View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Helsingin yliopiston digitaalinen arkisto

University of Helsinki

Faculty of Science

Chemistry Department

Funded by the Erasmus+ Programme of the European Union

Erasmus Mundus Joint Master Degree

"Advanced Spectroscopy in Chemistry"

MASTER THESIS

by

Andrea, Pruccoli

26-07-2018

PASSIVE SAMPLING OF CHEMICAL WARFARE AGENTS IN THE MARINE ENVIRONMENT

Research group: VERIFIN

Supervisor(s): Prof. Paula Vanninen and M.Sc. Martin Söderström

Reviewer(s): Prof. Paula Vanninen and Prof. Sirkka Liisa Maunu

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI
MATEMAATTIS-LUONNONTIETEELLINEN TIEDEKUNTA
MATEMATISK-NATURVETENSKAPLIGA FAKULTETEN
FACULTY OF SCIENCE

Tiedekunta – Fakultet – Faculty	Koulutusohjelma – Utbildningsprogram – Degree programme
Faculty of Science	Advanced Spectroscopy in Chemistry

Tekijä – Författare – Author

Andrea Pruccoli

Työn nimi – Arbetets titel – Title

Passive sampling of chemical warfare agents in the marine environment

Työn laji – Arbetets art – Level	Aika – Datum – Month and year	Sivumäärä – Sidoantal – Number of pages
Master's thesis	August, 2018	89

Tiivistelmä – Referat – Abstract

Dumping sites of chemical warfare agents related compounds have been created after the two last major wars. The assessment of the risks connected to these sites is a priority as they could threaten the human health directly, through incidents when the dangerous materials come directly into contact with a subject and indirectly, as the poisonous substances can affect the environment and enter the food chain.

Many techniques have been involved in the monitoring, for example sediment analysis and mussels bio-monitoring. To overcome the deficiencies of these techniques and to obtain a more complete overview of the situations, new ways of analysis are studied. In this thesis the passive sampler technique was studied as new method to monitor the chemical warfare agents dumping sites.

This technique has been often used in the environmental monitoring of air and water samples. In the specific this work focused its attention in the use of silicone sheets as passive samplers, investigating their effectiveness with the substances of interest: sulfur mustard derivatives, arsine related chemical warfare agents derivatives and α-chloroacetophenone. Furthermore, the extraction power of different solvents was tested and a theoretical study of the opposing phenomena that compete in the extraction process was carried out.

Finally, the theoretical uptake model was tested on the different substances verifying its validity and showing how the efficacy of the passive sampling technique depends on various factors like the sampler-water partition coefficient, the relative recovery from the sampler and the stability of the compound of interest. The recovery studies have shown how acetone is the best solvent with a wide variety of compounds, but its extraction power can be improved towards less polar compounds using a solution of acetone/ethyl acetate 9:1. The effectiveness of silicone sheets as passive samplers was demonstrated by the kinetic studies. Stable compounds with a high octanol-water partition coefficient (≥ 3) present the best results showing good agreement with the theoretical model.

The next step will be testing the silicone sheets near known dumpsites using performance reference compounds as in situ calibration.

Avainsanat – Nyckelord – Keywords

chemical warfare agents, chemical weapons, OPCW, passive sampling technique, passive samplers, silicone sheets, environmental monitoring, octanol-water partition coefficient.

Säilytyspaikka – Förvaringställe – Where deposited

University of Helsinki

Muita tietoja – Övriga uppgifter – Additional information

Abbreviations

CHEMSEA Chemical Munitions Search and Assessment

CN α-Chloroacetophenone

CW Chemical Weapon

CWA Chemical Warfare Agent

CWC Chemical Weapons Convention

DAIMON Decision Aid for Marine Munitions

DMMP dimethyl methylphosphonate

DMox adamsite oxide

DPA diphenylarsinic acid

GC-MS Gas Chromatography-Mass Spectrometry

GC-MS/MS Gas Chromatography-Tandem Mass Spectrometry

HCB Hexachlorobenzene

HD Sulfur Mustard

HELCOM Baltic Marine Environment Protection Commission

LC-MS/MS Liquid Chromatography-Tandem Mass Spectrometry

LDPE Low Density Polyethylene

LOD Limit of Detection

logK_{ow} Octanol-Water Partition Coefficient

MRM Multiple Reaction Monitoring

OPCW Organization for the Prohibition of Chemical Weapons

PRC Performance Reference Compound

PSD Passive Sampling Device

SS Silicone Sheet

TPA triphenylarsine

TPAox triphenylarsine oxide

VERIFIN Finnish Institute for Verification of the Chemical Weapons Convention

Contents

List of Figures

List of Tables

Introduction

The work for this thesis was host by the Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN) in the Department of Chemistry at the University of Helsinki and mainly funded by the Ministry for Foreign Affairs of Finland. VERIFIN was established in 1994, as continuation of the Chemical Weapons (CWs) research project started in 1973. The main task of VERIFIN is the development of identification methods for Chemical Warfare Agents (CWAs) and related chemicals supporting the disarmament of chemical weapons and assisting the Organization for the Prohibition of Chemical Weapons (OPCW). [7]

Daimon Project

In 2017, the EU INTERREG Baltic Sea Region Program 2014-2020 partly financed the Decision Aid for Marine Munitions (DAIMON) project. DAIMON is an international project that involves partners from Finland, Norway, Germany, Poland, Sweden, Netherlands, Lithuania and Russia to solve the situation concerning the munitions dumping sites in the area around the Baltic Sea.

The main aims are to increase the awareness and to evaluate the risks and benefits of various management options. Previous projects are used as a starting point. First of all, the EU BSRSPA Hazards flagship projects (assess the need to clean up chemical weapons) and Chemical Munitions Search and Assessment (CHEMSEA) that dealt mainly with the risk assessment of dumpsites located in the Bornholm and Gotland Deep. DAIMON will collaborate with the expert group of Baltic Marine Environment Protection Commission (HELCOM), on the environmental risks of hazardous submerged objects.

VERIFIN is an active partner in the DAIMON project with a long experience on

development of analysis methods and practical analysis of environmental samples for analytes related to CWs. VERIFIN is also a designated laboratory of the OPCW, acting as the National Authority of Finland for the Chemical Weapons Convention (CWC).

As there is not a general answer to the problem, each sites and situation is evaluated singularly, tools are being developed to support and facilitate these decisions. On the basis of the information gathered an artificial intelligent decision-aid software will be created for the relevant maritime authorities. This tool will propose an intervention strategy for the given case. Most importantly a wide net of information and people has been created in a way that make it possible to face the oncoming problems as a united entity using the shared knowledge of the problem.

Objectives of this study

This thesis contribution to this project is the first stage development of a new and more efficient way to monitor the dumping sites using the passive sampling technique.

Literature was used to collect information about the working principle of this technique and on previous cases in which it has been used. Specific silicone sheets were investigated and their capability to work as passive samplers for CWA related compounds.

The preparation of the sheets was studied and 20 of them were sent to be deployed near known CWA dumping sites in the Baltic Sea.

The other samples were instead used in the laboratory-controlled experiments to identify the best methodology that will be used later on the real samplers. A straightforward methodology was developed for the extraction of the compounds from the silicone sheet and for their analysis with Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).

In the end, the uptake model was tested in a real-life simulation experiment.

Brief hystory of CWAs

Since the beginning of human civilization peoples have always tried to find new and more effective ways to kill each other's. In these scenes the effectiveness of CWs has early been discovered. Yet more than 1000 years ago we have proofs of the use of CWs in actual war like the use of a mixture of pine resin, naphtha, quicklime, calcium phosphide, sulfur, or niter called 'Greek fire' used usually by the Roman Empire in naval battles as primitive form of flame thrower that was capable of setting on fire the enemies ships with flames that were impossible to extinguish with water that would have instead reacted violently with the quicklime, feeding the fire even more. [8]

It was only in the last century, thanks to the scientific and industrial development that the CWs production saw its full maturation.

Soon after the beginning of the first world conflict Germany started testing chemical weapons on the enemies' troops. After few practically failed attempts, one in the October of 1914 in which German forces used dianisidine chlorosulfate a lung irritant against the British army that came out unhurt as the chemical was inactivated by the explosive charge. The other in the January of 1915 during the battle of Bolimów in which the Germans released a great amount of xylyl bromide of the Russian army that didn't get affected as the chemical weapon was made ineffective by the extremely cold conditions of the winter not permitting an effective aerosol of the agent. [9]

In April of the same year the German troops carried out the first successful large-scale chemical weapon attack in the war at Ypres, Belgium. Using 170 metric tons of chlorine gas and ending more than 1000 lives. [10]

At this point both the allies and Germany discovered the atrocity of the chemical weapons while phosgene and Mustard gas were introduced in the conflict. These three last substances accounted the most for the deaths due to Chemical Weapons.

The prime act after the First World War to banish the use of chemical and biological weapons was the Geneva Protocol in 1925 but it didn't prohibit the production and the development of such weapons.

In Europe during the second conflict the use of chemical warfare agents on the field are much reduced yet Germany developed new phosphorous based CWs that have been extensively used on the prisoners of the concentration camps.

At the end of the Second World War while the Russians were marching toward

Berlin they discovered in Dyhernfurth a Sarin and Tabun plant from which they took the machinery back home.

As only Germany had yet discovered the new deadly nerve agents when the United States and the British discovering about the factory found by the Russians, used the knowledge of German scientists, starting the production and the stockpiling of the new weapons giving birth to a situation parallel to the one for nuclear weapons during the Cold War.

One of the main concern during the years following World War I and during and after World War II was on what to do with the major quantities of obsolete or damaged CW materials. The cheapest solution adopted was to dump them into the seas and oceans as it was believed that the great amount of water would dilute the threat making inoffensive the dangerous chemicals.

The procedure was often to sink the ships on which the chemicals were previously been loaded.[11] So, the ships usually settled on the seabed leaving the containers in which the dangerous material was stored practically intact and confined in a small area.

This procedure was carried out by all the forces embroiled in the conflicts. As the environmental safety wasn't considered in those years for many of the dumping sites there aren't even records of their construction.

This operation became increasingly rare during the 1960s as recognized environmental treat from the national environmental legislation and international environmental protection agreements. Even if it didn't end until 1972 with the Convention on Prevention of Marine Pollution by Dumping of Wastes and Other Matter (London Convention).

It is only on 13th January of 1993 that the Geneva Protocol of 1985 is augmented with the Chemical Weapons Convention (CWC) including the verification measures for the prohibition of development, production stockpiling and use of chemical weapons and their destruction. Now the dumping of chemical weapons in any body of water is banned by the Chemical Weapons Convention's Verification Annex which prohibits: 'dumping in any body of water, land burial or open pit burning'.[12]

Dumping sites

Many and diverse are the munitions dumped, varying in amount and type depending on the manufacturing period and location. Usually comprehending aircraft bombs, containers and encasements.

The threats correlated to the dumping sites are many and often hard to evaluate. They can be mainly divided in 3 categories. The risk correlated to munitions containing explosives charges that can still self-detonate even after spending a long period of time on the bottom on the ocean.

But also, the risk correlated to human activities for instance the dredging of the sea, the cables and pipe laying on the bottom of the sea and the legering or more in general fishing. These activities can disturb the seabed and result in the exposure to the chemical wastes that may cause serious injuries to the exposed.

The last threat is to the marine environment that is exposed to dangerous chemicals and their degradation products. Furthermore, this danger can directly affect humans as direct consumer of marine animals. Many CWA, related chemicals and their degradation products (Adamsite, Clark I, Clark II, Thriphenyarsine and Lewisite) contain arsenic, this element is highly toxic for the marine ecosystems as it presents in many different forms with uncertain toxicological significance. It has been reported to substitute nitrogen in many substrates involved in the phospholipid synthesis. [13]

Recently the presence of CWA has been confirmed in marine biota samples [14]. The oxidized form of Clark I and Clark II was found in fishes and crustaceans collected from a dumpsite near the Swedish coast.

Many have been the incidents over the years involving CWA waste.

Since 1995 the Baltic Sea has been the theatre for more that 100 episodes that saw involved mainly fishermen that had caught dumped chemical munitions in their nets unaware of the potential danger.

For these reasons the awareness has increased over the years and many attempts to identify, registers and monitor these underwater dumps, have been carried out.

Chapter 1

The Passive Sampling Technique

The passive sampling technique is a procedure usually used in environmental analysis that take advantage of accumulation devices, the Passive Sampling Devices (PSDs), to non-quantitatively collect the chemicals from the environment for a certain period of time, that can vary between hours to many days, with the aim of assessing their presence and concentration in that area. It has been in use in the monitoring of air quality since the early 1970s but only recently it has been extended for the monitoring of water quality standards levels. [6]

Many types of samplers have been developed over the years, like: silicone strip samplers, Low Density Polyethylene (LDPE) strip samplers, Chemcatchers, and many others.

1.1 Chemical uptake model

The diffusion process of the hydrophobic contaminants from the water to the passive sampler matrix is the driving force of the sampling process. Taking into consideration a silicon strip dipped into the water we can see that the analyte in water must travel through the water to reach the silicon matrix surface. This process, called convective transport, is facilitated when the motion of the particles is increased through agitation, increasing the temperature or lowering the viscosity of the fluid.[6] Close to the silicon layer the analyte is transported by molecular diffusion into the strip matrix. If the silicon layer is not clean but covered by a biofouled layer the diffusion process will start from there and the analyte will need more time to reach the silicon matrix as the

diffusion process is usually the limiting step of the process. Finally, the analytes are absorbed by the silicon matrix as shown in Fig.1.1. The absorption process is useful to understand the kinetics of analytes transfer to the passive samplers and to understand how the amount absorbed relates to the environmental concentration.

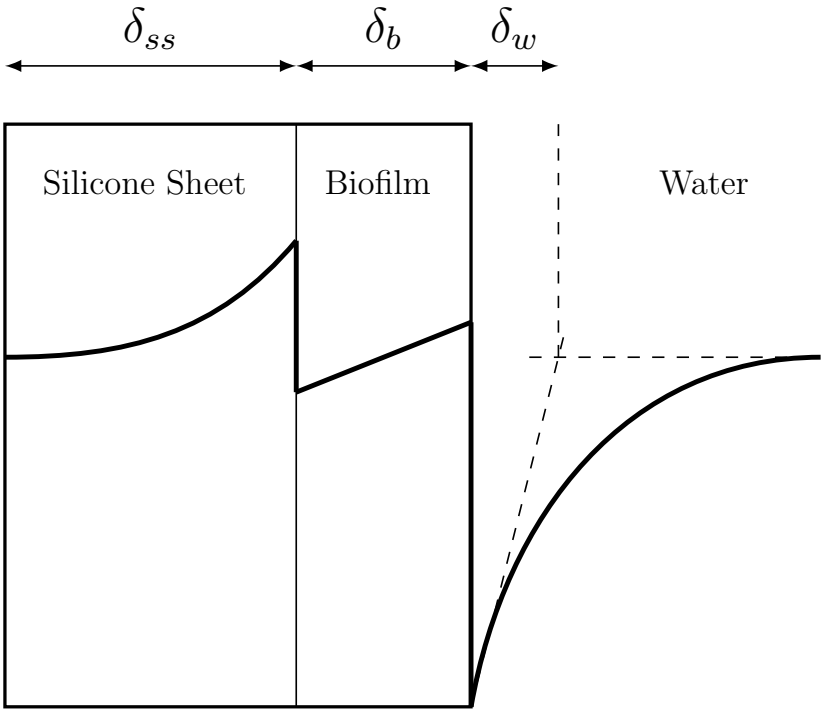


Figure 1.1: Representation of the three phases concentration profile.

The mass transfer process between two areas of different concentration is described mathematically by the first Fick's law of diffusion. That for a single dimension is:

$$j_i = -k_i \frac{dC}{dx} \tag{1.1}$$

That for an ideal solution becomes:

$$j_i = k_i \Delta C \tag{1.2}$$

Where:

- j_i: is the mass flux through the phase (i);
- dC/dx: is the gradient of concentration through space;
- k_i: is the conducivity or coefficient of diffusion;

- ΔC is the driving force of the diffusion process.

Assuming that the fluxes are equal on both sides of the silicone sheet and that at the interface with the water, the sorption equilibrium exists, the differential equation describing the uptake process, Fick's second law, is:[15]

$$\frac{dCs}{dt} = \frac{Ak_0}{Vs}\left(Cw - \frac{Cs}{K_{sw}}\right) \tag{1.3}$$

- C_s is the concentration in volume of the chemical in the passive sampler;
- C_w is the concentration in volume of the chemical in the water;
- V_s is the passive sampler volume;
- A is the passive sampler available surface area;
- K_{sw} is the sampler-water partition coefficient measured in volume.

k_0 can be described as the resistance to the mass transfer at which all phases contribute.

$$\frac{1}{k_0} = \frac{1}{k_w} + \frac{1}{k_b K_{bw}} + \frac{1}{k_s K_{sw}} \tag{1.4}$$

Where k_w, k_b, k_s are respectively the water boundary layer, the biofilm and the sampler membrane mass-transfer coefficients, while K_{bw} and K_{sw} are the biofilm-water and the sampler-water partition coefficients. The mass transfer coefficient (k_0), is equal to the ratio of diffusion (D) divided to the phase thickness (δ).

$$\frac{1}{k_0} = \frac{\delta_w}{D_w} + \frac{\delta_b}{D_b K_{bw}} + \frac{\delta}{D_s K_{sw}} \tag{1.5}$$

It is possible to simplify the Eq. 1.3 in the two extreme cases.

At short times the concentration in the silicone sheet is much lower than the one at equilibrium, $C_s \ll K_{sw} C_w^{eq}$, so the Eq. 1.3 becomes:

$$dC_s \simeq \frac{AK_0}{V_s} C_w dt \tag{1.6}$$

That integrated is:

$$C_s \simeq AK_0/V_s C_{w,TWA} t \tag{1.7}$$

As the concentration in water change during the uptake, $C_{w,TWA}$ is the time weighted average concentration in the water phase. Ak_0t can be defined as the apparent volume extracted during the time t, making Ak0 the apparent water sampling rate R_s. R_s links the passive sampling technique to the batch water extraction.

For long exposure times instead and a constant concentration in water C_w, the concentration of the analyte in the passive sampler, doesn't change with time.

$$C_w - \frac{C_s}{K_{sw}} = 0 \tag{1.8}$$

$$C_s = C_w K_{sw} \tag{1.9}$$

This equation gives the concentration of the analyte at equilibrium.

There is a third more general case in which considering the concentration of the analyte in water constant (C_w = constant), for example if there is a constant production of the chemical (leakage) or is the amount in water is high enough to not change significantly after the uptake into the passive sampler. We can solve the differential equation for C_s.

$$C_s = K_{sw} C_w [1 - e^{-k_e t}] + C_0 e^{-k_e t} \tag{1.10}$$

Where C_0 is the concentration at t = 0 and k_e is the elimination rate constant.

$$k_e = \frac{k_0 A}{K_{sw} V_s} = \frac{R_s}{k_{sw} V_s} \tag{1.11}$$

For a particular compound, k_e for the elimination and uptake process are the same. Using Performance Reference Compounds (PRCs) it is possible to calibrate the passive sampler in situ, determining the sampling rates of the compounds in those specific conditions.

When the initial concentration of the analyte in the silicone sheet is zero:

$$C_s = K_{sw} C_w [1 - e^{-k_e t}] \tag{1.12}$$

that for short periods of time becomes:

$$C_s = \frac{C_w R_s t}{V_s} \tag{1.13}$$

for PRCs $C_w = 0$ and $C_0 > 0$:

$$C_s = C_0 e^{-ket} \tag{1.14}$$

To determine the aqueous concentration from the amount absorbed into the passive sampler (N_s), the sampling rates (R_s) of the compounds and their sampler-water partition coefficient must be known.

$$C_w = \frac{N_s}{K_{sw} V_s [1 - e^{-R_s t/(K_{sw} V_s)}]} \tag{1.15}$$

Usually the sampling rate is determined in situ using PRCs. The PRCs are initially spiked into the silicon sheets before deployment. From the amount of PRCs that remain in the sampler after exposure, it is possible to determine the sampling rate Rs. [16]

Rs is usually estimated fitting the fraction of retained PRCs, (f) as a function of their K_{pw}:[17]

$$f = e^{-R_s t/(m K_{pw})} \tag{1.16}$$

The sampler-water partition coefficient is instead determined through equilibration experiments, in a similar way as the octanol-water partition coefficient. [18]

The samplers are often made of solid material for which the mass is a more reliable quantity than their volume, for this reason the concentration of the analyte in the sampler is often expressed in mass instead of volume. In this case it is more convenient to define a sampler-water partition coefficient as:

$$C_{w,m} = n_i / m_s \tag{1.17}$$

It is possible to convert Eq. 1.12 in mass as:

$$C_s = K_{sw} C_w [1 - e^{-k_e t}] \tag{1.18}$$

$$C_s = \frac{C_s^{eq}}{C_w^{eq}} C_w [1 - e^{-k_e t}] \tag{1.19}$$

$$\frac{n_s}{V_s} = \frac{n_s^{eq}}{V_s C_w^{eq}} C_w [1 - e^{-k_e t}] \tag{1.20}$$

$$\frac{n_s}{m_s} = \frac{n_s^{eq}}{m_s C_w^{eq}} C_w [1 - e^{-k_e t}] \tag{1.21}$$

Introducing the mass of the sampler (m_s) and erasing its volume becomes:

$$C_{s,m} = K_{sw,m} C_w [1 - e^{-k_e t}] \tag{1.22}$$

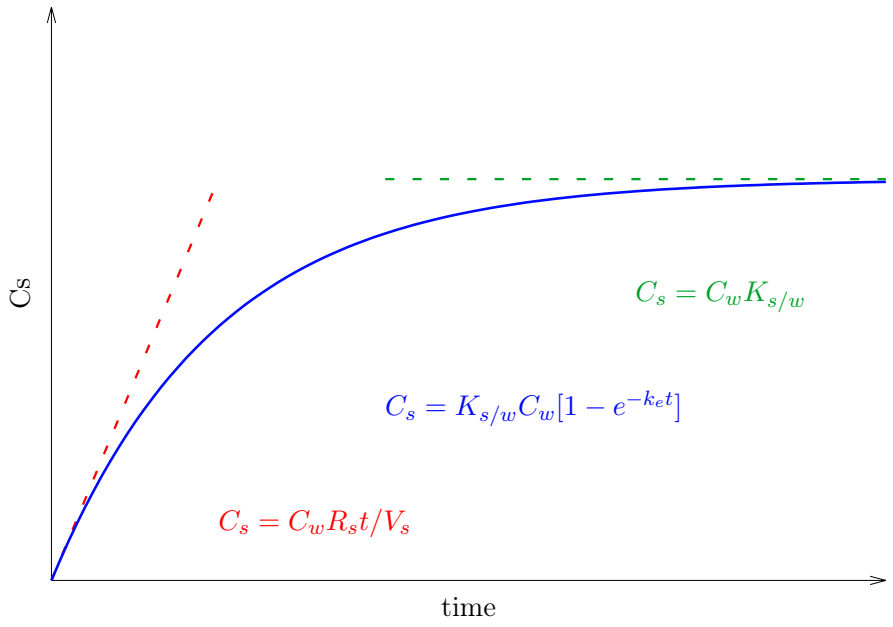


Figure 1.2: In the first stage of absorption the concentration of the analytes increases linearly 'linear uptake stage', after a while the concentration of the sampler reaches its equilibrium. Sampling rate usually increases at higher temperatures and greater flow rates. [19]

1.2 Passive sampling for the monitoring of dumping sites

The passive sampling technique has few practical advantages over direct analysis regarding pollution monitoring. Using direct analysis, it is necessary to collect multiple samples in different occasions to have an overview of the situation over time and it is often hard to take definitive decisions from the results as they may vary greatly from day to day depending on the pollution source and from the pollutants.[20]

The passive samplers allow the time-integrative determination of the pollutants after single deployment this mean that it is easier to determine a trend over time and possible pollution peaks from episodic and non-episodic events. Furthermore, while some analytes are present in concentration lower than the Limit of Detection (LOD)

to be determined using direct analysis, it often possible to detect them using PSDs as the analytes get concentrated in situ from large volume of water. [21]

As the compounds are recovered from the sampler body, matrix effects are reduced or completely avoided with solution that usually are ready to analyze and don't contain heterogeneous bodies (suspended particles, dissolved organic carbon, ...).[16]

Compared to other accumulation methods like biomonitoring, the uptake mechanism of passive samplers is less complex. Bioaccumulation is affected by complex processes like food-mediated transport, biotransformation and fluctuation of the physiological state of the organisms. [15][22]

Furthermore, comparing the different passive samplers, it has been found that silicone sheets provide a better agreement between hydrodynamic theory and experimental sampling rates compared for example with Chemcatchers. [23][15][24]

Regarding specifically the CWAs dumping sites it has been found that close to the sediment from which the status of the sites is usually assess, a desorption process occurs producing a layer of water close to the bottom with a concentration of CWAs related chemicals that is relatively stable and that reflect the status of the hazardous material occupying the site. This can produce the perfect conditions for passive sampling devices to be used to monitor the dumping site obtaining a better understanding of its conditions over time. [25]

1.3 Partition coefficients

The partition coefficient is a measure of solubility of a compound in phase in comparison to its solubility to another immiscible phase. It is calculated from the ratio of the concentration of the compound at equilibrium between the two phases.

