Investigation of Dicofol and Endosulfan Pesticide Levels In Tahtalı Dam Water or Drinking Water

By

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ABSTRACT

In this study, dicofol (2,2,2-trichloro-1,1-bis (4-chlorophenyl) ethanol) and endosulfan (6,7,8,10,10 - hexachloro - 1,5,5,1,6,9,9a - hexahydro - 6,9 - methano - 2,4,3 benzadioxathiepin 3-oxide) pesticide concentration levels in Tahtalı Dam Water were investigated. Endosulfan pesticide has two forms which are α-Endosulfan and β-Endosulfan. Dicofol and Endosulfan are both organochlorine pesticides.

Both of these pesticides are widely used for agricultural purposes in Tahtalı Dam Basin. These pesticides could be carried to the Tahtalı Dam Water, and therefore their concentrations should be controlled.

Another reason why these pesticides were selected was that their method of determination is not straightforward and a special determination technique has to be used. That is why these pesticides were not studied extensively for İzmir area.

For the determination of trace amount of above-mentioned pesticides, gas chromatography-mass spectrometry (GC-MS) was generally preferred as reported in most papers [1-3]. The GC-MS instrument in our laboratory has an Ion Trap (IT) mass analyzer. Operating in Selected Ion Storage (SIS) or Tandem mass (MS-MS) modes can increase the sensitivity and selectivity of this instrument. The matrix effect coming from the aqueous solution was eliminated by GC-SIS-MS and GC-MS-MS. Dicofol did not give stable peaks. So, Dicofol did not investigate in this study. The detection limits of the instrument are 0.083 μg/L for α-Endosulfan, and 0.662 μg/L for β-Endosulfan; therefore a preconcentration process was required because the studied concentrations are in 1-3 μg/L levels for surface water and 0.1 μg/L levels for drinking water.

Solid Phase Extraction (SPE) method was used for sample preconcentration. Gas chromatography (GC) - Mass spectrometry (MS) and Tandem mass spectrometry (MS–MS) were employed for the identification and quantification of Dicofol, α-Endosulfan, and β-Endosulfan pesticides. For SPE procedure ENVI-18 Disk was used, optimizing the extraction volume, pH and the salt concentration. In GC–MS–MS, the lowest detectable concentrations for the α-Endosulfan and β-Endosulfan were found as 0.083 ng/L and 0.662 ng/L, respectively. Recovery of α-Endosulfan for SPE was 112 (±0.002) % in 500 ml water samples spiked with 1 mg/L pesticides. Recovery of the β-Endosulfan for SPE was 132 (±0.008) % in 500 ml water samples spiked with 1 mg/L pesticides.

Water samples, which were collected between 01 August 2002 and 01 January 2003 by İZSU (İzmir Büyükşehir Belediyesi Su ve Kanalizasyon Genel Müdürlüğü), were analyzed using GC-MS system with tandem mass (MS-MS) mode after preconcentration process.

Both α-Endosulfan and β-Endosulfan were not found in detectable amounts in Tahtalı Dam Water although an enrichment technique -SPE- was used.

ÖZ

Bu çalışmada, Tahtalı Baraj suyunda, dicofol (2,2,2-trikloro-1,1-bis(4-klorofenil) ethanol) ve endosulfan (6,7,8,10,10-hegzakloro-1,5,5,1,6,9,9a-hegzahidro-6,9-methano-2,4,3-benzadiokzatiepin 3-oksit) pestisitlerinin derişim seviyeleri incelenmiştir. Endosulfan, α-Endosulfan ve β-Endosulfan olmak üzere iki forma sahiptir. Hem Dicofol hem de Endosulfan organoklorlu pestisitlerdir.

Bu pestisitlerin her ikisi de Tahtalı Baraj Havzasında yaygın olarak tarımsal amaçlarla kullanılmaktadır ve Tahtalı Baraj suyuna çeşitli yollarla taşınabilir. Bu yüzden derişimleri kontrol edilmelidir.

Bu pestisitlerin seçilmesinin diğer bir nedeni de, bunların eser miktarda doğrudan tayin yöntemlerinin olmaması ve özel tayin teknikleri gerektirmesidir. Bu nedenle söz konusu pestisitleri saptama çalışmaları İzmir bölgesinde yaygın olarak yapılmamıştır.

Çoğu makalede de bildirildiği gibi, yukarıda bahsedilen pestisitlerin tayininde Gaz Kromatografi - Kütle Spektrometrisi (GC-MS) cihazları genellikle tercih edilmektedir [1-3]. Laboratuvarımızdaki GC-MS cihazı İyon Kapanlı (IT) kütle analizörüne sahiptir. Bu cihazın hassasiyeti ve seçiciliği, Seçilmiş İyon Saklama (SIS) ve Tandem-Kütle (MS-MS) modlarında çalışılarak artırılabilir. Yine sulu çözeltilerden gelen matriks etkisi GC-SIS-MS ve GC-MS-MS modlarında çalışılarak giderilebilir. Cihazın saptama sınırı α-Endosulfan için 0.083 µg/L ve β-Endosulfan için de 0.662 µg/L' dir. Yüzey suyunda çalışma seviyesi 1-3 µg/L ve içme suyunda 0.1 µg/L olduğu için hala bir ön deriştirme basamağına ihtiyaç duyulmuştur.

Örneklerin ön deriştirilmesi amacıyla Katı Faz Özütleme (SPE) metodu kullanılmıştır. Dicofol, α-Endosulfan ve β-Endosulfan pestisitlerinin tanımlanması ve miktarlarının belirlenmesi için GC-MS ve MS-MS yöntemleri kullanılmıştır. ENVI-18 Disk kullanılarak yapılan SPE işlemi için hacim, pH ve tuz derişimi optimize edilmiştir. GC-MS-MS ile α-Endosulfan ve β-Endosulfan için en düşük saptama sınırı sırasıyla 0.083 ng/L ve 0.662 ng/L bulunmuştur. 500 ml su örneklerine eklenen 1 mg/L derişimindeki pestisitlerin SPE kullanılarak yapılan α-Endosulfana ait geri kazanım sonucu %112 (±0.002)'dir. Aynı şekilde SPE kullanılarak yapılan β-Endosulfana ait geri kazanım sonucu %132 (±0.008)'dir.

İZSU (İzmir Büyükşehir Belediyesi Su ve Kanalizasyon Genel Müdürlüğü) tarafından 01 Ağustos 2002 ile 01 Ocak 2003 tarihleri arasında toplanan su örneklerinin ön deriştirme işleminden sonra GC-MS sisteminde MS-MS modu ile analizleri yapıldı.

Ön deriştirme işlemi -SPE- yapılmış olmasına rağmen, Tahtalı Baraj suyunda, cihazın saptama sınırında ne α-Endosulfan ne de β-Endosulfan bulunamamıştır.

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CHAPTER 1

INTRODUCTION

Today, over 500 compounds are registered worldwide as pesticides or metabolites of pesticides [4]. Pesticides can be classified based on functional groups in their molecular structure (e.g. inorganic, organonitrogen, organohalogen, organophosphorus, organosulfur compounds, etc.), or their specific biological activity on target species (e.g. insecticides, fungicides, herbicides, acaricides, etc.) [4,5]. Herbicides are by far the most commonly used pesticides followed by insecticides, fungicides, and others. Pesticide use in agriculture has progressively increased after World War II, leading to increased world food production. Nevertheless, this use and additional environmental pollution due to industrial emission during their production have resulted in the occurrence of residues of these chemicals and their metabolites in food, water, and soil. Legislations were acted out in the USA, European Union (EU) and other countries to regulate pesticides in water, water supply, soil, and food.

The development and use of pesticides have played an important role in the increase of agricultural productivity. The majority of such substances are applied directly to soil or sprayed over crop fields and hence are released directly to the environment. Consequently, pesticides can enter as contaminants into natural waters either directly in applications or indirectly from drainage of agricultural lands. The amount and kind of pesticides in water of a given area depends largely on the intensity of production and kind of crops. However, transport of pesticides out of their area of application results in the presence and accumulation of these compounds in many parts of the hydrosphere. For example, atmospheric precipitation is an important route of transport of pesticides, resulting in contamination of environmental waters far away from agricultural areas. Substantial amounts of pesticides have been found in ice and water of polar regions [6,7], lakes [8], seawater [9], rainwater [8,10-12] or potable water [13,14].

Gas chromatography (GC) using the highly sensitive electron-capture detection (ECD) is an analytical technique of great importance especially in the determination of chlorinated hydrocarbon pesticide residues in environmental waters [12,15-17]. This is due not only to the sensitivity and specificity of ECD, but also to the power of GC for separating compounds of similar molecular structure. Consequently, multiresidue

analysis is the most common way of determining pesticides. Once the chromatographic separation is reached, information regarding the complexity (number of components), quantity (peak height or area) and identity (retention time) of the components in a mixture is provided. The certainty of identification based solely on retention time value is poor, even for not very complex samples, therefore a supplementary confirmation of the residues is necessary. Only when the identity is firmly established, the quantitative information from the chromatogram can be correctly interpreted without producing false (positive) results.

Spectroscopic techniques, conversely to chromatographic techniques, present a rich source of qualitative information from which component identity may be deduced with a reasonable degree of certainty. Thus, spectroscopic and chromatographic techniques provide complementary information about the concentration of the components and their identity in a sample.

Nowadays, GC interfaced to mass spectrometry (GC-MS) is the preferred analytical technique for the confirmation of trace compounds [1]. Generally, three modes of GC–MS operation are available for pesticide analysis: electron impact (EI), positive chemical ionization (PCI) and negative chemical ionization (NCI). GC-MS in the EI mode is commonly used in the determination of pesticides in water. Positive and negative chemical ionization modes are alternative methods, depending on the compounds they offer better selectivity and/or sensitivity than EI. For increasing the sensitivity, selected ion monitoring (SIM) is commonly used in the determination of pesticides in waters. This mode allows the analysis of trace amounts of pesticides but reduces the qualitative information. The use of tandem mass spectrometry (MS–MS) improves the selectivity of the technique with a drastic reduction of the background without losing identification capability. It enables analysis of pesticides at trace levels in the presence of many interfering compounds [18,19]. In spite of high sensitivity and selectivity of the technique a reduced number of reasearchers have applied this technique [20,21]. Evidently, the sensitivity is still not high enough to directly determine the trace amounts of pesticides in drinking and surface water samples at the level required by the European Community (EC). European Union (EU) Waters Directives are 0.1 μg/L for each pesticide, 0.5 μg/L for total amount in drinking water and 1-3 μg/L for surface water [22,23].

Due to these low presence levels, a preconcentration procedure for the analytes must be applied. Preconcentration of contaminants from water samples, and generally sample preparation steps, often require by extraction techniques, based on enrichment on liquid (liquid–liquid extraction) or solid (solid–liquid extraction) phases [24,25]. Extraction procedures, optimized prior to chromatographic separation, can be coupled on- or off-line to the analysis, which is mainly performed, by liquid chromatographic (LC), gas chromatographic (GC) or gas chromatography - mass spectrometric (GC-MS) methods [24-27].

1.1. Thesis Objective

In this study, investigation of Dicofol and Endosulfan pesticide levels in Tahtalı Dam Water, which is the most important drinking water supply in İzmir, were carried out. Study of the variation of Dicofol and Endosulfan amounts in Tahtalı Dam Water for a reasonable period was planned.

Mainly twenty pesticides are used for agricultural purposes in Tahtalı Dam Basin. Due to consumption of target pesticides in greater amounts compared to the others, the determination of Dicofol and Endosulfan pesticides and the examination of their levels in the Tahtalı Dam Water was studied.

According to European Community (EC) directives the tolerance levels of pesticides in drinking water are 0.1 μg/L for one pesticide and 0.5 μg/L for total pesticide concentrations. Therefore, sensitive analytical instruments and methods are required for the determination of these amounts.

For this purpose, Gas chromatography – Mass Spectroscopy (GC-MS) techniques are generally preferred as reported in most papers. The GC-MS instrument in our laboratory has an Ion Trap (IT) mass detector. Working in Selected Ion Storage (SIS) and Tandem (MS-MS) modes could increase the sensitivity and selectivity of this instrument. Nevertheless, a preconcentration process is still required. In this study Solid-Phase Extraction (SPE) methods was used for sample preconcentration process.

CHAPTER 2

PESTICIDES AND THEIR PROPERTIES

2.1 Pesticides

The name is derived from the Latin words *pestis* (pestilence, plague) and *caedere*-to kill. The word "pesticide" includes all chemicals that are used to kill or control pests. They include herbicides (kills weeds), insecticides (kills insects), fungicides (kills fungi), nematocides (kills nematodes), and rodenticides (kills small mammals).

Pesticides are most heavily used in agriculture but they are also heavily used in household as well as silvicultural applications.

The first mention of pesticides was made in 1763, when an extracted solution of tobacco was used to control the plant louse. Later, some other uses of pesticides were reported; for example, in 1865, in controlling the Colorado beetle by use of Paris green (copper-aceto-arsenite). However, the discovery of the insecticidal properties of DDT (4,4-dichlorodiphenyl trichloroethane) started the era of pesticide usage on a large scale. DDT (as shown in Figure 2.1.) was first synthesized by Zeidler in 1874, but Müller, who was looking for a mothproofing agent, did not observe its insecticidal properties until 1939.

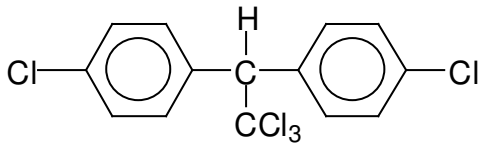


Figure 2.1 Molecular Structure of DDT

The use of the DDT in agriculture and forestry also produced spectacular results. Over the coming years many other pesticides were developed such as organophosphorus compounds, organochlorines, carbamates, and triazines. Pesticidal formulations usually contain one or more chemical agents which are biologically active in the mixture, along

with subsidiary substances and a non-active matrix. The technical pesticides are available as solid and liquid.

Of the pesticides that are used far less than 1% actually reaches a target organism; the rest ends up contaminating the air, soil, water, plants and animals. Furthermore while farmers use three times the amount of agricultural chemicals today than they did forty years ago we are still losing about 1/3 of our crops to pests; which is about the same as forty years ago. (Brady, 1996).

2.1.1 Concerns

Pesticides are of concern for human health because many are lipid soluble and as such they accumulate in our fatty tissues in a process called bioconcentration. Biomagnification is what happens when organisms eating contaminated organisms concentrate the pesticides and then in turn are eaten by other organisms. As a result those on the top of the food chain (all meat-eating humans) are most at risk because the concentration is magnified at each step of the food chain.

Furthermore because pesticides are designed to kill organisms due to their neurological or reproductive toxicity they also have many similar deleterious effects in humans, and many show adverse effects on the immune system at very low doses.

Pesticides have many ecological effects of concern as well. Ecological effects are often considered to be an early warning indicator of potential human health impacts.

In the environment pesticides can kill organisms, cause cancers, tumors and lesions in fish and wildlife, suppress the immune system, cause reproductive failure, damages on DNA, disrupt the endocrine (hormonal) system, and cause physiological birth defects (teratogenic effects) such as deformed beaks on birds or malformed reproductive organs such as observed in alligators exposed to DDT. Pesticides cause a dramatic decline in biodiversity in areas where used and cause a shift in the species balance in the plant communities, with cascading effects throughout the food chain. Some pesticides such as the carbamates have been shown to be highly toxic to earthworms. Many insecticides and fungicides have been shown to interfere with nitrogen fixing bacteria in the soil, which often contribute significantly to plant growth where nitrogen is limiting (nitrogen is usually limiting).

2.1.2 Historical Development of Pesticides (Stephenson and Solomon, 1993)

1500 BC

- Egyptians produced insecticides against lice, fleas and wasps.

1000 BC

- The Greek poet Homer referred to a pest-averting sulphur.

200 BC

- The Roman writer Cato advises vineyard farmers to burn bitumen to remove insects.

Early 1700's

- John Parkinson, author of 'Paradisus, The Ordering Of The Orchard' recommended a concoction of vinegar, cow dung and urine to be put on trees with canker.

1711

- In England, the foul smelling herb rue was boiled and sprayed on trees to remove canthraid flies.

1763

- In Marseilles, a mixture of water, slaked lime and bad tobacco was a remedy for plant lice.

1821

- London Horticultural Society advised that sulphur is the remedy for mildew on peaches.

1867

- The beginning of modern pesticide use.
- Colorado beetle invade US potatoes crops and arsenic is applied.
- Professor Millardet, a French professor, discovers a copper mixture to destroy mildew.

Late 1800's

- French vineyard growers have the idea of selective weed killers.

1892

- The first synthetic pesticide, potassium dinitro-2-cresylate, marketed in Germany.

Early 1900's

- Insecticides, fungicides and herbicides have all been discovered.
- Inorganic substances introduced.

1932

- Products to control house hold pests marketed.

1939

- The Second World War causes three discoveries: 1. the insecticide DDT.

2.1.3 Classification of Pesticides

Pesticide classification or grouping may be based on any of several criteria.

2.1.3.1 According to Chemical Structure

One of the most common means of classifying a pesticide is on the basis of similarities in chemical structure. Based on this mode of classification, there are 3 classes of pesticides commonly used in the structural pest control industry, the inorganic, botanical, and synthetic organic insecticides.

2.1.3.1.1 Inorganic Pesticides

Inorganic pesticides are typically derived from minerals or chemical compounds that occur as deposits in nature. Most of these compounds are quite stable and tend to accumulate in the environment. Some act as stomach poisons (borates and boric acid). Others are considered sorptive dusts (silica aerogel, diatomaceous earth) that absorb the waxy layer from the cuticle of pests. Many of the inorganic pesticides are relatively expensive and are only moderately effective in controlling insects and other pests. Common inorganic pesticides are silica aerogel, boric acid, borates, diatomaceous earth, cryolite, copper, mercury, and sulfur.

2.1.3.1.2 Botanicals

The botanical pesticides are extracted from various parts (stems, seeds, roots, flower heads) of different plant species. Botanical insecticides usually have a short residual activity and do not accumulate in the environment or in fatty tissues of warm blooded animals. Many botanical pesticides act as stomach poisons, although pyrethrins act mainly as a contact poison. Common examples of botanical pesticides are pyrethrins, sabidilla, rotenone, nicotine, ryania, neem, and limonene.

2.1.3.1.3 Synthetic Organic Insecticides

Synthetic organic insecticides do not naturally occur in the environment, but are synthesized by man. Since all these compounds have carbon and hydrogen atoms as the basis of their molecule (as do living plants and animals), they are referred to as organic compounds. The six basic types of synthetic organic insecticides are the chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, insect growth regulators, and microbial pesticides.

2.1.3.1.3.1 Chlorinated Hydrocarbons

This large group of insecticides varies considerably in toxicity to mammals. Most are only moderately toxic, however, a few are very toxic to mammals. The use of these materials has been severely criticized for their effect on the environment. Most chlorinated hydrocarbons are very stable and do not readily decompose in the environment. Most of these insecticides accumulate in the environment and in fatty tissues of birds and mammals. The use of most chlorinated hydrocarbons has been prohibited in the U.S.. Examples of the chlorinated hydrocarbons are DDT, BHC, dieldrin, chlordane, aldrin, endrin, heptachlor and methoxychlor.

