/slope (Equation 2)

**Note**: Slope: Angular coefficient of the analytical curve.

**Table 1.** Detection (LOD) and quantification (LOQ) limits, mean percentage recovery (% R), calibration equation* and coefficients of correlation (r$^2$) for the experimental solutions.

| Pesticide | Calibration equation | $r^2$ | % R | LOD (ng L$^{-1}$) | LOQ (ng L$^{-1}$) |
|---|---|---|---|---|---|
| Atrazine | y= 9.96.10$^5$x-1.16.10$^4$ | 0.997 | 99±1 | 8.11 | 24.58 |
| Ametryn | y=1.20.10$^6$x-2.83.10$^4$ | 0.998 | 95±2 | 6.76 | 20.49 |
| Malathion | y=1.88.10$^6$x-3.63.10$^4$ | 0.998 | 112±3 | 4.29 | 13.02 |
| Chlorpyrifos | y=1.14.10$^6$x-1.51.10$^4$ | 0.997 | 79±2 | 7.08 | 21.44 |

*Calibration equation was obtained by mixing the pesticides in synthetic water (100 NTU of turbidity) at concentrations of 0.48, 0.40, 0.32, 0.24, 0.16 and 0.08 mg L$^{-1}$.

*2.2.2 Determining optimum operating conditions for jar test*

The tests were carried out with the artificial sample mentioned in Section 2.1 using jar test Trade Lab Ambiental equipment to obtain the ideal condition of turbidity removal. Then, the pair of values "coagulant dosage × coagulation pH" was varied.

An aluminum sulphate solution (1% w/v) was used to facilitate the coagulation in the water and a calcium hydroxide solution (0.5% w/v) was used for adjusting the pH values[25].

### 2.2.3 Jar test procedures

Bench tests were performed in triplicate using the jar test method to reproduce the conventional water treatment systems, i.e., coagulation-flocculation and decantation, followed by filtration. The pesticides were added to synthetic water (pH corrected according to Section 2.2.2) at a concentration of 0.48 mg $L^{-1}$ for each compound, and their removal was verified according to Section 2.2.1.

The jar test was performed according to Brazilian standard NBR 12.216: 1992 and by the Practical manual of water analysis from the National Health Foundation to better represent the operations of the treatment plants of water, as follows:

Rapid mixture: dispersion of aluminum sulphate in 1 L of water sample to be treated, with a maximum speed of 100 rpm for 3 min;

Mechanized flocculation: total time of 10 min, with agitation speed of 50 rpm;

Decantation: the sample was left to decant for ~15 min, which corresponds to a sedimentation rate of 1.74 cm $min^{-1}$ (treatment plant with capacity of up to 1,000 m³ $day^{-1}$).

Filtration of the samples was done by gravity using 125 mm diameter filter paper. In the last step, chlorination, sodium hypochlorite was added in a dosage that resulted in 5 mg $L^{-1}$ of chlorine, a concentration as indicated by the Ministry of Health Ordinance N° 2914 of 12/12/2011. After this process, an aliquot of each test was removed and submitted to the analysis, as described in the following sections.

### 2.2.4 Acetylcholinesterase inhibitory activity

Determination of the AChE inhibitory activity was carried out according to the Ellman method, modified as follows[28]. This method is based on the amount of thiocholine released when AChE hydrolyses the substrate acetylthiocholine iodide. The product thiocholine reacts with Ellman's reagent (DTNB) to produce a yellow compound [5-thio-2-(nitrobenzoate)], which can be detected at 405 nm. In each well of a 96-well plate, 65 μL of PBS (0.2 mol·$L^{-1}$ phosphate buffer, pH 7.2), 10 μL

of sample* and 10 μL of AchE (1300 U $mg^{-1}$) were added. The mixture was kept at 37 °C for 3 h. Subsequently, 65 μL of 1.00 mmol $L^{-1}$ acetylcholine iodide and 65 μL of 1.00 mmol $L^{-1}$ Ellman's reagent (DTNB dissolved in 0.2 mol $L^{-1}$ phosphate buffer pH 7.2) were added. Then, the absorbance was measured at 405 nm using a microplate reader (ELX800 Biotec) in triplicate experiments. The enzymatic activity was calculated as a percentage of the velocities of each sample compared to the control. The inhibitory activity was calculated from one hundred percentage subtracted by the percentage of enzyme activity.

*Control - 10 μL of ultrapure water. Chlorine - 10 μL chlorine solution (5 mg $L^{-1}$). After filtration - 10 μL solution after filtration. Postchlorination - 10 μL solution after 30 min reaction time with chlorination.