1.3.1 Octanol-water partition coefficient

One of the most common partition coefficient is the Octanol-Water Partition Coefficient ($\log K_{ow}$) for which the two immiscible phases consist of octanol and water.

$$A_{water} \xleftrightarrow{K_{ow}} A_{octanol}$$

K_{ow} usually refers to the equilibrium of the species in an un-ionized form, when the diffusion process of the compound of interest reaches the equilibrium, it is possible to

calculate the coefficient from the ratio between the concentration of the compound in the two phases.

$$logK_{ow} = log([A]_o^{unionized}/[A]_w^{unionized}) \tag{1.23}$$

From this value it is possible to estimate the compound affinity to water and therefore, its hydrophobicity.

It is possible to determine the $logK_{ow}$ experimentally equilibrating a certain amount of compound in a known volume of octanol and water mixed together and determining its concentration using an appropriate analytical method. [26]

It is also possible to calculate the $logK_{ow}$ indirectly using the retention time in a HPLC column with substances with a similar retention time and known partition coefficient. The $logK_{ow}$ value can be extrapolated through linear regression using the known values. [27] This method is especially useful for those substances that are not stable in water.

The hydrophobicity or lipophilicity of a compound is an information of interest for pharmaceutical companies as from this information many characteristics of a drug can be predict. For this reason, during the years many have been the attempt to find an algorithm that will predict the $logK_{ow}$ in a theoretical way.

One of the theoretical methods is to parameterize the $logK_{ow}$ contributions of the different atoms of the overall molecule, producing a parametric model. It is one of the most general methods and it is capable of providing at least an estimation for a wide range of compounds. [28]

This method has been used to estimate the $logK_{ow}$ of the degradation form of CWAs degradation products containing arsine. The results are shown in Tab.2.2.

1.3.2 Silicone-water partition coefficient

The octanol water partition coefficient can give us an indirect information about the affinity of the molecules towards the silicone sheets, higher is the $logK_{ow}$, higher should be the affinity of the compound towards the silicon matrix in respect to the water. Another more direct measure of the relative affinity between the two phases is the silicon, water partition coefficient ($logK_{sw}$). In the same way as $logK_{ow}$, it is calculated as the ratio of the compound concentration in the two phases at the equilibrium.

$$logK_{sw} = log([A]_{silicone}/[A]_{water}) \tag{1.24}$$

As the silicon phase is a polymer, it is often preferred to use its concentration in mass instead of volume, as the mass of a polymer is more convenient to measure than its volume. [18] It has been demonstrated that there is a correlation between the $logK_{sw}$ and $logK_{ow}$. An estimation of $logK_{sw}$ can be calculated for nonpolar compounds as: [29]

$$logK_{sw} = log([A]_{silicone}/[A]_{water}) \tag{1.25}$$

$$logK_{sw}(LL^{-1}) = 0.86logK_{ow} - 0.13 \tag{1.26}$$

$$R2 = 0.78 \tag{1.27}$$

Depending on the manufacture and the polymerization process the silicone sheet may not be all polydimethylsiloxane, containing fillers and other agents that may affect the $logK_{sw}$ value. [18]

1.4 Silicone sheets

Many PSDs are available. From specifically designed devices, like the Chemcatcher, that can be tuned to be highly specific towards certain types of compounds to the much cheaper and readily available strips of different polymeric material like LDPE or Silicone.

The main difference between the different kinds of samplers, that is also the main feature that determine the effectiveness of the passive sampler over a certain class of substances, is its affinity towards them, Fig.1.3. This affinity is often calculated as polymer-water partition coefficient.

Higher is the affinity of analyte toward the sampler in respect to its affinity with the water, higher is the amount stored in the sampler at equal volumes, Eq. 1.24.

If the amount stored in the sampler is high enough, the analyte can be extracted and analyzed.

Silicon sheets for example work with substances in a $logK_{ow}$ range in between 3 and 10. This is because the silicon matrix is made of polysiloxane chains, Fig. 1.4.

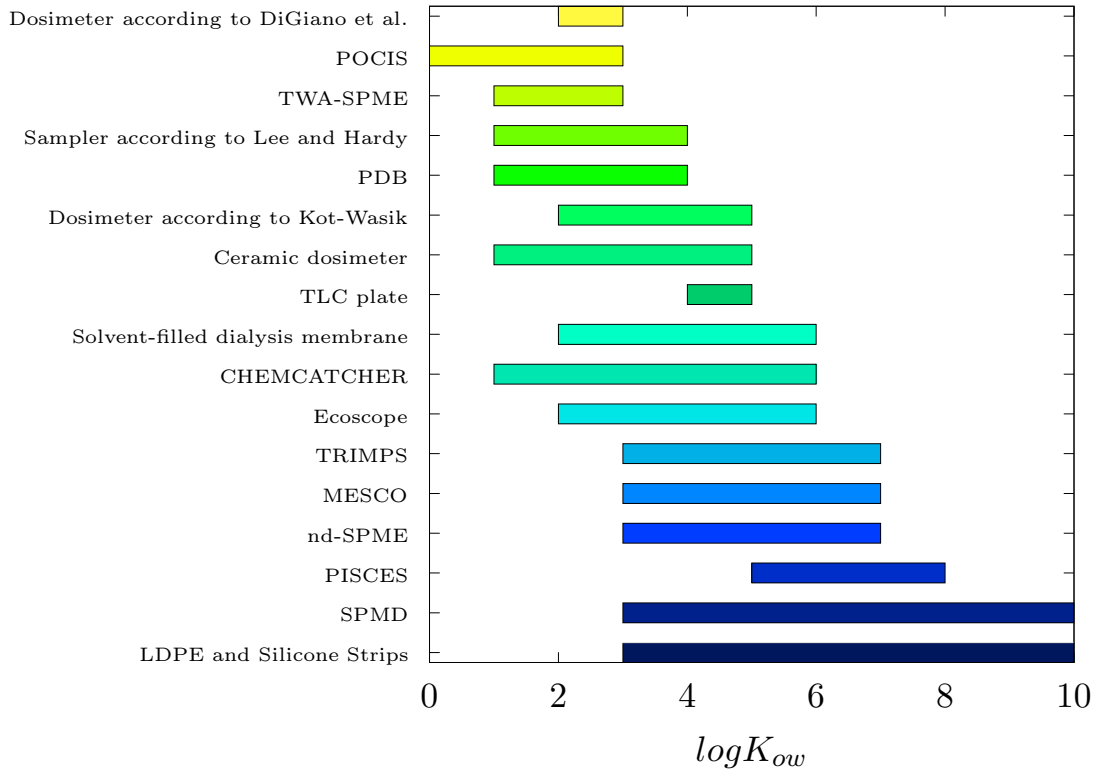


Figure 1.3: Typical $logK_{ow}$ ranges of organic compounds used with the most common passive samplers.[1][2][3][4][5][6]

Polysiloxane is a quite hydrophobic substance, with a good capacity of storing other hydrophobic compounds. Due to the strength of the silicon-oxygen bond, this material tends to be chemically inert, fact that make it suitable to be deployed in natural environments without risk of contamination. Substances with lower $logK_{ow}$ values can also be analyzed with sensitive enough methods like LC-MS/MS or GC-MS/MS as the kinetic model stays the same.

Due to its high working range, its cost effectiveness and because it is readily available and easy to prepare, silicone strips have been taken as main object of this study to develop a passive sampler for the detection of chemical weapons compounds and their degradation products in the marine environments.

1.4.1 Cohesive energy

After exposure the compounds trapped inside the passive sampler must be extracted to be analyzed.

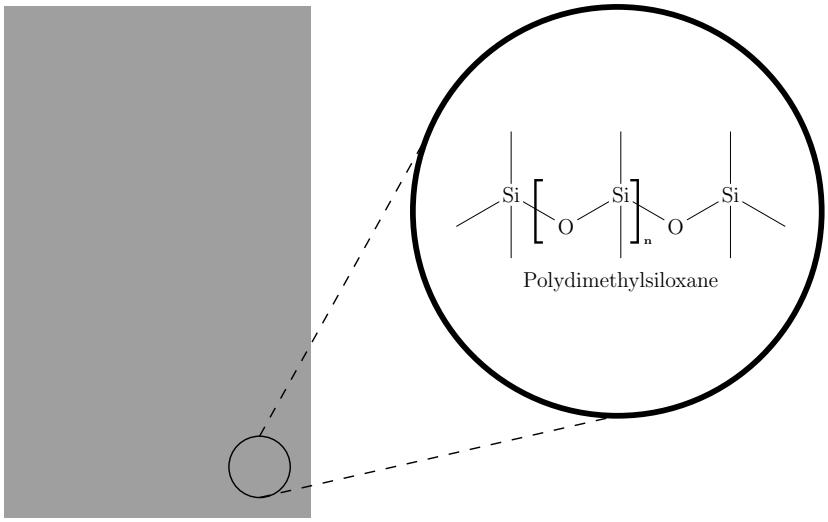


Figure 1.4: Silicone sheet matrix made of PDMS.

Many techniques have been used before depending also from the analytes of interest. The guidelines for passive sampling using silicone rubber samplers, [30], suggests two main techniques. The first method is to carry out a Soxhlet extraction. The silicone sheets are folded and inserted into the extraction chamber and the extraction is performed using methanol/acetonitrile (1:2v/v) for 8h. The inconvenience of this extraction is the fact that a Soxhlet apparatus is needed and that temperature sensitive compounds can degrade during the process. The other possibility is a cold extraction with 150 mL of methanol per 3 SS for 8h repeated once with fresh solvent.

But other methods have been used depending on the study, like for example pesticides have been back extracted from silicone sheets using sonication bath for 15 min in methanol/acetonitrile (50:50, v/v). [31]

The solvents used for these extractions are quite polar as non-polar solvents enter the silicone matrix causing swelling of the sheets.

To explain the swelling of the silicone when they are soaked in a non-polar solvent it is necessary to recall the solubility notion. Two compounds are soluble in each other when their intermolecular attractions are similar. It is possible to use the cohesive energy density, to quantify these interactions. The cohesive energy density is defined as:

$$c = -U/V \tag{1.28}$$

Where U is the molar internal energy (J/mol) while V is the molar volume (cm^3/mol).

This is the energy that a solute must overcome to insert himself between the molecules of the solvents. When two compounds have similar cohesive energies, they can dissolve in each other. Cross-linked polymers can't dissolve so if they are immersed into a solvent with similar cohesive energy, the molecules of the solvents will insert themselves between the polymeric chains causing the swelling of the whole structure. From the degree of swelling it is possible to measure the solubility of the polymer in that specific solvent.

The solubility can be related for a binary system to the cohesive energy through the Hildebrand-Scatchard equation:

$$\Delta H_m = V_m(\delta_1 - \delta_2)^2 \psi_1 \psi_2 \tag{1.29}$$

- Where ΔH_m is the entropy of mixing;
- V_m is the total volume of the mixture;
- δ_i is the solubility parameter of the compound i and it is equal to $\delta = \sqrt{c}$;
- ψ_i is the volume fraction of i in the mixture.

ΔH_m is always higher than zero unless $\delta_1 - \delta_2 = 0$, in which case the swelling should be maximal. The spontaneity of the process is regulated by the free energy ΔG_m.

$$\Delta G_m = \Delta H_m – T\Delta S_m \tag{1.30}$$

when $\Delta H_m = 0$,

$$\Delta G_m = -T\Delta S_m \tag{1.31}$$

As $T\Delta S_m$ is always positive for this type of process, ΔH_m determines the swelling depending on the type of solvent.

Even though the Hilderbrand-Scratchard equation gives an idea on how the polymer should interact with a solvent, it is not always perfect as the swelling depends a lot on the types of interactions. Luckily for poly(dimethylsiloxane) data have been collected and the swelling capability of each solvent has been determined, Tab. 1.1.

From the table it is possible to notice that the most polar solvents are the ones that less interact with the silicone matrix causing little or no swelling of the polymer. For

this reason, methanol and acetonitrile are the most used solvents used to back extract the compounds trapped inside the silicone sheets. If a non-polar solvent is instead used, the recovery from the extraction would be probably low as part of the solvent and with it the compounds of interest will be stay trapped inside the matrix of the passive sampler. The problem of using too polar solvent could instead be that if the interaction of the compounds with the passive sampler is too strong the solvent won't be able to break them giving low recovery.

For these reasons recovery studies with different solvents have been carried out.

Solvent	δ	S	μ_{ex}(D)	ref	rank	μ_t
perfluorotributylamine	5.6	1	0	10	32	1.7
perfluorodecalin	6.6	1	0	10	33	0.7
pentane	7.1	1.44	0	10	3	0.2
poly(dimethylsiloxane)	7.3	inf	0.6-0.9	8, 14	0	0
diisopropylamine	7.3	2.13	1.2	10	1	0
hexanes	7.3	1.35	0	10	8	0
n-heptane	7.4	1.34	0	10	10	0.1
triethylamine	7.5	1.58	0.7	8,10	2	0.2
ether	7.5	1.38	1.1	10	6	0.2
cyclohexane	8.2	1.33	0	10	11	0.9
trichloroethylene	9.2	1.34	0.9	10	9	1.9
dimethoxyethane(DME)	8.8	1.32	1.6	10	12	1.5
xylenes	8.9	1.41	0.3	10	4	1.6
toluene	8.9	1.31	0.4	10	13	1.6
ethyl acetate	9	1.18	1.8	8,10	19	1.7
benzene	9.2	1.28	0	10	14	1.9
chloroform	9.2	1.39	1	10	5	1.9
2-butanone	9.3	1.21	2.8	10	18	2
tetrahydrofuran(THF)	9.3	1.38	1.7	10	7	2
dimethyl carbonate	9.5	1.03	0.9	8,10	25	2.2
chlorobenzene	9.5	1.22	1.7	10	15	2.2
methylene chloride	9.9	1.22	1.6	10	16	2.6
acetone	9.9	1.06	2.9	8,12	22	2.6
dioxane	10	1.16	0.5	10	20	2.7
pyridine	10.6	1.06	2.2	10	23	3.3
N-methylpyrrolidone(NMP)	11.1	1.03	3.8	10	26	3.8
tert-butyl alcohol	10.6	1.21	1.6	8,12	17	3.3
acetonitrile	11.9	1.01	4	10	31	4.6
1-propanol	11.9	1.09	1.6	8,10	21	4.6
phenol	12	1.01	1.2	8,12	29	4.7
dimethylformamide(DMF)	12.1	1.02	3.8	8,10	27	4.8
nitromethane	12.6	1	3.5	10	34	5.3
ethyl alchol	12.7	1.04	1.7	8,12	24	5.4
dimethyl sulfoxide (DMSO)	13	1	4	10	35	5.7
propylene carbonate	13.3	1.01	4.8	10	30	6
methanol	14.5	1.02	1.7	8,12	28	7.2
ethylene glycol	14.6	1	2.3	8,12	36	7.3
glycerol	21.1	1	2.6	13,15	37	13.8
water	23.4	1	1.9	8,12	38	16.1

Table 1.1: Table of the cohesive energy of different solvents towards poly(dimethylsiloxane). δ is in unit of $cal^{0.5} = cm^{-1.5}$. S represents the swelling measured experimentally as $S = D/D_0$ where D is the length of the PDMS in the solvent and D_0 is its length when it is dry. Rank refers to the order of the solvent in decreasing swelling ability.[32] μ_{ex} is the solubility parameter calculated experimentally while μ_t is the solubility parameter calculated from thermodynamic data.

Chapter 2

Studied chemicals

The list of chemicals of interests for what concern the DAIMON project is quite broad, so it is necessary to establish few criteria to determine which substances are the most eligible to be analyzed using a passive sampling technique.

Due to their nature most of the CWAs are unstable in the water environment. They often form characteristic degradation products that may be use as identifiers of the parent compounds. The stability in water is an important factor to be considered but also its affinity for the PSD matrix in respect to water. For this reason, it is necessary to know the octanol water partition coefficient. Another desirable property is the ease of analysis of the compound, if the analyte is not easily analyzed after extraction from the PSD matrix all the process might be not convenient in respect to other kinds of analysis.

Even if some of the compounds of choice have a $logK_{ow}$ value lower than the recommended 3 for the silicon sheets passive samplers, it might be worth to test them anyway as it has been proved that silicon sheets can be used with $LogK_{ow}$ lower than 3 if the method is sensible enough to determine the lower absorbed concentration. [31]

2.1 Organophosphorus chemicals (Nerve agents)

Two are the main classes of nerve agents, the G-series and the V-Series. The compounds that belong to the G-series were the first nerve agents discovered. The most famous are GA (tabun), GB (sarin) and GD (soman) all discovered before 1944 during or prior to World War II. The second family are the more modern V-Series. They were born

after the 1950s while trying to develop a new class of organophosphorus pesticides. The most famous compound of this class is probably VX, that is one of the most toxic compound developed by mankind. All the nerve agents have in common a phosphorous group. Even if the newest class of nerve agents (V-series) is much more stable than the older G-series, in both the phosphorus bond present in the compounds is quite reactive and it is easily attacked by a nucleophilic reagent such as water. For this reason, nerve agents decompose in water resulting in non-toxic phosphoric acid. Nerve agents have not been selected to be studied with the passive sampling devices as they degrade in water leaving no characteristic trace behind.

2.2 Mustard derivatives

Sulfur Mustard (HD), also known as mustard gas or iprit is probably the most famous chemical warfare agent. Despite of its name when used as chemical weapon, it is not vaporized but instead dispersed as fine droplets. It acts as a strong blistering agent and it has also strong mutagenic and carcinogenic properties. Together with arsine containing compounds it is one of the main constituents in the dumping sites.

As most of the chemical warfare agents, sulfur mustard degrades in the environment. When in contact with water it will easily hydrolyze and oxidize producing several byproducts.[33]

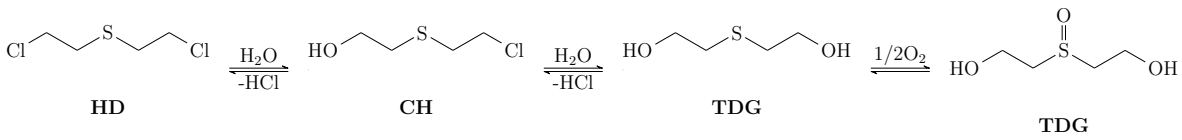


Figure 2.1: Sulfur mustard natural hydrolysis and oxidation.

After first hydrolysis and oxidation forming respectively thiodiglycol (TDG) and (TDGO) passing through multiple intermediate of sulfonium ions it ends up forming 1,4-Oxathiane and 1,4-Dithiane that can be further oxidized to 1,4-Dithiane oxide plus other minor byproducts Fig. 2.3 and 2.2. These cyclic degradation products have been often used as identifiers of possible sulfur mustard leakage.[34]

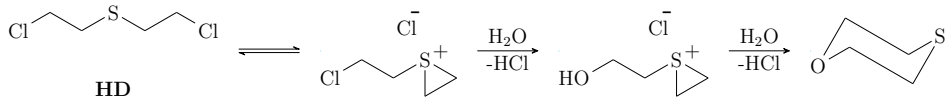


Figure 2.2: Sulfur mustard (HD) natural degradation into 1,4-Oxathiane.

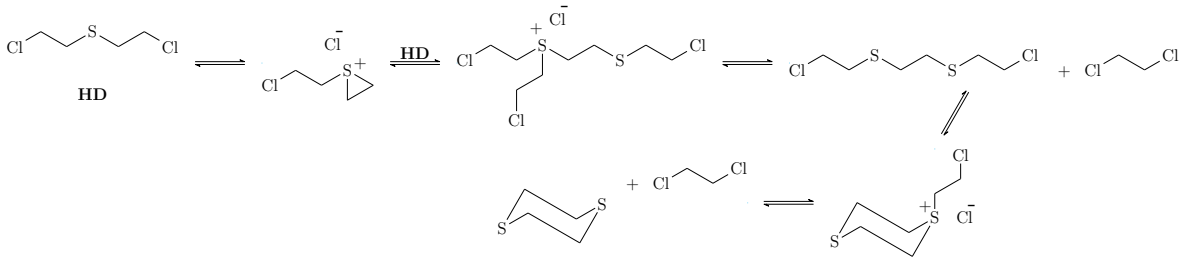


Figure 2.3: Sulfur mustard (HD) natural degradation into 1,4-Dithiane.

The degradation of sulfur mustard occurs rather quickly in water. The half-life of mustard has been calculated to be of 4 min in pure water at the temperature of 25°. [35]

The degradation process is usually slower in salt water than in fresh water. [36]

For this reason and also because the degradation process highly depends on the temperature, in the depth of the sea where the temperature can be close to zero it is possible that the compounds are found in solid form, a state that makes them harder to dissolve, reducing the hydrolysis rate.

Another factor that slow down the degradation process is that sulfur mustard molecules can react with each other, polymerizing into brittle lumps that slows down the dissolution process. [37]

Sulfur mustard was included into the analytes of interest being the parent compounds of the various degradation products even though its fast degradation will make it rather hard to detect. As seen before mustard gas has multiple degradation products but only few have been found in previous analysis of the dumping sites of this group only the ones that are analyzable directly with GC-MS without derivatization were included. The list of chosen mustard gas related chemical is shown in Tab. 2.1 and Fig.2.4.

TDG and TDGox weren't included due to their negative octanol water partition

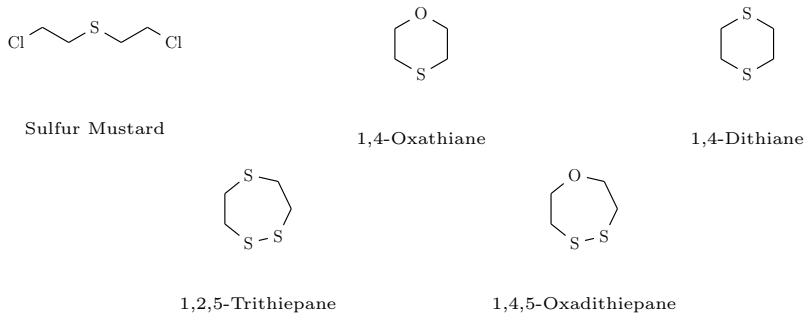


Figure 2.4: Structures of sulfur mustard related compounds analyzed.

coefficient (-0.62 - -0.77 for TDG and -0.94 - -2.11 for TDGox) that makes them interact rather weakly with the silicone matrix leading to small storage capacity of these compounds inside the passive sampler compared to their concentration in water. [38]

Name	Sulfur Mustard (HD)	1,4-Dithiane	1,4-Oxathiane	1,4,5-Oxathiepane	1,2,5-Trithiepane
Method	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS
$LogK_{ow}$	1.4-2.4	0.77	0.6	1.5	2.11

Table 2.1: Sulfur mustard related compounds analyzed.

