Organic Pollution in the Marine Environment of Tunisia

The book cover includes:

- The photo of the Bizerte lagoon, in which there are different shades of blue. I mean that the Bizerte lagoon is so beautiful and should be protected from pollution and ought to be kept beautiful. I took this photo during the sampling campaigns.
- The blue on the right side of the cover presents the marine environment.
- The red color on the book cover indicates the danger that comes to the marine environment.
- The orange color in the title of the book reveals the threat caused by organic compounds such as pesticides in the aquatic environment.
- The green color in the back of the photo shows the green chemistry that should be adopted to minimize the risk of the emission of pesticides into the aquatic environment.

Organic Pollution in the Marine Environment of Tunisia

By

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TABLE OF CONTENTS

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To HOURIA NECIBI my soul mate and mother
To all my family, professors, coworkers, students, and friends

Mouna

List of Tables

LIST OF FIGURES

ABBREVIATIONS LIST

ACN	:	Acetonitrile
ACP	:	Principal component analysis
ADBI	:	Celestolid
AHMI	:	Phantolide
AHTN	:	Tonalide
ATII	:	Traseolid
API	:	Industry Promotion Agency
ASE	:	Accelerated Solvent Extraction
GIC	:	Gas chromatography
CRM	:	Certified Reference Material
DCM	:	Dicloromethane
DDD	:	Dichlorodiphenyldichloroethane
DDE	:	Dichlorodiphenyldichloroethylene
DDT	:	Dichlorodiphenyltrichloroethane
DGPA	:	Directorate General of Fisheries and Aquaculture
DPMI	:	Cashmeran
ECD	:	Electron capture detector
ELL	:	Liquid liquid extraction
ES	:	Soxhlet extractor
PAH	:	polycyclic aromatic hydrocarbons
HCB	:	Hexachlorobenzene
HHCB	:	Galaxolid
Hex	:	Hexane
IAEA	:	International Atomic Energy Agency
IC	:	Internal calibration
ICQ	:	Internal calibration with QuEChERS
IUPAC	:	International Union of Pure and Applied Chemistry
K_{ow}	:	Octanol / water partition coefficient
LOD	:	Limit of detection
LOQ	:	Limit of quantification
MAE	:	Microwave assisted extraction
MM	:	Musk mosken

MK	**:**	Musk keton
MX	**:**	Musk xylem
MS	**:**	Synthetic musk
MX D15	**:**	Musk xylen deuterated
WHO	**:**	World Health Organization
P	**:**	Pressure
PCB	**:**	Polychlorinated biphenyls
PES	**:**	Suspended particles
UNEP	**:**	United Nations Environment Program
PNEC	**:**	Concentration that does not pose a danger
POP	**:**	Persistent Organic Pollutants
OCP	**:**	Organochlorine pesticides
PSU	**:**	Practical unit of salinity
PTC	**:**	value at which the concentration of the pollutant is considered toxic
QUEChERS	**:**	Quick, Easy, Cheap, Effective, Rugged and Safe .
RSD	**:**	Relative standard deviation
S	**:**	Solubility
SDSE	**:**	Simultaneous solvent extraction and distillation
SPME	**:**	Micro solid phase extraction
SPE	**:**	Solid phase extraction
SRM	**:**	Standard Reference Material
TEC	**:**	value below which the concentration of a pollutant is considered non-toxic
TOC	**:**	total organic carbon

INTRODUCTION

Aquatic ecosystems are important reservoirs of pollutants, some of which are toxic, and their discharge into the environment may pose a threat to their balance and to human health. The pollution sources of water are diversified and affect so many countries, including Tunisia. There are industrial, agricultural, domestic, and natural sources of pollution, which can be direct or indirect, individual or diffuse. The main types of pollutants that reach the aquatic environment are either organic compounds, such as polycyclic aromatic hydrocarbons, pesticides, medicines, dioxins, chlorophenols, phthalates, or inorganic compounds, which include metal, nitrogen, and, phosphorus compounds.