2.1.3.1.3.2 Organophosphates

The organophosphates are an extremely large and diverse group of insecticides. Their toxicity to mammals range from extremely toxic to some of the least toxic pesticides known. Most organophosphates are not persistent and will break down to non-toxic materials in one to 30 days, depending on the compound. Organophosphates do not accumulate in fatty materials and do not accumulate in food chains. These compounds act mainly as contact insecticides although they may also act as stomach poisons and fumigants. Common organophosphates are malathion, chlorpyrifos (Dursban), diazinon, dichlorvos (Vapona), acephate (Orthene), and propetamphos (Safrotin).

2.1.3.1.3.3 Carbamates

The carbamate compounds are also a large group of insecticides. As a rule, these compounds are slightly more persistent in the environment than the organophorphrous compounds, but do not accumulate in the environment or fatty tissues of mammals. Most carbamates are only moderately toxic to mammals. They mainly act as contact insecticides with some stomach activity. Common carbamate insecticides are carbaryl (Sevin) and propoxur (Baygon).

2.1.3.1.3.4 Pyrethroids

Pyrethroid Pesticides were developed as a synthetic version of the naturally occurring pesticide pyrethrin, which is found in chrysanthemums. They have been modified to increase their stability in the environment. Some synthetic pyrethroids are toxic to the nervous system.

2.1.3.1.3.5 Insect Growth Regulators (IGR's)

Insect growth regulators are chemicals that affect the ability of insects to growth and mature normally. They are based on and often mimic the growth hormones that occur naturally within the insect's body. Because mammals do not molt like insects do, most insect growth regulators are not very toxic to man and domestic animals. Common insect growth regulators are methoprene (Precor), hydroprene (Gentrol, Gencor), fenoxycarb (Torus), and hexaflumuron (Sentricon)

2.1.3.1.3.6 Microbial Pesticides

Microbial pesticides are formulated disease organisms of pests, many of which are grown in large quantities in manufacturing plants. Some of the microorganisms available for pest control are bacteria, fungi, and nematodes. Some of the bacterial pesticides available are *Bacillus thuringiensis* variety *kurstaki* (Thuricide, Javelin) for control of caterpillars and *Bacillus thuringiensis* variety *israelensis* (Teknar, Vectobac) for control of mosquitoes. Some of the fungi available for pest control are *Metarhyzium*

(Biopath) for control of cockroaches. Some of the nematodes available for pest control are *Steinernema feltiae* (Vector) for flea control.

2.1.3.1.3.7 Other Synthetic Organic Insecticides

Other synthetic organic insecticides are

- phenylpyrazoles,
- neonicotinoids and nicotine,
- spinosyns,
- juvenile hormones,
- furnigant,
- Bt Microbials,
- pyrrole compounds,
- thiadizine,
- pyrazolines, and
- pheromones.

2.1.3.2 According to use

According to use, pesticides are classified as follows:

- insecticides (insect killers)
- herbicides (plant killers)
- fungicides (controlling fungi)
- molluscicides (controlling molluscs)
- nematicides (controlling nematodes)
- rodenticides (controlling rodents)
- bacteriocides (bacteria killers)
- defoliants (removing plants leaves)
- acaricides (killers of ticks and mites)
- wood preservatives
- repellents (substances repugnant to pest)
- attractants (substances attracting insects, rodents and other pests)
- chemosterilants (substances inhibiting reproduction of insects)

2.1.4. Usage Purposes and Areas of Pesticides

Pesticides are used mostly in agriculture to control the pest (insects, rodents), fungi and weeds. In health protection, pesticides are used mainly to control the mosquitoes that carry diseases, particularly malaria. Pesticides are used in homes to control insects, rodents, etc. Other applications are: to control pest in forestry, for wood and textile preservation, and also to control the excessive growth of undesirable plants in water reservoirs.

2.1.5. General Properties of Pesticides

In general, pesticides should have the following properties:
high toxicity to pests,
low toxicity to other organisms, principally to water organisms and to people,
adequate stability so that they fulfill their goal before degrading,
great ability for degradation so that after completing their task they will disappear in the environment with minimal harm.
Two properties of the pesticides are most important. Their toxicity and degradation.

2.1.6. Degradation of Pesticides

Decomposition of pesticides in the environment is now one of the main considerations when deciding their approval by the regulating authorities. Degradation is mainly by biochemical methods, but chemical and photochemical (under the influence of sunlight) degradation also occurs. Biodegradation of pesticides is partly correlated with their solubility in water. Those organic pesticides, which readily dissolve in water, hydrolyze rapidly in water, and in general they degrade easily. The same pesticides are quickly washed out from the soil by rainwater and enter river waters. The solubility of some pesticides is given in Table 2.1 [28].

Table 2.1 Solubility of Some Pesticides

		Compound	Solubility, mg/L
	Organo-	DDT	0.0012
	Chlorine	Aldrin	0.01
		Heptachlor	0.056
I		Methoxychlor	0.10
N		Dieldrin	0.18
S		**Dicofol**	**0.8**
E		Endrin	0.23
C		**Endosulfan**	**0.32**
T		Parathion	24.0
I	**Organo-**	Disulfon	25.0
C	**Phosphorus**	Diazinon	40.0
I		Chlorfenvinfos	145.0
D		Malathion	145.0
E		Methyl demeton	330.0
S		Dichlorvos	10000.0
		Dimethoate	2500.0
	Carbamates	Carbaryl	40.0
		Carbofuran	700.0
	Herbicides	Aldicarb	6000.0
		Simazine	5.0
		Propazine	8.0
		Diuron	42.0
		2,4,5-T	280.0
		2,4-D	890.0
		Trifluralin	0.300
		Diquat	70.0%
		Dalapon	80.0%

Pesticides can be classified into four groups of various persistences. Relative persistence of some pesticides in natural water is given in Table 2.2 [29].

Table 2.2 Relative Persistence of Some Pesticides in Natural Waters

Readily degradable; half-life less then 2 weeks	**Slightly persistent; half-life 2-6 weeks**	**Moderately Persistent; half-life 6 weeks-6 months**	**Persistent; half-life more than 6 months**
Captan	Chloramben	Carbofuran	DDT
Carbaryl	Chlorpropham	Carboxin	γ-HCH
Chlorpyrifos	Dalapon	Chlordane	Aldrin
Dicrotophos	Diazinon	Chlorfenvinfos	Dieldrin
Endotol	Dichlorvos	Chloroxuron	Heptachlor
2,4-D	**Dicofol**	Dimethoate	Isodrin
Fenitrothion	Disulfoton	Diphenamid	Monocrotophos
Malathion	**Endosulfan**	Diuron	Benomyl
Methiocarb	Fenuron	Ethion	
Methylparathion	MCPA	Fensulfothion	
Parathion	Methoxychlor	Linuron	
Phophamidon	Monuron	Prometion	
Propoxur	Phorate	Propazine	
	Propham	Simazine	
		Toxaphene	
		Trifluralin	

The persistent pesticides such as DDT, γ-HCH (Hexachlorocyclohexane), dieldrin, endrin and others have only slight solubility in water. However, they usually dissolve in fats, and for this reason they accumulate in the body tissue of birds, fish and mammals, and threaten the health of the organism. Because of the high persistence of pesticides, their consumption is decreasing in many countries.

The degradation process depends on temperature, water, pH and biota. The pH of the water is a significant factor, because very often hydrolysis is one stage of the biodegradation. A rise in temperature increases the rate of the chemical reaction and activity of microorganisms taking part in the biodegradation. In addition, the evaporation rate of pesticides to the atmosphere increases with the rise in temperature. The most significant factor though is the presence of microorganisms capable of degrading the particular pesticide and the time that has elapsed to allow the

microorganisms to adapt to the presence of the material. The half-life of some pesticides in the environment is presented in Table 2.3 [30].

Table 2.3 The Half-Life of Some Pesticides in the Environment

		Compound	Half-life(days)
	Organo-	Aldrin	365
	Chlorine	Heptachlor	250
I		Methoxychlor	120
N		Dieldrin	1000
S		**Dicofol**	**60**
E		Endrin	4300
C		**Endosulfan**	**50**
T	**Organo-**	Parathion	14
I	**Phosphorus**	Disulfon	30
C		Diazinon	40
I		Chlorfenvinfos	35
D		Malathion	1
E		Methyl demeton	7
S		Dichlorvos	0.5
		Dimethoate	7
	Carbamates	Carbaryl	10
		Carbofuran	50
	Herbicides	Aldicarb	30
		Simazine	60
		Propazine	135
		Diuron	90
		2,4,5-T	30
		2,4-D	10
		Trifluralin	60
		Diquat	1000
		Dalapon	30

2.1.7. Toxicity of Pesticides

Pesticides by definition are toxic substances. They are designed to kill or to harm insects, rodents, weeds, fungus, etc. It is intended that the pesticides should be toxic in selective way; they should kill only the pest organism and be harmless to non-

target organisms, including humans. To achieve this goal is difficult, and pesticides are always, to various extents, harmful to the environment and to people.

Pesticides may be divided into five classes according to toxicity to warm-blooded animals, as shown in the LC_{50} values, in mg/kg of organism weight (Table 2.4.)[31].

Table 2.4 Oral Acute Toxicity Classes of Pesticides for Mammals

Class	**LC_{50}, mg/kg***
I	Below 50
II	51-150
III	151-500
IV	501-5000
V	Above 5000

*LC50 (Lethal Concentration) represents the concentration of pesticides that will kill half of a group of test animals from a single exposure by either the dermal, oral or inhalation routes.

Pesticides belonging to class I and class II are classified as toxic substances. Pesticides in classes III and IV are harmful substances. Pesticides in class V can be regarded as harmless.

The toxicity of pesticides to living organisms differs, and depends on the particular organisms, the environmental conditions, on the methods of applications, the form the pesticide is in (liquid or powder), etc. The toxicity of pesticides to water organism is usually high, particularly to insect's life, as many pesticides are designed to kill insects.

The toxicity to the water organisms depend on the temperature, ionic strength, concentration and character of suspended solids, and on the commercial form of the pesticide. Pesticides are rapidly adsorbed onto suspended solids, and their toxic effect is then usually diminished. Generally, the toxicity is lower in turbid water than in clear water for a given concentration of pesticide. Pesticides may be divided into four classes of toxicity to fish according to their LC_{50} values expressed as a concentration of pesticide in water (Table 2.5.) [31].

Table 2.5 Toxicity Classes of Pesticides for Fish

Class	LC_{50}, mg/L*
I	Below 0.5
II	0.5 - 5.0
III	5.1 – 50
IV	Above 50

*LC50 (Lethal Concentration) represents the concentration of pesticides that will kill half of a group of test animals from a single exposure by either50 the dermal, oral or inhalation routes.

2.2. Introduction Routes of Pesticides into Water

Generally, pesticides are introduced into water by the following routes, surface runoff, transport through soil; soil erosion, direct introduction into water when pesticides are sprayed onto crops or forest from planes, in waste waters from plants producing pesticides, in waste water from washing the equipment used for pesticides spraying, in municipal sewage (fungicides, bacteriocides or insecticides when controlling flies at sewage works), by direct application to control aquatic plants and insects, in waste water from manufacturers using pesticides, (e.g. textiles, carpet mothproofing).

After the pesticides are introduced into water, they degrade more rapidly than their predecessor compounds, but are still present in measurable quantities in receiving river and in the water supply. To protect aquatic organisms and human health, almost every country and some official organizations determine upper limit of concentration of pesticides in water. For instance, according to European Community (EC) directives, a pesticide residue must not be present at a concentration greater than 0.1 μg/L in drinking water and requirements for surface water are 1-3 μg/L

CHAPTER 3

DICOFOL AND ENDOSULFAN PESTICIDES AND THEIR PROPERTIES

3.1. DICOFOL

Dicofol is an organochlorine miticide used on a wide variety of fruit, vegetable, ornamental and field crops. It is produced as emulsifiable concentrate and wettable powder formulations [32]. Dicofol has little effect on insects.

Dicofol is manufactured from DDT. In 1986, use of dicofol was temporarily canceled by the EPA because of concerns raised by high levels of DDT contamination [33]. Modern manufacturing processes can produce technical grade dicofol which contains less than 0.1 % DDT. Dicofol causes hyperstimulation of nerve transmissions along nerve axons. This hyperstimulation is thought to be related to inhibition of ATPases in the central nervous system [34].

3.1.1 Genaral Properties of Dicofol

Trade names include Acarin, Cekudifol, Decofol, Dicaron, Dicomite, Difol, Hilfol, Kelthane, and Mitigan. The EPA has classified dicofol as toxicity class II - moderately toxic, and toxicity class III - slightly toxic, depending on the formulation. Products containing dicofol bear the Signal Word WARNING or CAUTION, depending on the formulation.

3.1.2. Physical Properties

Chemical Class/Use: Organochlorine miticide.
CAS (Chemical Abstracts Services) Number: 115-32-2
CAS Name: 4-chloro-alpha-(4-chlorophenyl)-α-(trichloromethyl)benzene-methanol
IUPAC Name: 2,2,2-trichloro-1,1-bis(4-chlorophenyl) ethanol

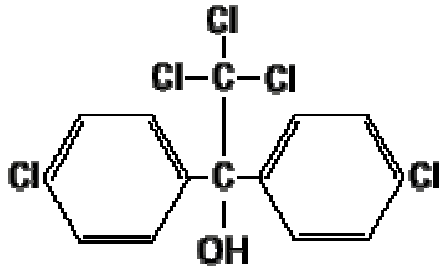


Figure 3.1 Molecular Structure of Dicofol

Molecular Formula: $C_{14}H_9Cl_5O$

Relative Molecular Mass: 370.5

Appearance: Pure dicofol is a colourless solid. Technical product (95% pure) is brown viscous oil and is composed of 80-85% p,p'-dicofol and 15-20% o,p'-dicofol.

Water Solubility: Practically insoluble; 0.8 ppm at 25°C [35].

Solubility in Other Solvents: Soluble in most organic solvents [36].

Melting Point: 78.5 - 79.5°C for pure dicofol [36,37] ; 50°C (122 degrees F) for technical dicofol [35].

Vapor Pressure: Negligible at room temperature; < 0.00001 mm Hg at 20°C/68°F [35], 3.9 x 10 to the minus 7 power mm Hg at 25°C [35,38].

Adsorption Coefficient: 5000 (estimated) [39].

Partition Coefficient: 4.2788 [33,40].

3.1.3. Uses Of Dicofol

Dicofol is used to kill crop-feeding mite pests such as the red spider mite. It is a contact poison which kills the pest after being ingested and picked up from the surface of the crop.

52 685 kg or L Dicofol was used in Turkey in 2002.(This has been gathered from data of Tarım ve Köy İşleri Bakanlığı).

3 346 kg or L Dicofol was used in İzmir in 2003.(This has been gathered from data of İzmir Tarım İl Müdürlüğü).

3.1.4. Toxicological Effects

Acute toxicity:

Dicofol is moderately toxic to practically nontoxic and may be absorbed through ingestion, inhalation, or skin contact. Symptoms of exposure include nausea, dizziness, weakness, and vomiting from ingestion or respiratory exposure, skin irritation or rash from dermal exposure, and conjunctivitis from eye contact. Poisoning may affect the liver, kidneys, or the central nervous system. Overexposure by any route may cause nervousness and hyperactivity, headache, nausea, vomiting, unusual sensations, and fatigue. Very severe cases may result in convulsions, coma, or death from respiratory failure [40,41]. Dicofol is a moderate skin and eye irritant [40,42]. Since dicofol is stored in fatty tissues, intense activity or starvation may mobilize the pesticide, resulting in the reappearance of toxic symptoms long after actual exposure [42]. The oral LD_{50} for dicofol in rats is 575 to 960 mg/kg, in rabbits and guinea pigs is 1810 mg/kg, and in mice is 420 to 675 mg/kg. The dermal LD_{50} in rats is 1000 to 5000 mg/kg, and in rabbits is between 2000 and 5000 mg/kg. The inhalation LC_{50} (4-hour) in rats is greater than 5 mg/L [33,40,43].

Chronic toxicity:

In a 2-year dietary study with rats, liver growth, enzyme induction, and other changes in the liver, adrenal gland, and urinary bladder were observed at doses of 2.5 mg/kg/day and above. Effects on the liver, kidney, and adrenals, and reduced body weights were observed at doses of 6.25 mg/kg/day and above in a 3-month dietary study with mice [40]. When dicofol was fed to rats for 3 months, fewer than half of the animals survived a 75 mg/kg/day dose. Liver enzyme induction was observed at 75 mg/kg/day and above. Decreased body weights, decreased cortisone levels, and toxic changes in the liver, adrenal glands, and kidneys were noted at 25 mg/kg/day. Similar results were observed in a 3-month feeding study with mice [41]. When dogs were fed dicofol for 3 months, 2 two out of 12 survived at 25 mg/kg/day. Poisoning symptoms and effects on the liver, heart, and testes were observed at the 7.5 mg/kg/day dose [41]. When dicofol was fed to dogs, 4.5 mg/kg/day for 1 year caused toxic effects on the

liver. Long-term dermal exposure of rats to dicofol as an emulsifiable concentrate formulation also produced toxic effects on the liver [41].

Reproductive effects:

Reproductive effects in rat offspring have been observed only at doses high enough to also cause toxic effects on the livers, ovaries, and feeding behavior of the parents. Rats fed diets containing dicofol through two generations exhibited adverse effects on the survival and/or growth of newborns at 6.25 and 12.5 mg/kg/day [41].

Teratogenic effects:

No teratogenic effects were observed when rats were given up to 25 mg/kg/day on days 6 through 15 of pregnancy [41].

Mutagenic effects:

Five separate laboratory tests have shown that dicofol is not mutagenic [40,41].

Carcinogenic effects:

No evidence of carcinogenicity was observed in when rats were fed up to 47 mg/kg/day for 78 weeks. A 2-year oncogenicity study in mice showed an increased incidence of liver tumors in male mice at dietary concentration levels of 13.2 and 26.4 mg/kg/day [40]. It is unlikely that dicofol poses a carcinogenic risk to humans.

Organ toxicity:

Chronic exposure to dicofol can cause damage to the kidney, liver, and heart. Prolonged or repeated exposure to dicofol can cause the same effects and symptoms as acute exposure [42]. Prolonged or repeated skin contact can cause moderate skin irritation and/or sensitization of the skin [40].

Fate in humans and animals:

Dicofol is converted in rats to the metabolites 4,4'-dichloro-benzophenone and 4,4'-dichlorodicofol [44,45]. Studies of the metabolism of dicofol in rats, mice, and rabbits have shown that ingested dicofol is rapidly absorbed, distributed primarily to fat, and readily eliminated in feces. When mice were given a single oral dose of 25 mg/kg dicofol, approximately 60% of the dose was eliminated within 96 hours, 20% in the urine, and 40% in the feces. Concentrations in body tissues peaked between 24 and 48 hours following dosing, with 10% of the dose found in fat, followed by the liver and other tissues. Levels in tissues other than fat declined sharply after the peak. When rats were given a single oral dose of 50 mg/kg of dicofol, all but 2% of the dose was eliminated within 192 hours, with peak concentrations in body tissues occurring between 24 and 48 hours after dosing [41].