2.3 Arsine compounds

Thanks to the intrinsic toxicity of arsine containing compounds, they have been extensively used during WWI as chemical warfare agents. Clark I (diphenylchloroarsine, DA), Clark II (diphenylcyanoarsine, DC) and Adamsite (10-chloro-5,10-dihydrophenarsazinine, DM) are part of this family, Fig.2.5. They were found quite effective for trench warfare during WWI, as their main effect was to cause eyes and respiratory system irritation followed by violent vomiting. [39]

Another compound in this family is triphenylarsine (TPA), one of the main constituents of the arsine oil. To change the physical properties of a CWA mixture often additives where added. For example, often Mustard gas was mixed with arsenic containing compounds, around 37%, to create a more viscous substance, capable of withstanding cold environments, this substance was known as "winter mustard". [40]

Arsine oil is one of these mixtures, containing 50% phenyldichloroarsine, 35% diphenyl-chloroarsine, 5% triphenylarsine and 5% of trichloroarsine. [38]

For this reason, these compounds are often found together in the dumpsites. Still, it is often impossible to analyze them in their original form as they tend to degrade rather quickly in aqueous environments.

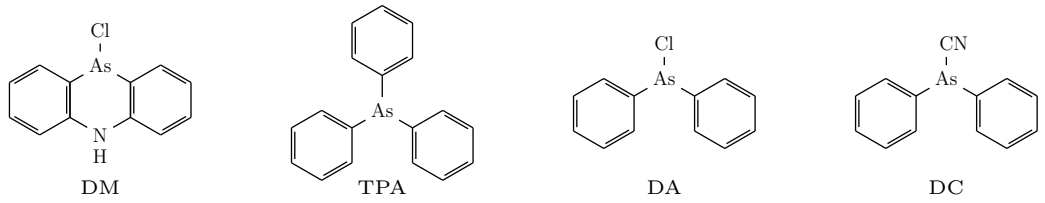


Figure 2.5: Most common chemical warfare agents containing arsine

Like many others arsenic-containing chemicals, Clark I and Clark II degrade into the same hydrolysis and oxidation products. As shown in Fig.2.6, Clark I and Clark II degrade into diphenylarsinous acid (DPA[OH]) that dimerizes and oxidizes into bis(diphenylarsinic)oxide (BDPAO), the oxidation can go further forming diphenylarsinic acid (DPA).[38].

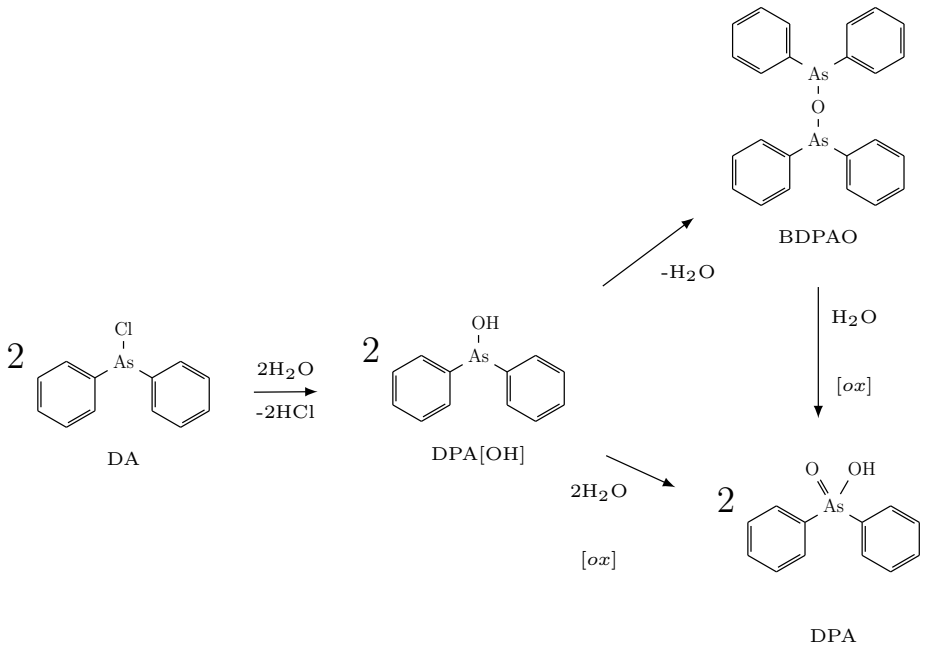


Figure 2.6: Clark I (DA) natural hydrolysis and oxidation into diphenylarsinous acid (DPA[OH]) that further degrades into bis(diphenylarsinic)oxide (BDPAO) and diphenylarsinic acid (DPA).[14]

Adamsite also hydrolyzes and oxidizes in a similar way as described for Clark I and Clark II into adamsite oxide (DMox). The degradation products of these arsine-containing compounds retain the toxicity of their parent compounds.[41]

It has been reported that these compounds degrade quite quickly in water, for this reason the degradation products are useful indicator of the dumping site condition, Fig. 2.2. [42]

These compounds have less tendency to group into lumps as sulfur mustard does, while instead they are found widely spread on the sea floor.[25]

Of these arsenic-related compounds, triphenylarsine was thought to be the most stable. It has been reported to be highly resistant to hydrolysis and oxidation. Yet during some recovery studies it has been found to oxidize rather quickly to triphenylarsine oxide (TPAox) in acqueous environments.[33]

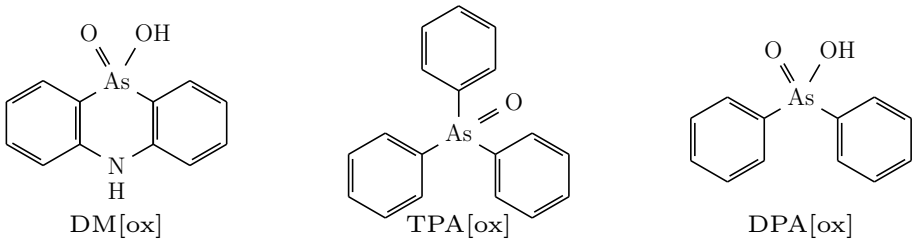


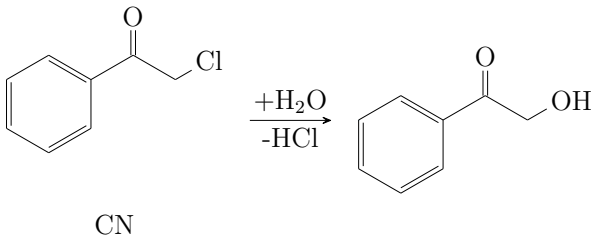
Figure 2.7: Common degradation products of arsine containing CWAs.

Name	DMox	DPA	TPA	TPAox
Method	LC-MS/MS	LC-MS/MS	GC-MS/MS	LC-MS/MS
$LogK_{ow}$	1.1-2.1*	1.5-2.5*	5.97	3.5-4.7*

Table 2.2: Arsine compounds analyzed. K_{ow} values calculated with XLOGP3 and SILICOS-IT at http://www.swissadme.ch/index.php*

2.4 Other chemical warfare agents

Mustard gas and Arsine related chemicals are the major pollutants present in the dumping sites, yet other chemicals like α-Chloroacetophenone (CN) has been found to contaminate the surrounding areas. This compound has been used in solution as riot control agent thanks to its tear gas effect. It is still in use as tear gas even if it has been often substituted for less toxic alternatives.



CN

Figure 2.8: CN natural degradation into 2-hydroxyacetophenone and hydrochloric acid.

α-Chloroacetophenone degrades slowly hydrolyzing, at ambient conditions into the nontoxic hydrochloric acid and α-hydroxyacetophenone Fig. 2.8, [43]. Due to its abundance, its ease of analysis and to its temporary stability it has been included in the study Tab. 2.3.

Name	α-Chloroacetophenone
Method	GC-MS/MS
$LogK_{ow}$	1.93

Table 2.3: Other chemicals analyzed.

Chapter 3

Experimental Part

From here starts the experimental section regarding the studies of the CWAs using the passive samplers that have been chosen.

3.1 Solvents and materials

The solvents used during the different procedures are listed in the Tab. 3.1. Before its utilization, each solvent was transferred from the main bottle to a laboratory portion bottle, to avoid contamination and facilitate the use.

In addition to the standard laboratory equipment a number of disposable materials have been used during the different procedures. The disposable materials are listed in Tab. 3.2.

Chemicals	Use	Manufacturer	Purity
Acetone	Solvent	Sigma Aldrich	≥99.8%
Dichloromethane	Solvent	VWR	HPLC grade
Ethyl Acetate	Solvent	Honeyweii	≥99.7%
Methanol	Solvent	Fisher	HPLC grade
Ultrapure Water	Solvent	In-house	18.2μs/cm (conducivity)
Formic acid	LC eluent	Merck	≥98%

Table 3.1: Technical information about the solvents used.

Material	Manufacturer	Details	Procedure
Disposable syringe	Braun	1 mL ± 0.01 mL, plastic, sterile disposable syringe for LC samples filtration.	LC samples preparation
Disposable Syringe-driven filter unit	Millex	Filter unit, HPLC certified 0.20μm pores. Low protein binding hydrophilic (PTFE) membrane.	LC samples preparation
Disposable scalpel	Swann-Morton	Sterile disposable scalpels.	SS extraction
Filter paper	GE Healthcare	Whatman, hardened, diameter 90 mm.	SS extraction

Table 3.2: Disposable materials used during the samples preparation.

3.2 Standards and chemicals

All the standard solutions have been prepared from the solid standards both purchased or prepared by the synthesis laboratory. The solution prepared from the standard solutions were diluted in acetone. The water was purified using Milli-Q (Merck Millipore, 0.22 μm filter) equipment. The prepared standards from the solid substances are listed in Tab.3.3. Due to its low solubility DMox standard solution was prepared by adding 11.0 mg of the solid standard to 3950 μL of a MeOH/H2O, 75:25 solution with 50 μL of NaOH 1M as a basic environment will facilitate the dissolution of the compound deprotonating the acid group. The solution was then gently warmed and agitated until complete dissolution of the solid.

Standard	CAS	Solvent/volume	Manufacturer	Concentration
Sulfur Mustard	505-60-2	Dichloromethane/1mL	In-house	11.3mg/mL
1,4-Dithiane	505-29-3	Dichloromethane/1mL	In-house	16.2mg/mL
1,4-Oxathiane	15980-15-1	Dichloromethane/1mL	In-house	19.5mg/mL
1,4,5-Oxadithiepane	3886-40-6	Dichloromethane/1mL	In-house	14.2mg/mL
1,2,5-Trithepane	6576-93-8	Dichloromethane/1mL	In-house	23.0mg/mL
CN	532-27-4	Dichloromethane/1mL	Fluka 98%	13.3mg/mL
TPA	603-32-7	Dichloromethane/1mL	Fluka 96.5%	25.3mg/mL
TPA[ox]	1153-05-5	Acetone/1mL	Sigma 97%	17.9mg/mL
DPA	4656-80-8	Methanol/1mL	Envilytix GmbH	12.7mg/mL
DM[ox]	4733-19-1	MeOH/H2O 75:25 3950μL + [NaOH]=1M 50μL	Envilytix GmbH 99.8%	2.75mg/mL
HCB	118-74-1	Dichloromethane/1mL	Sigma 99%	25mg/mL
DMMP	756-79-6	Dichloromethane/1mL	Sigma 97%	2μg/mL

Table 3.3: Standard solutions prepared.

From the standard solutions three main solutions have been prepared using different

chemicals. The chemicals used in the 3 solutions are listed in tab.3.4

	Mustard	1,4-Dithiane	1,4-Oxathiane	1,4,5-Oxadithiepane	1,2,5-Trithiepane	CN	TPA	DPA	TPA[ox]	DM[ox]
G01	x	x	x	x	x	x	x			
G02								x	x	x
G03	x	x	x	x	x	x		x	x	x

Table 3.4: Solutions prepared from the standard chemicals.

3.3 Instrumentation and parameters

3.3.1 GC-MS instrument

The method and condition used for the gas chromatographic analysis are listed in the Tab. 3.5, 3.6 and 3.7. The full scan method is the standard method to analyze CWA related chemicals and identify them with the NIST database. This method was used to determine the retention times and to identify the compounds of interest. A single ion monitoring method was developed to increase the sensitivity of the instrument towards the analytes. A solution of [G01] = 10μg/mL was prepared from the standard solutions. This solution was then analyzed in full scan with Gas Chromatography-Mass Spectrometry (GC-MS). From the resulting spectrum the different compounds were identified using the NIST library. For each compound a quantifier (Q) and two qualifiers (q) ions were selected. As quantifier the most specific ion with also the highest response was selected followed by two qualifiers. Pure solvent was used as matrix as the extracted from the silicone sheets is usually clean. The ions monitored in SIM mode are shown in Tab. 3.8.

GC-MS	
GC	Agilent Technologies 6890N
MS	Agilent Technologies 5975N
Column	DB-5MS, 30 m x 250 μn x 0.25 μm

Table 3.5: Names and manufacture of the GC-MS instrument.

GC parameters for GC-MS/MS		
GC	Full scan	SIM
Injection mode	splitless	
Splitless time	1 min	
Injection volume	1μL	
Injection temperature	250°C	
Carrier gas	He	
Flow pressure	0.487 bar	
Temperature program	1 min at 40 °C 10 °C/min to 300°C 5 min at 300 °C	

Table 3.6: Conditions used for the GC instrument.

MS parameters for GC-MS		
Method	Full scan	SIM
Ionization	EI	
Electron energy	70eV	
Transfer line temperature	290°C	

Table 3.7: Conditions used for the MS instrument.

Analyte	Quantifier ion, Q(m/z)	Qualifier ions, q(m/z)
1,4-Oxathiane	46	61,104
1,4-Dithiane	120	46,61
Sulfur Mustard	109	63,158
1,4,5-Oxadithiepane	136	60,64
CN	105	51,77
1,2,5-Trithiane	152	87,124
TPA	152	227,306
HCB	284	286,288

Table 3.8: Ions selected to be monitored in SIM mode.

3.3.2 GC-MS/MS

The SIM method developed for GC-MS isn't sensitive enough to detect the substances retrieved from the silicone sheets during the kinetic study. To increase even more the sensitivity towards the compounds of interest, a multiple reaction monitoring method (MRM) was developed using a triple quadrupole mass spectrometric instrument. The informations about the instrument, the GC and the MS/MS apparatus are listed respectively in Tab. 3.9, Tab. 3.10 and Tab. 3.11. A full scan chromatogram was recorded on a 10 ng/mL solution containing the chemicals of interest, to detect the time segments of the various substances and to identify the best quantifier and qualifier ions that is going to be used in the Multiple Reaction Monitoring (MRM) mode, the results obtained are listed in Tab. 3.12. An overview of triple quadruple working principle is summarized in Fig. 3.1

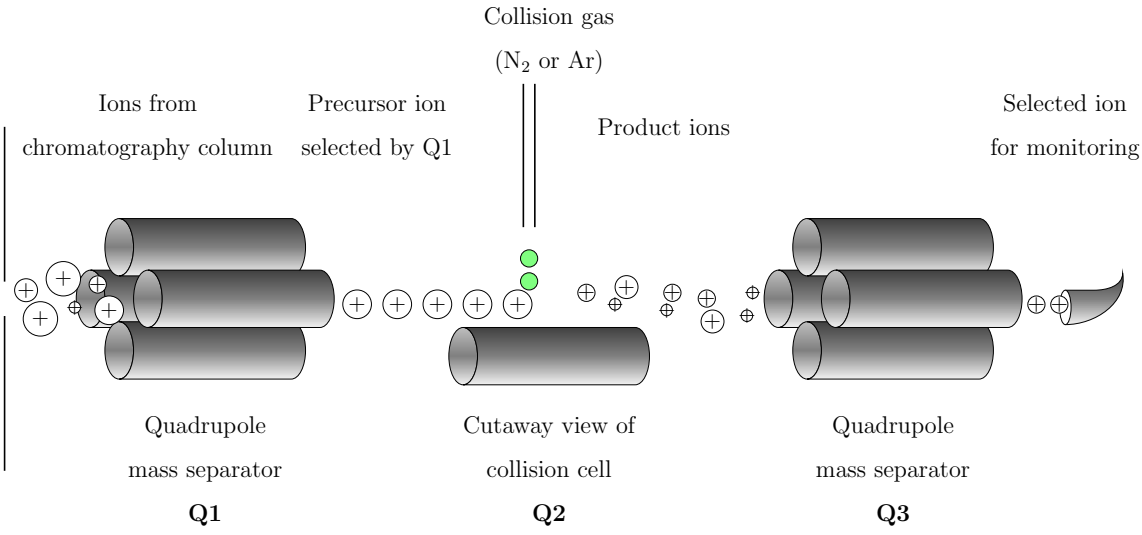


*Figure 3.1: Simplified view of triple quadrupole working principle. In which the first quadrupole (***Q1***) let through only the specified ion depending on its m/z. The second quadrupole (***Q2***) instead works as a collision cell to produce fragments of the selected precursor ions. Finally the third quadrupole (***Q3***) send only the selected ions to be monitored to the detector.*

GC-MS/MS	
GC	Agilent Technologies 7890A
MS	Agilent Technologies 7010 Triple Quad
Column	DB-5MS, 30 m x 250 μn x 0.25 μm
Autosampler	Agilent Technologies 7693

Table 3.9: Names and manifacture the GC-MS/MS instrument.

GC parameters for GC-MS/MS		
Method	Full scan	MRM method
Injection mode	splitless	
Splitless time	1 min	
Injection volume	1μL	2μL
Injection temperature	250°C	
Carrier gas	He	
Flow pressure	0.487 bar	
Temperature program	1 min at 40 °C 10 °C/min to 290°C 9 min at 290 °C	1 min at 40 °C 10 °C/min to 290°C 10 min at 290°C

Table 3.10: GC parameters of the methods used with the GC-MS/MS instrument.

MS parameters for GC-MS/MS		
Method	Full scan	MRM method
Ionization	EI	
Electron energy	70eV	
Transfer line temperature	290°C	
Ion source temperature	230°C	
Data acquisition mode	Full scan	MRM
Scan range	40-500 m/z	50-350 m/z

Table 3.11: MS parameters of the methods used with the GC-MS/MS instrument.

Analyte	Precursor ions (m/z)	Product ions (m/z)	Collision energy (V)	Time segment (min)
1,4-Oxathiane	104	61	10	0.00
	104	46	17	
	74	46	2	
1,4-Dithiane	120	61	10	7.00
	120	46	30	
	92	46	10	
Mustard	160	109	7	9.30
	109	73	5	
	109	63	12	
1,4,5-Oxadithiepane	136	92	5	9.30
	136	64	15	
	89	35	22	
CN	154	105	0	11.00
	154	77	25	
	105	77	17	
	105	51	35	
1,2,5-Trithiepane	152	92	5	12.20
	152	87	7	
	124	60	15	
HCB	284	249	25	16.35
	284	214	35	
	284	142	50	
TPA	306	152	7	21.00
	152	77	20	
	152	51	37	

Table 3.12: Parameters used for the MRM method for the substances analyzed with GC-MS/MS.

3.3.3 LC-MS/MS instrument

LC-MS/MS instrument parameters are listed in the Tab. 3.13, Tab. 3.14 and Tab. 3.15. The optimization parameters for the MRM method are listed in Tab. 3.16

MRM method was developed to increase the sensitivity of analysis of TPA[ox], DPA, DM[ox] in LC-MS/MS. Two solutions for each compound were prepared at 10 μg/mL and 0.05 μg/mL level from the standard solutions. A solution of all three compounds, G02, was also prepared at the same concentrations. Having the three compounds in one solution makes the method development faster but if overlapping between the peaks is found, the single compound solutions are necessary to obtain the separated peaks and to develop the method. [G02] at 10μg/mL was then tested to determine the retention times of the different compounds and no overlapping was found. In total 3 transitions were selected for each compound. MRM method at different collision energy, 10, 20, 30, 40, 50eV was tested on the [G02] solution at 0.05μg/mL to determine the ones that give the highest intensity for the ions of interest. The use of the more diluted concentration is necessary in the MRM method otherwise the signal would be saturated when monitoring only one fragment.

LC-MS	
LC	Waters Acquity UPLC H-class
MS	Water Acquity UPLC BEH C18 1.7μm, 2.1 x 100 mm
Column	Waters C18 1.7μm (2.1x100) mm

Table 3.13: Informations regarding the LC-MS/MS instrument.

LC parameters	
Injection volume	5 μL
Flow rate	0.6 mL/min
Column temperature	40 °C
Mobile phase A	0.1% HCOOH in H2O (v/v)
Mobile phase B	0.1% HCOOH in MeOH (v/v)
Gradient	1% B and 99% A for 0.6min From 1% to 100% (B) from 0.6 to 2.3 min 100% B from 1.7 min
Total run time	5.5 min

Table 3.14: LC parameters used.

MS parameters	
Ionization mode	ESI+
Capillary voltage	3.5kV
Source temperature	120°C
Desolvatation gas	N2
Desolvatation gas flow rate	1000 L/h
Desolvatation temperature	500°C
Collision gas	Argon
Mass resolution	0.75 amu

Table 3.15: MS parameters for LC-MS/MS instrument.

Analyte	Precursor ion (m/z)	Cone voltage (V)	Product ions (m/z)	Collision energy (V)
DM[ox]	276	30	230 (Q) 154 (q) 127 (q)	20 40 50
DPA	263	30	152(Q) 141(q) 128(q)	30 20 20
TPA[ox]	323	30	227(Q) 154(q) 77(q)	40 40 30
DMMP	125	30	63(Q) 93(q)	20 15

Table 3.16: Parameters, optimized collision energy and Cone voltages for the compounds analyzed with LC-MS/MS.

3.4 Silicone sheets

A piece of food grade, UV-resistent, silicone sheet, 1200x1000 mm wide and 0.5 mm thick, was purchased from ETRA. [44]

Name	Code	Dimensions (mm)	Temperature range (C)	Density	Colour
Silicone sheet	NM60	1200x1000x0.5	-50 to +200	1.2 g/cm^3	Translucent

Table 3.17: Silicone sheet technical information from the manufacture.

3.5 Passive sampler preparation

The passive samplers are prepared from a silicone rubber sheet from which multiple sheets where cut to a size of 5.5 x 9.0 cm giving an exposed surface area of around $100cm^2$. On field, usually the sheets are used as 3 replicates at a time to reach the common surface area for a passive sampler that is around $300 - 600cm^2$. But more sheets can be used to improve sensitivity. [30]

3.5.1 Cleaning

During the polymerization of the polysiloxane some of the monomers don't reach the polymers dimensions and remain trapped inside the polymer matrix as oligomers. These oligomers can interfere with the chemical analysis that are going to be carried out on the passive sampler, for example they can remain stuck inside the liquid chromatographic column or can daub the chromatographic liner of the gas chromatography instrument.

These impurities can be removed from the samplers through a Soxhlet extraction with ethyl acetate that should be carried out for at least 100 h. [30]

Ethyl acetate is used as it is a quite apolar solvent, capable of penetrating inside the silicone matrix removing the oligomers quantitatively.

The extraction is carried out in a in series Soxhlet apparatus shown in Fig. 3.2. The rounded flasks are filled with 200mL of ethyl acetate, each 7 silicone sheets are placed in each extraction chamber enclosed between 2 cotton wool disks, reaching 21 silicone sheets cleaned per cycle. The extraction is carried out throughout 13 days for

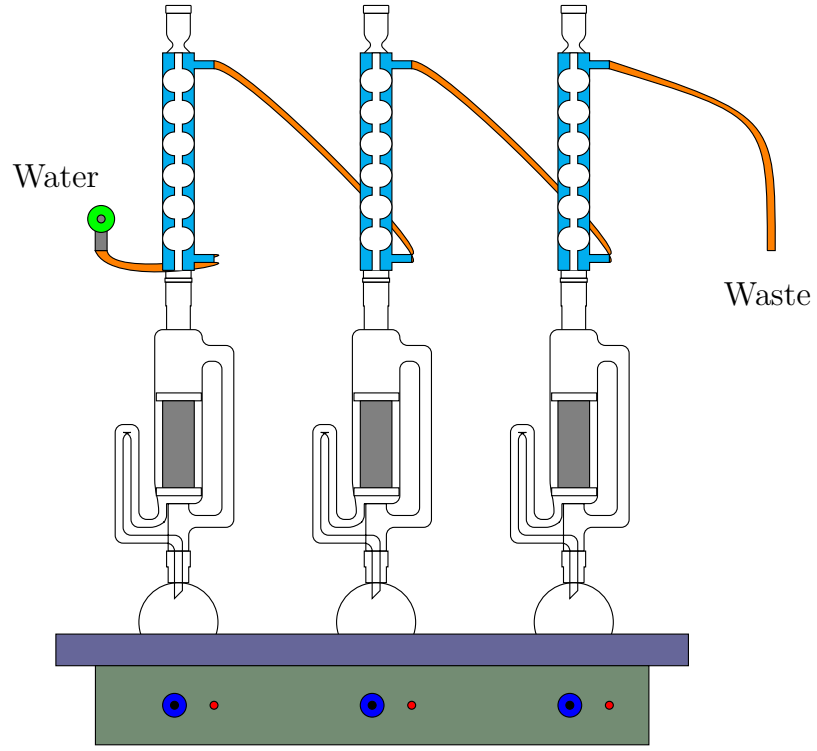


Figure 3.2: Soxhlet apparatus.