A large proportion of compounds of human or natural origin tend to accumulate in sediments and, in some cases, to concentrate in aquatic food in the aquatic environment. They are introduced into water courses in particulate, dissolved, and colloidal forms by industrial, urban, and agricultural activities, as well as by atmospheric transport. The polychlorinated biphenyls (PCBs) and the organochlorine pesticides (OCPs), which are found in concentrations of traces in water, are examples of toxic substances that are hydrophobic, and tend to accumulate in the sediment phase. Despite the prohibition of the use of some of these pollutants in North America and Europe for over 40 years and in Tunisia since 1980, they continue to pose problems because of their persistence in the environment. Sediments can therefore represent an important source of contaminants for the communities of organisms that reside there. Measures regulatory (prohibition or restriction of use of some chemical compounds) are also applied in order to protect humans and their environment. The Tunisian government has reacted against the degradation of aquatic environments by signing, in particular, the protocol of the Stockholm convention on persistent organic pollutants. PCBs, aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), and toxaphene are all prohibited under this protocol.

The Bizerte lagoon is the second largest lagoon in Tunisia. This lagoon has experienced a fleet of fishing and aquaculture related to the presence of three sectors. The human population around the lagoon is estimated at 163,000 inhabitants (2004 census), with a significant percentage in the city of Bizerte. The other main cities are in the region of Menzel Bourguiba, Menzel Abderrahmen and Menzel Jemil. This lagoon is one of the most economically important areas in Tunisia. It is heavily influenced by anthropogenic factors such as oil refineries, ceramics, metallurgy, ship building and air output. The organochlorine pesticides (OCPs) are persistent organic pollutants (POPs) and are well known for their chronic toxicity, their persistence, and their bioaccumulation.

Contamination with organochlorine pesticides and polychlorinated biphenyl compounds has spread worldwide and continues to be detected in a wide range of environmental media, including water, sediment, suspended particulate matter, and fish. Small sediment particles have a very large capacity of absorption and accumulation of pesticide. Pesticides released into the aquatic environment through various mechanisms are easily adsorbed on the particles and then incorporated into sediments. Thus, the concentration of organochlorine pesticides and polychlorinated biphenyls in surface sediments provide information about recent contamination, while concentrations of OCPs and PCBs in sediment cores can provides information on the contamination in the last decades. Gas chromatography is the technology used for the analysis of these organic pollutants.

The emerging contaminants are natural and synthetic chemicals, which are not commonly monitored in the environment. These contaminants are likely interred to the environment and cause negative effects to human health and the environment. Among these pollutants, synthetic musks (SM) have recently been identified as a new type of emerging marine contaminant. These compounds have become indispensable in our modern society and are used in a wide range of purposes as additives in perfumes, lotions, sunscreens, deodorants, antiseptics, and laundry detergents. Today, Tonalide (AHTN) and Galaxolide (HHCB) are the two main products widely used in cosmetics and cleaning. Because of their octanol–water coefficient (log $K_{ow} \geq 5$) and lipophilic character, the SM are easily absorbed by suspended particles and, optionally, are deposited and accumulated in the sediments. The assessment of the impact of human activities on the marine environment requires analysis of sediment samples, which represent the ultimate sink for hydrophobic pollutants. The presence of SMs in coastal and marine environments has previously been reported in different environmental matrices such as water, mussels, fish, sludge, and sediments. Anastassiades has developed a QuEChERS procedure to extract pesticides from fruits and vegetables. The method replaces many complex analytical steps commonly used in traditional methods, and provides high quality with a low time of analysis (10 min extraction time), a small amount of solvent, minimum of glassware, little work, and low cost sample analysis.

The first chapter of this book was a literature review of the three studied families of organic contaminants: organochlorine pesticides, synthetic musks, and polychlorinated biphenyls. This chapter presents the physicochemical properties and the sources of interception in the environment. It also revealed the presence and development of these compounds in the various environmental compartments.

The second chapter is the presentation of the Bizerte lagoon; it focuses on the presentation of the Bizerte lagoon and the industrial areas. Different matrices were studied in this work; the water, the suspended particulate matter (SPM), sediments, and sediment cores. The samples were collected from stations spread over the lagoon. The sampling, collection, preservation, and storage techniques for each type of matrix have been presented in this chapter. The sediment samples (surface and core) were lyophilized and homogenized before being stored for subsequent analysis. This chapter covers all the theoretical and experimental methods, used for the extraction of various types of organic pollutants. The origin of each method, the advantages and disadvantages, as well as the different detection, injection, and analysis methods were presented. In this chapter, the different analytical protocols used for the analysis of the different samples collected from the Bizerte lagoon have been detailed.