3.1.5. Ecological Effects

Effects on birds:

Dicofol is slightly toxic to birds. The 8-day dietary LC_{50} is 3010 ppm in bobwhite quail, 1418 ppm in Japanese quail, and 2126 ppm in ring-necked pheasant. Eggshell thinning and reduced offspring survival were noted in the mallard duck, American kestrel, ring dove, and screech owl [40].

Effects on aquatic organisms:

Dicofol is highly toxic to fish, aquatic invertebrates, and algae. The LC_{50} is 0.12 mg/L in rainbow trout, 0.37 mg/L in sheepshead minnow, 0.06 mg/L in mysid shrimp, 0.015 mg/L in shell oysters, and 0.075 mg/L in algae [40].

Effects on other organisms:

Dicofol is not toxic to bees [33].

3.1.6. Environmental Fate

Breakdown in soil and groundwater: Dicofol is moderately persistent in soil, with a half-life of 60 days [39,45]. Dicofol is susceptible to chemical breakdown in moist soils [46]. It is also subject to degradation by UV light. In a silty loam soil, its photodegradation half-life was 30 days. Under anaerobic soil conditions, the half-life for dicofol was 15.9 days [45]. Dicofol is practically insoluble in water and adsorbs very strongly to soil particles. It is therefore nearly immobile in soils and unlikely to infiltrate groundwater. Even in sandy soil, dicofol was not detected below the top 3 inches in standard soil column tests. It is possible for dicofol to enter surface waters when soil erosion occurs [39,45].

Breakdown in water: Dicofol degrades in water or when exposed to UV light at pH levels above 7. Its half-life in solution at pH 5 is 47 to 85 days. Because of its very high absorption coefficient (K_{OC}), dicofol is expected to adsorb to sediment when released into open waters [46].

Breakdown in vegetation: In a number of studies, dicofol residues on treated plant tissues have been shown to remain unchanged for up to 2 years [45].

3.2. ENDOSULFAN

Endosulfan is an organochlorine insecticide and acaricide, and acts as a contact poison in a wide variety of insects and mites. Endosulfan is effective against a wide range of insects and certain mites on cereals, coffee, cotton, fruit, oilseeds, potato, tea, vegetable and other crops[47]. It can also be used as a wood preservative.
Endosulfan is sold as a mixture of two different forms of the same chemical (alpha- and beta-endosulfan). Its color is cream to brown and it smells like turpentine [48].

Endosulfan is a highly toxic substance. The World Health Organisation (WHO) classifies endosulfan in Category II (moderately hazardous). The US Environmental Protection Agency (US EPA) classifies it as a Category 1b (highly hazardous) pesticide[49]. Short-term toxicity is high, and influenced by the solvents and emulsifiers used to dissolve it [50]. Endosulfan is easily absorbed by the stomach, by the lungs and through the skin, meaning that all routes of exposure can pose a hazard[51]. Exposure to endosulfan may result from, for example: breathing air near where it has been sprayed; drinking water contaminated with it; eating contaminated food; touching contaminated soil; smoking cigarettes made from tobacco with endosulfan residues; or working in an industry where endosulfan is used[52]. Proper protective clothing (safety goggles, gloves, long sleeves, long pants, respirator) is needed to prevent poisoning when handling endosulfan[53].

3.2.1 Genaral Properties of Endosulfan

Trade or other names for the product include Afidan, Beosit, Cyclodan, Devisulfan, Endocel, Endocide, Endosol, FMC 5462, Hexasulfan, Hildan, Hoe 2671, Insectophene, Malix, Phaser, Thiodan, Thimul, Thifor, and Thionex. Endosulfan is a highly toxic pesticide in EPA toxicity class I. It is a Restricted Use Pesticide (RUP). Labels for products containing endosulfan must bear the Signal Words DANGER - POISON, depending on formulation.

3.2.2. Physical Properties

Chemical Class/Use: Organochlorine insecticide and acaricide.
CAS (Chemical Abstracts Services) Number: 115-29-7

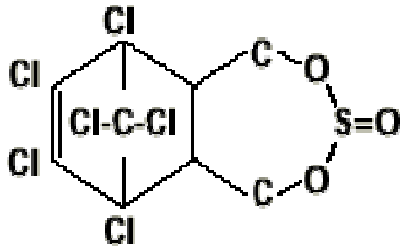


Figure 3.2 Molecular Structure of Endosulfan

CAS Name: Alpha-isomer, 959-98-8; beta-isomer, 33213-65-9

IUPAC Name: 6,7,8,10-10-hexachloro-1,5,5,1,6,9,9a-hexahydro-6,9-methano-2,4,3 benzadioxathiepin 3-oxide

Molecular Formula: $C_9H_6CI_6O_3S$

Relative Molecular Mass: 406.96

Appearance: Pure endosulfan is a colourless crystal. Technical grade is a yellow-brown color[33].

Water Solubility: 0.32 mg/L at 22°C [33]

Solubility in Other Solvents: Soluble in toluene and hexane.

Melting Point: Technical material, 70-100°C [33]

Vapor Pressure: 1200 mPa at 80°C [33]

Adsorption Coefficient: 12,400 [39]

Partition Coefficient: Not Available

3.2.3. Uses Of Endosulfan

Endosulfan is effective against a wide range of insects and certain mites on cereals, coffee, cotton, fruit, oilseeds, potato, tea, vegetable and other crops.

144 238 kg or L Endosulfan was used in Turkey in 2002.(This has been gathered from data of Tarım ve Köy İşleri Bakanlığı).

32 557 kg or L Endosulfan was used in İzmir in 2003.(This has been gathered from data of İzmir Tarım İl Müdürlüğü).

3.2.4. Toxicological Effects

Acute toxicity:

Endosulfan is highly toxic via the oral route, with reported oral LD_{50} values ranging from 18 to 160 mg/kg in rats, 7.36 mg/kg in mice, and 77 mg/kg in dogs [33,44]. It is also highly toxic via the dermal route, with reported dermal LD_{50} values in rats ranging from 78 to 359 mg/kg [33,44]. Endosulfan may be only slightly toxic via inhalation, with a reported inhalation LC_{50} of 21 mg/L for 1 hour, and 8.0 mg/L for 4 hours [44]. It is reported not to cause skin or eye irritation in animals [44]. The alpha-isomer is considered to be more toxic than the beta-isomer [44]. Animal data indicate that toxicity may also be influenced by species and by level of protein in the diet; rats which have been been deprived of protein are nearly twice as susceptible to the toxic effects of endosulfan [44]. Solvents and/or emulsifiers used with endosulfan in formulated products may influence its absorption into the system via all routes; technical endosulfan is slowly and incompletely absorbed into the body whereas absorption is more rapid in the presence of alcohols, oils, and emulsifiers [44]. Stimulation of the central nervous system is the major characteristic of endosulfan poisoning [54]. Symptoms noted in acutely exposed humans include those common to the other cyclodienes, e.g., incoordination, imbalance, difficulty breathing, gagging, vomiting, diarrhea, agitation, convulsions, and loss of consciousness [44]. Reversible blindness has been documented for cows that grazed in a field sprayed with the compound. The animals completely recovered after a month following the exposure [44]. In an accidental exposure, sheep and pigs grazing on a sprayed field suffered a lack of muscle coordination and blindness [44].

Chronic toxicity:

In rats, oral doses of 10 mg/kg/day caused high rates of mortality within 15 days, but doses of 5 mg/kg/day caused liver enlargement and some other effects over the same period [44]. This dose level also caused seizures commencing 25 to 30 minutes following dose adiministration that persisted for approximately 60 minutes [44]. There is evidence that administration of this dose over 2 years in rats also caused reduced

growth and survival, changes in kidney structure, and changes in blood chemistry [44,54].

Reproductive effects:

Rats fed doses of endosulfan of 2.5 mg/kg/day for three generations showed no observable reproductive effects, but 5.0 mg/kg/day caused increased dam mortality and resorption [44,54]. Female mice fed the compound for 78 weeks at 0.1 mg/kg/day had damage to their reproductive organs [55]. Oral dosage for 15 days at 10 mg/kg/day in male rats caused damage to the semeniferous tubules and lowered testes weights [37,44]. It is unlikely that endosulfan will cause reproductive effects in humans at expected exposure levels.

Teratogenic effects:

An oral dose of 2.5 mg/kg/day resulted in normal reproduction in rats in a three-generational study, but 5 and 10 mg/kg/day resulted in abnormalities in bone development in the offspring [44,54]. Teratogenic effects in humans are unlikely at expected exposure levels.

Mutagenic effects:

Endosulfan is mutagenic to bacterial and yeast cells [54]. The metabolites of endosulfan have also shown the ability to cause cellular changes [44,54]. This compound has also caused mutagenic effects in two different mammalian species [54]. Thus, evidence suggests that exposure to endosulfan may cause mutagenic effects in humans if exposure is great enough.

Carcinogenic effects:

In a long-term study done with both mice and rats, the males of both groups experienced such a high mortality rate that no conclusions could be drawn [55]. However, the females of both species failed to develop any carcinogenic conditions 78 weeks after being fed diets containing up to about 23 mg/kg/day. The highest tolerated

dose of endosulfan did not cause increased incidence of tumors in mice over 18 months, and a later study also showed no evidence of carcinogenic activity in mice or rats [44,55]. It appears that endosulfan is not carcinogenic.

Organ toxicity:

Data from animal studies reveal the organs most likely to be affected include kidneys, liver, blood, and the parathyroid gland [54].

Fate in humans and animals:

Endosulfan is rapidly degraded into mainly water-soluble compounds and eliminated in mammals with very little absorption in the gastrointestinal tract [44]. In rabbits, the beta-isomer is cleared from blood plasma more quickly than the alpha-isomer, with reported blood half-lives of approximately 6 hours and 10 days, respectively [44], which may account in part for the observed differences in toxicity. The metabolites are dependent on the mixture of isomers and the route of exposure [44]. Most of the endosulfan seems to leave the body within a few days to a few weeks.

3.2.5. Ecological Effects

Effects on birds:

Endosulfan is highly to moderately toxic to bird species, with reported oral LD_{50} values in mallards ranging from 31 to 243 mg/kg [33,56], and in pheasants ranging from 80 to greater than 320 mg/kg [56]. The reported 5-day dietary LC_{50} is 2906 ppm in Japanese quail [57]. Male mallards from 3 to 4 months old exhibited wings crossed high over their back, tremors, falling, and other symptoms as soon as 10 minutes after an acute, oral dose. The symptoms persisted for up to a month in a few animals [56].

Effects on aquatic organisms:

Endosulfan is very highly toxic to four fish species and both of the aquatic invertebrates studied; in fish species, the reported 96-hour LC_{50} values were (in ug/L):

rainbow trout, 1.5; fathead minnow, 1.4; channel catfish, 1.5; and bluegill sunfish, 1.2. In two aquatic invertebrates, scuds (G. lacustris) and stoneflies (Pteronarcys), the reported 96-hour LC_{50} values were, respectively, 5.8 ug/L and 3.3 ug/L [58]. The bioaccumulation for the compound may be significant; in the mussel (Mytelus edulis) the compound accumulated to 600 times the ambient water concentration [42].

Effects on other organisms:

It is moderately toxic to bees and is relatively nontoxic to beneficial insects such as parasitic wasps, lady bird beetles, and some mites [33,42].

3.2.6. Environmental Fate

Breakdown in soil and groundwater: Endosulfan is moderately persistent in the soil environment with a reported average field half-life of 50 days [39]. The two isomers have different degradation times in soil. The half-life for the alpha-isomer is 35 days, and is 150 days for the beta-isomer under neutral conditions. These two isomers will persist longer under more acidic conditions. The compound is broken down in soil by fungi and bacteria [33]. Endosulfan does not easily dissolve in water, and has a very low solubility [33,39]. It has a moderate capacity to adhere or adsorb to soils [39]. Transport of this pesticide is most likely to occur if endosulfan is adsorbed to soil particles in surface runoff. It is not likely to be very mobile or to pose a threat to groundwater. It has, however, been detected in California well water [46].

Breakdown in water: In raw river water at room temperature and exposed to light, both isomers disappeared in 4 weeks [46]. A breakdown product first appeared within the first week. The breakdown in water is faster (5 weeks) under neutral conditions than at more acidic conditions or basic conditions (5 months) [46]. Under strongly alkaline conditions the half-life of the compound is 1 day. Large amounts of endosulfan can be found in surface water near areas of application [54]. It has also been found in surface water throughout the country at very low concentrations [46].

Breakdown in vegetation: In plants, endosulfan is rapidly broken down to the corresponding sulfate [33]. On most fruits and vegetables, 50% of the parent residue is

lost within 3 to 7 days [33]. Endosulfan and its breakdown products have been detected in vegetables (0.0005-0.013 ppm), in tobacco, in various seafoods (0.2 ppt-1.7 ppb), and in milk [46].

CHAPTER 4

GAS CHROMATOGRAPHY (GC), MASS SPECTROMETRY (MS) AND THEIR COMBINATION (GC-MS)

4.1. Introduction

GC and MS are complementary techniques that together create a powerful and versatile analytical method. Separation of the volatile components of a mixture by GC is a technology that was first described in 1952 [59], and it was immediately recognized as an indispensable tool for the analysis of organic compounds. Of particular importance in the evolution of GC toward modern instruments was the introduction of capillary chromatographic columns, which improved the resolution of GC separations by several orders of magnitude. However, there are two significant limitations of GC as a qualitative and quantitative analytical technique. The first limitation is the necessity for analytes to be sufficiently volatile and thermally stable to vaporize at practical temperatures. A second limitation is the specificity of GC detectors, which can range from very nonspecific [e.g. thermal conductivity, flame ionization detectors (FIDs)], to highly specific (mass spectrometer).

GC/MS combines the resolving capabilities of GC with the unique structural information from MS, making it the hybrid analytical method of choice for qualitative analysis of suitably volatile organic compounds. Quantitative applications of GC/MS are more complicated, and typically require internal standards. The ability to resolve the components of complex mixtures, and yielding qualitative information about organic molecules, makes GC/MS an attractive technique for environmental and biomedical applications.

MS has limited standalone applications, since specimen purity is essential. MS methods for measuring low-boiling compounds require a procedure that will volatilize enough molecules to be detected. There are several approaches to MS measurement of nonvolatile compounds, including liquid chromatography/MS interfaces, fast atom bombardment (FAB), electrospray, thermospray, and matrix-assisted laser desorption/ionization (MALDI). All of these methods incorporate techniques that

ultimately produce vapor-phase molecules that are subsequently fragmented in the mass spectrometer's ion source.

4.2. Gas Chromatography

In gas chromatography, the sample is vaporized and injected onto the head of chromatographic column. Elution is brought about by the flow of an inert gaseous mobile phase.

A typical gas chromatograph (as shown in Figure 4.1 [60]) comprises three fundamental components: an injection system, a chromatographic column, and a detector. In most cases, specimens for GC analyses are dissolved in a volatile solvent, although neat or gaseous specimens can also be used. Most GC injection systems are designed to vaporize liquid specimens, and they accomplish this by heating the injector body to a temperature above the boiling point of the solvent and analyte. In older GC designs, the sample was injected directly into the chromatographic column, which was preheated. However, introduction of capillary chromatographic columns, which have bores half a millimeter or less in diameter require innovative injector designs.

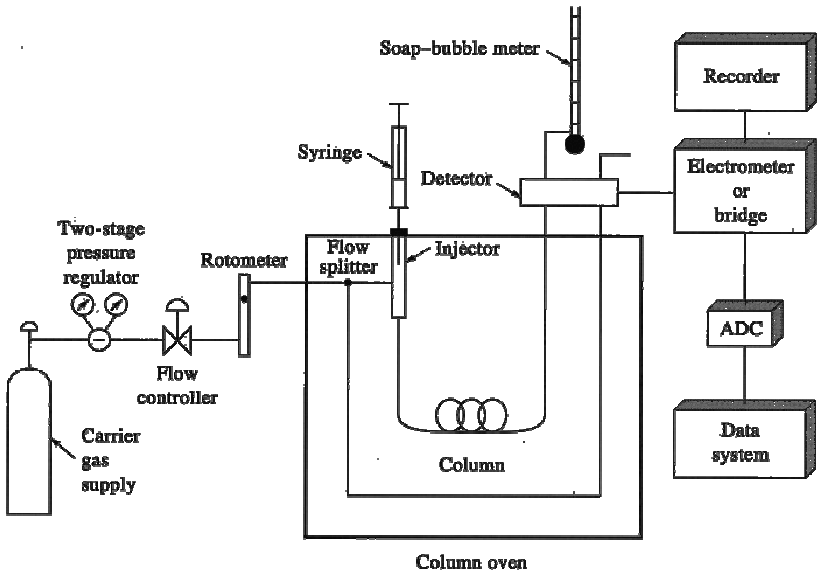


Figure 4.1 Schematic of a Gas Chromatograph

The challenge was to avoid peak broadening due to leakage of residual sample into the capillary column over an extended period of time. One microliter of specimen, when volatized occupies a considerable volume within the injector body, and the small inside diameter of the capillary column cannot accommodate the large volume of vapor.

One approach to minimizing the injection bandwidth is to constantly purge the injector body so that only a small amount of the vapor has the opportunity to enter the capillary column – this technique is called *split* injection. The split ratio (amount of specimen entering the column versus the amount purged) typically varies from 1 : 10 to 1 : 99. A limitation of split injection is the loss of analytical sensitivity, since a smaller amount of specimen enters the column and detector. In some cases, the loss of analytical sensitivity is not problematic, and may even be beneficial, especially when analyte concentration is high and the detector's range of linear response is limited.

Another approach to capillary column injectors is *splitless*. In a splitless injection, the injector body is kept hot enough to vaporize the specimen and solvent, but the column temperature remains below the boiling point of the solvent. As the vaporized specimen enters the capillary column, it condenses and therefore the bandwidth is minimized. After a sufficient period of time (usually about 60s), the injector body is purged and the column is warmed up to re-vaporize the specimen and begin the chromatography. Splitless injections are technically more complex and involve more variables than split injections, but a significantly greater amount of specimen is delivered to the capillary column, resulting in better analytical sensitivity.

On-column injections with capillary columns are also possible, and require specially designed syringes fitted with needles that terminate with a length of very small capillary, which fits inside the chromatographic column. Because of the fine capillary point, the syringes are delicate, and generally not compatible with autosampler mechanisms. For sufficiently volatile compounds, vapor may be injected into the gas chromatograph using an airtight syringe. Raoult's law states that the mole fractions contained in the vapor phase above a liquid are determined by the respective vapor pressures of the constituents of the liquid, which in turn are proportional to their relative concentrations. Therefore, the vapor in equilibrium with a liquid can be used to quantify volatile constituents in the liquid – this technique is called *headspace analysis*. Headspace sampling offers several advantages over conventional liquid injections: the vapor is substantially free of nonvolatile constituents that may form residue inside the injector; the injection bandwidth is considerably reduced; and specimen delivery is more nearly quantitative. Headspace analysis is only useful for highly volatile compounds such as low-molecular-weight alcohols.