8 h a day. The apparatus is turned off during the night for safety reasons, taking care of the fact that the solvent would fully cover the silicone sheets while the instrument is off.

After cleaning the silicone sheets are collected from the Soxhlet apparatus and placed on a clean aluminium foil surface. After the complete evaporation of the solvent the sheets are weighted and stored in a clean plastic bag. The silicone sheets prepared have an average mass of $m_s = 2.9 \pm 0.2\ g$.

3.5.2 Spiking

Silicone sheets spiking

To assess the recoveries of the different substances using different solvent the silicone sheets were spiked before the extraction.

Before spiking the silicone sheets were positioned on a foil paper separating one to the other with at least 1 cm of space.

For each solvent the spiking was carried out in triplicate with a blank and a standard sample with a theoretical 100% yield. A total of 5 sheets per solvent were obtained.

Solution	Concentration ($\mu g/mL$)	Volume spiked (μL)	Water volume (mL)	Experiment
G01,G02	100	100	30	Degradation study
G03	300	100	30000	Kinetic study

Table 3.18: Solution spiked in water.

A solution of the compounds to spike at around 100 $\mu g/mL$ in acetone was prepared and used to spike 100 μL on each sheet previously wetted with around 1 mL of acetone. After the spiking the sheets were let dry under the fume hood for at least 30 min.

Water spiking

A degradation study and a kinetic study were carried out spiking a specific amount of solution into the water, Tab. 3.18.

3.5.3 Extraction

Silicone sheets extraction

After leaving the Silicone Sheet (SS) drying they were cut into square pieces of around 2 cm of side, using a disposable scalpel, to increase the contact with the solvent avoiding the sheets to stick to the walls of the vials and to increase the extraction efficiency.

They were transferred in glass vials and an extraction with 25 mL of the respective solvent was carried out overnight, around 15 h and then repeated with fresh solvent for 6 h. Leading to a total volume of around 50 mL. In parallel to the spiked sheets the extraction was also performed on two non-spiked sheets that represented the blank and the standard.

After each extraction the extracted from each sheet was filtrated on filter paper and transferred into a 50 mL volumetric flask. The solutions were then brought to 50 mL volume with the respective solvents.

	MeOH	MeOH/Acetone50:50	Acetone	Acetone/EtOAc 9:1	Acetone/EtOAc 8:2
G01	x	x	x	x	x
G02			x	x	x

Table 3.19: Solvents tested and respective compounds groups.

- G01: sulfur mustard (HD), 1,4-dithiane, 1,4-oxathiane, 1,4,5-oxadithiepane, 1,2,5-trithiepane, α-chloroacetophenone (CN) and triphenylarsine (TPA).
- G02: adamsite oxide, diphenylarsinic acid and triphenylarsine oxide

Water extraction

In the degradation study the samples containing G01 needed to be analyzed with GC. Before the analysis 5 mL of the water samples were extracted using 5 mL x 2 of Ethyl acetate in a two steps cold extraction.

3.5.4 GC samples preparation

Kinetic study and recovery study GC samples

From the 50 mL volumetric flasks 10 mL of solution was collected into a Turbo vap tubes and then brought to less than 500 μL for the samples containing ethyl acetate and acetone and less than 100 μL for the solutions containing methanol, using a Turbo vap evaporator. The samples are then reconstituted to 1 mL using acetone into a volumetric flask. With the exception of the standards samples that were spiked after evaporation with 20 μL of the solution at 100 $\mu g/mL$ and brought to 1 mL into a volumetric flask.

Degradation study G01 samples

The 10 mL of Ethyl acetate from the liquid-liquid extraction were collected together, evaporated and adjusted to 1mL into a volumetric flask. The extraction was conducted in triplicate and a standard solution was prepared spiking 16.7 μL of G01 in Ethyl acetate bringing to volume to 1 mL in a volumetric flask.

Evaporation study samples

In a 10 mL evaporation vial, 10 mL of acetone were spiked with 20 μL of G01 (2 μg) in 3 different samples. The blank solution was prepared using just plain acetone while the standard solution was prepared spiking 20 μL of G01 (2 μg) in 1mL of acetone. After evaporation of the 10 mL solution and recostitution to 1 mL, the samples were analyzed with GC-MS.

3.5.5 LC samples preparation

Kinetic study and recovery study LC samples

Similarly, as in GC samples preparation 10 mL of solution was collected into a Turbo vap tubes, 500 μL of ultrapure water were added to each tube to avoid complete evaporation. Afterwards the solutions were dried to less than 500 μL, using a Turbo vap evaporator. The samples are then reconstituted to 1 mL using ultrapure water into a volumetric flask. The standards samples were spiked after evaporation with 20 μL of the solution at 100 $\mu g/mL$ and brought to 1 mL into a volumetric flask. Before transferring into the 1.5 mL LC vials the samples were filtrated with disposable syringes and filters as the LC column is quite sensitive to small particles.

Degradation study G02 samples

1mL of water, from the 30 mL disposable vial, was filtrated with a disposable filter and syringe and collected into a 1 mL vial. 3 samples were prepared for each water sample. The standard was prepared spiking 5 μg of G02 into 15 mL of the blank sample from which 1 mL was filtrated and used as standard sample.

3.6 Kinetic study setup

The kinetic study was carried out in a 30 L rectangular, four walls, glass tank, Fig. 3.3. The tank was filled with 30L of tap water and spiked with 100μL of G03, 0.3mg/mL solution. The system was then let equilibrate for 24 h under continuous stirring.

6 metallic rings were used to secure 3 SS each, for a total of 18 SS, using plastic strips. The rings were then attached to a metallic rod.

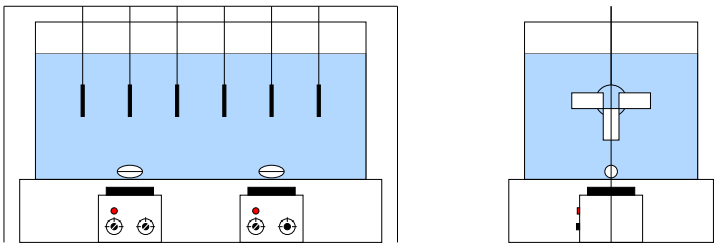


Figure 3.3: Schematic view of the experimental setup for the 8 days experiment.

After the equilibration time the 18 SS were deployed inside the water. The SS were positioned in a way for which they were fully immersed and that each of them was distant enough from the other and from the tank surfaces to avoid contact. The sheets were then extracted from the system at precise time in groups of 3, after 6, 12, 24, 48, 96 and 192 h subsequent to the first deployment. The recovered sheets were then extracted as described in the samples extraction section using acetone as solvent.

3.7 Qualitative and quantitive analysis

Depending on the purpose of the experiment two different methods were used to assess the amount of substance related to the instrument response. For the solvent recoveries studies an external standard was used while for the kinetic study an internal standard was used.

3.7.1 External standard calibration

In the recoveries study the signal from the samples was evaluated as ratio against the signal produced by an external standard prepared from the parent solution used. This method is highly affected by the sample preparation procedure and by the instrument volume injections variations. In this case this method is acceptable as the values obtained are compared to each other and on each measure an uncertainty is evaluated using 3 samples.

3.7.2 Internal standard calibration

Internal calibration is more precise and usually more accurate than external calibration. For GC analysis Hexachlorobenzene (HCB) is used as internal standard while for LC analysis dimethyl methylphosphonate (DMMP) is used instead.

A calibration curve was built using 7 solutions of concentrations between 0.1 and 50 ng/mL from the compounds standard solutions adding at each of them the internal standard to reach a concentration of 25 ng/mL, as shown in Tab.3.20.

The solutions at different concentrations were prepared from a stock solutions of G03 (3.4) at 100 μg/mL. From the stock solution, 3 standards solutions at $[G03]_1 = 1$, $[G03]_2 = 0.1$ and $[G03]_3 = 0.01\mu$g/mL were prepared using consecutive dilution. These

solutions were further diluted to prepare the standard solution for the calibration curve and are shown in Tab.3.21.

Internal standard	C0 ($\mu g/mL$)	V(μL)	Analysis
HCB	2.5	10	GC
DMMP	2	12.5	LC

Table 3.20: Internal standards for calibration.

Standard solution	V_x(μL)	C_i(ng/mL)	V_i(mL)	Solvent	Analysis
$[G03]_3$	10	0.1	1	Acetone	GC-MS/MS
	20	0.2			
	50	0.5			
$[G03]_2$	10	1		Acetone	GC-MS/MS
	20	2			
	50	5			
$[G03]_1$	10	10		Water	LC-MS/MS
	25	25			
	35	35			
	50	50		Water	LC-MS/MS

Table 3.21: Solutions used for the preparation of the standard calibration curve.

Chapter 4

Results and Discussion

In this Chapter the results obtained from the experimental part are discussed. At first the results obtained from the analysis of the compounds by GC-MS/MS and LC-MS/MS are shown and the procedure of how the Quantifier and qualifier ions have been chosen is shown. The second section regards the recovery study results, used to determine the best solvent for the back extraction of the analytes from the SSs.In the third section, the results of two complementary studies that have been used to investigate further the recoveries obtained are shown. The first is a degradation study to determine the effect of the exposure to water of the different compounds while the second is a recovery study regarding the evaporation step of the sample preparation.The chapter ends with the kinetic studies of the various compound collected by the SSs in water.

4.1 Compounds fragmentation

The compounds analyzed by GC-MS are eluted in an order that reflect their K_{ow} as shown in Fig.4.1. All the peaks are well separated except Sulfur Mustard and 1,4,5-Oxadithiepane that elute at the same time. From the full spectrum the TIC spectrum of the different compounds were analyzed to develop the SIM method. An example for the evaluation of TIC mass spectrum of CN, Fig.4.2 is described next. The strong fragmentation caused by EI lead to the almost complete loss of the parent ion signal at m/z 154 while creating many fragments with the most intense at m/z 105 selected as Quantifier as it also is quite selective probably due to the fact that it contains a

chlorine atom (154-49(CH_2Cl^+) = 105). The ions at m/z 77 and at 51 that recall the typical fragmentation of benzene ring compounds ($C_6H_5^+$) and ($C_4H_3^+$) have instead been selected as qualifiers, respectively. The mass spectrum of the other compounds can be found in Appendix A.

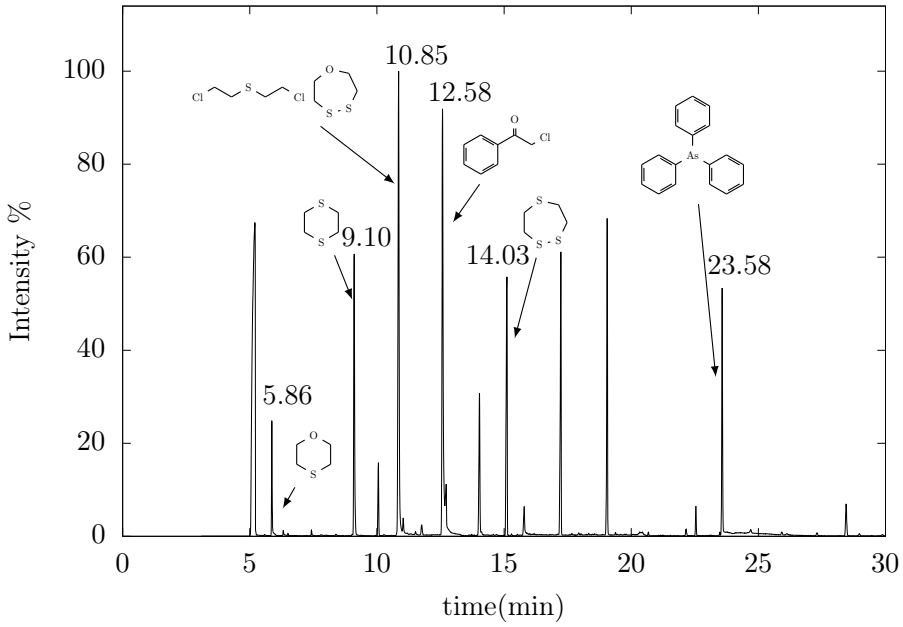


Figure 4.1: GC-MS full spectrum of G01 in acetone. 1,4-Oxathiane (5.86 min), 1,4-Dithiane (9.10 min), 1,4,5-Oxadithiepane and Sulfur Mustard (10.85 min), α-Chloroacetophenone (12.58 min), 1,2,5-Trithiepane (14.03 min) and Triphenylarsine (23.58 min).

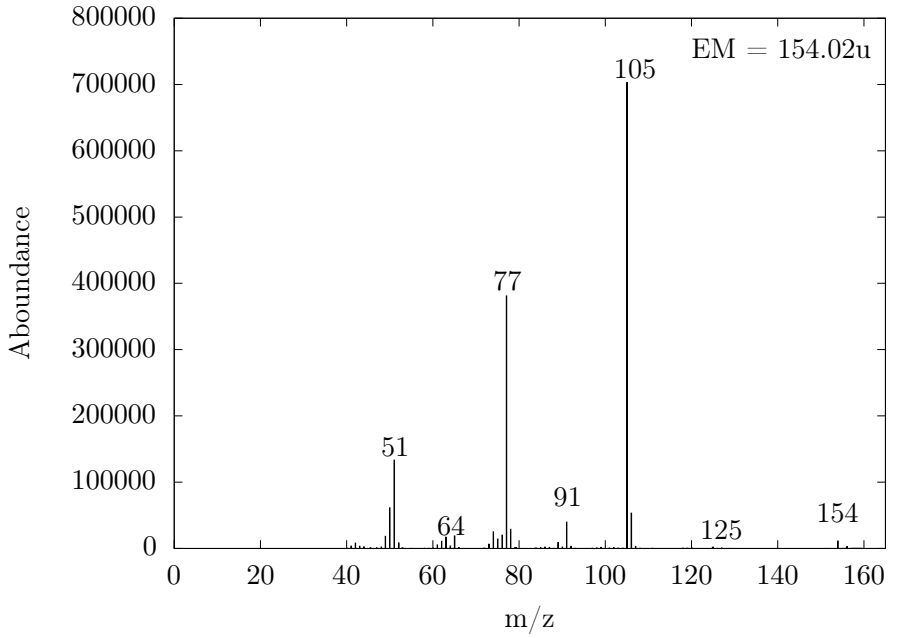


Figure 4.2: Mass spectrum of CN.

The total ion chromatogram of the compounds analyzed by LC-MS is shown in Fig.4.3. The tree compounds are eluted in close proximity, but the peaks are well separated, and the elution order reflect the predicted K_{ow}. ESI in positive mode produces protonated parent ions (EM+1u) as shown in the MS spectrum of TPA[ox], fig.4.4.

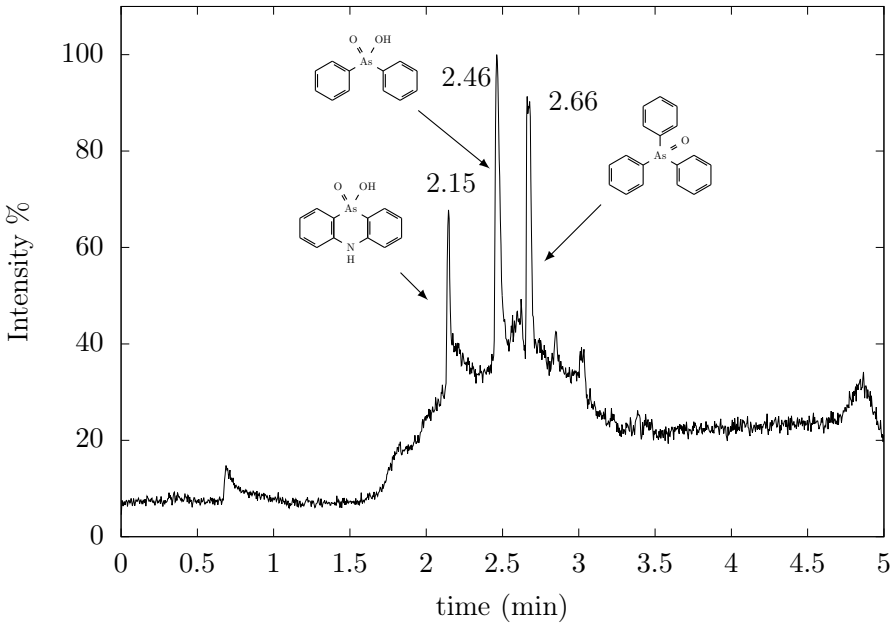


Figure 4.3: LC-MS full spectrum of G02 in acetone. DM[ox] (2.15 min), DPA (2.46 min) and TPA[ox] (2.66 min).

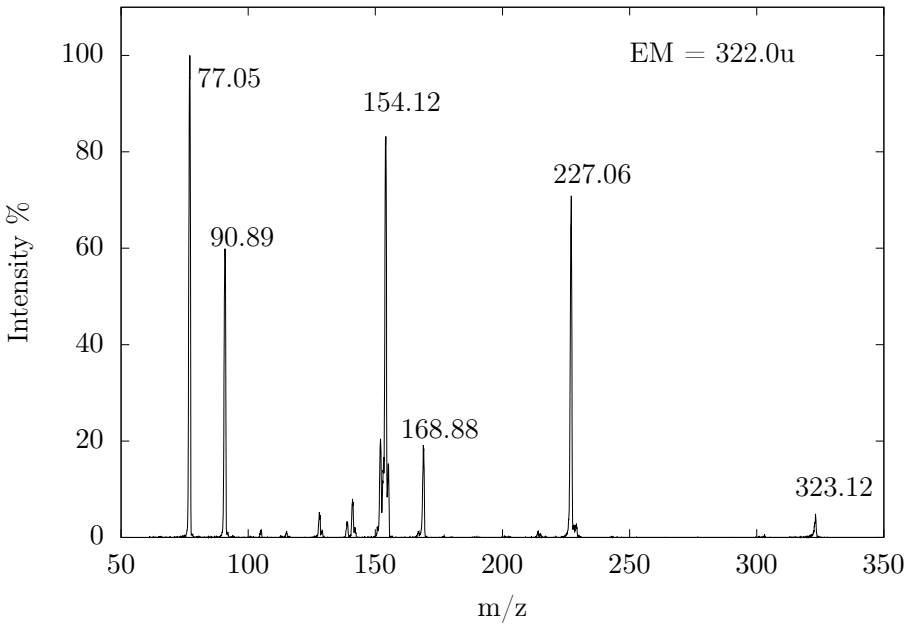


Figure 4.4: Mass spectrum of TPA[ox].

The peak at highest intensity for TPA[ox] is at m/z 77 corresponding to the phenyl cation ($C_6H_5^+$). This peak wasn't chosen as quantifier as it is a quite common fragmentation for compounds containing a benzene ring. Same goes for the fragment at m/z 154 that comes from the condensation of 2 benzene rings ($C_{12}H_{10}^+$). Usually peaks of high intensity and corresponding to large fragments are better qualifier ions as they are usually more selective. For this reason, the peak at m/z 227 was set as quantifier.

Similar procedure was used for the MRM analysis with GC-MS/MS. As the conditions and the instrument change, the retention times change as well as shown in Tab. 4.1 but the elution order remains the same.

Analyte	GC-MS (min)	GC-MS/MS (min)	LC-MS/MS (min)	LogK_{ow}
1,4-Oxathiane	5.86	5.87		0.6
1,4-Dithiane	9.10	8.81		0.77
Mustard	10.85	10.44		1.4-2.4
1,4,5-Oxadithiepane	10.85	10.43		1.5
CN	12.58	12.03		1.93
1,2,5-Trithiepane	14.03	13.36		2.11
TPA	23.58	22.21		5.97
DM[ox]			2.15	1.1-2.1
DPA			2.46	1.5-2.5
TPA[ox]			2.66	3.5-4.7

Table 4.1: Elution times obtained by the different methods for the compounds analyzed and their octanol-water partition coefficient.

4.2 Silicon sheets recovery study

A recovery exam was carried out to assess the best solvent for the extraction of the compounds of interest from the silicone sheets and to determine the extraction yield of the process.

The first recovery study was carried out using compounds with a wide range of polarities (sulfur mustard (HD), 1,4-dithiane, 1,4-oxathiane, 1,4,5-oxadithiepane, 1,2,5-trithiepane, that are all HD derivatives but also α-chloroacetophenone (CN) and triphenylarsine (TPA)), between 0.6 and 5.97. Methanol, acetone and a solution of the two were chosen as solvents of choice as methanol shouldn't cause any swelling but being quite polar should be less effective with the most lipophile compounds while acetone can cause little swelling in the silicone sheets but being less polar should be more effective in the extraction of the most lipophilic compounds.

The second recovery study was carried out on a wider list of compounds adding three compounds to the most lipophilic range, adamsite oxide, diphenylarsinic acid and triphenylarsine oxide. Acetone and ethyl acetate where studied this time to check the efficiency of less polar solvents. A solution at 9:1 acetone/ehtylacetate and 8:2 acetone/ethyl acetate where studied. Increasing further the percentage of ethyl acetate would lead to a degree of swelling too high to make the recovery efficient.

The efficiency of the recovery was determined as percentual ratio between the peak area of the compound extracted from the silicone sheets and the peak area of the compound obtained from the standard solution.

$$r = A_{j,i}/A_{std,i} * 100 \tag{4.1}$$

Where r is the percentage value of recovery, $A_{j,i}$ is the peak area of the compound i in the sample j and $A_{std,i}$ is the area of the compound i in the standard.

The results are shown in the Tab.4.2, with their relative standard deviation calculated on the triplicate samples.

Substance	$r_{Acetone}$	$s_{Acetone}$	r_{MeOH}	s_{MeOH}	$r_{MeOH/Acet50:50}$	$s_{MeOH/Acet50:50}$	$r_{Acet/EtOAc9:1}$	$s_{Acet/EtOAc9:1}$	$r_{Acet/EtOAc8:2}$	$s_{Acet/EtOAc8:2}$
1,4-Oxathiane	14.17	1.64	3.96	0.68	10.07	1.80	7.98	2.24	8.05	1.82
1,4-Dithiane	38.62	6.00	27.29	5.16	29.90	4.74	28.76	3.34	29.96	5.13
Mustard	61.36	9.42	50.16	5.15	54.13	5.29	54.59	4.82	52.31	7.18
1,4,5-Oxadithiepane	58.43	11.17	67.68	7.46	50.11	11.36	54.01	4.58	52.87	6.76
CN	77.33	2.40	50.35	7.17	49.60	2.13	78.19	5.39	69.08	5.43
1,2,5-Trithiepane	97.67	2.79	94.92	9.04	86.50	7.29	105.23	5.02	105.85	10.95
TPA	30.64	2.92	8.89	2.03	14.37	1.62	22.85	2.85	19.69	2.31

Table 4.2: Recoveries obtained from the extraction of the different compounds from the silicone sheets with different solvents, r and relative standard deviation, s.