Chapter III focuses on the contamination of surface water by PCBs and OCPs in the Bizerte lagoon. We were first interested in the analysis, distribution, and evaluation of the possible sources of these compounds. The results were then compared with those in the literature. Chapter VI focuses on assessing the level of contamination of suspended particulate matter and surface sediments by the OCPs. This chapter also studied their impact on the aquatic ecosystem of the Bizerte lagoon. Chapter V investigates the vertical distribution of OCPs in the different layers of the sediment cores. This chapter presents the vertical distribution of these compounds and provides useful information for the ecological restoration in the lagoon. Chapter VI focuses on the validation of a new method for the analysis of synthetic musk by the QuEChERS extractor. In this chapter, we also studied the application of this method to assess the state of contamination of the surface sediments of the lagoon by these contaminants.

CHAPTER I

ORGANIC POLLUTION IN TUNISIA

I. Pesticides in Tunisia

I .1 Agricultural activities

Tunisia is an agricultural country, and its agricultural land is estimated at 4 316 million hectares. The agriculture intensification has resulted in the development of "intensive" cropping systems, based on the use of pesticides and the search for high yields. The number of products marketed is 725 and more, corresponding to 330 active substances (Table 1.1). Data from the National Statistics Institute (INS 2008) shows that the average annual imports of pesticides between 1999 and 2006 amounted to 3.750 tons per year (Figure 1.1). These quantities are nearly 5 kg/ha used mainly in agriculture (95%) for a treated area of 761.000 ha/year. The quantity of pesticides used in France amounts to 110.000 tons per year for a cultivated area of 295.000 km ^{2} (3.7 kg / ha). According to the General Directorate of Fisheries and Aquaculture, the use of these quantities is divided between cereals 63%, arboriculture 16% and market gardening 21% (Kane 2008).

Table 1.1 Number of active substances and commercial formulations for each type of pesticide imported into Tunisia (PAN Africa 2006).

Pesticides	Active substances	Commercial formulations
Fungicides	143	312
Insecticides	102	259
Herbicides	76	135
Rat poison	9	19
Total	330	725

In addition to the annual imports of pesticides, obsolete stocks (1.200 tons) present a real threat to human health and the environment. The situation has worsened because the country does not have adequate destruction facilities of hazardous waste, and suffers from a lack of training in the proper storage and use of pesticides.

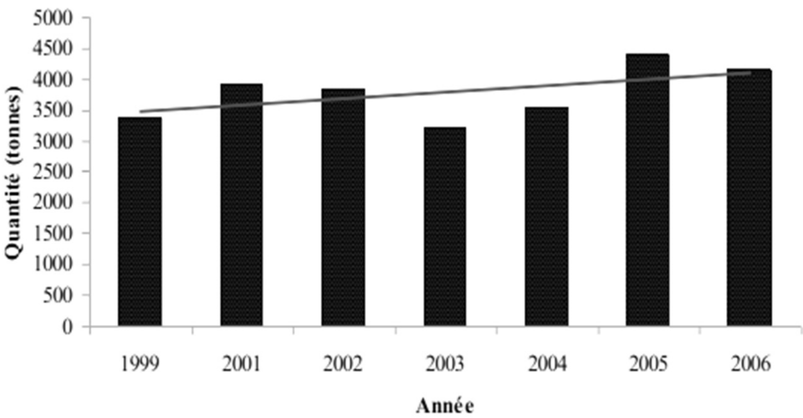

Fig. 1-1. Evolution of imports of pesticides in Tunisia between 1999 and 2006 (PAN Africa 2006).

I.2 Regulations

Tunisia is not a producer of pesticide products, either for agricultural use, or for public health and hygiene needs. These products are exclusively imported from abroad. The import and use of the 9 pesticides; persistent organic pollutants (POPs) are prohibited.[1]

Tunisia signed the Stockholm Convention on 23 May 2001 and parliament ratified it on 9 Mars 2004. As a part of this convention, Tunisia is obliged to prohibit any production and use of POPs and take the necessary steps to remove and eliminate the POPs that are already in the country (in storage areas and in the natural environment). The current problem is essentially related to, emissions of contaminated products (dioxins, furans) and the management of waste from these substances.