GC column performance improved dramatically with the introduction of fused-silica capillary columns, a technology derived from fiber optics. Resolution equivalent

to several hundred thousand theoretical plates is commonly achievable with capillary GC columns. Microprocessor control of the GC oven temperature has enhanced the ability to program temperature changes, improving both the resolution and speed of GC analyses. In most GC columns the stationary phase is a liquid and the analytical method is therefore gas–liquid chromatography, following the widely used convention of specifying the state of both stationary and mobile phases in the names of chromatographic applications. Gas–solid chromatography applications also exist, but are less common. The liquid stationary phase may be coated on a solid support or chemically bonded to the inner wall of a fused silica capillary column (‘‘bonded phase’’ columns).

The choice of GC detector depends on the type of compound that is to be measured, the sensitivity that is required, and the degree of selectivity necessary to avoid significant interference. Thermal conductivity detectors have moderate sensitivity, but are not selective. FIDs have better sensitivity, and respond mostly to hydrocarbon compounds. Nitrogen–phosphorus detectors are specific for nitrogen- and phosphorus-containing compounds, and are very sensitive. Electron capture detectors can measure chlorine-containing compounds in subpicogram amounts. The properties and performance characteristics of various GC detectors are summarized in Table 4.1 [61].

Table 4.1 Performance Characteristics of Common GC Detectors

Detector	Detection Limit	Linear Range	Application
Thermal conductivity	0.5 ng	10^5	Universal
Flame ionization	10 pg	10^7	Hydrocarbons
Electron capture	0.05 pg	10^4	Halides
Thermionic (nitrogen – phosphorus)	0.1 pg	10^3	N, P
Mass spectrometer	10 pg	10^6	Universal

The versatility and ruggedness of GC makes this analytical method an attractive choice for the measurement of easily vaporized compounds.

4.3. Mass Spectrometry

Mass spectrometry is a spectrometric method, which does not involve the absorption or emission of electromagnetic radiation. Sample in a molecular or atomic state is converted into ionic particles that are fragments and then analyzed by measuring the mass-to-charge ratio of ions. It is an extremely sensitive, versatile and important analytical method.

In Molecular Mass Spectrometry, analyte is vaporized and bombarded with a stream of electrons that lead to the loss of an electron by the analyte and the molecular ion $M^{\bullet +}$ is formed as shown below;

$$M + e^{-} \longrightarrow M^{\bullet +} + 2e^{-}$$

The charged species $M^{\bullet +}$ is the molecular ion. As indicated by the dot, the molecular ion is a radical ion that has the same molecular weight as the molecule. The collision between energetic electrons and analyte molecules usually transfer enough energy to the molecules to leave them in an excited state. Relaxation then often occurs by the fragmentation of molecular ion to produce ions of lower masses.

Several instrumental techniques have been devised to separate and measure charged particles based on their mass. A typical mass spectrometer consists of four components: an inlet system, an ion source, a mass analyzer, and a detector, which are shown in Figure 4.2 [60].

The inlet system must ensure that a pure compound is delivered to the ion source. Therefore, chromatographic systems are a popular choice for a mass spectrometer inlet system. The ion source is where the compound is ionized, a process that is ordinarily followed by decomposition of the analyte into unique, charged fragments.

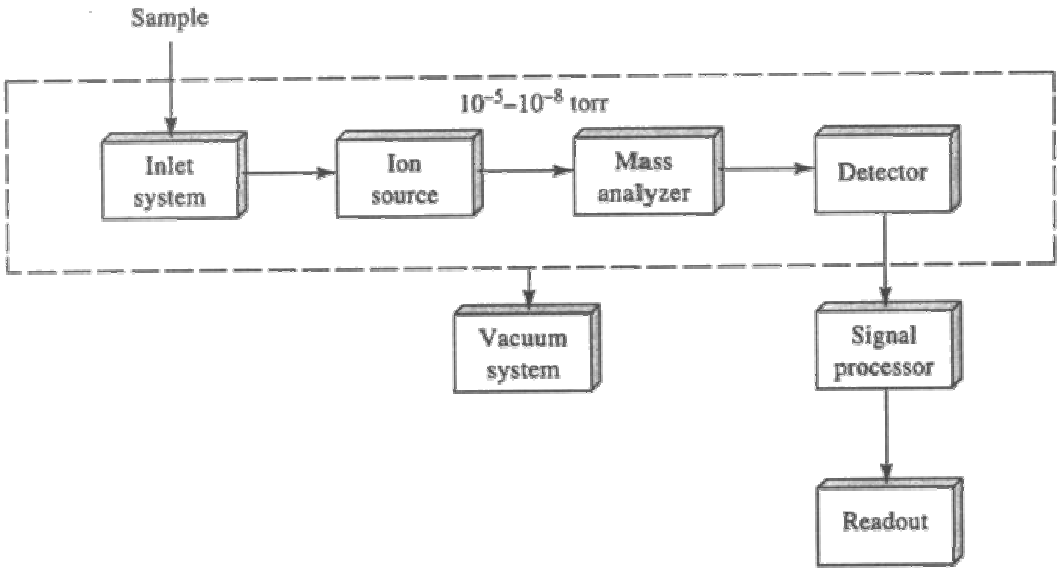


Figure 4.2 Components of a Mass Spectrometer

The mass analyzer sorts the charged fragments and the detector measures the number of charged fragments of any given mass. Since a mass spectrum (sometimes called a mass fragmentogram) uniquely identifies a compound based on its fragmentation pattern, superimposition of the fragments from a second compound in the ion source would make the spectrum uncertain. Therefore, the inlet system for a mass spectrometer must deliver pure compound to the ion source in order for the mass spectrometer to be useful for qualitative analysis. Inlet systems for MS include GC, liquid chromatographs, and several methods for vaporization and ionization of nonvolatile compounds.

The ion source in a mass spectrometer usually operates under a vacuum – the presence of oxygen and nitrogen may affect ionization and contribute interfering fragments to the mass spectrum – so a pressure differential exists between the ion source and the inlet system. This pressure differential is difficult to maintain when the inlet system is pressurized, as are gas and liquid chromatographs. Several devices have been created to remove the mobile phase as it elutes from the chromatographic system so that only analyte enters the ion source; examples are vacuum jet separators for packed-column GC systems, and moving-belt solvent evaporators for high-performance liquid chromatographs.

Capillary GC columns can usually terminate at the entrance to the ion source since the minimal carrier gas flow can be removed efficiently by the mass

spectrometer's vacuum system. When solid sampling systems for nonvolatile analytes are used, the pressure differential is less of a concern because the sampling system can operate under vacuum. Solid sampling inlet systems include MALDI, FAB, thermospray, and electrospray.

In a MALDI system, the analyte is embedded into a pure crystalline matrix. When a laser is directed at the crystal, analyte and crystal molecules are ejected. FAB is a similar technique, except that high-energy beams of inert atoms, such as argon, are used to initiate molecular ejection. In electrospray ionization, the analyte is dissolved in an organic solvent, and passed through an electrically charged capillary. Small clusters of analyte/solvent form in the capillary, and become charged. As the clusters are accelerated through a series of lenses, the solvent is gradually removed, resulting in smaller and smaller clusters. When the clusters reach a certain size, coulombic forces cause them to explode, and the resulting fragments are measured in the mass analyzer. Thermospray ionization is a similar technique, except that the capillary is heated, and solvent evaporates quickly after the analyte/solvent aerosol exits the capillary. In both electrospray and thermospray applications, nonvolatile analytes are stranded in the vapor phase as solvent is removed, and can therefore enter the mass analyzer and be measured. These solid sampling techniques are particularly useful for high molecular weight compounds, which include proteins and nucleic acids. The ion source of a mass spectrometer shatters the analyte molecules so that their fragments can be separated and measured.

Most mass spectrometers use a high-energy flux of electrons to ionize molecules the method is called electron impact ionization. Most reference mass spectra are generated by electron impact ionization. There are circumstances, though, when electron impact ionization does not produce satisfactory spectral uniqueness or analytical sensitivity, in this case other ionization methods may be preferable. One alternative method is chemical ionization, in which the ion source is pressurized with a reagent gas such as methane. The electron flux ionizes the reagent gas, which in turn interacts with the analyte to produce charged species. This approach is particularly useful for generating negatively charged ions.

Fragments may also be produced by collisional dissociation, where analyte molecules (or fragments) are accelerated and collide with inert gas molecules to produce fragments. This technique is often used in mass spectrometers that have multiple mass analyzers, and the collisionally induced fragments are therefore called daughter ions

since they are produced after initial ionization and passage through the first-stage mass analyzer.

There are several types of mass analyzers, and some instruments combine multiple mass analyzers. Time-of- flight mass spectrometers incorporate a simple design in which fragments are separated based on their velocities as shown in Figure 4.3 [62].

Magnetic sector mass spectrometers separate fragments based on the degree to which they are deflected in a magnetic field. Magnetic sector instruments are very sensitive, but cost and complexity is high (Figure 4.4 [60]). Instruments that incorporate two magnetic sector mass analyzers can achieve very high resolution, and are useful for making accurate mass measurements. Mass measurements with accuracy to 0.0001 amu are usually sufficient to determine the exact empirical formula of a parent ion or fragment.

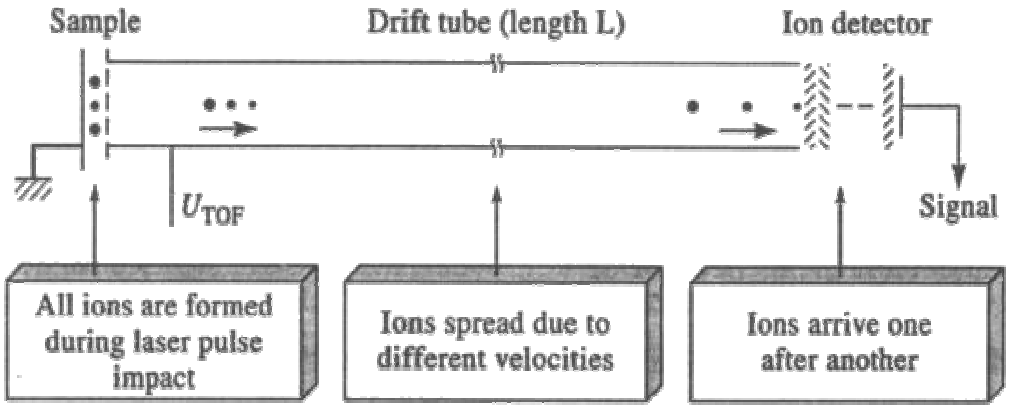


Figure 4.3 A Time-of-flight Mass Spectrometer

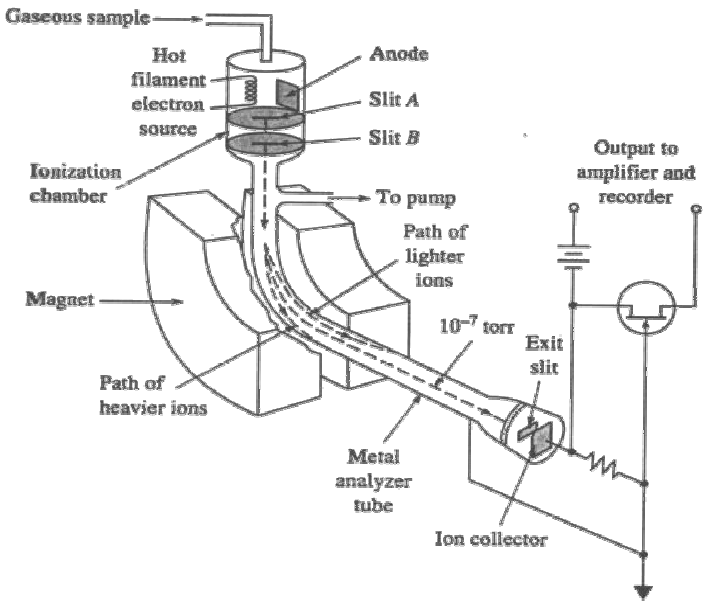


Figure 4.4 A Magnetic Mass Spectrometer

The most popular mass analyzer is the quadrupole as shown in Figure 4.5 [60], which uses a combination of static and oscillating (radio-frequency) electromagnetic fields to separate the ions produced in the ion source. Quadrupole instruments are relatively inexpensive, have <1.0 amu resolution, and have detection limits for most compounds in the picogram range. Multiple quadrupole instruments have also been designed, their principal advantage being the ability to analyze mixtures of compounds. A variation on the quadrupole mass analyzer is the ion trap mass spectrometer as shown in Figure 4.6 [63].

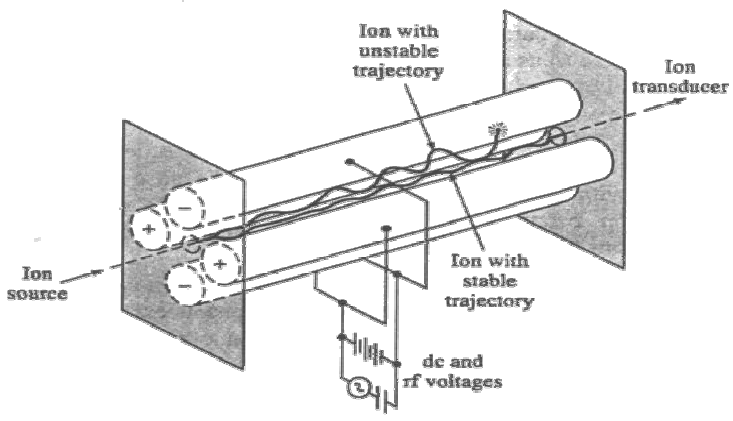


Figure 4.5 A Quadrupole Mass Spectrometer

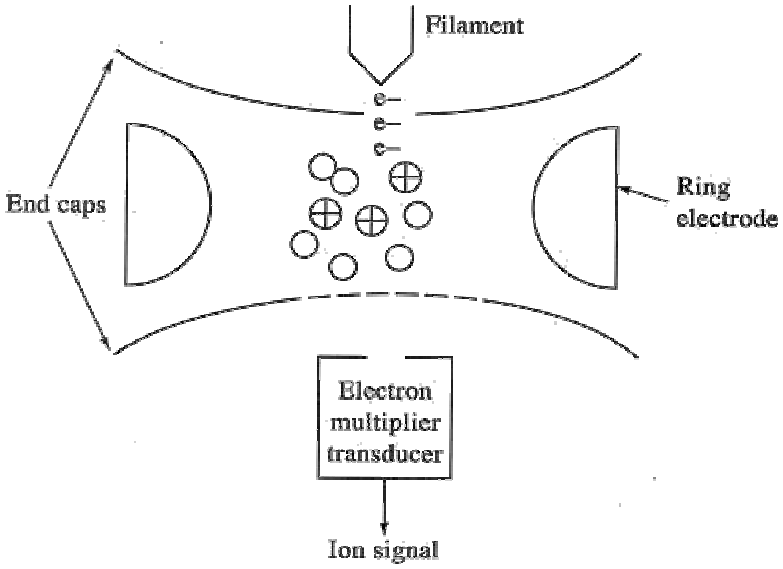


Figure 4.6 Ion Trap Mass Spectrometer

The principal difference between a quadrupole analyzer and an ion trap is that the former filters ions by creating an oscillating electromagnetic path through which the ions travel, whereas an ion trap keeps the ions with the oscillating electromagnetic field. An advantage of the ion trap mass spectrometer is its sensitivity, since ions of a particular mass can be accumulated, then released to the detector – the ion yield is greater than that achievable by the quadrupole design. Ion trap instruments cost about the same as quadrupole instruments, and are more sensitive, but also have two disadvantages: mass spectra obtained in ion trap instruments do not always correspond closely with reference spectra generated by quadrupole or magnetic sector instruments; and ion trap instruments are, generally, less precise for quantitative analysis than are quadrupole instruments. Nevertheless, ion trap mass spectrometers are used in many of the same applications as quadrupole instruments. Multiple mass analyzer instruments using ion traps have also been designed; usually the ion trap accumulates a particular ion, and a quadrupole is used to subsequently measure the daughter ions. Most mass spectrometers use an electron multiplier tube as the detector, although the design may be modified with dynodes in order to measure both positive and negative ions.

4.3.1. Ion Trap

The quadrupole ion trap mass analyzer (Figure 4.7.) consists of three hyperbolic electrodes: the ring electrode, the entrance endcap electrode and the exit endcap electrode. These electrodes form a cavity in which it is possible to trap and analyze ions. Both endcap electrodes have a small hole in their centers through which the ions can travel. The ring electrode is located halfway between the two-endcap electrodes.

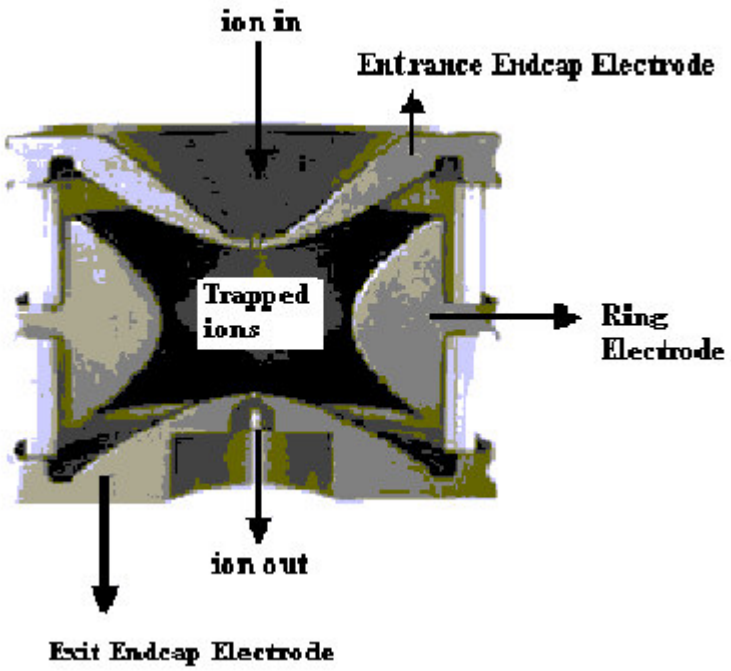


Figure 4.7 A Schematic Diagram of an Ion Trap Mass Spectrometer

Ions produced from the source enter the trap through the inlet focusing system and the entrance endcap electrode. Various voltages are applied to the electrodes to trap and eject ions according to their mass-to-charge ratios. The ring electrode RF potential, and a.c. potential of constant frequency and variable amplitude, is applied to the ring electrode to produce a 3D quadrupolar potential field within the trapping cavity. This will trap ions in a stable oscillating trajectory confined within the trapping cell. The nature of the trajectory is dependent on the trapping potential and the mass-to-charge ratio of the ions.