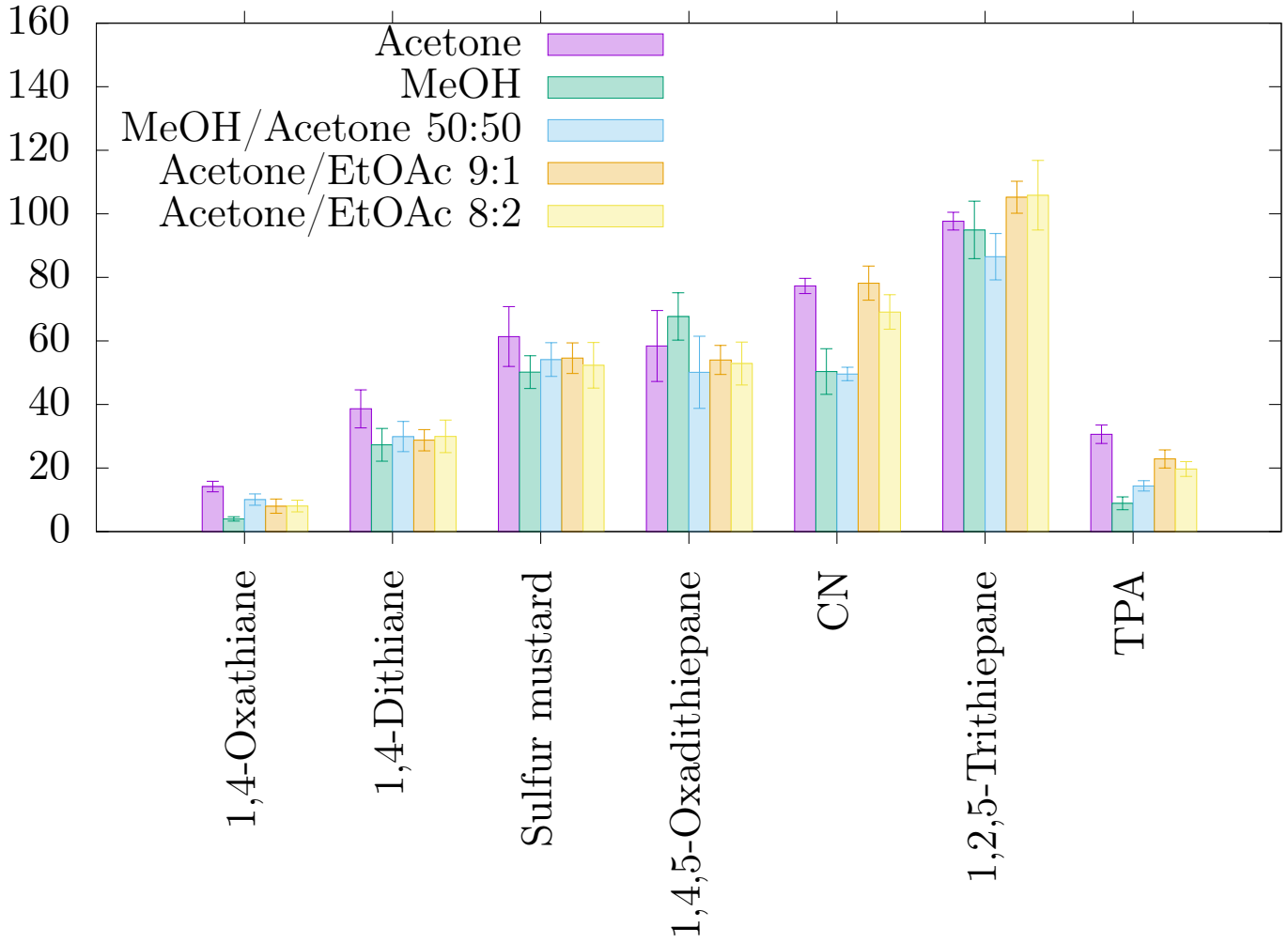


Figure 4.5: Histogram displaying the recoveries obtained for the compounds analyzed with GC-MS, using different solvents with error bars calculated from the standard deviation.

Substance	$r_{Acetone}$	$s_{Acetone}$	$r_{Acet/EtOAc9:1}$	$s_{Acet/EtOAc9:1}$	$r_{Acet/EtOAc8:2}$	$s_{Acet/EtOAc8:2}$
DM[ox]	17.37	2.18	14.54	3.85	13.80	1.94
DPA	24.38	3.65	23.68	6.40	19.66	1.19
TPA[ox]	85.62	2.33	109.35	7.03	95.61	5.98

Table 4.3: Recoveries obtained for the compounds analyzed with LC-MS/MS, r and respective standard deviation, s.

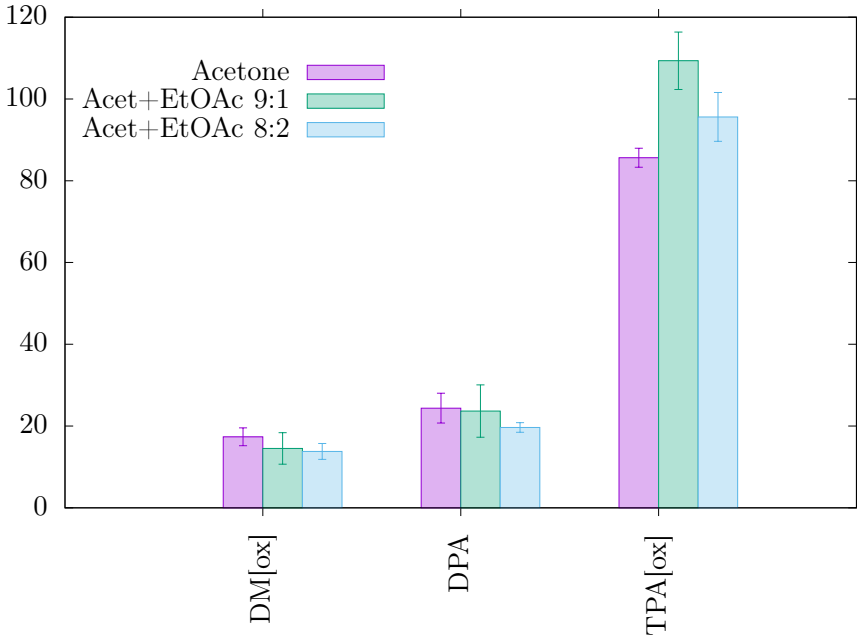


Figure 4.6: Histogram displaying recoveries obtained for the compounds analyzed with LC-MS/MS, using different solvents with error bars calculated from the standard deviation.

Methanol and its mixture are the extracting solution that provides the least amount of swelling with that said the results from their recoveries are comparable to the other solvents only for the compounds with the low partition coefficient while they provide the worst results when the partition coefficient increases. As expected the solutions containing ethyl acetate provide the best recoveries at high partition coefficients. The best compromise between this wide range of compounds turns out to be Acetone. The polarity and the swelling don't seem to fully explain the different recoveries obtained for the different compounds, for this reason further studies were carried out with the intent to determine possible losses through the procedure.

4.3 Small scale experiments

4.3.1 Degradation study

A degradation study was carried out to determine the possible loss of analyte during the exposure time in water. The solutions containing G01 were analyzed with GC-MS while the ones containing G02 where analyzed using LC-MS/MS. The uncertainty on the measure was calculated as standard deviation calculated on the three repetitions of the experiment. From the recoveries of G01, Fig.4.7, it is possible to notice how sulfur mustard and its degradation products are highly affected by the aqueous environment. Sulfur mustard fully degrades and its degradation products present a less than 100% recovery indicating a possible further degradation. Even if 1,4,5-Oxathiepane presents a full recovery this value is not significant as it might come from other sources and not only from the initial spiking. CN is the only compound presenting a 100% recovery, in fact the hydrolysis of this compound is rather slow at almost neutral condition. Furthermore, although TPA was predicted to be quite stable in water, it presents a quite strong degradation probably oxidizing to TPA[ox]. Arsine compounds degradation products present a full recovery making them persistent for at least 24h in water environment, Fig.4.8. Their stability was predicted as they are the end degradation products of the respective CWAs compounds.

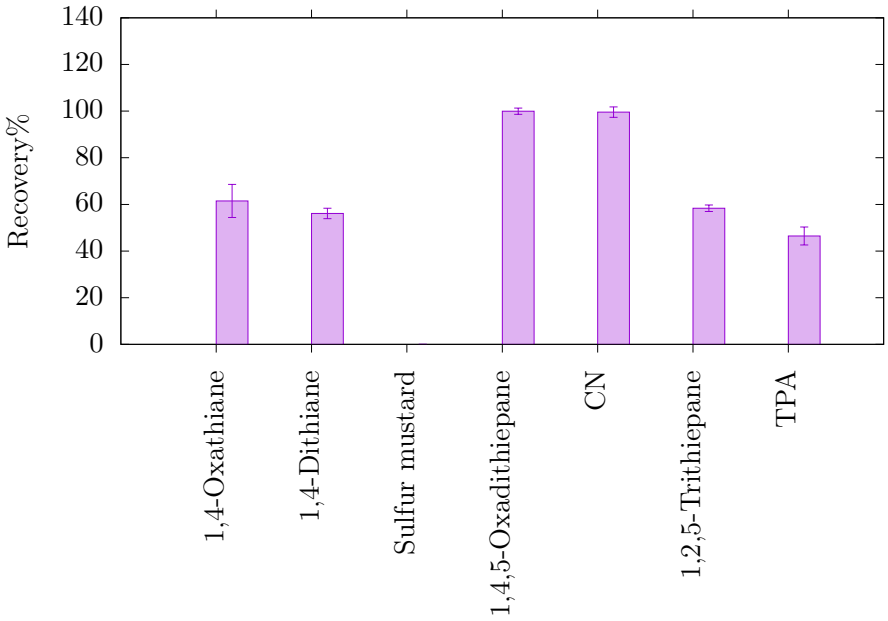


Figure 4.7: Recoveries obtained for the compounds analyzed with GC-MS, after 24h of water exposure with error bars calculated from the standard deviation..

Substance	r	s
1,4-Oxathiane	61.50	7.10
1,4-Dithiane	56.13	2.25
Mustard	0	0
1,4,5-Oxadithiepane	99.95	1.34
CN	99.57	2.22
1,2,5-Trithiepane	58.37	1.39
TPA	46.47	3.83

Table 4.4: Recoveries obtained for the compounds analyzed with GC-MS after 24h of water exposure, r and respective standard deviations, s.

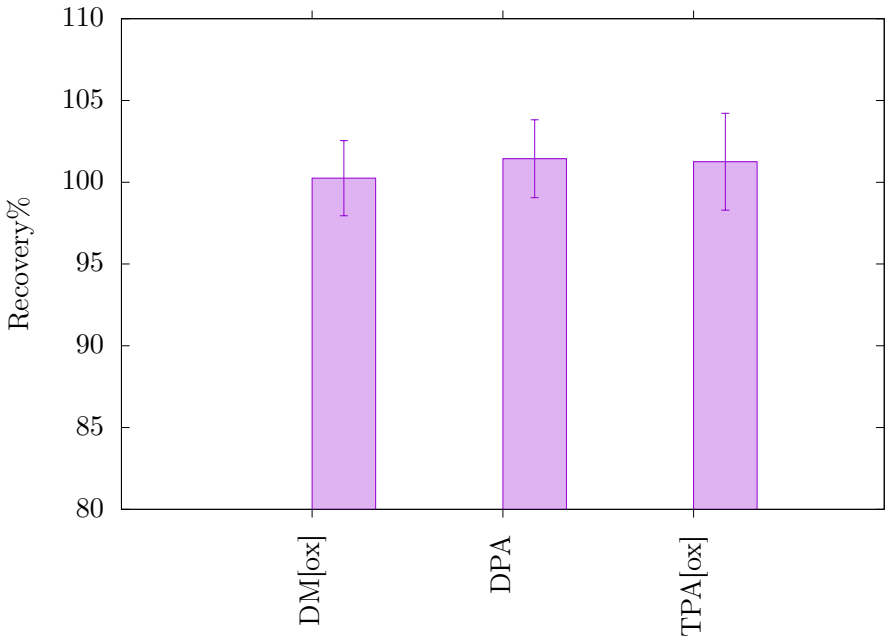


Figure 4.8: Recoveries obtained for the compounds analyzed with LC-MS/MS, after 24h of water exposure, with error bars calculated from the standard deviation..

Substance	r	s
DM[ox]	100.25	2.30
DPA	101.44	2.38
TPA[ox]	101.26	2.96

Table 4.5: Recoveries obtained for the compounds analyzed with LC-MS/MS, after 24h of water exposure, r and relative standard deviations.

4.3.2 Evaporation study

Volatile substances can be lost during the evaporation of the solvent. To determine if the recoveries found are affected by the evaporation step of the procedure, an evaporation study was carried out on G01 solution as it contains small molecules.

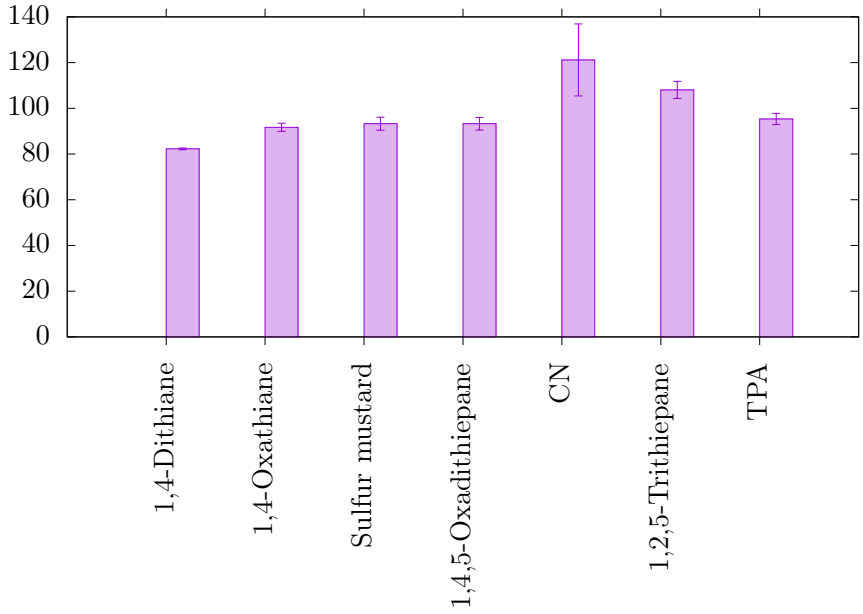


Figure 4.9: Recoveries obtained for the compounds analyzed with GC-MS, after concentration through evaporation, with error bars calculated from the standard deviation.

Substance	r	s
1,4-Dithiane	82.27	0.38
1,4-Oxathiane	91.70	1.79
Mustard	93.28	2.85
1,4,5-Oxadithiepane	93.25	2.75
CN	121.16	15.74
1,2,5-Trithiepane	108.05	3.71
TPA	95.31	2.45

Table 4.6: Recoveries obtained from the evaporation step of the sample preparation procedure, r and relative standard deviations, s.

All the compounds present a recovery above 90% apart from 1,4-Dithiane that presents a recovery of 82.3 ± 0.4 %. 1,4-Dithiane is also the most volatile compound in the group but it is possible that its low recovery is due to photodegradation, Fig. 4.9.[45]

4.4 Kinetic study

To determine the capability of the silicone sheets as passive sampler for the compounds of interest, the uptake model was tested.

After reaching the 8 days all the sheets were recovered from the water. Following the methodology described in Chapter 3, for each SS a GC and a LC sample were prepared. Analyzing those samples with tandem mass spectroscopy and comparing the obtained signal with the calibration curve it was possible to determine the amount of compound retrieved from each SS. Using the amount of compound and the exposure time of each SS it was possible to follow the uptake of the compound through time. A model was then built from the results obtained, using the model uptake equation:

$$n_s(t) = m_s C_w k_1/k_2(1 - e^{-k_2 t}) \tag{4.2}$$

Where:

- n_s(ng) is the amount sampled during the exposure time (t);
- m_s(Kg) is the mass of the sampler;
- C_w(ng/L) is the concentration of the compound in water;
- $k_1/k_2 = K_{sw}$ (L/Kg) is the silicone water partition-coefficient.

A theoretical value for the amount sampled was calculated, taking random initial values for k_1 and k_2. The model was then optimized varying the values of k_1 and k_2 until the sum of the differences squared of the theoretical and experimental values for the amount sampled was minimized. The calibration curves used to determine the amount sampled for all compounds are shown in Appendix B.

$$y_{min} = \sum_i (n^e_{s,i} - n^t_{s,i})^2 \tag{4.3}$$

where:

- y_{min} is the function to minimize;
- $n^e_{s,i}$ is the experimental value of the amount sampled;
- $n^t_{s,i}$ is the theoretical value calculated from the model.

The data obtained from 1,4-dithiane and 1,4-oxathiane don't follow the uptake model, Fig.4.10. The two compounds possess the smallest octanol-water partition coefficient in the group of compounds analyzed and this can cause high variability in the data recorded and the fact that these two mustard degradation products can undergo further degradation can contribute to the non-ideal uptake.[45]

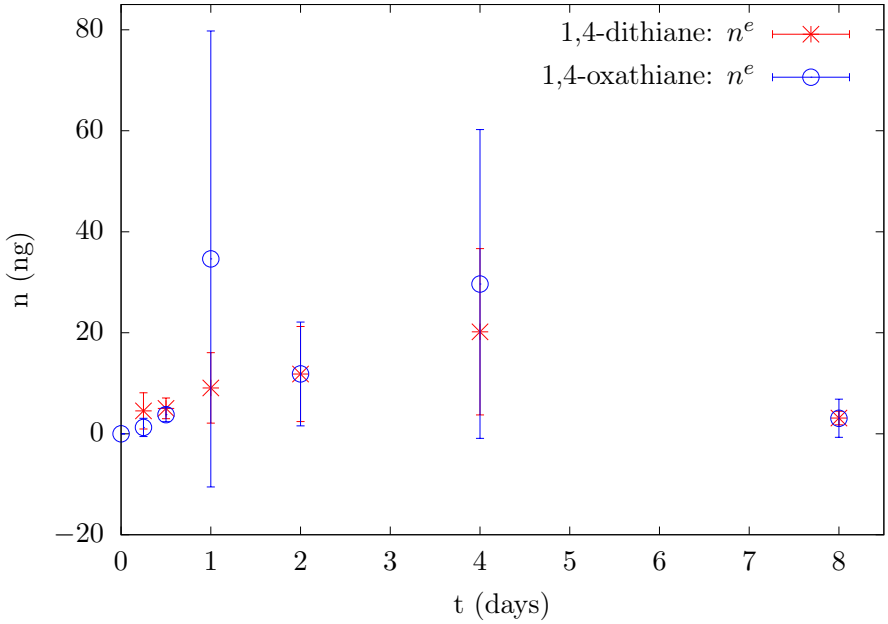


Figure 4.10: Uptake results for 1,4-dithiane and 1,4-oxathiane.

	1,4-oxathiane		1,4-dithiane	
t(days)	n^e(ng)	s_e(ng)	n^e(ng)	s_e(ng)
0	0	0	0	0
0.25	1.26	1.80	4.52	3.58
0.5	3.77	1.37	5.03	2.04
1	34.62	45.15	9.07	6.98
2	11.84	10.29	11.83	9.41
4	29.66	30.58	20.20	16.46
8	3.07	3.78	3.12	1.34

Table 4.7: Average value for the amount of 1,4-dithiane and 1,4-oxathiane retrieved, n^e, after a time period t of exposure in spiked water from three SSs and relative standard deviation s_e.

Mustard gas quickly degrades into water but either because the small amount left in the water or the initial amount before complete degradation, part of it is stored in the SSs. The model related to the uptake of sulfur mustard is quite fast reaching the equilibrium value just after 6 h. The variability correlated to the concentration retrieved is the smallest compared to the other compounds even when compared in relative terms, Fig.4.11.

The fact that sulfur mustard degrades rather quickly makes it hard to correlate the amount found in the SSs to its concentration into water. It is anyhow possible to use the SSs as a qualitative instrument to determine the presence of this compound.

1,4,5-Oxadithiepane and 1,2,5-trithiepane present a quick uptake kinetic reaching the equilibrium concentration just after 12-24 h as their $logK_{ow}$ is lower than 3. The rise in concentration of 1,2,5-trithiepane can be due to others compounds degradation, as for example 1,4-Dithiane.[46] Both 1,4,5-oxadithiepane and 1,2,5-trithiepane can be used to determine the possible presence of sulfur mustard leakage.

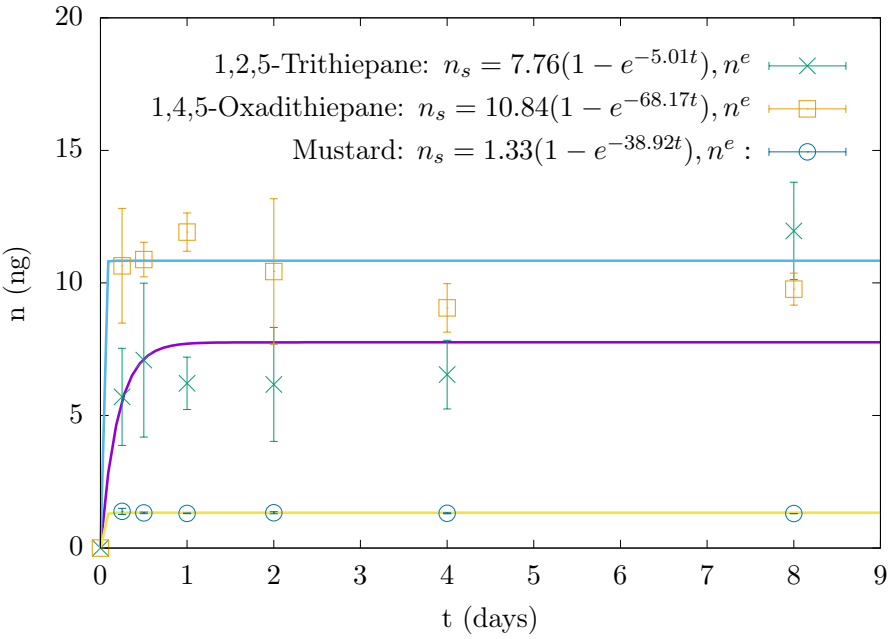


Figure 4.11: Silicone sheets uptake results for 1,2,5-trithiepane, 1,4,5-oxadithiepane and sulfur mustard.

	Mustard		1,2,5-trithiepane		1,4,5-oxadithiepane		CN	
t(days)	n^e(ng)	s_e(ng)	n^e(ng)	s_e(ng)	n^e(ng)	s_e(ng)	n^e(ng)	s_e(ng)
0	0	0	0	0	0	0	0	0
0.25	0.28	0.12	5.70	1.83	10.65	2.16	112.96	15.49
0.5	0.27	0.03	7.09	2.90	10.88	0.65	120.98	10.96
1	0.26	0.01	6.22	0.99	11.92	0.72	115.75	12.20
2	0.27	0.04	6.17	2.15	10.43	2.75	127.26	25.55
4	0.26	0.02	6.54	1.29	9.06	0.91	91.76	19.73
8	0.26	0.00	11.96	1.84	9.77	0.60	24.38	1.76

Table 4.8: Average values for the amount of CN, 1,2,5-trithiepane, 1,4,5-oxadithiepane and sulfur mustard retrieved, n^e, after a time period t of exposure in spiked water from three SSs and relative standard deviation s_e.

CN initially present a high response and a good kinetic with relatively small variability, but after 2 days its concentration starts to decrease probably due to its degradation into hydrochloric acid and α-chloroacetophenone, as shown in Fig.2.8. Using in situ calibration its concentration can still be determined but if long deployment times are used, its degradation kinetic must be included in the model.