The most important section of legislation covering waste management in Tunisia is Law No. 96-41 of 10 June 1996 relating to waste and the control of its management and disposal. This important law, which represents a major achievement in terms of environmental protection in Tunisia, has been consolidated by implementing texts (decrees) dealing with the management of some categories of hazardous waste (kane 2008, APEK 2005).

As a result, decree n°2000-2339 issued on October 5, 2000, and established the national list of hazardous waste, specifically mentions obsolete pesticides. Whatever their nature, waste contaminated by PCBs, dioxins and furans is considered being hazardous waste, the management and disposal of which must meet very specific requirements and specifications set out in the law on waste (kane 2008, APEK 2005).

Although the OCPs and PCBs have never been produced in Tunisia and their import and use are prohibited, these compounds continue to exist in different matrices of the marine environment: sediment, biota, and even breast milk. Table 1.2 summarizes the pesticide compounds, their levels, and their analytical methods.

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[1] ♦ 1980 for the dieldrin and heptachlor.

♦ 1984 for the DDT (dichlorodiphenyltrichloroethane), the aldrin, chlordane, the endrin, and toxaphene.

♦ The Mirex has never been approved or authorized in Tunisia.

Since 1980, Tunisia has banned the use of any organochlorine product, as well as the approval of these products (kane 2008, APEK 2005).

Table 1.2 Presentation of pesticides in different matrices from Tunisia.[2]

Location	Matrices	Analytical methods	Concentration of pesticides	References
Northern Tunisia	Breast milk	LLE- GC–ECD	∑ 3 DDT : 0.058–31.325 (ng g^{-1}) HCB : 0.003–3.127 (ng g^{-1}) Dieldrin : ND – 0.529 (ng g^{-1})	(Ennaceur 2007, 325–329)
Bizerte Lagoon	Fish	ES- GC – ECD	∑ 12 PCB : 164–642 (ng g^{-1}) ∑ 7 OCPs : 52.9–265 (ng g^{-1})	(Ben Ameur 2013, 2372–2380)
Tunisia	Butter	ES- GC -MS	∑ 8 PCB : 11.81 (ng g^{-1}) ∑ 6 DDT: 7.8 (ng g^{-1}) ∑ 3 HCH: 6.41 (ng g^{-1}) HCB : 2.340 (ng g^{-1})	(Kalantzi 2001, 1013-1018)
Tunisia	Breast milk	LLE- GC – ECD	∑ 8 PCB : 1- 154 (ng g^{-1}) ∑ 3 DDT : 8-7060 (ng g^{-1}) HCB : 1-727 (ng g^{-1}) Dieldrin : 1-713 (ng g^{-1})	(Ennaceur 2008, 86–93)

[2] Levels of OCPs and PCB in fish and sediment are presented with more details in the research of Barhoumi et al 2013 and Ben Ameur et al. 2013.

Bizerte City	Breast milk	LLE- GC – ECD	∑ 3 DDT : 125.8–4574.8 (ng g^{-1}) HCB : 24.1–1470.2 (ng g^{-1}) Dieldrin : ND – 62 (ng g^{-1}) ∑ 8 PCBs : 16.4-1360.2 (ng g^{-1})	(Ben Hassine, 2012, 369–377)
Bizerte Lagoon	Sediment	ASE- GC – ECD	Σ 10 PCBs : 0.8 to 14.6 (ng g^{-1}) Σ 4 OCPs : 1.1 to 14.0 (ng g^{-1})	(Barhoumi 2013, 6290-6302)

II The pesticides

a) Definitions

Pesticides or plant protection products are defined as substances whose chemical properties contribute to the protection of crops and harvested products from fungus attacks of pests, insects, and mites of rural rodents or even destroying weeds or "weeds".

They reveal from Directive 91/414 / EC (EC 1997). These are compounds containing one or more mineral or organic, synthetic, or natural chemical substances. The formulations are generally composed of one or more active substances and one or more adjuvants. The active substance exerts a general or specific action on harmful organisms or on plants; it is this which gives the product the desired effect.

The adjuvant meanwhile, is a substance free of biological activity considered sufficient in practice, but able of changing the physical properties, chemical or biological products. It enhances the efficiency, safety of the product and its ease of use (Couteux 2006).

b) Classification

- **First system**

The first classification system is based on the target to be checked. There are mainly three main families of activities, which are herbicides, fungicides, and insecticides.