During detection, the electrode system potentials are altered to produce instabilities in the ion trajectories and thus eject the ions in the axial direction. The ions are ejected in order of increasing mass-to-charge ratio, focused by the exit lens and detected by the ion detector system.

GC-(IT)MS system has two analysis modes for sensitive and selective analysis. These are MS-MS (Tandem Mass Spectrometry) and SIS (Selected Ion Storage) modes.

MS-MS (Tandem Mass Spectrometry) Mode: Ion Trap Tandem Mass Spectrometry (MS-MS Mode) for electron ionization consists four basic operation steps;
Ion formation and matrix ion ejection,
Parent ion isolation,
Product ion formation,
Product ion mass scanning.
The utility of the MS-MS technique derives from the following;
optimally filling an ion trap with the selected parent ion,
obtaining qualitative structural information about the sample by forming the product ion spectrum,
increasing the signal-to-noise ratio by eliminating interfering matrix ions in the product ion spectrum during isolation.

SIS (Selected Ion Storage) Mode: SIS eliminates unwanted ions by ejecting them from the ion trap. Given the optimum number of ions that can be stored in the ion trap, SIS enriches the sample ions relative the unwanted matrix ions and ejects the latter throughout ionization. Working in SIS mode, the unwanted ions are ejected from the ion trap and selectivity is increased.

4.4. Combined Gas Chromatography and Mass Spectrometry

The combination of GC and MS is one of the most useful and versatile analytical configurations available for measuring organic molecules. Although in principle any gas chromatograph and mass spectrometer could be combined, the most popular configuration nowadays is a capillary gas chromatograph with a split/splitless injector and a quadrupole mass spectrometer or ion trap using electron impact ionization.

Most quadrupole and magnetic-sector mass spectrometers are offered with accessories that permit interfacing with gas chromatographic equipment. The simplest mass detector for use in GC is the ion trap detector (ITD).

In this instrument, ions are created from the eluted sample by electron impact or chemical ionization and stored in a radio-frequency field. The trapped ions are then

ejected from the storage area to an electron multiplier detector. The ejection is controlled so that scanning on the basis of mass-to-charge ratio is possible. The ion trap detector is remarkably compact and less expensive than quadrupole instruments.

Gas chromatograph / mass spectrometer instruments have been widely applied to analyze pesticides in water [64,65], because of its high specificity and sensitivity. Other attractive technique for determination is gas chromatography – tandem mass spectrometry (GC–MS–MS). The tandem MS technique allows highly specific MS analyses, with the possibility of directly analyzing complex environmental samples without extensive clean-up steps. The last generation of low-cost benchtop ion trap instruments can operate in the MS–MS mode: a specific ion, formed by electron ionization, is isolated in the ion trap and subsequently dissociated, increasing its collisions with the GC carrier gas molecules. Product ions are detected after this step, ejecting these ions from the trap by applying a radio frequency (RF) voltage ramp to the trap electrodes. Few applications of GC–MS–MS in pesticide analysis are reported [2,3] and its use is limited to residue confirmation [66]. The recent application of the MS–MS function in ion trap instruments could in the future increase the number of applications, considering its ease of use and the relatively low cost of the instruments.

CHAPTER 5

MATERIALS AND METHOD

5.1. Chemicals and Reagents

Standards of the Dicofol and Endosulfan pesticides were obtained from Riedel-de Haën® (Germany) with purity higher than 98%. The internal standard (I.S.), pentachloronitrobenzene (99% purity) was obtained from Aldrich. Each of pesticide stock standard solutions (1000 mg/L) were prepared by exact weighing and dissolving them in dichloromethane and stored in a freezer (-18 °C). GC quality solvents of dichloromethane, and methanol were purchased from Fluka, and Riedel-de Haën®, respectively. Organic-free water was prepared by Barnstead / Thermolyne EASYpure UV System (Dubuque, IOWA, USA). Solid Phase Extraction Disks (ENVI™ -18 DSK 47mm) and NaCl were obtained from Supelco (Sigma-Aldrich) and Carlo Erba (Italy), respectively.

5.2. Calibration Set

Intermediate stock standard solutions (10 mg/L) of endosulfan were prepared from 1000 mg/L stock standard solutions. From this 10 mg/L standard pesticide solution, eight calibration solutions (from 0.003 to 5 mg/L) were prepared in dichloromethane. Pentachloronitrobenzene internal standard solution (1 mg/L) was prepared in dichloromethane and 50 µl of this solution was added to each 1.0 ml calibration solutions prior to chromatographic quantifications. All solutions were stored frozen in the dark at -18 °C until use.

5.3. GC–MS analysis

Star 3400 *Cx* Gas Chromatograph - Saturn 2000 Ion Trap Mass Spectrometer from Varian Instruments (USA) was used for analysis. The gas chromatograph was equipped with a split / splitless programmed temperature injector SPI/1078 operated in the splitless mode and a DB5-MS (30mX0.25mm I.D.), film thickness 0.25 µm capillary

column was employed. The ion trap mass spectrometer was operated in the EI mode and the MS–MS option was used.

Varian Saturn GC/MS Workstation controlled the system.

GC conditions were as follows: initial column temperature 90°C, then increased at 10°C/min to 250°C (kept 4.00 min); carrier gas He (99.999%) at a flow-rate of 1 ml/min; manifold, transfer-line and trap temperatures were 40, 280 and 200°C, respectively; injection volume was 1 µl.

GC–MS conditions were: solvent delay 4 min; 70 eV of electron impact energy; scan rate 1 scan/sec; scanned-mass range 50–300 m/z in segment 2, 50-400 m/z in segment 3 and 4. The mass spectrometer was calibrated weekly.

For GC–MS–MS and GC-MS (SIS Mode), the sample was injected under the gas chromatographic conditions described for GC–MS. The MS–MS and MS (SIS) parameters are shown in Appendix A.

5.4. Sampling

All 5 L of water samples were collected by İZSU from Tahtalı Dam in Seferihisar/İZMİR and Tahtalı Dam Water Treatment Plant in Görece/İZMİR. These samples were supplied twice a month between August 2002 and January 2003 by İZSU. Collected water samples were acidified and stored in refrigerator at 4 ^{0}C until they were used for analysis.

5.5. Analysis of Water Samples Using Solid Phase Extraction (SPE) Preconcentration Method

Trace level of pesticides were preconcentrated using the ENVI™ -18 DSK Solid Phase Disk [glass fiber embedded with surface-modified silica (C18 bonded phase)]. Passing 5 ml of dichloromethane, 5 ml of methanol, and 5 ml of pesticide-free water in sequence, under low vacuum, activated the SPE disk.

Once activated 500 ml of the spiked or real sample water, with the prior addition of 10 g/l of NaCl and adjusted to pH 5, was passed through the SPE disk at a flow-rate of approximately 75-100 ml/min using a vacuum system. Then the SPE disk was dried for 15 minutes under vacuum. The elution was carried out by adding 5 ml of dichloromethane under low vacuum.

The eluate was collected in a tube, and then all elution solvent was evaporated under nitrogen gas stream. After this evaporation process, exactly 500µl of dichloromethane and 25µl of internal standard (Pentachloronitrobenzene) was added. And then 1 µl of this solution was injected to the GC-MS system.

Tahtalı Dam water samples were filtered through Filtrak® filter paper (black band) before preconcentration.

The analytical procedure can be summarized as follows:

ENVI™ -18 DSK Solid Phase Disk

Preconditioning: 5 ml dichloromethane, 5 ml methanol, and 5 ml pesticides-free water

Filtration: 500 ml water sample for solid phase extraction

Drying: 15 min under vacuum, 15 min air

Elution: 5 ml dichloromethane

Elution solvent evaporated under N_2 gas

Redissolved in exactly 500µl dichloromethane

Add Pentachloronitrobenzene (I.S.) (25µl)

Inject 1 µl [GC–MS system under MS–MS, and SIS modes

CHAPTER 6

RESULTS AND DISCUSSION

Several parameters were compared while analyzing a sample with GC-MS. Two organochlorine pesticides were analyzed, Dicofol and Endosulfan. Endosulfan consists of α- and β- isomers in the ratio of approximately 7:3.

First the peaks of Dicofol, α-Endosulfan, and β-Endosulfan had to be found. Figure 6.1 shows our first injection of 1.0 mg/L standard pesticide mixture solution in GC-MS.

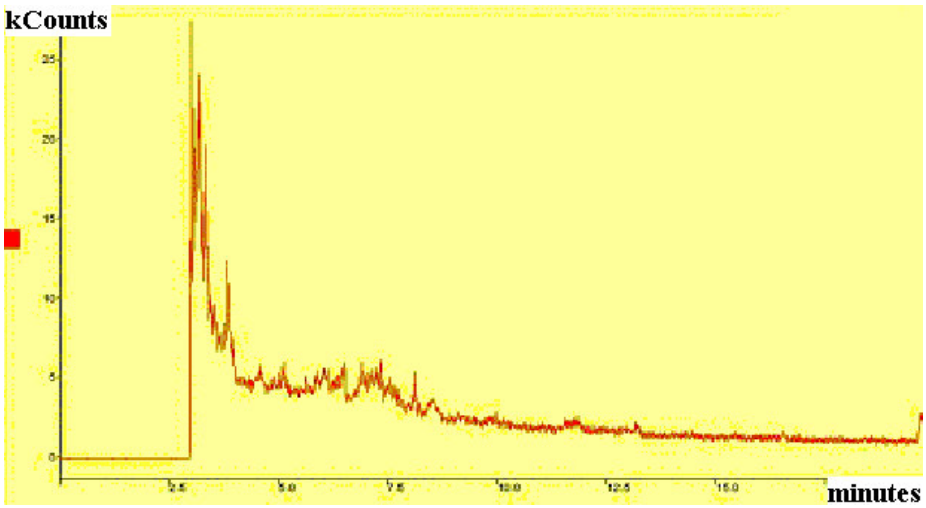


Figure 6.1 GC-MS Chromatogram of 1.0 mg/L Standard Pesticide Mixture Solution

In Figure 6.1 library search was made and the peaks of Dicofol, α-Endosulfan and, β-Endosulfan were not found. The time of analysis was increased and again 1.0 mg/L standard pesticide mixture solution was injected into GC/MS. Figure 6.2 shows this chromatogram.

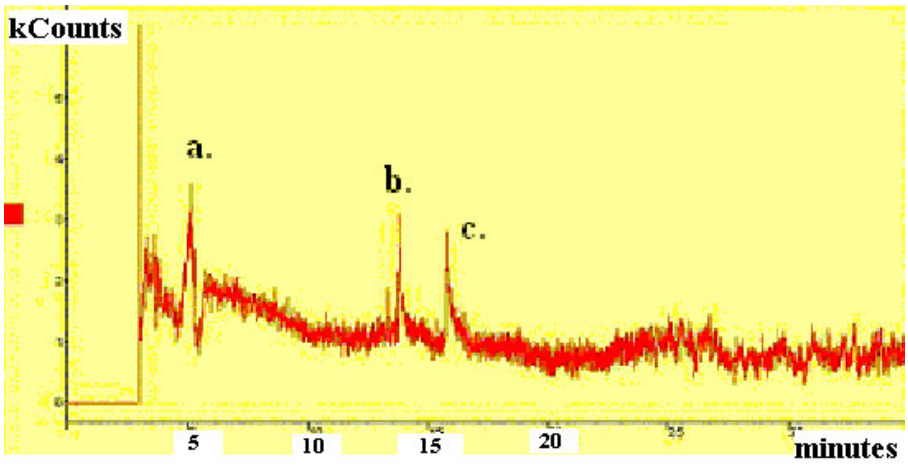


Figure 6.2 GC-MS Chromatogram of 1.0 mg/L Standard Pesticide Mixture Solution [a= Dicofol; b = α-Endosulfan; c = β-Endosulfan]

The peaks of Dicofol, α-Endosulfan and, β-Endosulfan appeared. In Figure 6.2 three peaks can be seen.

To recognize these peaks 5.0 mg/L standard Dicofol solution, 5.0 mg/L standard Endosulfan solution, and 5.0 mg/L standard pesticide mixture solution were injected respectively. Figure 6.3 shows 5.0 mg/L standard Dicofol solution chromatogram, Figure 6.4 shows 5.0 mg/L standard Endosulfan solution, and 5.0 mg/L standard pesticide mixture solution chromatogram.

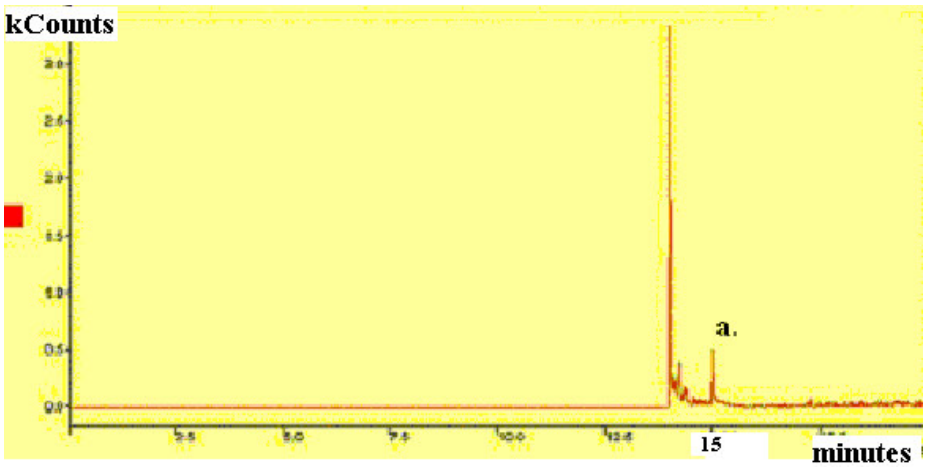


Figure 6.3 GC-MS Chromatogram of 5.0 mg/L Standard Dicofol Solution [a= Dicofol]

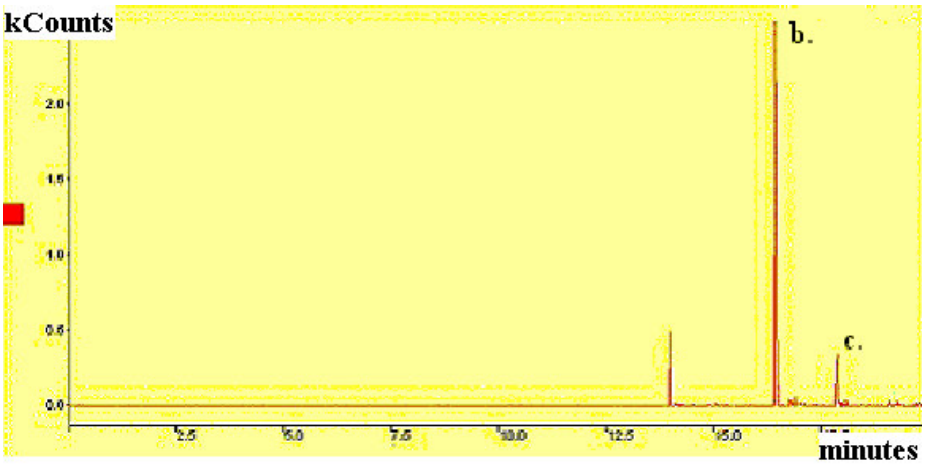


Figure 6.4 GC-MS Chromatogram of 5.0 mg/L Standard Endosulfan Solution [b = α-Endosulfan; c = β-Endosulfan]

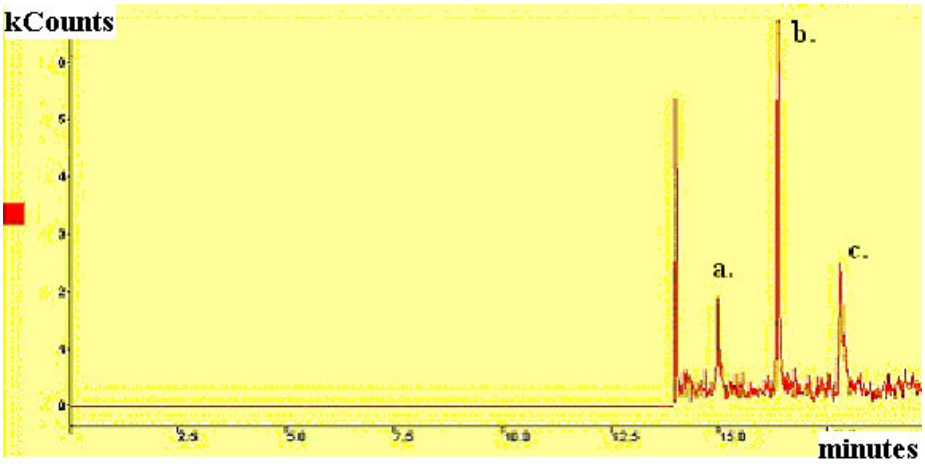


Figure 6.5 GC-MS Chromatogram of 5.0 mg/L Standard Pesticide Mixture Solution [a= Dicofol; b = α-Endosulfan; c = β-Endosulfan]

After the peaks were recognized the best conditions of GC-MS had to be found for Dicofol and Endosulfan.

Measurements under best conditions Dicofol did not give stable peaks. Dicofol readily degrades to 4, 4'-dichlorobenzophenone when exposed to a higher pH, light or a higher temperature [67], but only the parent compound is included in the pesticide residue definition [68]. But Dicofol was never recognized as one peak in our study.

Dicofol will be investigate in our future studies. Only Endosulfan was investigated in our study. The optimum conditions was found for α-Endosulfan and β-Endosulfan.

6.1 Comparison of Solvents

Two different solvents have been used which are dichloromethane and acetone. Figure 6.6 shows the chromatogram of 10.0 mg/L standard pesticide mixture solution with both acetone and dichloromethane solutions.

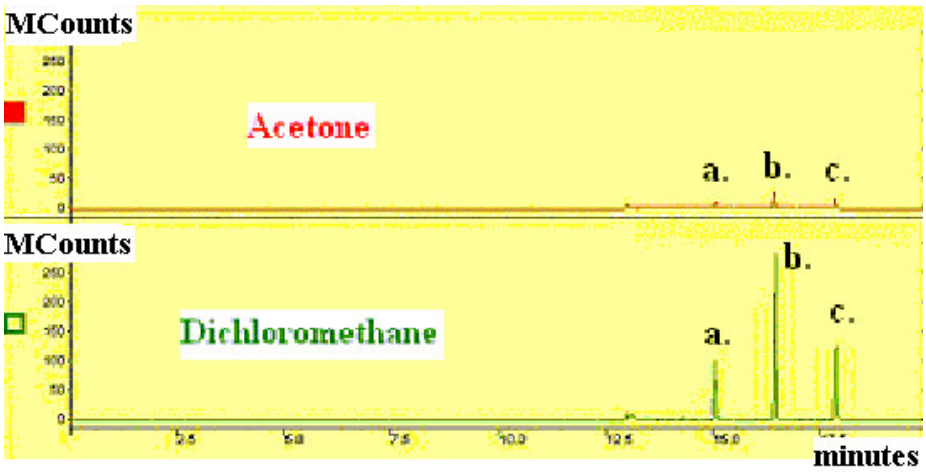


Figure 6.6 GC-MS Chromatogram of 10.0 mg/L Standard Pesticide Mixture Solution
[a= Dicofol; b = α-Endosulfan; c = β-Endosulfan]

With dichloromethane solution the chromatogram appeared better with greater peak sizes. So we had to choose the dichloromethane as a solvent.