## 3. Results and discussion

### 3.1 Determining optimum operating conditions for jar test

The optimal conditions for the treatment of 1 L of water at 100 NTU were using 20 mL of aluminum sulphate solution (1% w/v) and pH 10.5, or 15 mL of calcium hydroxide solution (0.5% w/v), conditions which provided a turbidity of 0.24 NTU and 0.0 uH apparent color. All results considered in this study meet the standards for turbidity and apparent color, set forth in Brazilian legislation from the Ministry of Health for drinking water standards. Therefore, these were the parameters used in the pesticide removal test in the simulation of conventional water treatment.

### 3.2 Pesticide removal

As shown in Figure 2, it was observed that after filtration, the pesticides were not removed efficiently. The organophosphorus compounds were removed in a higher percentage (malathion: 62.21 ± 0.01%, chlorpyrifos: 43.8 ± 0.9%) than the triazines (atrazine: 10.8 ± 0.6%, ametryn: 14.8 ± 0.3%). As the flake formation phenomenon in the treatment of water is carried out by electrostatic attraction, it is inferred that the organophosphorus compounds have been removed in greater percentage due to the presence of the phosphate group, which would allow a greater attraction to the

particles, and consequently, greater efficiency in the decantation/filtration process.
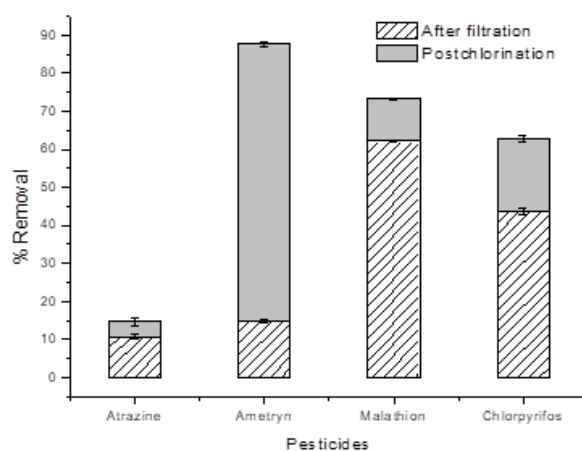


**Figure 2.** Average percentage of pesticide removal after treatment.

After the chlorination, the removal of pesticides increased, and lower and higher percentages were found for atrazine (15 ± 1%) and ametryn (87.7 ± 0.5%), respectively. The organophosphorus malathion and chlorpyrifos were eliminated by 73.2 ± 0.2% and 62.9 ± 0.8%, respectively. Despite the increased removal of pesticides, according to

Figure 3, two by-products were detected in the post-chlorination step. According to the NIST library, the first by-product is malaoxon and the second is an ametryn-derived compound, as shown in Figure 3B. Li *et al.*[29] reported that the oxidation of malathion by chlorination generates malaoxon.
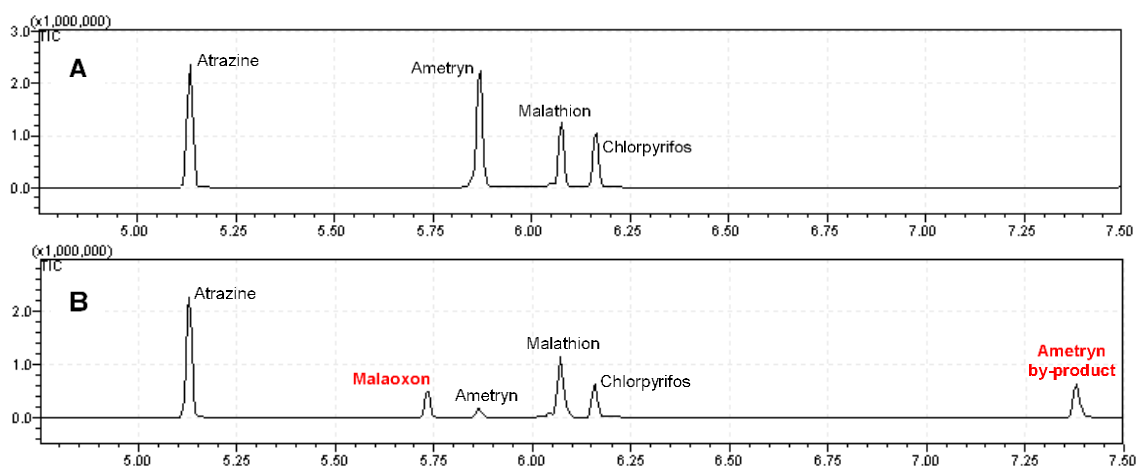