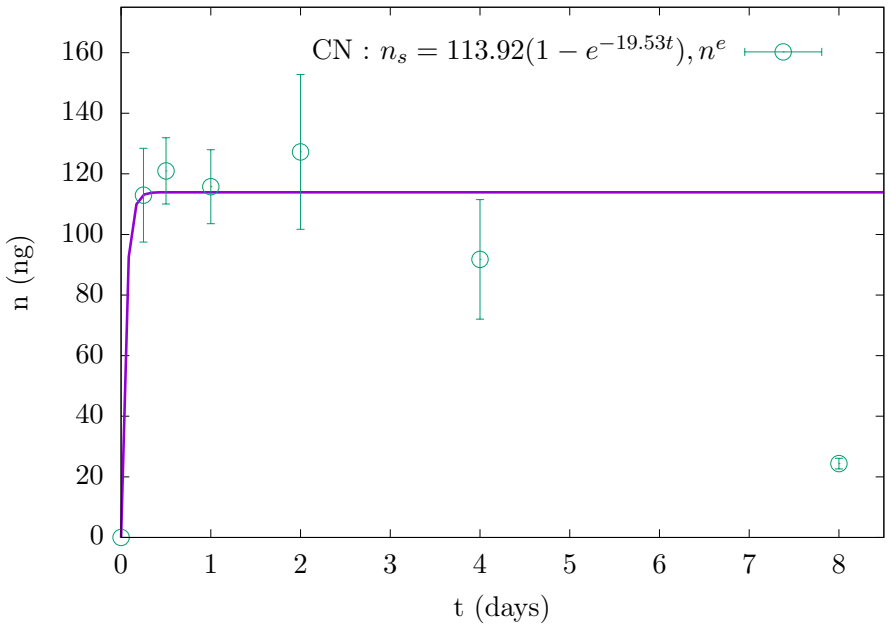


Figure 4.12: Silicone sheets uptake results for α-chloroacetophenone.

DM[ox] and DPA don't present a significant trend in the amount retrieved from the SSs showing high variability and amount retrieved that are often lower than the calibration range, Fig.4.13.

TPA[ox] instead present the longest kinetic with an equilibration time between 4 and 8 days, Fig.4.14. This trend was predicted as TPAox has a $logK_{ow}$ higher than 3, that in the group of compounds studied is second only to TPA.

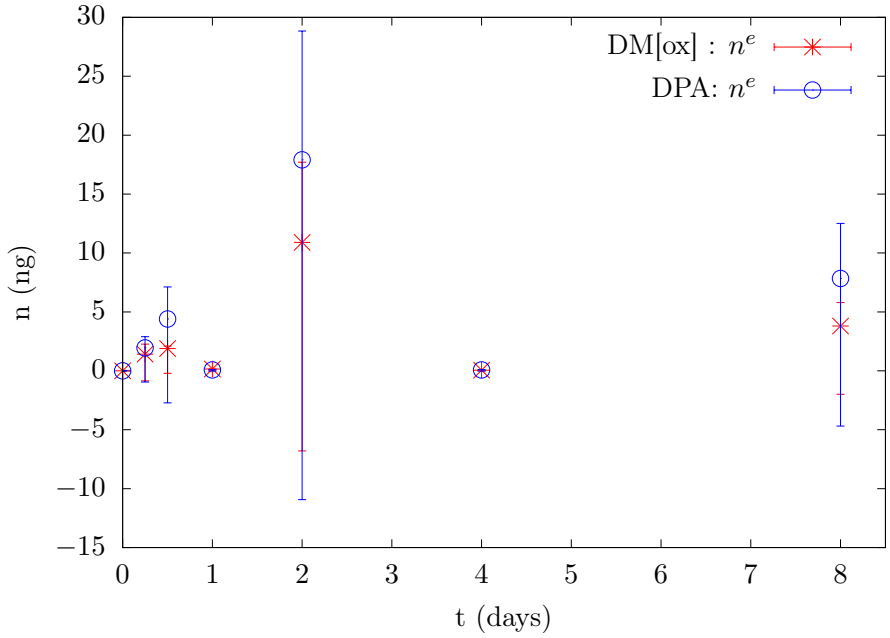


Figure 4.13: Silicone sheets uptake results for DM[ox] and DPA.

	DM[ox]		DPA		TPA		TPA[ox]	
t(days)	n^e(ng)	s_e(ng)	n^e(ng)	s_e(ng)	n^e(ng)	s_e(ng)	n^e(ng)	s_e(ng)
0	0	0	0	0	0	0	0	0
0.25	1.41	2.27	1.95	2.90	10.87	2.15	3.90	0.15
0.5	1.90	2.10	4.40	7.11	8.60	3.03	4.40	1.30
1	0.15	0.15	0.08	0.10	14.05	1.45	5.63	1.80
2	10.90	17.70	17.91	28.84	17.12	8.65	7.96	2.78
4	0.05	0.042	0.08	0.10	38.58	15.42	13.76	2.65
8	3.80	5.79	7.83	12.52	38.30	6.63	13.33	0.92

Table 4.9: Average value for the amount of DM[ox], DPA, TPA and TPA[ox] retrieved, n^e, after a time period t of exposure in spiked water from three SSs and relative standard deviation s_e.

Even if it wasn't directly spiked, TPA was found probably as an impurity of TPA[ox]. Its kinetic resemble the one of TPA[ox] and the amount retrieved is quite high probably due to its high $logK_{ow}$.

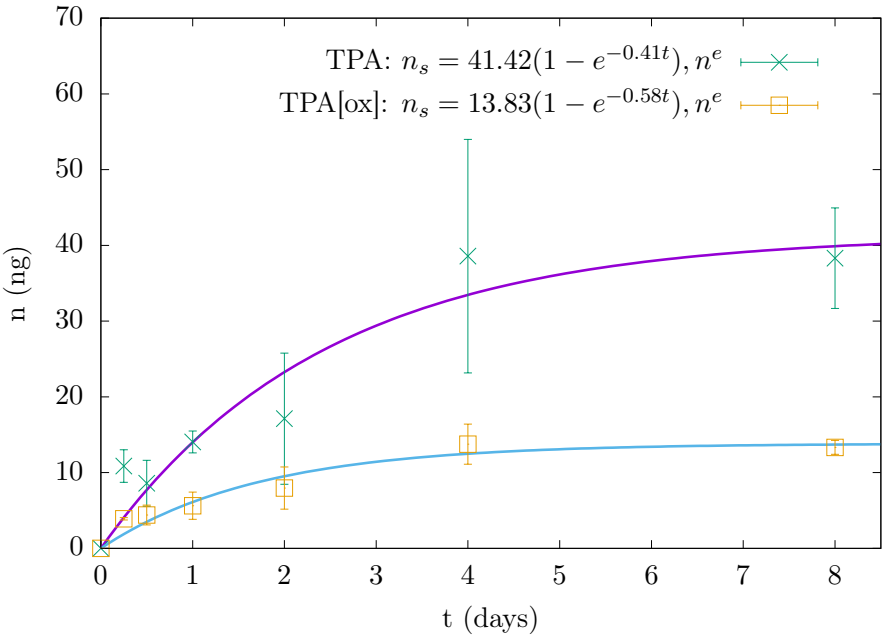


Figure 4.14: Silicone sheets uptake results for TPA and TPA[ox].

Conclusion

In this work, SSs have been tested as possible passive sampler for the monitoring of undersea CWAs dumping sites. The method developed is capable of determining a wide variety of compounds (Tab.3.3). The method of extraction of the compounds from the silicone sheets can be extended to other types of passive samplers, after optimizing the extraction solvent for the matrix of choice. In this case, for the silicone matrix it was found that what it works best with the wide variety of compounds analyzed is acetone. If the interest is focused on more apolar compounds like CN, 1,2,5-trithiepane and TPA[ox] a more apolar solution can be used. It is suggested to use a mixture of acetone/ethyl acetate 9:1 as more apolar mixtures would cause the excessive swelling of the silicone matrix producing low recoveries. As passive samplers are best suited for stable compound like pesticides, a degradation study in water was carried out. The 24 hours degradation study shown that only sulfur mustard presented a full degradation. The only compounds that remained intact where 1,4,5-oxathiepane, CN and the arsine related compounds degradation products DM[ox], DPA and TPA[ox].

The most important features to determine the suitability of SS towards different compounds are their persistence in the environment, their octanol-water partition coefficient and their recovery from the sampler matrix. The kinetic study shown how substances with low recovery and partition coefficient lower than 2 like 1,4-dithiane, 1,4-oxathiane, DM[ox] and DPA present an high variability between samples that make it hard to build a kinetic model. For these substances a qualitative determination can be carried out but their quantitation becomes rather hard. To improve the possibility of determining these compounds the extraction with other solvents can be tested to improve recoveries but also other passive sampler can be tested. For example Chemcatcher should work for a range of octanol-water partition coefficient that is lower than the one for SSs. By using passive samplers with an higher affinity for the compounds

of interest an higher amount is stored at equilibrium possibly reducing the variability between samples. Compounds with a medium octanol-water partition coefficient, between 1.5 and 3 and good recoveries presented a fast kinetic reaching the equilibrium in less than a day.

Despite its degradation it was possible to build a model for sulfur mustard, the amount retrieved from the samplers was steady for the whole experiment but evidently low. While 1,2,5-trithiepane and 1,4,5-oxadithiepane presented a good kinetic and it could be possible to use them as sulfur mustard indicators.

The hypothesis that the compounds stored inside the silicone matrix are protected by further degradation was disproved by CN as from its kinetic is evident that even if its equilibrium concentration is quickly reached after less than a day, after 4 days the concentration stored inside the SSs started decreasing. This phenomenon, if not due to the direct degradation inside the silicone sheets, can be due to the back extraction from the sampler into the water as the concentration into the water start decreasing when the compound undergoes hydrolysis.

The longest kinetic were achieved by TPA and TPA[ox], the compounds with the highest octanol-water partition coefficients, higher than 3, and good recoveries. These two compounds presented the best conditions to be used with the passive sampling technique with silicone sheets.

The next step would be to determine the exact silicone-water partition coefficient of the compounds of interest to be able to carry out on field test using silicone sheets spiked with PRCs near CWAs dumping sites. From the on field experiment it will be possible to determine the suitability of the silicone sheets in monitoring the dumping sites. Other passive sampler should also be tested in particular Chemcatcher should be more sensitive to compounds with a $\log K_{ow} < 3$ for which silicone sheets are not sensitive when their concentration is too low. It would also be very helpful to have a standardize distribution of conditioned and pre-cleaned SSs, as these two steps are the most time consuming taking away time to what should be the focus of the experiment. [16]

Bibliography

[1] J. K. Kingston, R. Greenwood, G. A. Mills, G. M. Morrison, and L. Björklund Persson, "Development of a novel passive sampling system for the time-averaged measurement of a range of organic pollutants in aquatic environments," *Journal of Environmental Monitoring*, vol. 2, pp. 487–495, 2000.

[2] K. Booij, F. Smedes, and E. M. van Weerlee, "Spiking of performance reference compounds in low density polyethylene and silicone passive water samplers," *Chemosphere*, vol. 46, pp. 0–1161, 2002.

[3] D. A. Alvarez, J. D. Petty, J. N. Huckins, T. L. Jones-Lepp, D. T. Getting, J. P. Goddard, and S. E. Manahan, "Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments," *Environmental Toxicology and Chemistry*, vol. 23, pp. 1640–0, 2004.

[4] D. A. Vroblesky and W. T. Hyde, "Diffusion samplers as an inexpensive approach to monitoring vocs in ground water," *Groundwater Monitoring & Remediation*, vol. 17, pp. 177–184, 1997.

[5] C. J. LeBlanc, W. M. Stallard, P. G. Green, and E. D. Schroeder, "Passive sampling screening method using thin-layer chromatography plates," *Environmental Science & Technology*, vol. 37, pp. 3966–3971, 09 2003.

[6] B. Vrana, I. J. Allan, R. Greenwood, G. A. Mills, E. Dominiak, K. Svensson, J. Knutsson, and G. Morrison, "Passive sampling techniques for monitoring pollutants in water," *Trends in Analytical Chemistry*, vol. 24, pp. 845–868, 2005.

[7] "Verifin homepage." www.helsinki.fi/verifin/. Accessed: 2018-05-18.

[8] J. Haldon, "Greek fire revisited: recent and current research," in *Byzantine style, religion, and civilization: in honour of Sir Steven Runciman*, pp. 290–325, Cambridge: Cambridge University Press, 2012.

[9] J. H. Corey, K. S. Jeffery, and A. H. Benjamin, *Chapter 2: History of Chemical Warfare*. Office of The Surgeon General Department of the Army, United States of America and US Army Medical Department Center and School Fort Sam Houston, Texas, 2008.

[10] A. Palazzo, *Seeking Victory on the Western Front*. University of Nebraska Press Lincoln and London, 2000.

[11] A. V. Kaffka, *Sea-Dumped Chemical Weapons: Aspects, Problems and Solutions Special Study on the Sea Disposal of Chemical Munitions by the United States*, vol. 10.1007/978-94-015-8713-6. Springer, 1996.

[12] "Opcw." opcw.org. Accessed: 2018-06-03.

[13] D. Philips and M. Depledge, "Metabolic pathways involving arsenic in marine organisms: A unifying hypothesis," *Marine Environmental Research*, vol. 17, pp. 1–12, 1985.

[14] H. Niemikoski, M. Södeström, and P. Vanninen, "Detection of chemical warfare agentrelated phenylarsenic compounds in marine biota samples by lc-hesi/ms/ms," *Analytical Chemistry*, no. 20, pp. 11129–11134, 2017.

[15] K. B. James N. Huckins, Jim D. Petty, *Monitors of Organic Chemicals in the Environment Semipermeable Membrane Devices*. Springer, 1 ed., 2006.

[16] B. S. Chepchirchir, A. Paschke, and G. Schüürmann, "Passive sampling for spatial and temporal monitoring of organic pollutants in surface water of a ruralurban river in kenya," *Science of the Total Environment*, no. 601-602, pp. 453–460, 2017.

[17] K. Booij and F. Smedes, "An improved method for estimating in situ sampling rates of nonpolar passive samplers," *Environmental Science & Technology*, vol. 44, pp. 6789–6794, 09 2010.

[18] K. Booij, F. Smedes, and J. Ian Allan, "Guidelines for determining polymer-water and polymer-polymer partition coefficients of organic compounds," *ICES Techniques in Marine Environmental Sciences*, no. 61, pp. 1–37, 2017.

[19] F. Smedes and K. Booij, "Guidellines for passive sampling of hydrophobic contaminants in water using silicone rubber samplers," *ICES Techniques in Marine Environmental Sciences*, no. 52, pp. 1–19, 2012.

[20] R. Lohmann, K. Booij, F. Smedes, and B. Vrana, "Use of passive sampling devices for monitoring and compliance checking of pop concentrations in water," *Environmental Science and Pollution Research*, pp. 1885–1895, 10 2011.

[21] G. Schulze, T. Streck and A. Paschke, "Sampling and conservation," *Water Chemistry and Microbiology*, no. 3, pp. 132–152, 2011.

[22] R. Greenwood, G. Mills, and B. Vrana, *Passive sampling techniques in environmental monitoring*, vol. 48 of *Comprehensive analytical chemistry*. Elsevier, 1 ed., 2007.

[23] P. T. Rusina, S. Foppe, M. Koblizkova, and J. Klanova, "Calibration of silicone rubber passive samplers: Experimental and modeled relations between sampling rate and compound properties," *Environmental Science & Technology*, vol. 44, pp. 362–367, 01 2010.

[24] B. Vrana, A. M. Graham, M. Kotterman, P. Leonards, K. Booij, and R. Greenwood, "Modelling and field application of the chemcatcher passive sampler calibration data for the monitoring of hydrophobic organic pollutants in water," *Environmental Pollution*, vol. 145, pp. 0–904, 2007.

[25] J. Beldowski, R. Been, and E. K. Turmus, *Towards the Monitoring of Dumped Munitions Threat (MODUM): A Study of Chemical Munitions Dumpsites in the Baltic Sea*. NATO Science for Peace and Security Series C: Environmental Security, Springer, 2017.

[26] J. Dearden and G. Bresnen, "The measurement of partition coefficients," *molecular informatics*, no. 7, pp. 133–144, 1988.

[27] K. Valko, "Application of high-performance liquid chromatography based measurements of lipophilicity to model biological distribution," *Journal of Chromatography A*, no. 1037, pp. 299–310, 2004.

[28] R. Wang, Y. Fu, and L. Lai, "A new atom-additive method for calculating partition coefficients," *Journal of Chemical Information and Modeling*, no. 37, pp. 615–621, 1997.

[29] E. L. DiFilippo and R. P. Eganhouse, "Assessment of pdms-water partition coefficients: Implications for passive environmental sampling of hydrophobic organic compounds," *Environmental Science & Technology*, vol. 44, pp. 6917–6925, 2010.

[30] F. Smendes and K. Booij, "Guidelines for passive sampling of hydrophobic contaminants in water using silicone rubber samplers," *ICES Techniques in Marine Environmental Sciences*, vol. 52, p. 20 pp, 06 2012.

[31] A. Martin, C. Margoum, J. Randon, and M. Coquery, "Silicone rubber selection for passive sampling of pesticides in water," *talanta*, no. 4, pp. 306–313, 2016.

[32] J. N. Lee, C. Park, and G. M. Whitesides, "Solvent compatibility of poly(dimethylsiloxane)-based microfluidic devices," *Analytical Chemistry*, vol. 75, pp. 6544–6554, 12 2003.

[33] N. B. Munro, S. S. Talmage, G. D. Griffin, L. C. Waters, A. P. Watson, J. F. King, and V. Hauschild, "The sources, fate, and toxicity of chemical warfare agent degradation products," *Environmental Health Perspectives*, vol. 107, pp. 933–974, 12 1999.

[34] J. A. Tørnes, A. M. Opstad, and B. A. Johnsen, "Determination of organoarsenic warfare agents in sediment samples from skagerrak by gas chromatography-mass spectrometry," *Science of The Total Environment*, vol. 356, pp. 235–246, 2006.

[35] R. Tilley, "The hydrolysis of bis(2-chloroethyl) sulfide (sulfur mustard) in aqueous mixtures of ethanol, acetone and dimethyl sulfoxide," *Australian Journal of Chemistry*, vol. 46, pp. 293–300, 1993.

[36] O. G. Bizzigotti, H. Castelly, A. M. Hafez, H. Smith, and M. T. Whitmire, "Parameters for evaluation of the fate, transport, and environmental impacts of chemical agents in marine environments," *Chemical Reviews*, vol. 109, pp. 236–256, 01 2009.

[37] H. Sanderson, P. Fauser, M. Thomsen, and P. B. Sørensen, "Pbt screening profile of chemical warfare agents (cwas)," *Journal of Hazardous Materials*, vol. 148, pp. 210–215, 2007.

[38] T. Missiaen, M. Södeström, I. Popescu, and P. Vanninen, "Evaluation of a chemical munition dumpsite in the baltic sea based on geophysical and chemical investigations," *Science of The Total Environment*, vol. 408, pp. 3536–3553, 2010.

[39] N. R. Council, *Systems and Technologies for the Treatment of Non-Stockpile Chemical Warfare Material*. Washington, DC: The National Academies Press, 2002.

[40] T. Hellström and M. Ödalen, "Long-time behaviour of mustard gas dumped in the bornholm basin," *University of Gothenburg*, no. Report no.6, 2013.

[41] F. Francken and A. M. Hafez, "A case study in modeling dispersion of yperite and clark i and ii from munitions at paardenmarkt, belgium," *Marine Technology Society Journal*, vol. 43, pp. 52–61, 10 2009.

[42] S. Hanaoka, K. Nomura, and S. Kudo, "Identification and quantitative determination of diphenylarsenic compounds in abandoned toxic smoke canisters," *Journal of Chromatography A*, vol. 1085, pp. 213–223, 09 2005.

[43] U. S. Army, *Transportable treatment systems for non-stockpile chemical warfare materiel: environmental impact statement*. 2001.

[44] "Etra." Etra.fi. Accessed: 2018-05-26.

[45] F. S. Timothy C. Marrs, Robert L. Maynard, *Chemical Warfare Agents: Toxicology and Treatment*. Wiley, 2 ed., 2007.

[46] C. A. Brevett, K. B. Sumpter, and R. G. Nickol, "Kinetics of the degradation of sulfur mustard on ambient and moist concrete," *Journal of Hazardous Materials*, vol. 162, pp. 281–291, 2009.

Appendix A

MS spectra

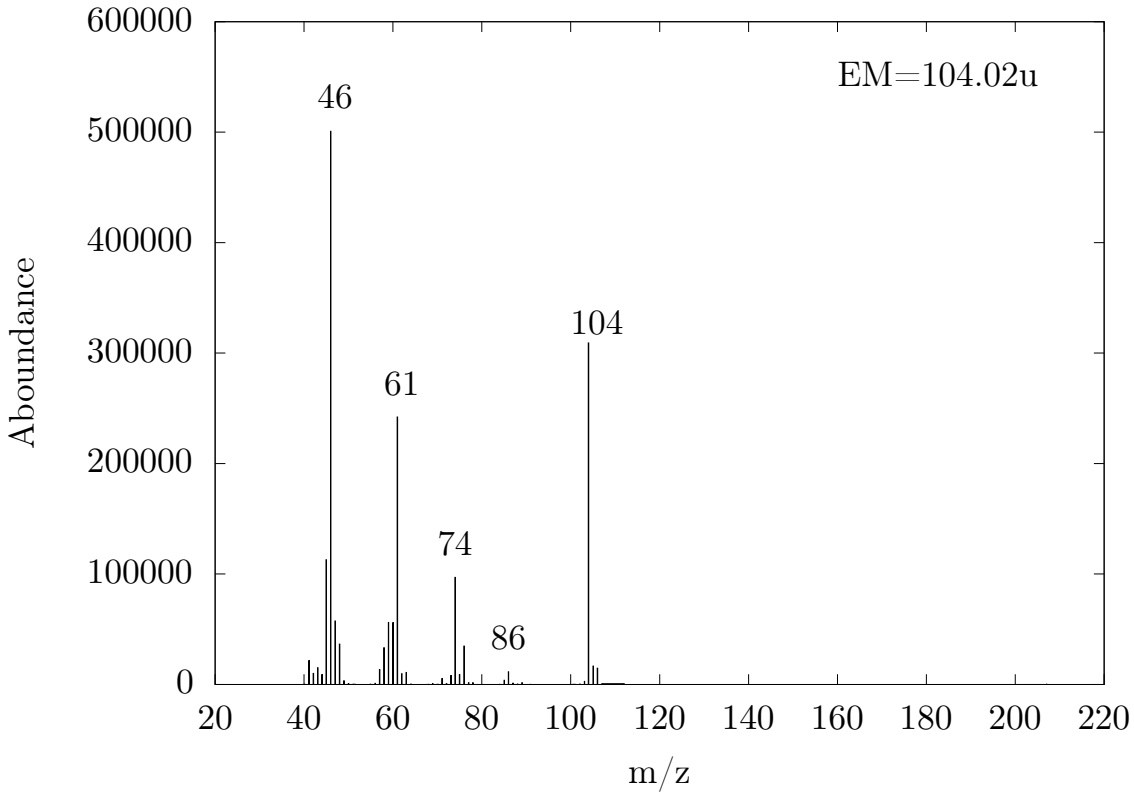


Figure A.1: Mass spectrum of 1,4-oxathiane.

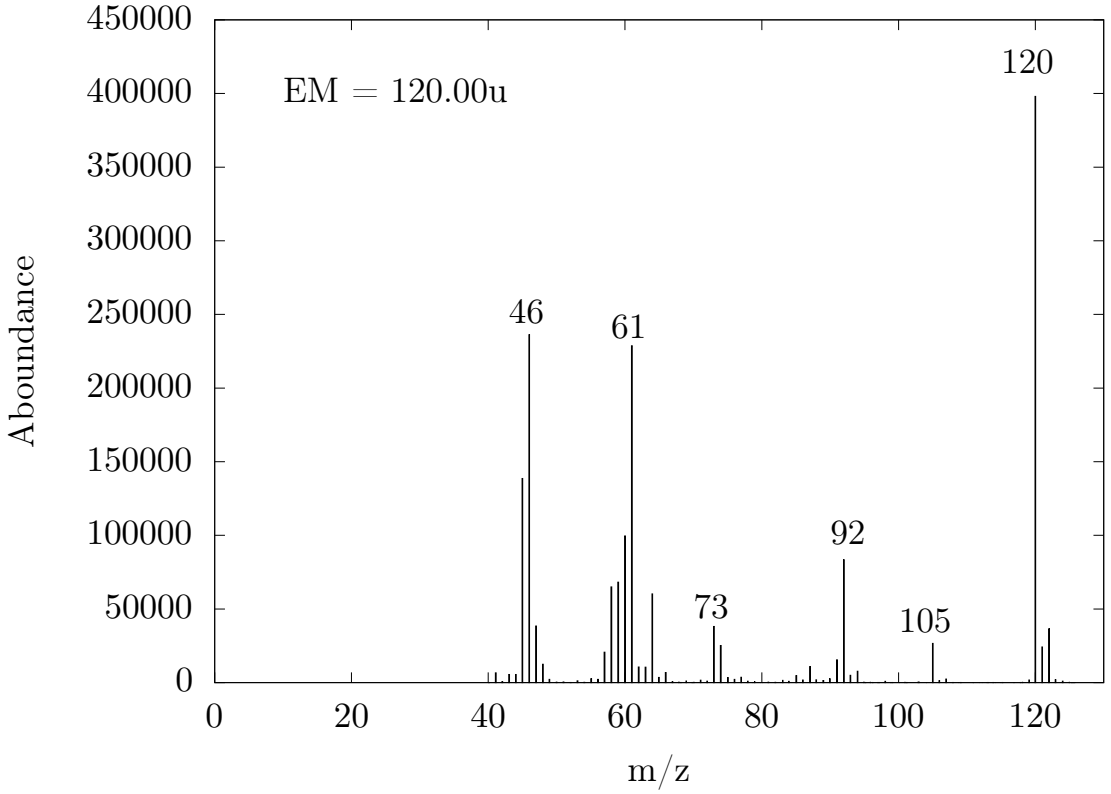


Figure A.2: Mass spectrum of 1,4-dithiane.