6.2 Comparison of Column Temperature Programs

Different column programs have been tried. In our study two of them are compared; one step column temperature program, and three steps column temperature program. Table 6.1 shows these programs and their conditions.

Table 6.1 Column Temperature Programs

One Step Column Temperature Program		**Three Steps Column Temperature Program**			
Initial Temperature: 90°C	**Rate:** 10°C/min.	**Initial Temperature:** 70°C	**Rate$_1$:** 50°C/min.	**Rate$_2$:** 10°C/min.	**Rate$_3$:** 50°C/min.
Hold Time: 0.00 min.	**Final Temp.:** 250°C	**Hold Time:** 3.00 min.	**Temp$_1$:** 150°C	**Temp$_2$:** 180°C	**Final Temp:** 250°C
	Hold Time: 11 min.		**Hold Time$_1$:** 3 min.	**Hold Time$_2$:** 5 min.	**Hold Time$_3$:** 4.40 min.

Figure 6.7 shows the chromatogram of 10.0 mg/L standard pesticide mixture solution. One Step Temperature Program and Three Step Temperature Program has given almost the same results, but One Step Temperature Program had the shorter analysis time. So, One Step Temperature program was chosen.

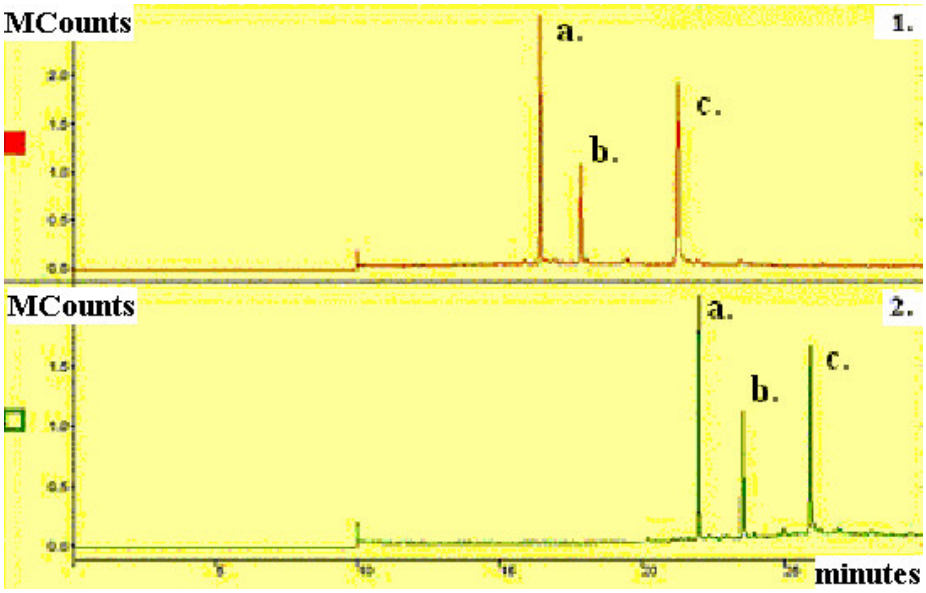


Figure 6.7 GC-MS Chromatogram of 10.0 mg/L Standard Pesticide Mixture Solution

[a= Dicofol; b = α-Endosulfan; c = β-Endosulfan]

[1=One Step Column Temperature Program; 2=Three Steps Column Temperature Program]

6.3 Comparison of Injector Temperature Programs

Three different injector programs have been compared; injector temperature program 1, injector temperature program 2, and injector temperature program 3. Table 6.2 shows these programs and their conditions.

Table 6.2 Injector Temperature Programs

Injector Temperature Program 1	Injector Temperature Program 2	Injector Temperature Program 3
Initial temperature: 280°C	**Initial temperature:** 70°C	**Initial temperature:** 45°C
Hold Time: 0.00 min.	**Hold Time:** 0.50 min.	**Hold Time:** 0.20 min.
	Rate: 100°C/min.	**Rate:** 40°C/min.
	Final temperature: 280°C	**Final temperature:** 280°C
	Hold Time: 17.40 min	**Hold Time:** 10.00 min

Figure 6.8 shows the GC-MS chromatogram of 10.0 mg/L standard pesticide mixture solution. The better result is the first spectrum Injector Program 1. This measurement has no temperature program. In Injector Program 1 α-Endosulfan and β-Endosulfan peaks size are Mcount dimensions, but in Injector Program 2 and Injector Program 3 peaks sizes are kCount dimension. So Injector Program 1 was chosen as an injector temperature program.

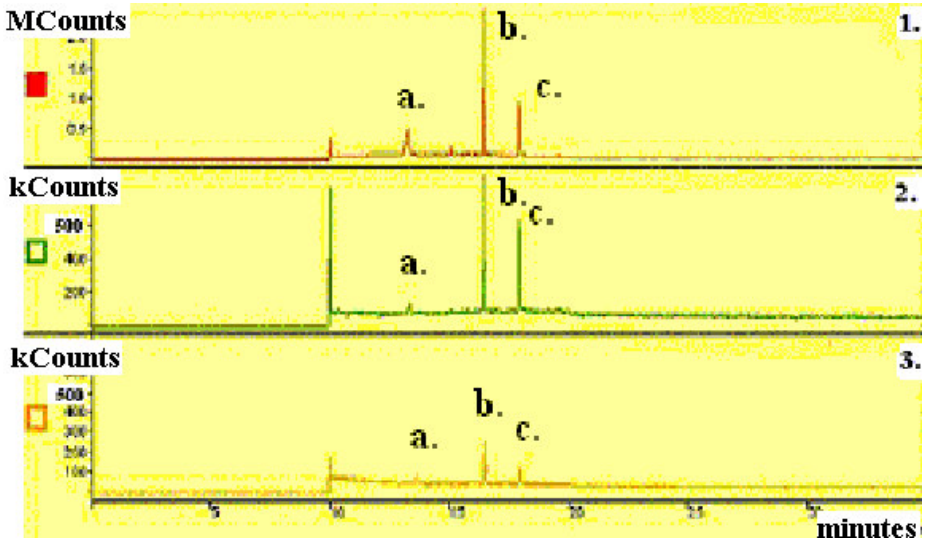


Figure 6.8 GC-MS Chromatogram of 10.0 mg/L Standard Pesticide Mixture Solution

[a= Dicofol; b = α-Endosulfan; c = β-Endosulfan]

[1= Injector Program 1; 2= Injector Program 2; 3= Injector Program 3]

6.4 Comparison of GC-MS Modes

In this study, three different methods were used for identification and quantification of α-Endosulfan and β-Endosulfan pesticides.

6.4.1 GC-MS Mode

First method was GC-MS full scan mode. This mode was used for identification of the α-Endosulfan and β-Endosulfan pesticides. Standard Endosulfan pesticide solutions were injected under full scan mode. Total ion GC-MS chromatogram (Figure 6.9) and mass spectra of α-Endosulfan and β-Endosulfan pesticides were obtained (Figure 6.10 and 6.11).

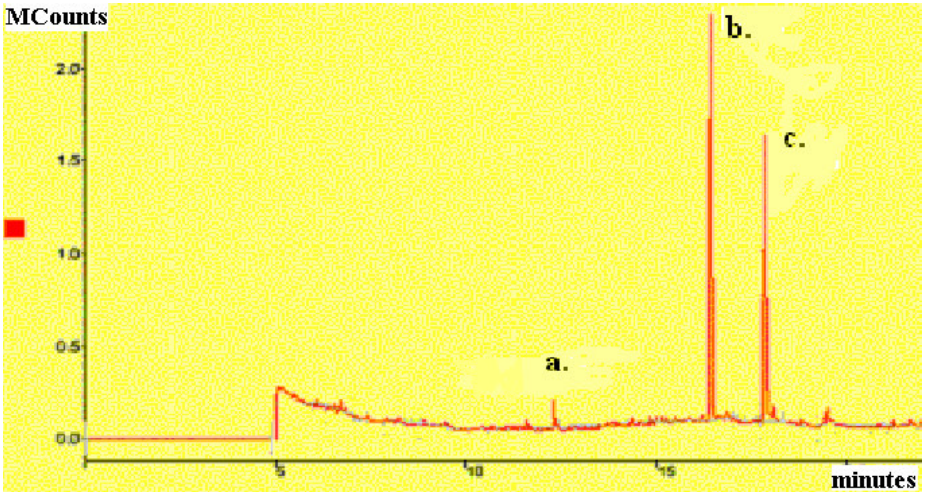


Figure 6.9 Total Ion GC-MS Chromatogram of 10.0 mg/L Standard Endosulfan Pesticide Solution

[a= Pentachloronitrobenzene (Internal Standard); b = α-Endosulfan; c = β-Endosulfan]

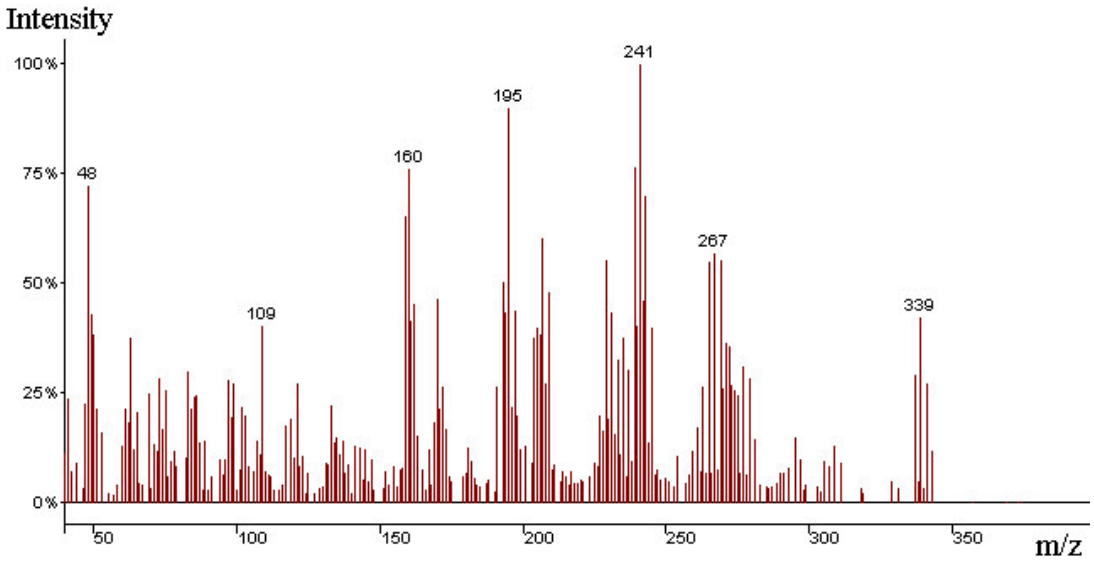


Figure 6.10 Mass Spectrum of α-Endosulfan

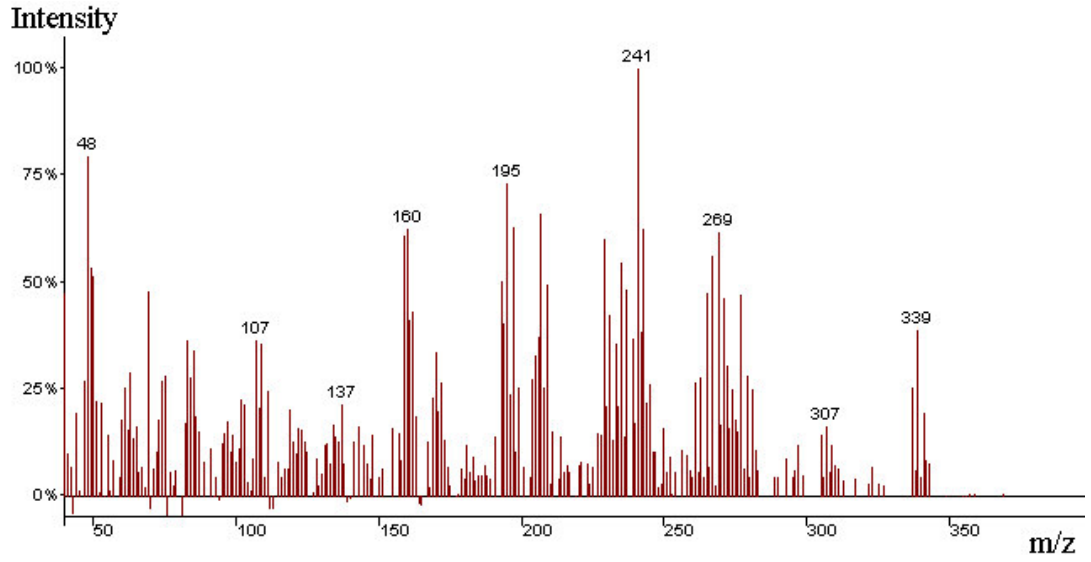


Figure 6.11 Mass Spectrum of β-Endosulfan

Obtained mass spectra of these pesticides were almost the same in the mass spectrum library (appendix B). MS full scan mode was used because it gives structural information about the target pesticides to be identified. However it was limited sensitivity.

6.4.2 GC-SIS Mode

SIS eliminates unwanted ions by ejecting them from the ion trap. Given the optimum number of ions that can be stored in the ion trap, SIS enriches the sample ions compared to the unwanted matrix ions and ejects the latter throughout ionization. Figure 6.12 shows that using GC-MS with SIS mode; selectivity of the technique improves, but with SIS mode some valuable qualitative information could be lost.

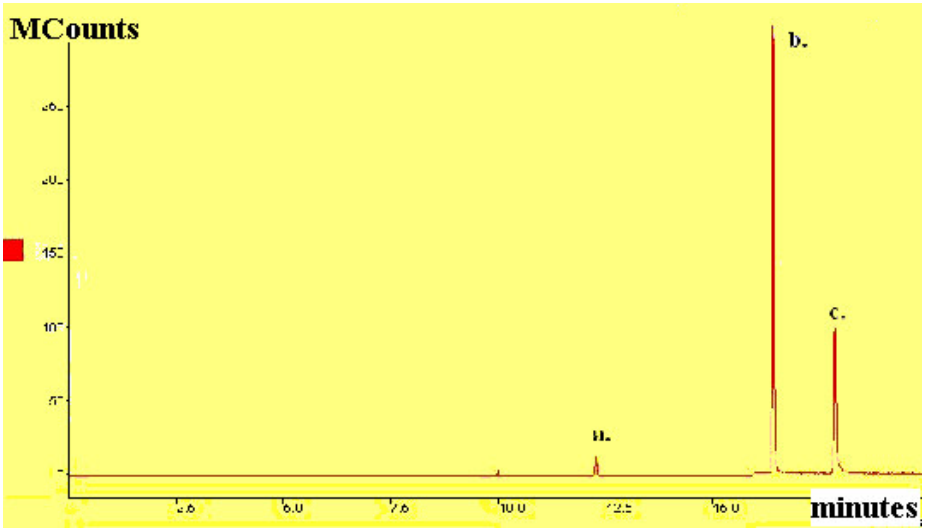


Figure 6.12 GC-MS (SIS Mode) Chromatogram of 10.0 mg/L Standard Endosulfan Pesticide Solution

[a= Pentachloronitrobenzene (Internal Standard); b = α-Endosulfan; c = β-Endosulfan]

6.4.3 GC-MS-MS Mode

Using tandem mass spectrometry (GC-MS-MS) mode; selectivity of the technique improves with a drastic reduction of the background and without losing identification capability. Also, the tandem mass technique allows highly specific MS analyses, with possibility of directly analyzing complex environmental samples without extensive clean-up steps. The MS-MS parameters are shown in Table 6.3. Tandem mass spectrometry (GC-MS-MS) chromatogram is shown in Figure 6.13.

Table 6.3 MS-MS Parameter

Pesticides	Activation Time (min)	m/e Range	Major Fragment Ion (m/e)	Excitation Amplitude (V)	Excitation Storage Level (m/e)
α-Endosulfan	4.00 – 5.25	50 – 300	241	84	80
β-Endosulfan	5.25 – 7.75	50 – 300	241	84	80

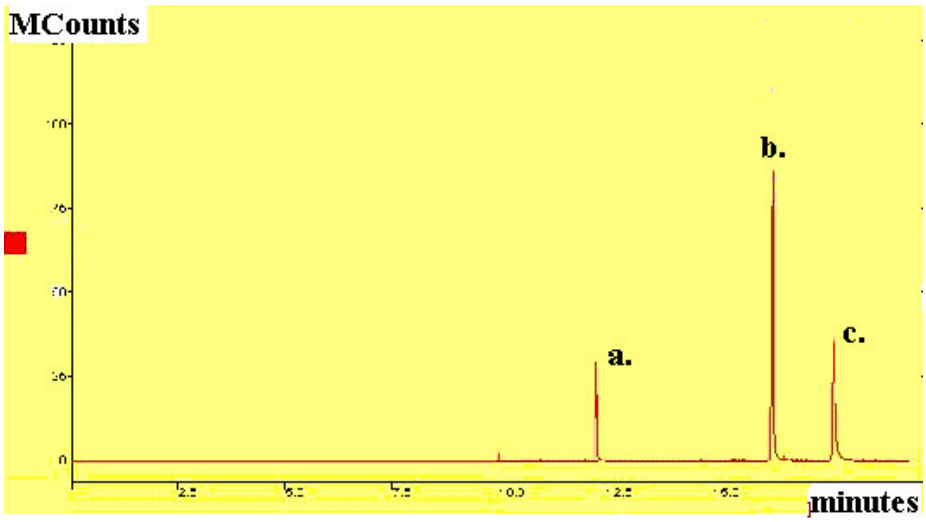


Figure 6.13 GC-MS-MS Chromatogram of 10.0 mg/L Standard Endosulfan Pesticide Solution

[a= Pentachloronitrobenzene (Internal Standard); b = α-Endosulfan; c = β-Endosulfan]

Under these situations, GC-Tandem Mass (MS-MS) mode was used for analyzing the real water samples from Tahtalı Dam.

6.5. Calibration Results

The instrument calibration for GC-MS-MS was performed by injecting standard solutions of each pesticide at levels ranging from 0.03 to 10.0 mg/L. The results are shown in Table 6.2 GC chromatograms for the lowest and highest concentration of standard solution are shown in Figure 6.14 and Figure 6.15. Good linearity of the response was found for α-Endosulfan and β-Endosulfan pesticides at concentration belonging to cited interval, with determination coefficients (or correlation coefficient)

higher than 0.996. The calibration plots for α-Endosulfan and β-Endosulfan pesticides are shown in Figure 6.16 to Figure 6.17.