**Figure 3**. GC-MS chromatogram scan mode: A – After filtration; B – Post-chlorination.

Using the ametryn-derived fragmentation analysis (Figure 4), we found the molecular ion with a mass to charge ratio of 243, the molecular ametryn ion is m/z = 227, resulting in an increase equal to 16 in its mass value. This difference is consistent with the oxidation product of ametryn, derived from the reaction with sodium

hypochlorite, generating ametryn sulfoxide, as reported by Lopez and collaborators[30]. This by-product formation justifies the marked difference in the removal of ametryn compared with atrazine, due to the methyl sulfide group (R-S-CH$_3$), which is susceptible to reaction with sodium hypochlorite.
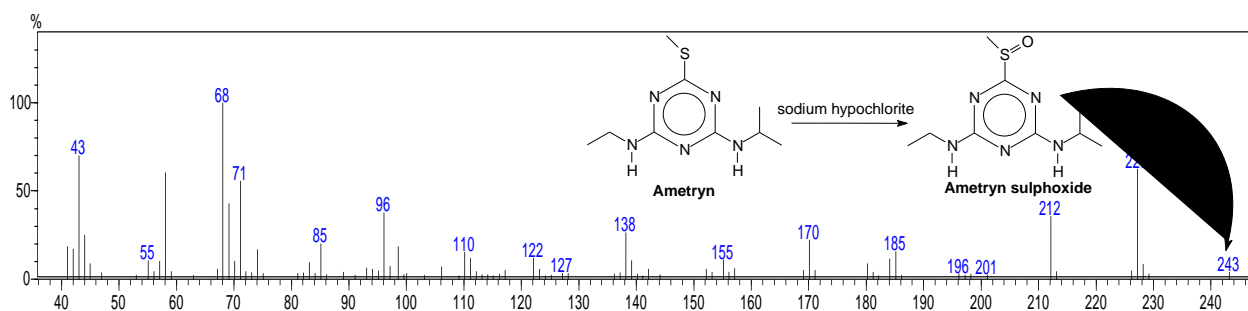
**Figure 4**. GC-MS mass spectra for ametryn by-product.

There are other studies on water treatment plant simulation that note that the conventional model is not efficient for the removal of pesticides, such as Soares *et al.*[21], whose objective was to verify the removal of endosulfan, ethylenethiourea and 1,2,4-triazole. At the end of the experiments, they verified that 54% of endosulfan, 11% of ethylenethiourea and 18% of 1,2,4-triazole were removed[21]. Li *et al.*[29] also found that after the treatment process, the organophosphorus diazinon and tolcoflos-methyl (initial concentration of 50 µg L$^{-1}$) were removed by *ca*. 63% and 49%, respectively, after filtration. These results are very similar to those found in the removal of the organophosphorus pesticides malathion and chlorpyrifos presented here.

### 3.3 Acetylcholinesterase inhibitory activity

According to Table 2, it is observed that the sample containing chlorine did not show significant difference to the control, in other words, chlorine was not the capable of inhibiting the acetylcholinesterase enzyme. Alternatively, it is known that organophosphates have the ability to inhibit such enzymes[31–33], which explains the inhibition generated after filtration, i.e., this treatment was not effective in removing such compounds. There are not studies demonstrating that atrazine and ametryn alone are capable of inhibiting AChE. However, during toxicity bioassays, the atrazine in combination with chlorpyrifos decreased significantly the acetylcholinesterase activity as compared to chlorpyrifos only treatments[34]. Studies show that atrazine enhances the uptake and facilitates the biotransformations of chlorpyrifos to chlorpyrifos-oxon[35].

**Table 2**. Acetylcholinesterase activity (AChE).

| Sample | % AChE activity |
|---|---|
| Control | 100 ± 2 |
| Chlorine | 100 ± 1 |
| After filtration | 96 ± 1 |
| Post-chlorination | 51 ± 2 |

After chlorination, 50% enzyme inhibition was found, which is an indication that the compounds formed were more toxic than their parents. Based on Figure 3B, we observed the formation of two by-products, malaoxon and ametryn sulfoxide. It is known that the oxidation of organophosphorus generates more toxic products, the oxons, which are more efficient in inhibiting AChE[27–29]. In this perspective, we expected that the enzyme inhibition would be accentuated, since malaoxon was generated after the chlorination. The effect of ametryn sulfoxide is still unknown, however this will be investigated in the next step of this work.

### 4. Conclusions

Based on the results, we verified that the conventional water treatment was not effective in removing atrazine, ametryn, malathion and chlorpyrifos pesticides. Organophosphorus compounds were removed in higher percentages than the triazines.

It was observed that the post-chlorination water became more toxic, because the enzyme acetylcholinesterase activity reached at 50%, indicating that the malaoxon was one of the responsible by-products. It is not yet known if the ametryn oxidation by-product, ametryn sulfoxide, is capable of inhibiting this enzyme, this action will be verified in future experiments by testing only this by-product.

Based on the toxicity of pesticides and their post-chlorination by-products, water quality control measures and treatment systems improvement are necessary to allow further removal of these contaminants at levels that are not a hazard to the population.

## 5. Acknowledgments

## 6. References

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