✓**Herbicides**: represent the most widely used pesticides in the world, all crops combined. They are intended to eliminate plants that compete with plants to be protected by slowing their growth. Herbicides have different modes of action on plants; they can disrupt the regulation of hormones, "auxins" (the main hormone acting on the increase in cell size), of photosynthesis, or else inhibit cell division, of the synthesis of lipids, of cellulose or of amino acids.

✓**Fungicides:** allow them to fight the proliferation of plant diseases caused by fungi or bacteria. They can act differently on plants, either by inhibiting the respiratory system or cell division, or by disrupting the biosynthesis of sterols, amino acids, proteins or the metabolism of carbohydrates.

✓**Insecticides:** are used to protect plants against insects. They intervene by eliminating them or preventing their reproduction, different types exist: neurotoxicants, growth regulators, and those acting on respiration cellular.

In addition to these three main families mentioned above, others can be cited as examples: acaricides, against mites; nematicides, against the group of nematode worms; rodenticides, against rodents; maupicides, against moles; molluscicides, against slugs and snails or corvicides, and corvifuges, respectively against crows and other crop pest birds.

- **Second system**

The second classification system takes into account the chemical nature of the active substance mainly is compound of plant health products. Given the variety of physicochemical properties of pesticides available on the market, there are a very large number of chemical families. The oldest and the main chemical groups are organochlorines, organophosphates, carbamates, triazines and substituted ureas. This second classification system does not allow a compound to be defined systematically. Some pesticides can, in fact, be composed of several chemical functionalities. They can then be classified into one or more chemical families.

II. 1 Organochlorine pesticides (OCPs)

The organochlorine pesticides (OCPs) include various groups of chemicals, which have the tendency to share some structural characteristics. They have an aliphatic radical or an aromatic ring structure, which is strongly substituted with chlorine atoms (Shen 2005, 742-768). Table 1.3 shows the concentrations of PCBs and OCPs in different matrices as well as the analytical methods used.

Table 1.3 Locations, matrices, analytical methods and concentrations of PCBs and OCPs in different locations from the world.

Location	Matrice	Analytical method	Concentration	References
Nakdong river (Korea)	Sludge	ES- GC–MS	22 OCPs : 5.45-31.2 µg kg^{-1} 14 PCBs : ND -13 156 ng kg^{-1}	(Ju 2009, 441–447)
Pangani river (Tanzania)	Surface sediment	ES- GC-MS	8 OCPs : 245–10.230 pg g^{-1} 28 PCBs : 357–11.000 pg g^{-1}	(Kihampa 2013, 186–197)
Egypte	Drink water	LLE- GC –ECD	11 PCBs : ND – 11.21 µg L^{-1}	(Eissa 2013, 694-700)
Fatima do Sul City (Brazil)	Underground water	SPME- GC - ECD	18 OCPs : 0.761- 615 ngL^{-1}	(Junior 2007, 1833–1841)
Eastern Cape (South Africa)	Sea water	LLE - GC	15 OCPs : ND - 450 ng L^{-1}	(Awofolu 2003, 323-330)
	Surface sediment	ES- GC	15 OCPs : ND- 184 10^{3} ng kg^{-1}	
Northwest Atlantic Coast	Seal	ASE-GC – ECD	10 PCBs : 5.7-151 µg g^{-1} DDT : 1.4–57.5 µg g^{-1} Dieldrin : 3–1060 ng g^{-1}	(Shaw 2005, 1069–1084)
Chaohu Lake (Chine)	Water	SPE- GC- ECD	1.6–1.678 6 ng L^{-1}	(Liu, 2013,2033–2045)
	Suspended particulate matter	SE- GC-ECD	18.6–1.046 8 ng g^{-1}	
	Surface sediment	SE- GC- ECD	0.9–36.9 ng g^{-1}	
Lebanon	Mineral water	SPE- GC–MS	67 pesticides: 1.7 – 31.8 ng L^{-1}	(Kouzayha 2013, 503–509)
Mexique	Mineral water	LLE- GC-EC D	7 PCBs : 0.035-0.039 mg L^{-1}	(Salinas 2010, 372–376)