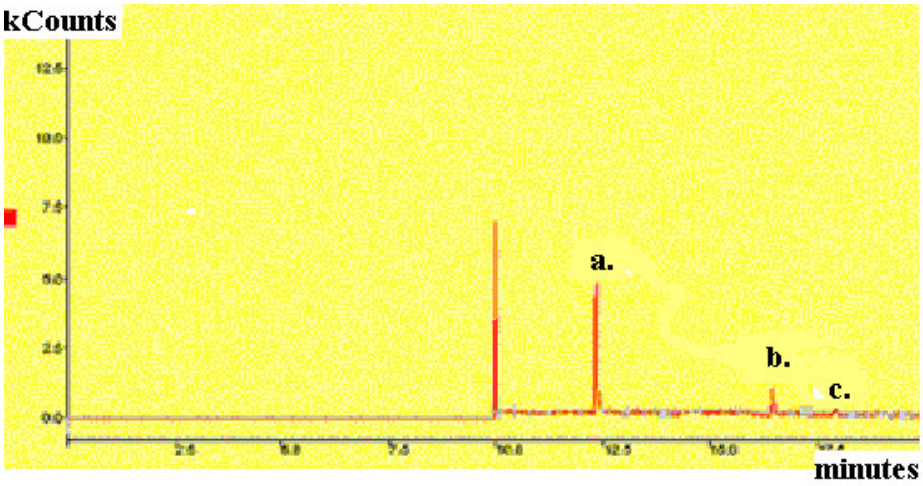


Figure 6.14 Chromatogram obtained with GC-MS-MS mode 0.03 mg/L of Endosulfan Pesticides Standard Solution.
[a= Pentachloronitrobenzene (Internal Standard); b = α-Endosulfan; c = β-Endosulfan]

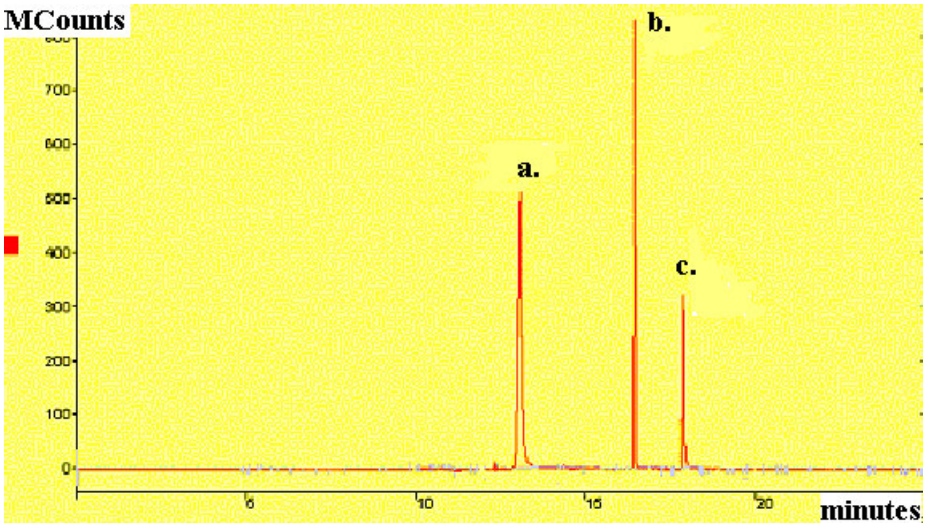


Figure 6.15 Chromatogram obtained with GC-MS-MS mode 10.0 mg/L of Endosulfan Pesticides Standard Solution.
[a= Pentachloronitrobenzene (Internal Standard); b = α-Endosulfan; c = β-Endosulfan]

Table 6.4 Retention Time Windows (RTWs) [a] and Calibration Data of GC-MS-MS Methods[b]

Pesticide	RTW[a] (min)	Precursor Ion	Studied Ion	Linear Range (mg/L)	r^2	RSD (%)	LOD[c] (µg/L) (Before preconcentration)	LOD[c] (µg/L) (After preconcentration)	LOQ[d] (µg/L) (Before preconcentration)	LOQ[d] (µg/L) (After preconcentration)
α-Endosulfan	16.46- 16.47	195	195	0.03 – 10	0.999	0.187	0.083	0.000083	0.276	0.000276
β-Endosulfan	17.90-17.92	195	195	0.03 - 10	0.996	0.087	0.662	0.000662	2.205	0.002205

[a] Retention time windows (RTWs), defined as retention time of analyte averages ± 3 standard deviation of retention times.
[b] Calibration data for GC-MS-MS obtained using relative areas of the Internal Standard (I.S.)
[c] LOD (limit of detection)
[d] LOQ (limit of quantitation)

Detection limit (LOD) (Signal-to-Noise Ratio S/N = 3) and quantitation limit (LOQ) (S/N = 10) were calculated on the values of the blank at the retention times of analytes (ten injections). They were low enough to allow the analysis of pesticides in water samples at the levels required by the EU Drinking Water Directive (0.1 µg/L individually, 0.5 µg/L in total).

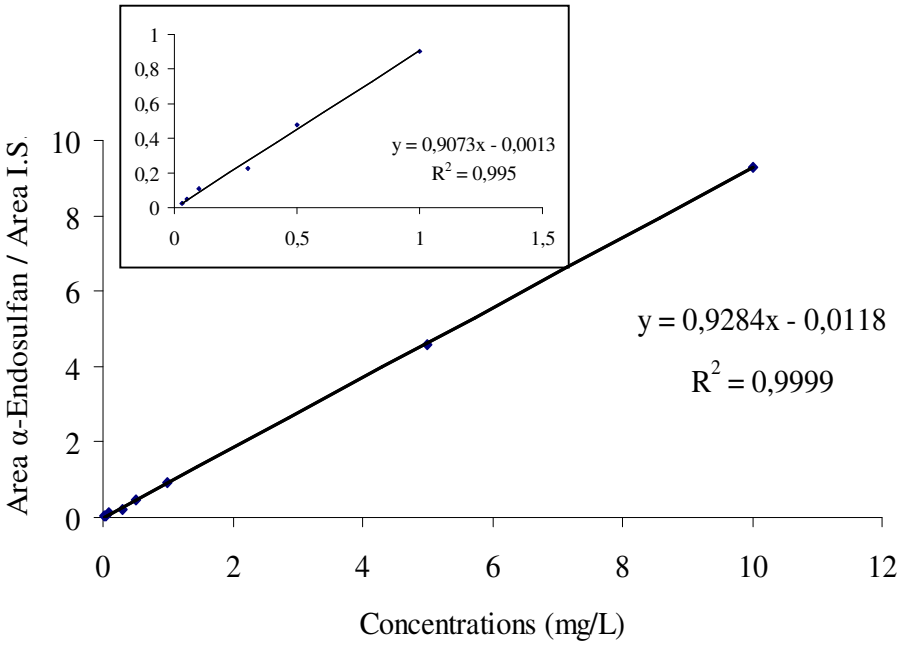


Figure 6.16 Calibration Plot for α-Endosulfan for Concentration Range of 0.03 mg/L - 10.0 mg/L

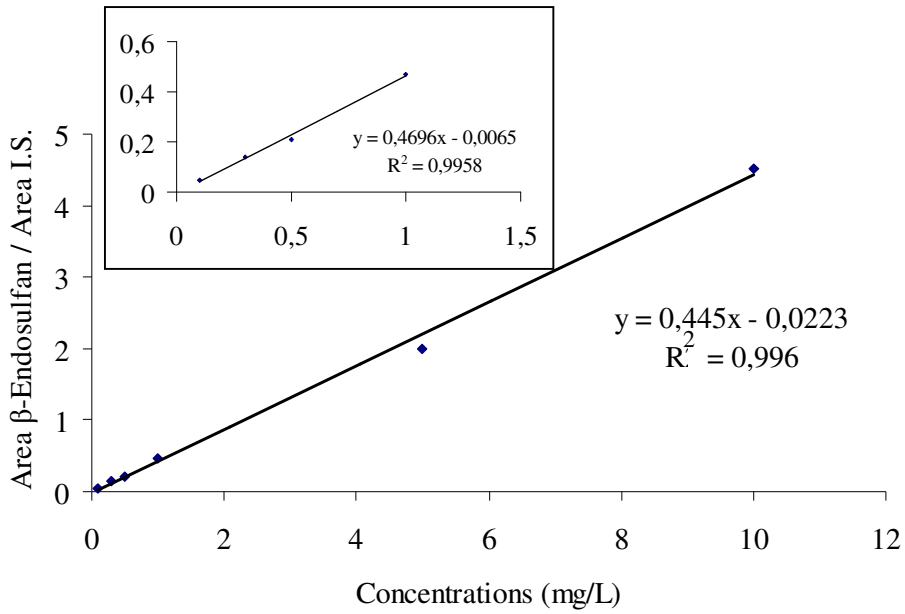


Figure 6.17 Calibration Plot for β-Endosulfan for Concentration Range of 0.03 mg/L - 10.0 mg/L

6.6. Solid Phase Extraction (SPE)

In the solid phase extraction process, ENVI™-18 DSK 47mm Solid Phase Extraction Disks were used. For each trial, three 500 ml aliquots of pesticide free water samples spiked with 1 µg/L of each target pesticide were used to study the extraction efficiency of the analytes. Eluent volume was 0.5 ml. So water samples preconcentrated 1000 times.

Three parameters pH, salt (NaCl) effect, and sample volume were studied for the recovery efficiency of the target pesticides.

The effects of three different pH values were tested; pH of pesticide free water was adjusted to 4.0, 5.0, and 6.0 by adding hydrochloric acid and NaOH before the preconcentration step. Good recoveries were obtained for α-Endosulfan and β-Endosulfan at pH 5. Recovery results are shown in Table 6.5, and Figure 6.18.

Table 6.5 Effect of pH on Recoveries in the Solid Phase Extraction Process

Pesticides	**pH**		
	4	**5**	**6**
	***Recovery %**	***Recovery %**	***Recovery %**
α-Endosulfan	82±0.002	112±0.002	97±0.001
β-Endosulfan	121±0.003	132±0.008	124±0.006

*n=3

Recoveries of α-Endosulfan and β-Endosulfan for solid phase extraction were 112 (±0.002) % and 132 (±0.008) % in 500 ml water samples spiked with 200 ng/L pesticides at pH 5.

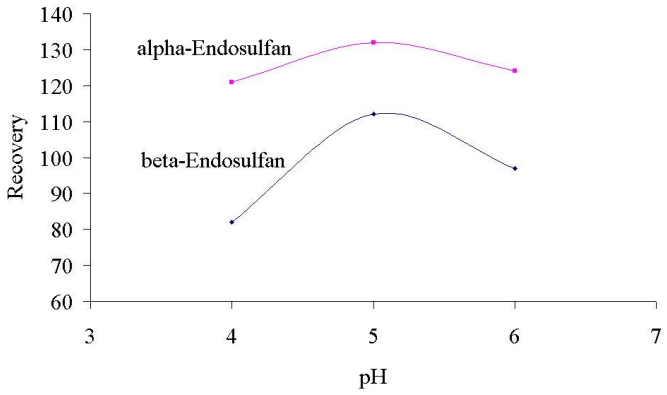


Figure 6.18 Effect of pH on The Recovery of Target Pesticides

Another parameter tested was the addition of salt (NaCl) at four different concentrations 0, 10, and 20 g/L [69]. The results as figured in Table 6.4 and Figure 6.19 show an improvement in the recoveries of target pesticides when 10 g/L of NaCl was added and so this concentration was chosen for further studies. Addition of NaCl affects the increase of ionic strength of the solution to decrease the solubility of analytes.

Table 6.6 Effect of Salt (NaCl) on Recoveries in the Solid Phase Extraction Process[1]

Pesticides	**Salt (NaCl) g/L**		
	0	**10**	**20**
	***Recovery %**	***Recovery %**	***Recovery %**
α-Endosulfan	67±0.008	112±0.002	31±0.004
β-Endosulfan	113±0.002	132±0.008	91±0.203

*n=3

1 These values were obtained at pH 5

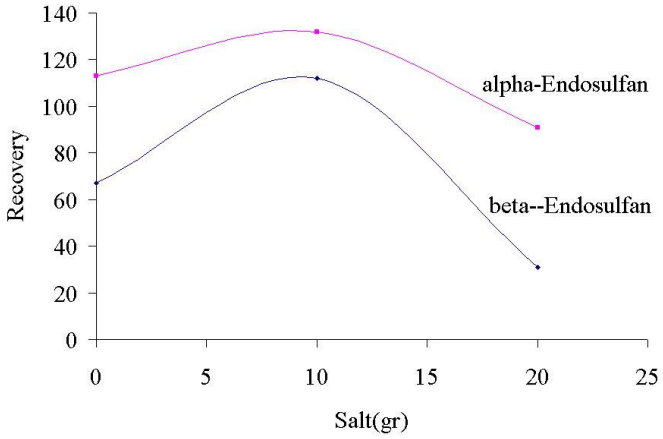


Figure 6.19 Effect of Salt Addition on the Recovery of Target Pesticides

Also, the next step was to study the recoveries of pesticides at different sample volumes. 250, 500 and 1000 ml of pesticide free water samples were spiked with different amounts of pesticides so that the pesticide concentration was always the same. In Table 6.5 recoveries for each pesticide obtained with GC-MS-MS is shown.

Table 6.7 Recoveries of Solid Phase Extraction of Pesticides at Different Sample Volumes[1]

Pesticides	Sample Volume(ml)		
	250	**500**	**1000**
	***Recovery %**	***Recovery %**	***Recovery %**
α-Endosulfan	88±0.009	112±0.002	83±0.005
β-Endosulfan	122±0.0009	132±0.009	118±0.002

*n=3

1 These values were obtained at pH 5

As seen from Table 6.7 an Figure 6.20, when the extraction volumes were increased, recoveries of pesticides decreased. Optimum a volume of 500 ml was chosen for further studies.

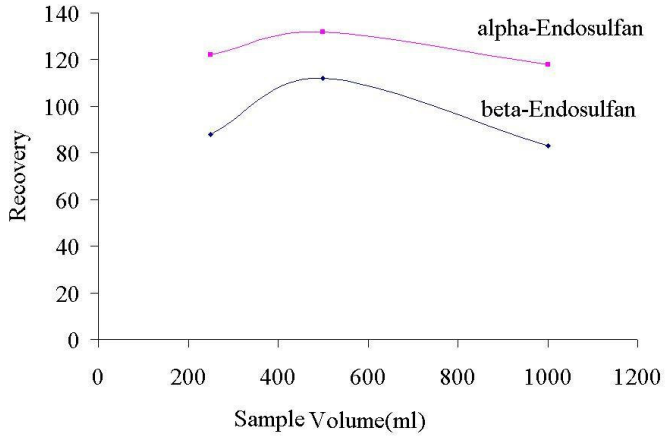
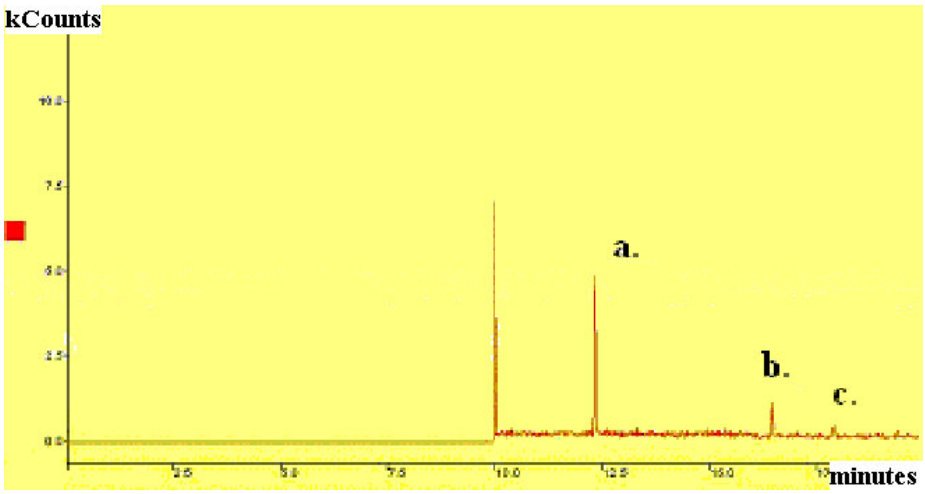


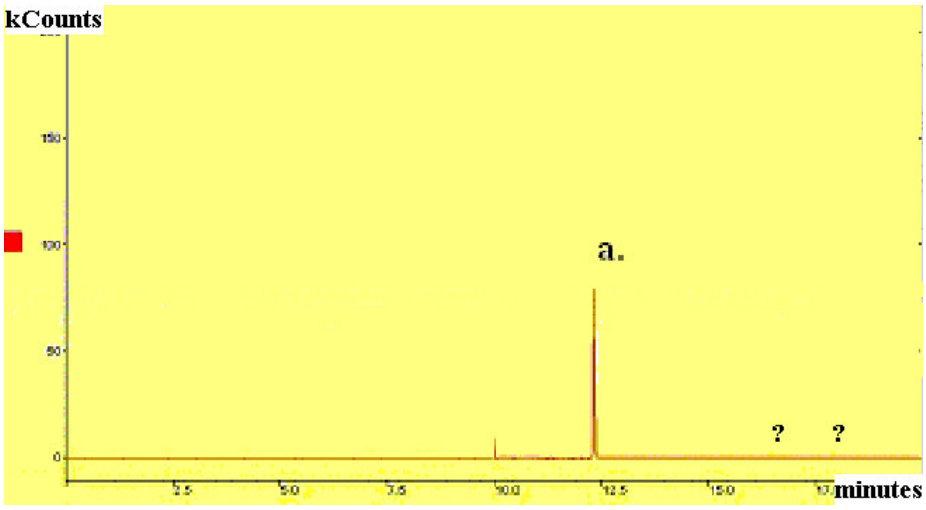
Figure 6.20 Effect of Sample Volume on the Recovery of Endosulfan Pesticide

6.7. Real Sample Analysis

Water samples which were analyzed were collected between 01 August 2002 and 01 January 2003 by İZSU. Solid Phase Extraction method was used to analyze all the water samples. Obtained results are below the detection limit for each pesticide. A typical chromatogram obtained with standard sample and a real sample from Tahtalı Dam Water are shown in Figure 6.21.



1



2

Figure 6.21 Chromatogram **1** obtained with GC-MS-MS mode 0.05 mg/L of standard pesticide solution, Chromatogram **2** obtained with GC-MS-MS mode after SPE step of 500 ml of water sample

[a= Pentachloronitrobenzene (Internal Standard); b = α-Endosulfan; c = β-Endosulfan]

In Figure 6.21 chromatogram 2 was obtained with GC-MS-MS mode from real water sample after SPE, whereas chromatogram 1 was obtained from 0.05 mg/L standard pesticides solution. Comparison of these two chromatograms show that the real sample does not give any related peaks.

As a result analysis of water samples collected between 01 August and 01 January 2003 shows that α-Endosulfan and β-Endosulfan pesticides are not present at detectable levels in Tahtalı Dam Water.