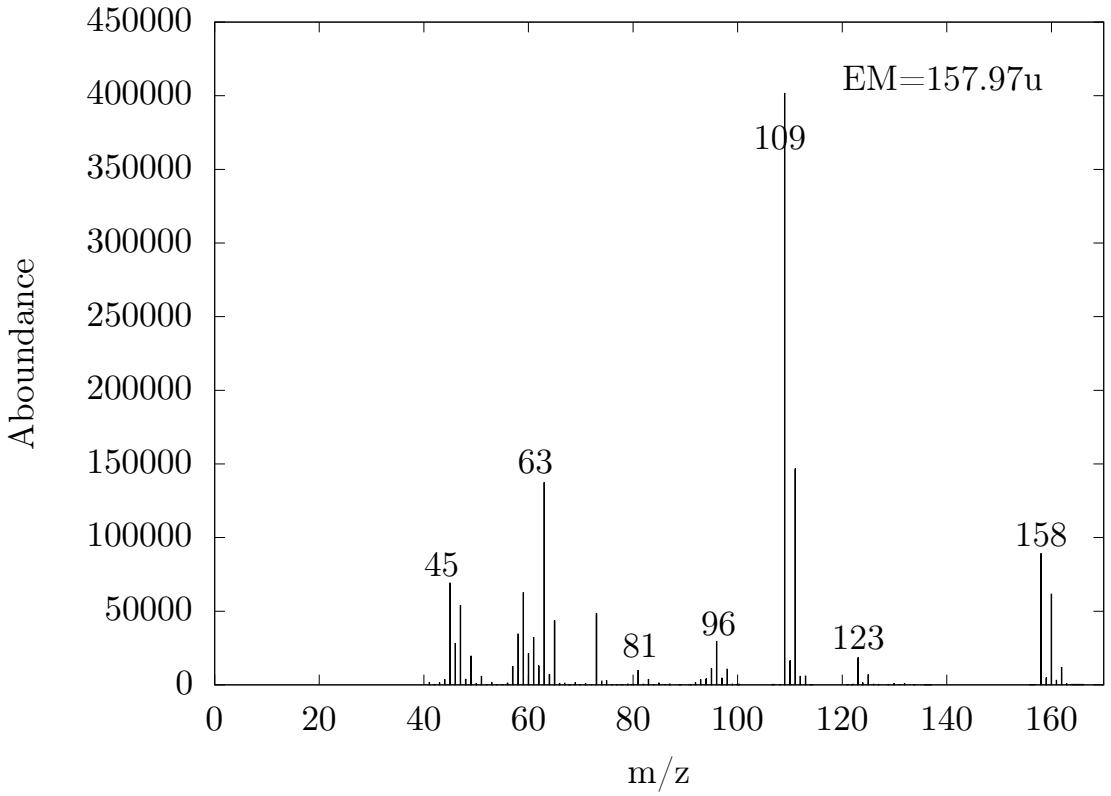


Figure A.3: Mass spectrum of sulfur mustard.

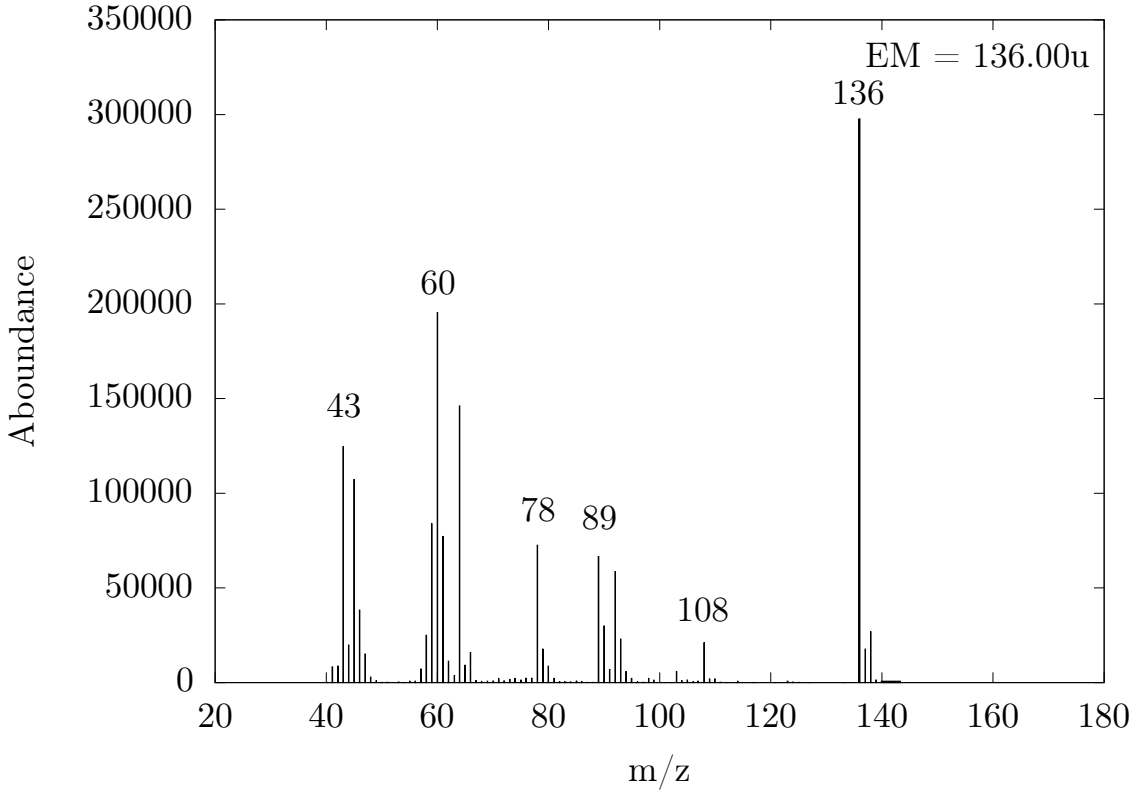


Figure A.4: Mass spectrum of 1,4,5-oxadithiepane.

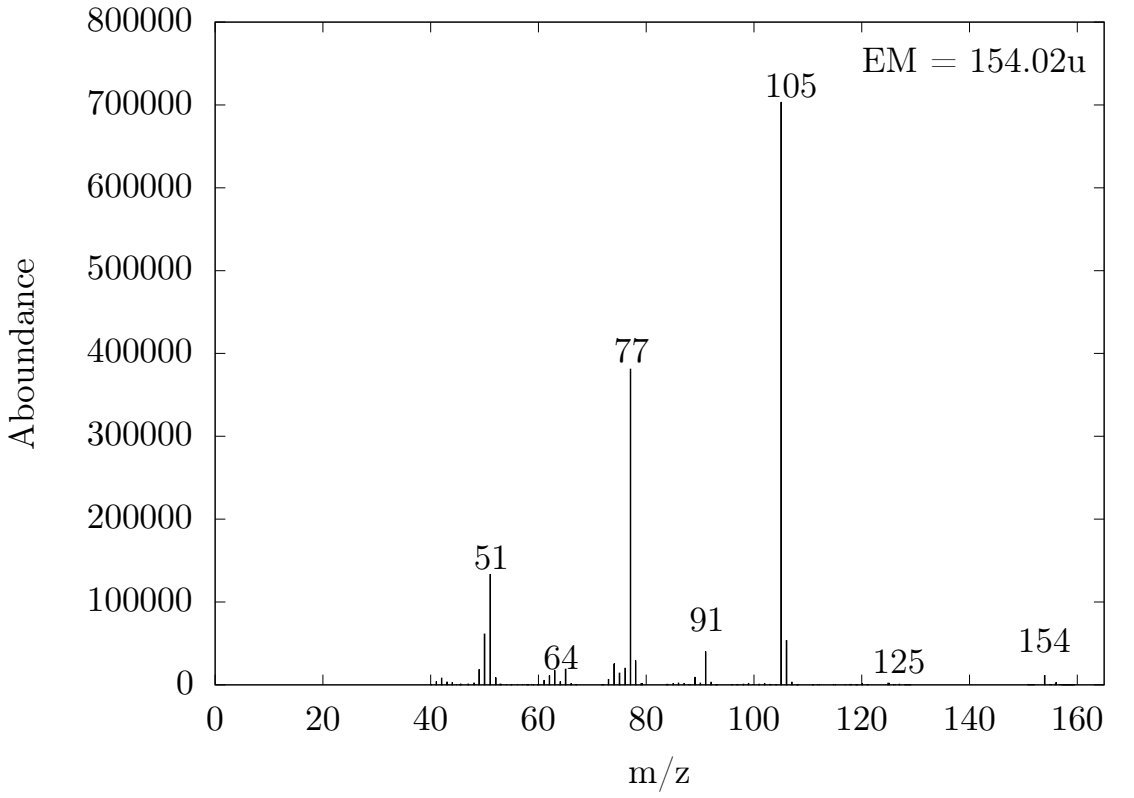


Figure A.5: Mass spectrum of CN.

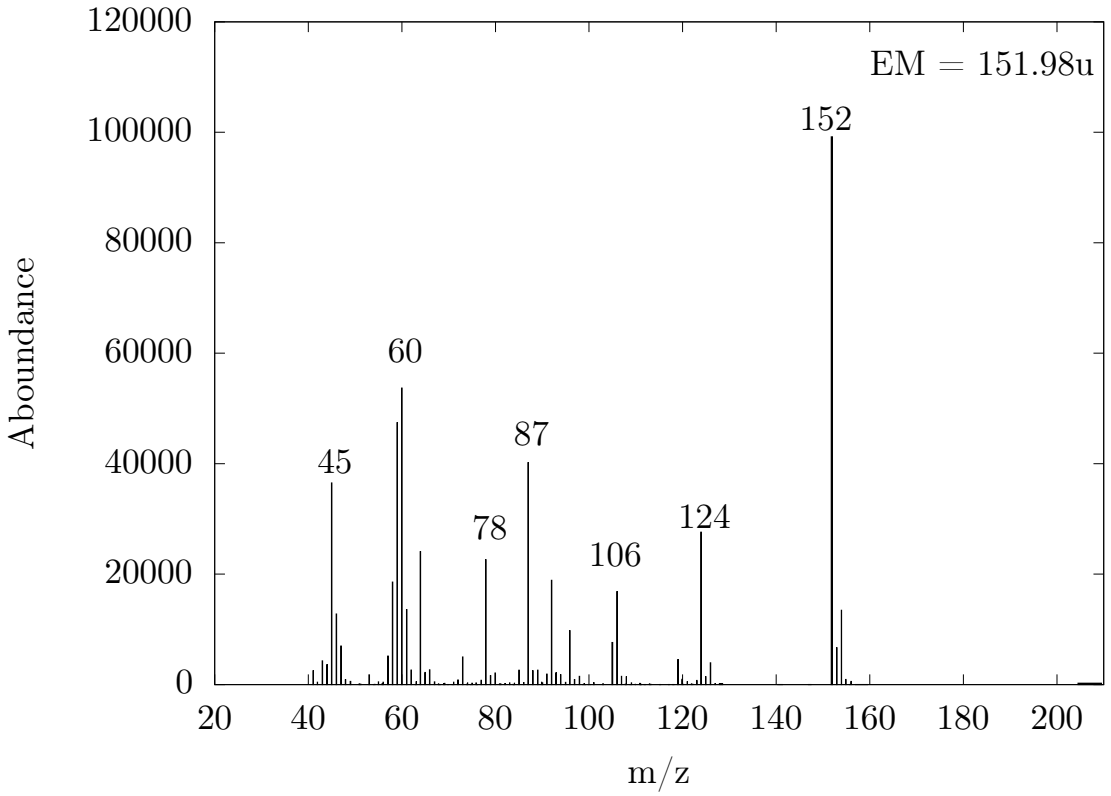


Figure A.6: Mass spectrum of 1,2,5-trithiepane.

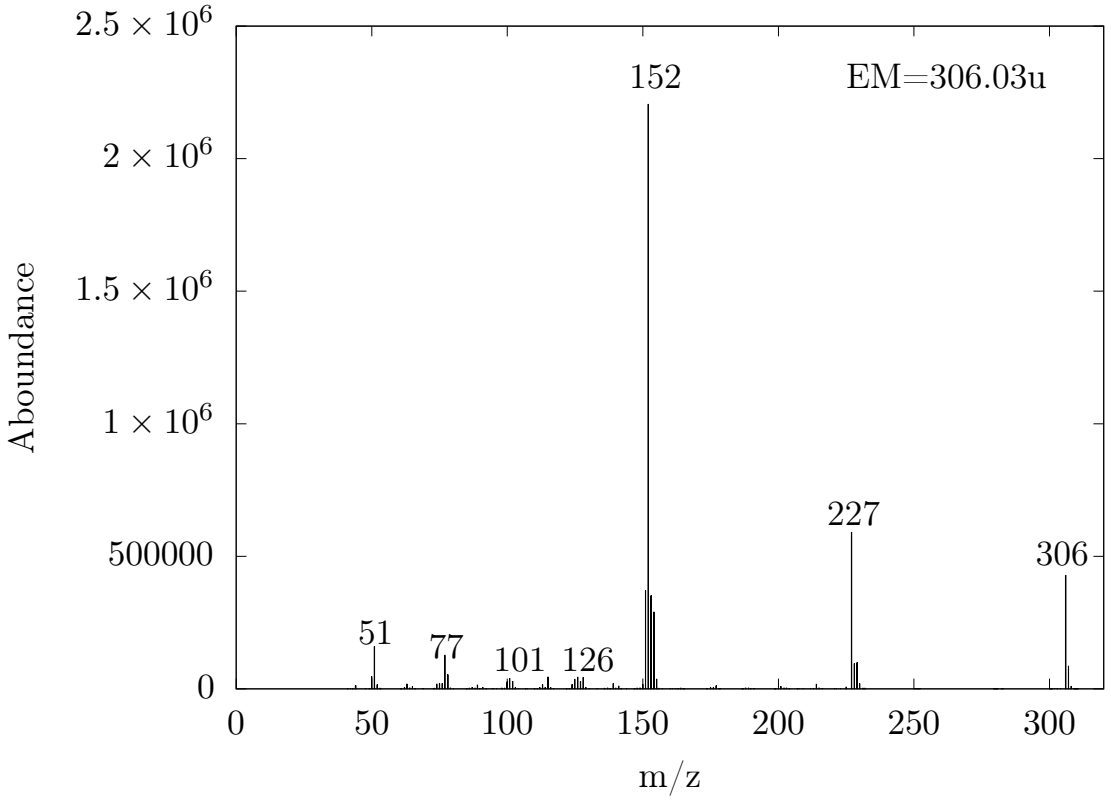


Figure A.7: Mass spectrum of TPA.

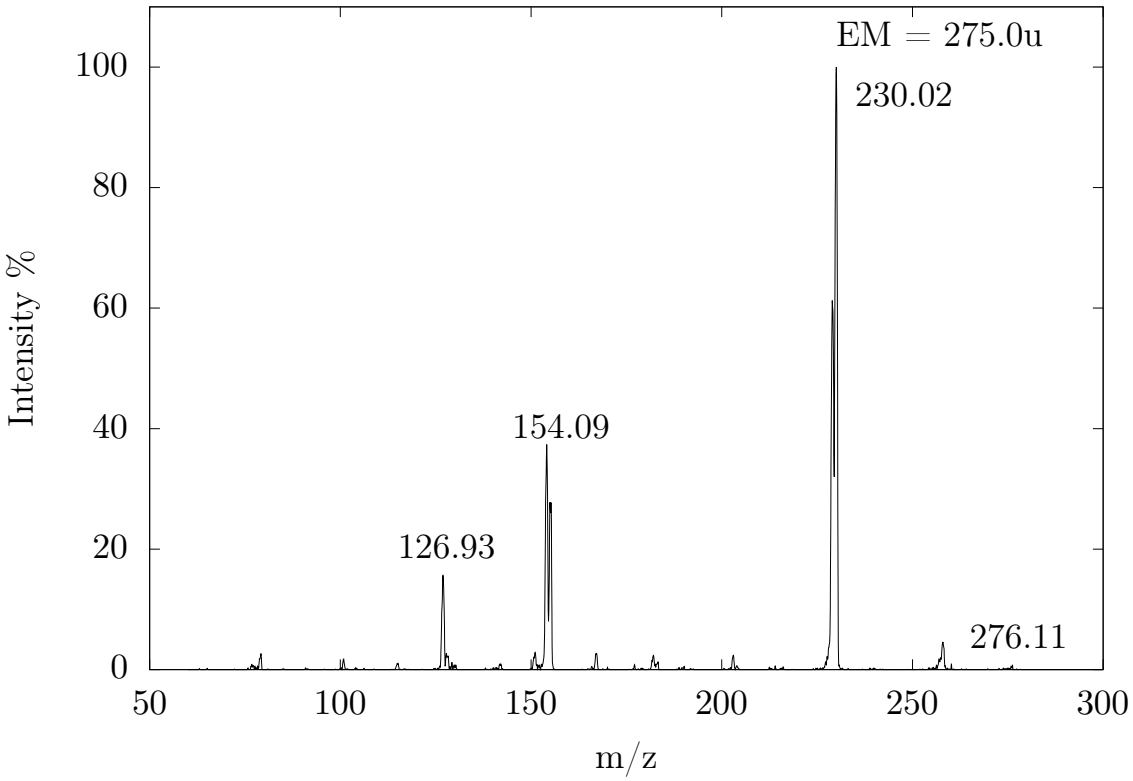


Figure A.8: Mass spectrum of DM[ox].

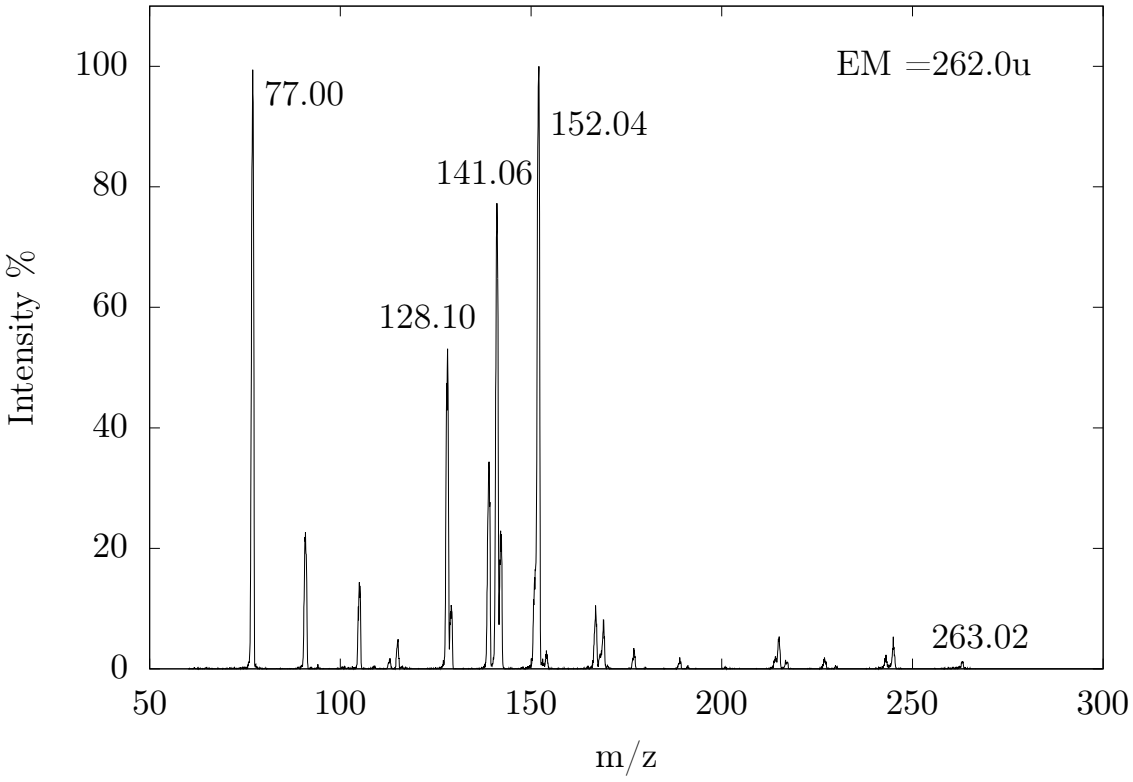


Figure A.9: Mass spectrum of DPA.

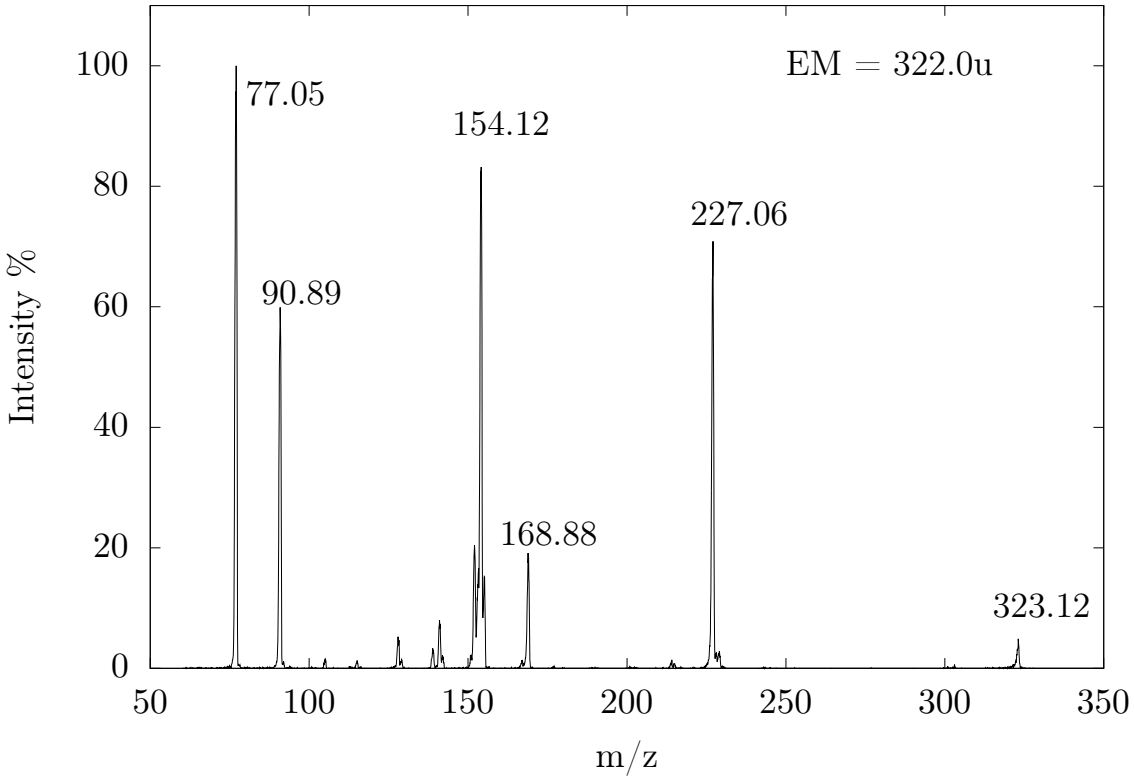


Figure A.10: Mass spectrum of TPA[ox].