Río Xanaes river (Argentina)	Water milfoil	ES- GC –ECD	OCPs 11 : ND- 4.5 ng L^{-1}	(Schreiber 2013, 466–473)
	Surface water	SPE - GC- ECD	OCPs 11 : ND- 5 µg kg^{-1}	
United states	Drink water	SPE- GC–ECD	PCBs 209 : <9.3-186.6 ng L^{-1}.	(Palmer 2011, 487–499)
Pond Thau (France)	Air	ES- GC –MS	Σ 7 PCBs : 31–57 pg m^{-3},	(Castro- Jiménez 2008, 123-135)
	Surface sediment	ASE- GC –MS	Σ 7 PCBs : 2.5–33 ng g^{-1}	
	Mussel	ASE- GC – MS	Σ 7 PCBs : 10–39 ng g^{-1}	
Venice lagoon (Italy)	Air	ES- GC-MS	Σ 60 PCBs: 44- 600 pg m^{-3}	(Manodori 2006,449–458)

Accordingly, most of these compounds are poorly soluble and semi-volatile. In May 2001, the Stockholm convention on POP which was adopted by the United Nations Program for Environment (UNEP) highlighted the need to control the global contamination produced by toxic chemical compounds in the environment.[3] Several studies have shown the presence of OCPs and PCBs in the various components of the marine ecosystem: surface water, suspended particulate matter, and sediments. It has been proven in different studies the presence of these persistent organic pollutants in the plant and even in living tissue with concentrations more or less important (Satphathy 2008, 1062-71).

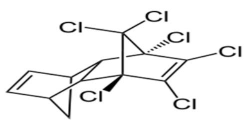
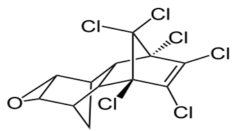
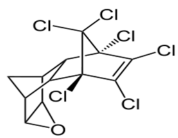
II.1.1 The aldrin, the dieldrin and endrin

The aldrin was synthesized for the first time by J. Hyman and Co. Denver, in 1948. Aldrin is an insecticide which can undergo oxidation to form dieldrin. Both insecticides have been commonly used for termite control in cotton and corn since the 1950s (EC 1997, 13-14).

Due to its harmful effects, the use of dieldrin is limited controlling termites, insects and pests. It was included to POPs in 1995 because of its many characteristics such as hydrophobicity, bio-molecularity and persistence in the environment. It is less phytotoxic, but increased exposure can cause cancer and it can act as a neurotoxicant in humans. Figure 1.2 shows the synthesis of aldrin and dieldrin.

[3] It has an agreement that promotes regulations on the production and use of OCPs such as Aldrin, dieldrin, endrin, heptachlor, chlordane, hexachlorobenzene, mirex, toxaphene, PCBs, and DDT around the world. While most developed countries have already banned or limited the production and use of these compounds, some developing countries still use OCPs in agriculture (Gao 2008, 1097–1103).

Table 1.4 Physicochemical characteristics of aldrin, the dieldrin and endrin (Shen 2005, 742-768, Zitko 2002, 47-90).

Commercial name	**Aldrin**	**Dieldrin**	**Endrin**
Biological properties	Termiticide	Insecticide, Termiticide	Insecticide
Name according to IUPAC	1,2,3,4,10,10- Hexachloro 1,4,4a, 5,8,8a-hexahydro-1,4: 5,8-dimethanonaphthalene.	3,4,5,6,9,9-Hexachloro-1a, 2,2a, 3,6,6a, 7,7a-octahydro-2,7: 3,6-dimetanonapth [2,3-b] oxirene.	3,4,5,6,9,9, -Hexachloro-1a, 2,2a, 3,6,6a, 7,7a-octahydro-2,7: 3,6-dimethanonaphth [2,3-b] oxirene .
Chemical formula	$C_{12}H_8Cl_6$	$C_{12}H_8Cl_6O$	$C_{12}H_8Cl_6O$
Chemical structure			
Half-life	<0.4 days (air); 1.1-3.4 years (water and soil)	1.1-4.2 days (air); up to 5 years in the ground	1.3-4.2 days (air); (1.1-3.4 years in water and 12 years in soil)
LD50 (mg Kg $^{-1}$)	39	46	18
Molecular Mass (g mol $^{-1}$)	364.92	380.91	380.92
Case No	309-00-2	60-57-1	72-20-8