To extend the sampling time and also to confirm our first results, another water sample collected at 18 October 2004 was analyzed both by us and also by Ege Üniversitesi İlaç Araştırma-Geliştirme Merkezi. No detectable amount of α-Endosulfan and β-Endosulfan pesticides were found in both set of analysis. This confirms our early results.

All these studies lead the conclusion that α-Endosulfan and β-Endosulfan pesticides are not present at detectable levels in Tahtalı Dam Water.

CHAPTER 7

CONCLUSION

1. GC-(IT)-MS is a suitable technique for analyzing trace amounts of organic compounds.
2. During this research study GC-(IT)-MS instrument was used for analysis of Dicofol and Endosulfan organochlorine pesticides which could be present in Tahtalı Dam Water.
3. Dicofol did not give stable peaks. This is also mentioned in some literature. This can be due to the standard that we obtained. Due to time limitation we plan to investigate Dicofol in our future studies.
4. Both α-Endosulfan and β-Endosulfan were not found in detectable amounts in Tahtalı Dam Water although an enrichment technique - Solid Phase Extraction (SPE) - was used.
5. Other enrichment techniques could be used, but SPE performs better separation especially for samples with big matrix effects.
6. Solid Phase Extraction (SPE) is also preferable for environmental reasons because amount of polluting extraction solutions are minimized.
7. Soil and sediment analysis can complement our study. Although, they degrade reasonably fast in water, these pesticides are widely used in Tahtalı Dam Basin and the probability of finding them in soil or sediment may be higher.

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APPENDIX A

SATURN GC/MS WORKSTATION – METHOD LISTING

A.1. 3400 GC Method Report

GC Injector

Injector Type : Temperature Programmable

GC Injector Oven On? : Yes

Inital GC Injector Temperature : 280 ˚C

Inital GC Injector Hold Time : 0.00 minutes

GC Column

Column Oven On? : Yes

Inital GC Column Temperature : 280 ˚C

Inital GC Column Hold Time : 0.00 minutes

GC Column Temperature Program 1

Final Temperature : 280 ˚C

Rate : 20.0 ˚C/min.

Hold Time : 2.50 min

GC Relays

Relay Time Program : Use

Initial Relay States : -----

Relay Initial Conditions at Run End? : No

Relay Program 1

Relay Time : 0.01 State 1---

Relay Program 2

Relay Time : 1.00 State ----

A.2. MS Method Report

Segment Number 1

Description : FIL/MUL DELAY

Emission Current :	10 microamps
Mass Defect :	0 mmu/100u
Count Threshold:	1 counts
Multiplier Offset :	0 volts
Cal Gas :	OFF
Scan Time :	1.000 Sec.
Segment Start Time :	0.00 Min.
Segment End Time :	10.00 Min.
Segment Low Mass :	40 m/z
Segment High Mass :	650 m/z
Ionization Mode :	NONE
Ion Preparation Technique :	NONE

Segment Number 2

Emission Current :	80 microamps
Mass Defect :	0 mmu/100u
Count Threshold:	1 counts
Multiplier Offset :	300 volts
Cal Gas :	OFF
Scan Time :	1.000 Sec.
Segment Start Time :	10.00 Min.
Segment End Time :	13.00 Min.
Segment Low Mass :	50 m/z
Segment High Mass :	300 m/z
Ionization Mode :	EI/AGC
Ion Preparation Technique :	MS/MS
Target TIC :	20000 counts
Prescan Ionization Time :	1500 microseconds

Background Mass : 45 m/z

RF Dump Value : 650 m/z

MS/MS Ion Preparations

Ionization Parameters :

- Ionization Storage Levels : 48 m/z
- Ejection Amplitude : 20.0 volts

Isolation Parameters :

- Parent Ion Mass : 241.0 m/z
- Isolation Window : 3.0 m/z
- Low-edge Offset : 6 steps
- High-edge Offset : 2 steps
- High-edge Amplitude : 30.0 volts
- Isolation Time : 5 milliseconds

Dissociation Parameters :

- Waveform Type : NON-RESONANT
- Excitation Storage Level : 80.0 m/z
- Excitation Amplitude : 84.00 volts
- Excitation Time : 20 milliseconds

Segment Number 3

Emission Current : 50 microamps

Mass Defect : 0 mmu/100u

Count Threshold: 0 counts

Multiplier Offset : 300 volts

Cal Gas : OFF

Scan Time : 1.000 Sec.

Segment Start Time : 13.00 Min.

Segment End Time : 20.00 Min.

Segment Low Mass : 45 m/z

Segment High Mass : 650 m/z

Ionization Mode : EI/AGC

Ion Preparation Technique : MS/MS

Target TIC : 20000 counts

Prescan Ionization Time : 100 microseconds

Background Mass : 45 m/z

RF Dump Value : 650 m/z

MS/MS Ion Preparation :

Ionization Parameters :

Ionization Storage Levels : 48 m/z

Ejection Amplitude : 20.0 volts

Isolation Parameters :

Parent Ion Mass : 241.0 m/z

Isolation Window : 3.0 m/z

Low-edge Offset : 6 steps

High-edge Offset : 2 steps

High-edge Amplitude : 30.0 volts

Isolation Time : 5 milliseconds

Dissociation Parameters :

Waveform Type : NON-RESONANT

Excitation Storage Level : 80.0 m/z

Excitation Amplitude : 84.00 volts

Excitation Time : 20 milliseconds

Segment Number 4

Emission Current : 50 microamps

Mass Defect : 0 mmu/100u

Count Threshold: 0 counts

Multiplier Offset : 200 volts

Cal Gas : OFF

Scan Time : 1.000 Sec.

Segment Start Time : 7.75 Min.

Segment End Time : 10.00 Min.

Segment Low Mass : 50 m/z

Segment High Mass :	400 m/z
Ionization Mode :	EI/AGC
Ion Preparation Technique :	SIS
Target TIC :	10000 counts
Prescan Ionization Time :	100 microseconds
Background Mass :	50 m/z
RF Dump Value :	650 m/z

SIS Ion Preparation :

Mass Range 1 : 294 to 296

APPENDIX B

GC / MS MASS SPECTRA LIBRARY

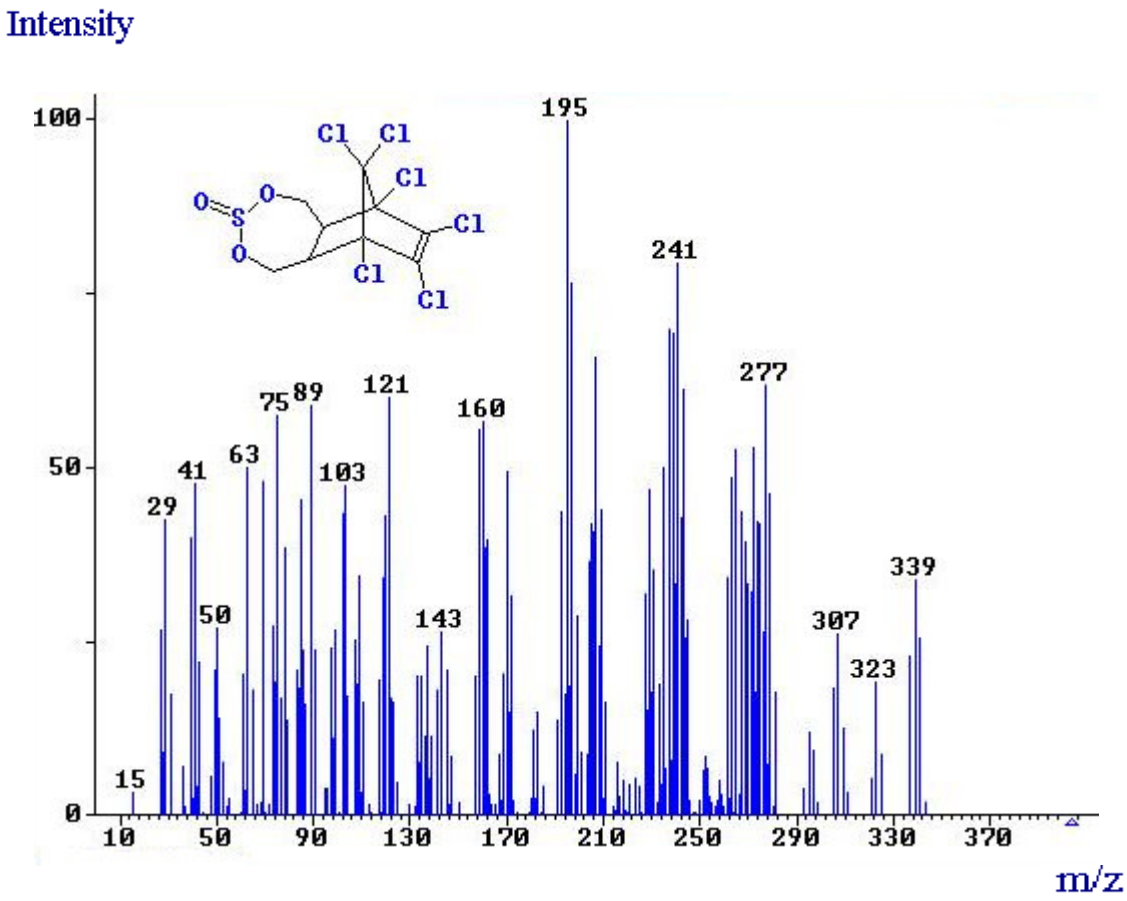

Figure B.1. Mass Spectrum of α-Endosulfan (from NIST Pesticides Library)

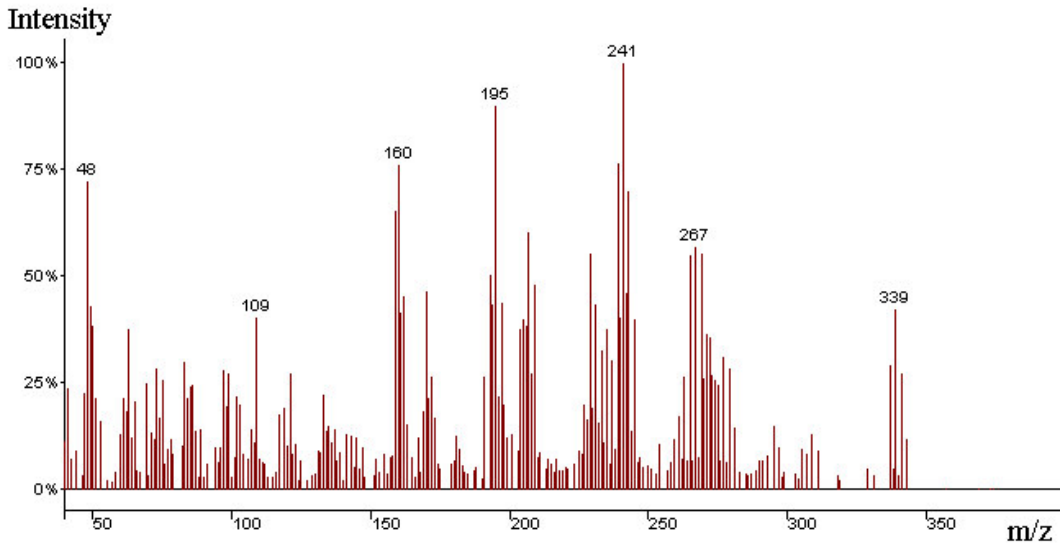

Figure B.2. Mass Spectrum of α-Endosulfan

This mass spectrum (Figure B.2.) was obtained using Varian 3400 CX Gas Chromatograph - Saturn 2000 Mass Spectrometer instrument.

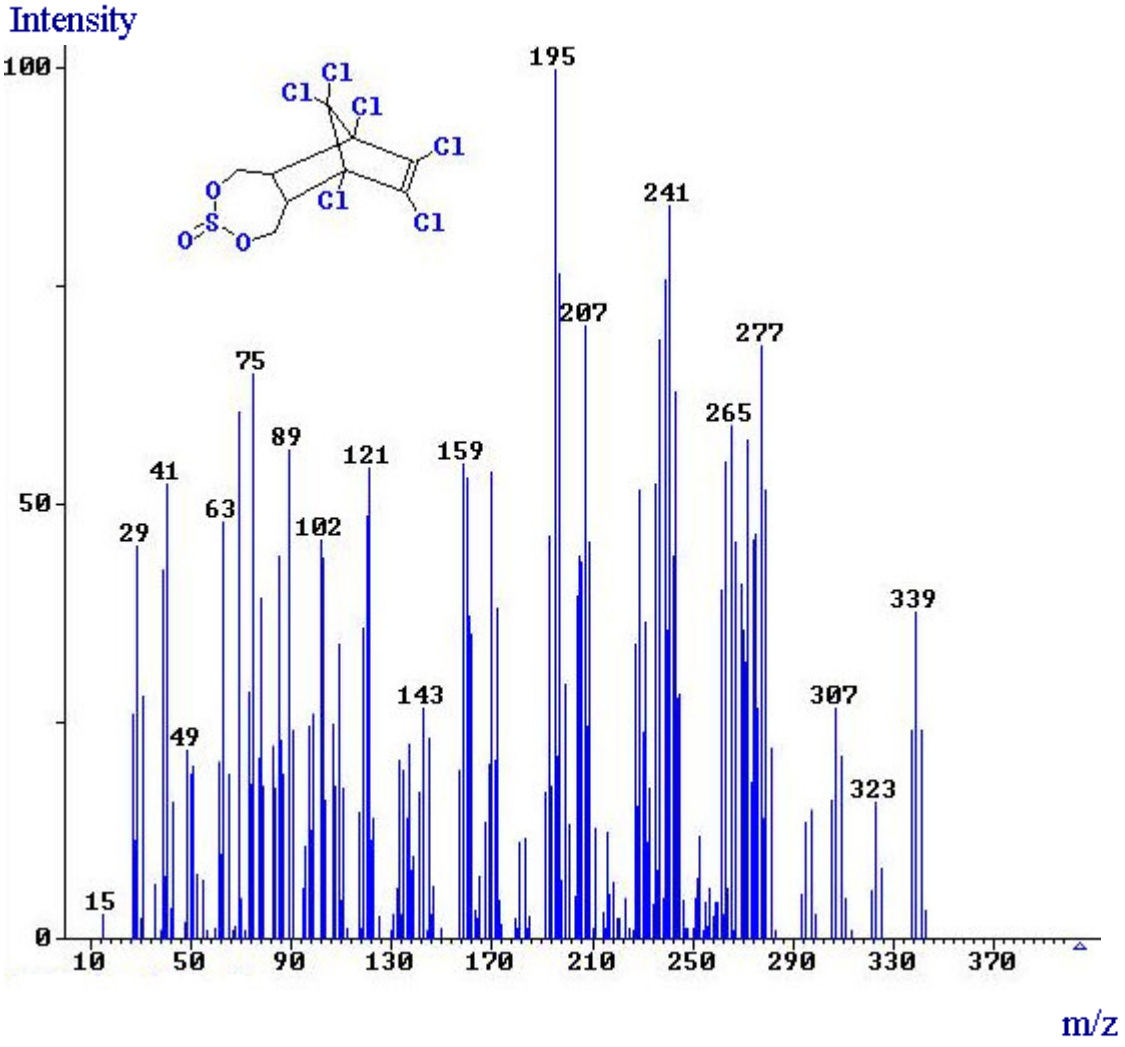

Figure B.3. Mass Spectrum of β-Endosulfan (from NIST Pesticides Library)

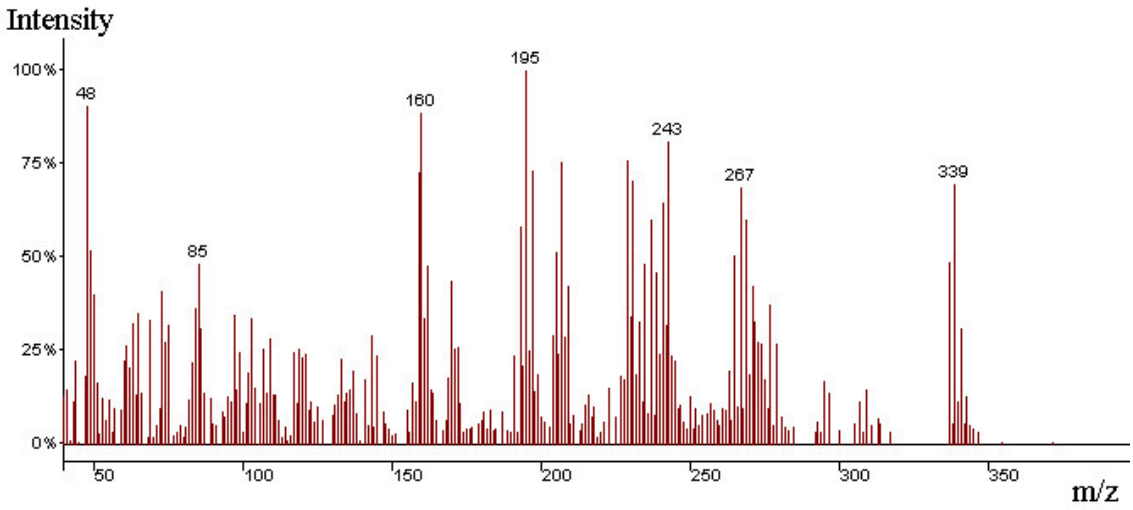

Figure B.4. Mass Spectrum of β-Endosulfan

This mass spectrum (Figure B.4.) was obtained using Varian 3400 CX Gas Chromatograph - Saturn 2000 Mass Spectrometer instrument.

APPENDIX C

General Informatıon ABOUT Tahtalı Dam

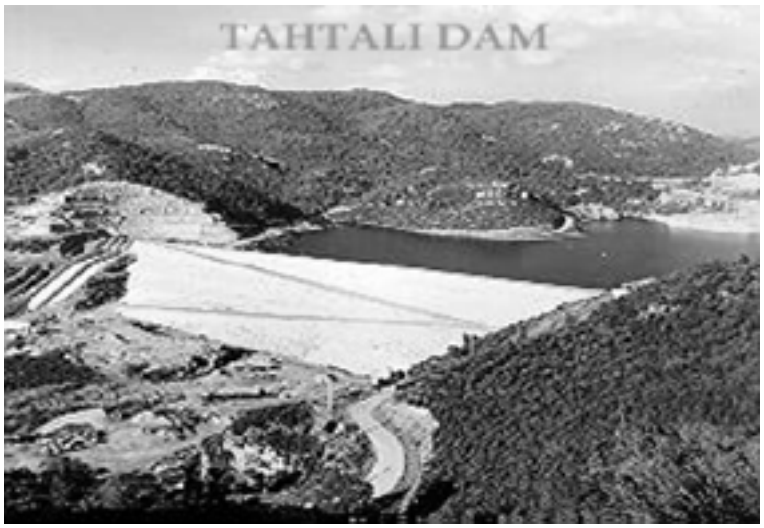

Figure C.1. General View Of Tahtalı Dam

- Location: Seferihisar / İzmir / TÜRKİYE,
- Construction started in 1986 and was completed in 1996,
- Used as a Domestic and industrial water supply,
- Volume: 297,200,000 m^3,
- Annual domestic water: 205,000,000 m^3.