Appendix B

Calibration curves

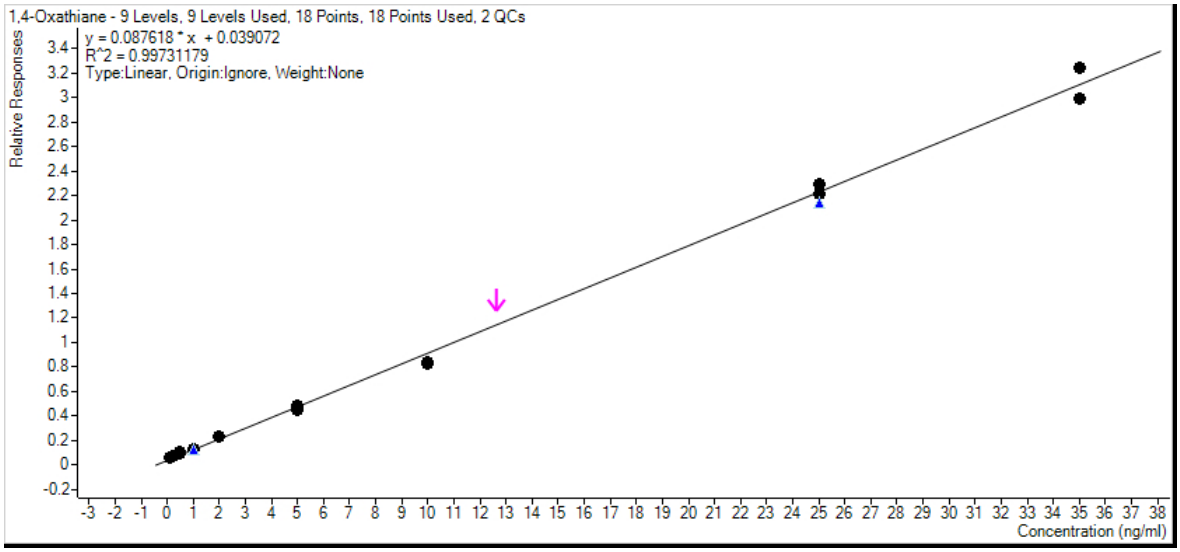


Figure B.1: Calibration curve of 1,4-oxathiane.

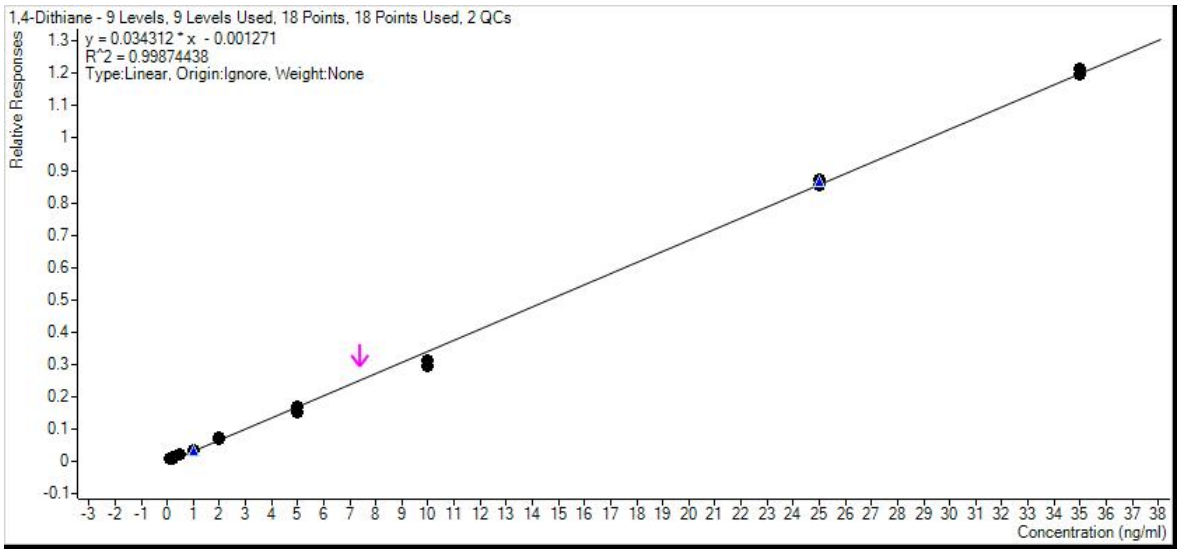


Figure B.2: Calibration curve of 1,4-dithiane.

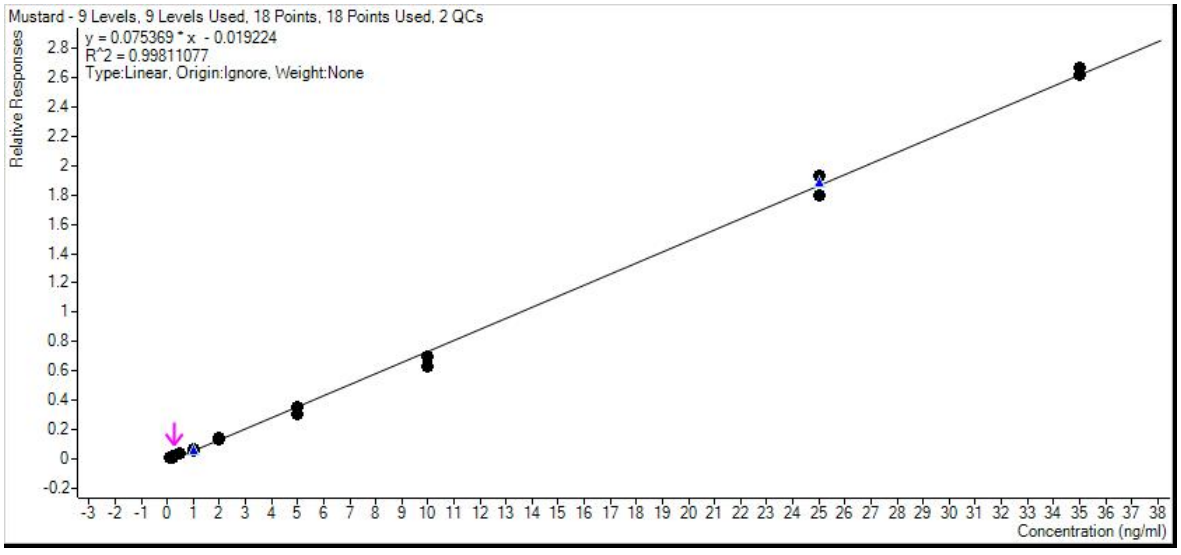


Figure B.3: Calibration curve of sulfur mustard.

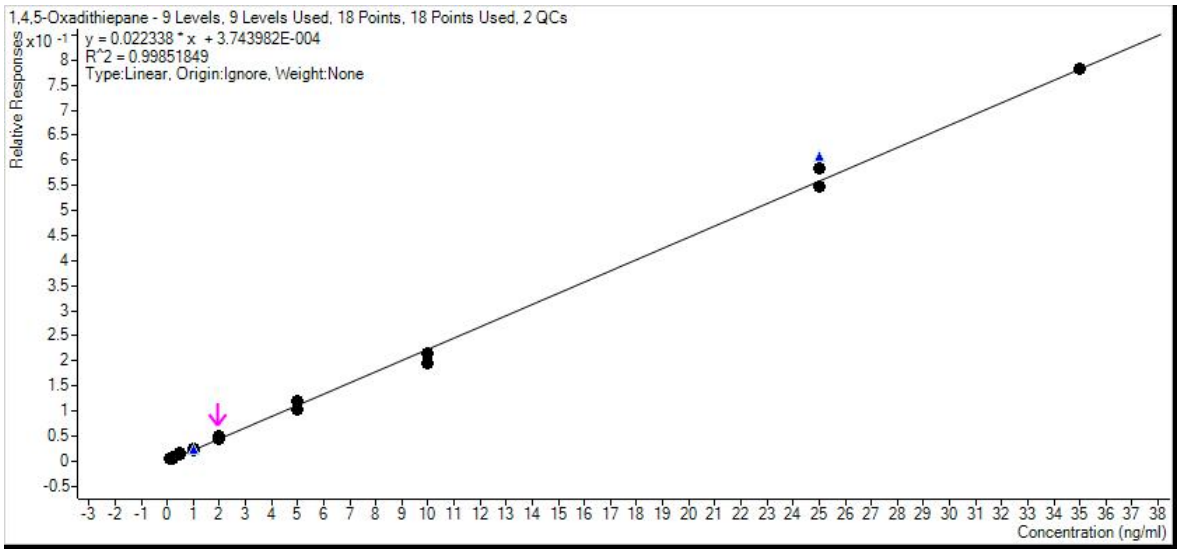


Figure B.4: Calibration curve of 1,4,5-oxadithiepane.

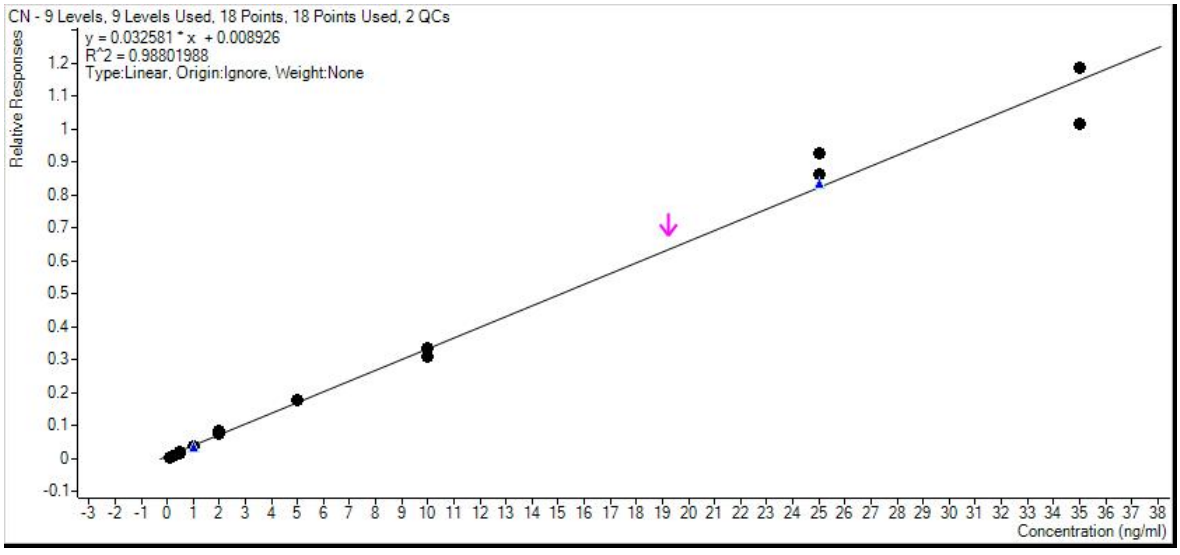


Figure B.5: Calibration curve of CN.

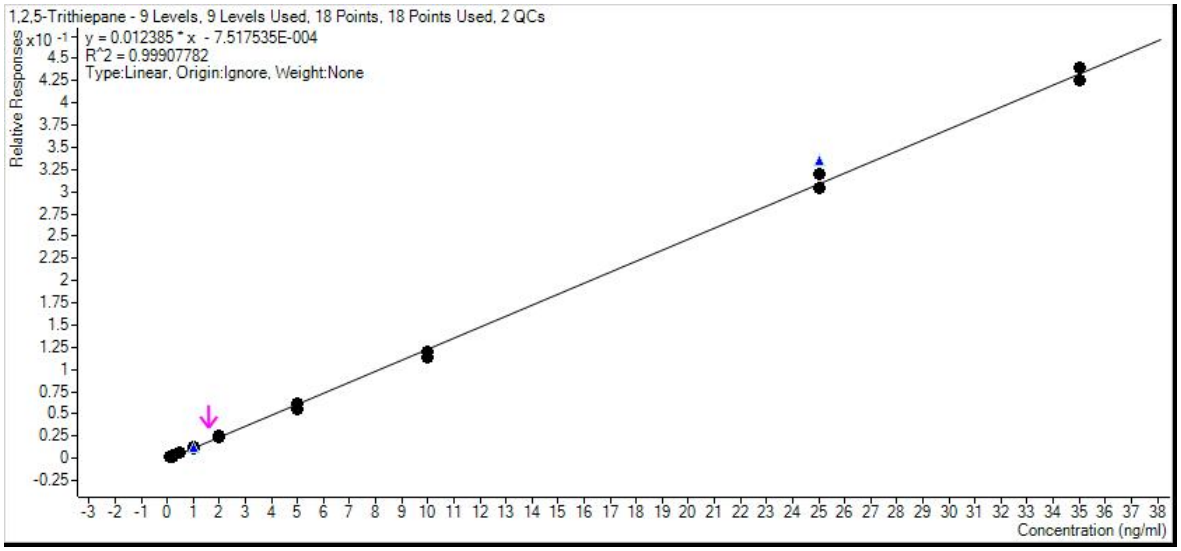


Figure B.6: Calibration curve of 1,2,5-trithiepane.

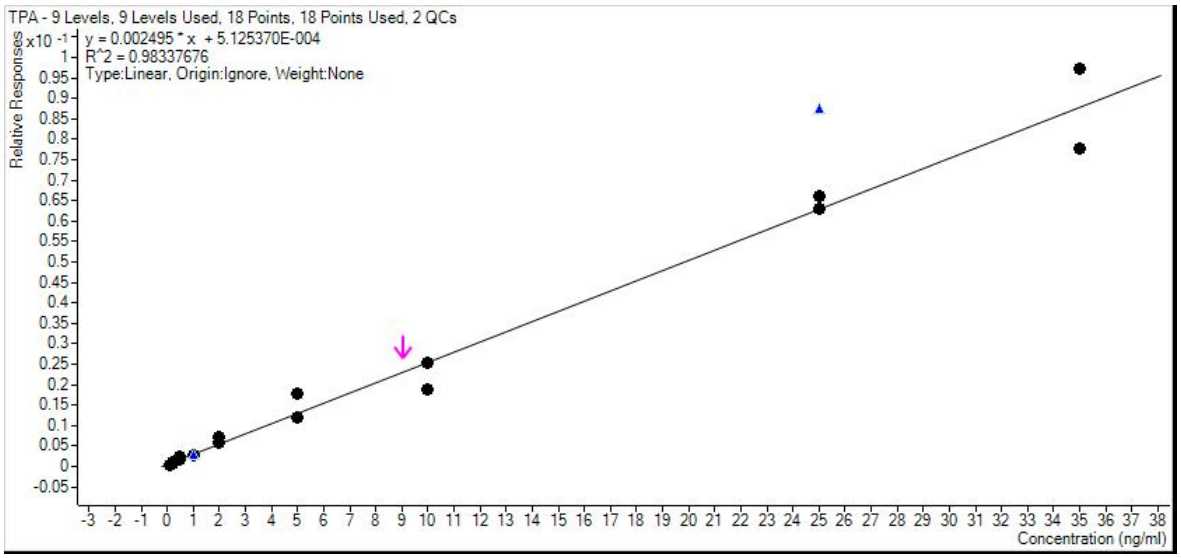


Figure B.7: Calibration curve of TPA.

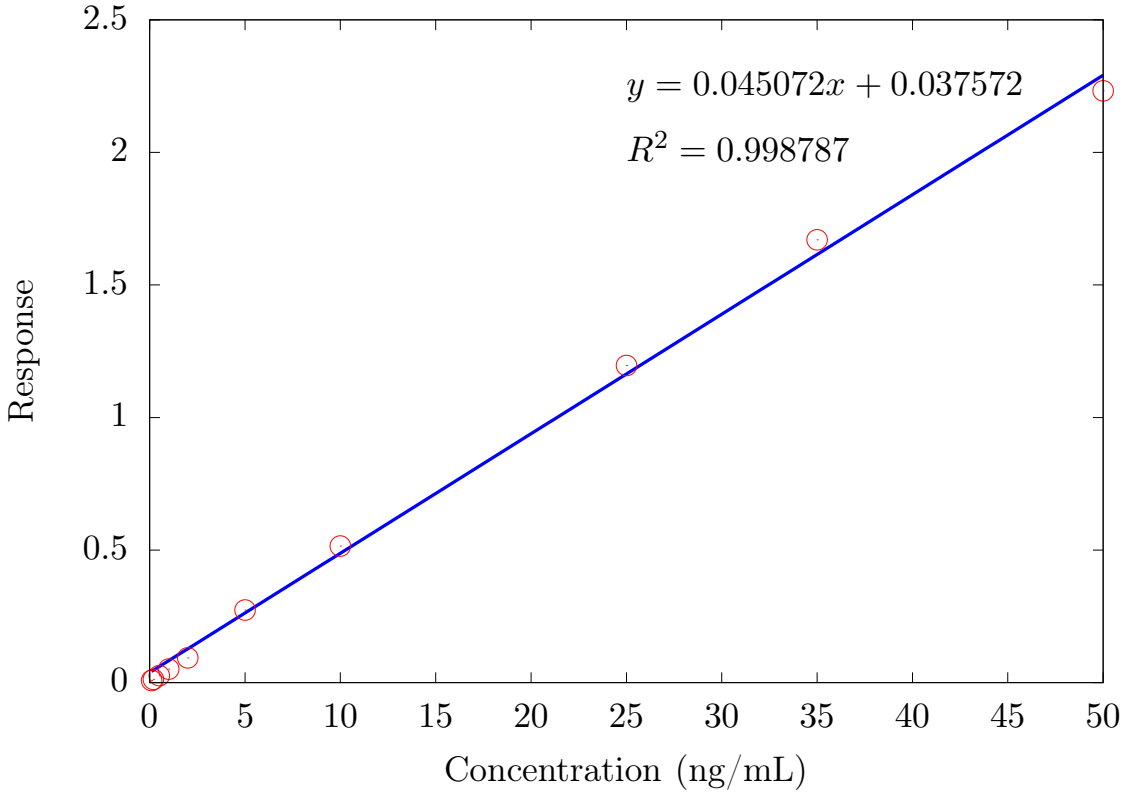


Figure B.8: Calibration curve of DM[ox].

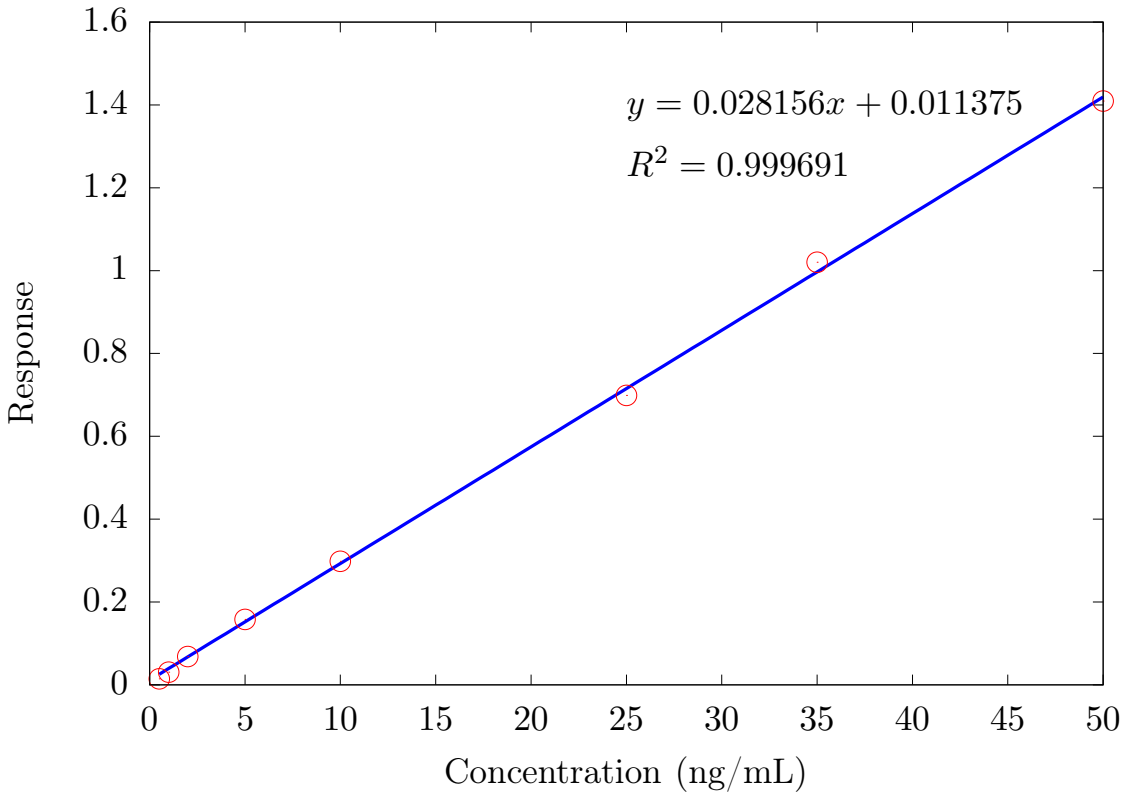


Figure B.9: Calibration curve of DPA.

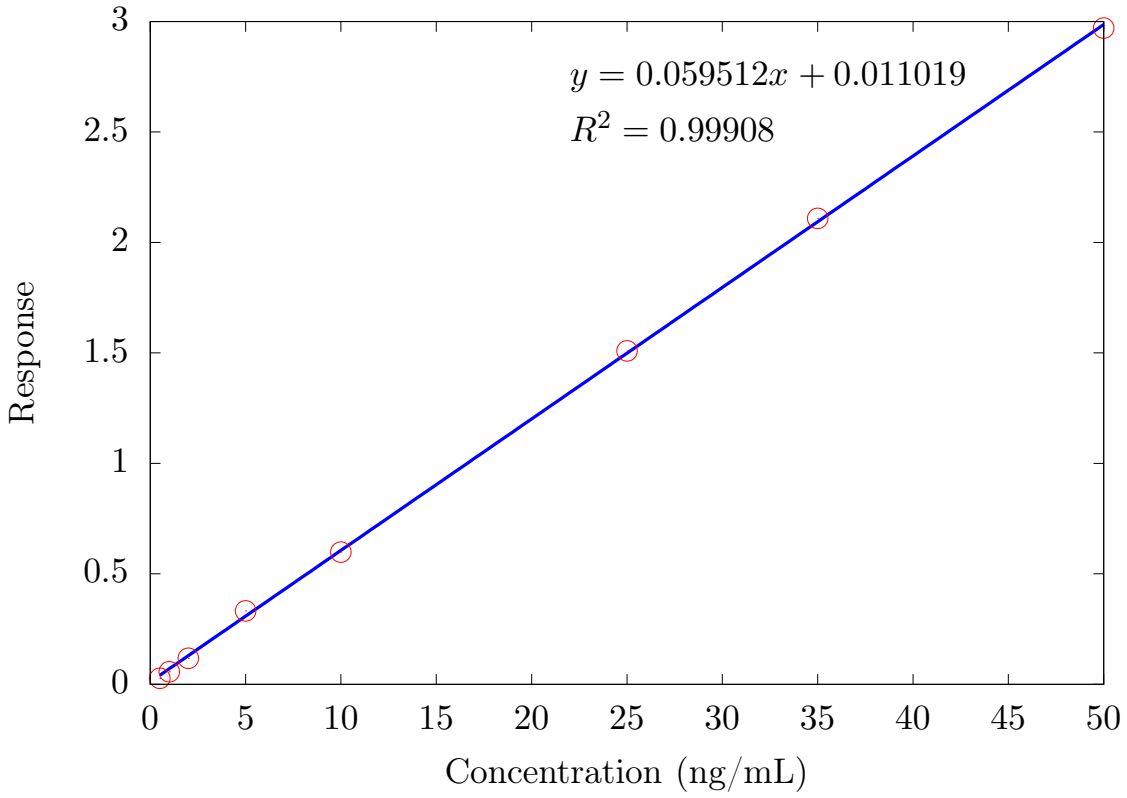


Figure B.10: Calibration curve of TPA[ox].