Investigation of Dichlorvos (DDVP) and Trifluralin Pesticide Levels In Tahtalı Dam Water

By

Murat ERDOĞAN

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ABSTRACT

In this study, dichlorvos (Dimethyl 2,2-dichlorovinyl phosphate - DDVP) and trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidin) pesticide concentration levels in Tahtalı Dam Water were investigated. Dichlorvos is an organophosphorus pesticide, whereas Trifluralin is a dinitroaniline pesticide.

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 was not straightforward and special determination technique has to be used. That is why these pesticides were not studied extensively for İzmir area.

For the determination of the above-mentioned pesticides, gas chromatography-mass spectrometry (GC-MS) was generally preferred as reported in most papers [1,2,3]. The GC-MS instrument in our laboratory has an Ion Trap (IT) mass detector. 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. The detection limits of the instrument are 0.8 μg/L for trifluralin and 10.5 μg/L for dichlorvos, 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.

Liquid-Liquid Extraction (LLE) and Solid Phase Extraction (SPE) methods were used for sample preconcentration. Gas chromatography (GC) - Mass spectrometry (MS) and Tandem mass spectrometry (MS–MS) were employed for the identification and quantification of Trifluralin and Dichlorvos (DDVP) pesticides. For Solid Phase Extraction procedure ENVI-18 Disk was used, optimizing the extraction volume, pH and the salt concentration. Liquid-Liquid extraction procedure was also used, optimizing the extraction volume. In GC–MS–MS, the lowest detectable concentrations for the Trifluralin and Dichlorvos were found as 0.8 ng/L and 10.5 ng/L, respectively. Recovery of Dichlorvos for Liquid-Liquid Extraction and Solid Phase Extraction were 86.0 (±5.4) % and 63.0 (±5.7) % in water samples spiked with 200 ng/L pesticides. Recovery of the Trifluralin for Liquid-Liquid Extraction and Solid Phase Extraction

were 90.8 (±9.4) % and 107.5 (±4.5) % in water samples spiked with 200 ng/L pesticides.

Water samples, which were collected between 01 June 2002 to 30 September 2002 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. Analysis of samples showed no detectable Trifluralin and Dichlorvos levels in Tahtalı Dam Water.

ÖZ

Bu çalışmada, Tahtalı Baraj suyunda, diklorvos (Dimetil 2,2-diklorovinil fosfat) (DDVP) ve trifluralin (α,α,α-trifloro-2,6-dinitro-N,N-dipropil-p-tolidin) pestisitlerinin derişim seviyeleri incelenmiştir. Diklorvos organofosforlu, trifluralin de dinitroanilin pestisitidir.

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

Bu pestisitlerin seçilmesinin diğer bir nedeni de, bunların 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 [1,2,3]. Laboratuvarımızdaki GC-MS cihazı İyon Kapanlı (IT) kütle dedektörüne sahiptir. Bu cihazın hassasiyeti ve seçiciliği, Seçilmiş İyon Saklama (SIS) ve Kütle-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 trifluralin için 0,8 µg/L ve diklorvos için de 10,5 µg/L' dir. Yüzey suyunda çalışma seviyesi 1-3 µg/L ve içme suyunda 0,1 µg/L olduğu için hala bir önderiştirme basamağına ihtiyaç duyulmuştur.

Örneklerin önderiştirilmesi amacıyla Sıvı-Sıvı Özütleme (LLE) ve Katı Faz Özütlemesi (SPE) metotları kullanılmıştır. Trifluralin ve diklorvos pestisitlerinin tanımlanması ve miktarlarının belirlenmesi için GC-MS ve MS-MS yöntemleri kullanılmıştır. ENVI-18 Disk kullanılarak yapılan Katı Faz Özütlemesi işlemi için hacim, pH ve tuz derişimi optimize edilmiştir. Sıvı-Sıvı Özütlemesi işlemi içinde hacim optimize edilmiştir. GC-MS-MS ile trifluralin ve diklorvos için en düşük saptama sınırı sırasıyla 0,8 ng/L ve 10,5 ng/L bulunmuştur. Su örneklerine eklenen 200 ng/L derişimindeki pestisitlerin Sıvı-Sıvı Özütlemesi ve Katı Faz Özütlemesi kullanılarak yapılan diklorvosa ait geri kazanım sonuçları % 86,0 (±5,4) ve % 63,0 (±5,7)' tür. Aynı şekilde Sıvı-Sıvı Özütlemesi ve Katı Faz Özütlemesi kullanılarak yapılan trifluraline ait geri kazanım sonuçları % 90,8 (±9,4) ve % 107,5 (±4,5)' tir.

İZSU (İzmir Büyükşehir Belediyesi Su ve Kanalizasyon Genel Müdürlüğü) tarafından 01 Haziran 2002 ile 30 Eylül 2002 tarihleri arasında toplanan su örneklerinin önderiştirme işleminden sonra MS-MS modu ile GC-MS sisteminde analizleri yapıldı. Örneklerin analizinde, Tahtalı Baraj Suyunda ölçülebilir seviyede Trifluralin ve Diklorvos bulunmamıştır.

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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 residues [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 determination of pesticides in water, and positive and negative chemical ionization modes are alternative methods, which depending on the compounds, 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 papers 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) and European Union (EU) Waters Directives of 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 levels, a preconcentration procedure for the analytes must be applied. Preconcentration of contaminants from water samples, and generally sample preparation steps, are often accomplished 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 [24,25,26,27].

1.1. Thesis Objective

In this study, investigation of Dichlorvos (DDVP) and Trifluralin pesticide levels in Tahtalı Dam Water, which is the most important drinking water supply in İzmir were carried out. Study of the variation of Dichlorvos (DDVP) and Trifluralin 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 DDVP and Trifluralin pesticides and the examination of their levels in the Tahtalı Dam Water was studied.

According to the literature and some official organizations [World Health Organization (WHO), USA Environmental Protection Agency (EPA)], 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, Liquid-Liquid Extraction (LLE) and Solid-Phase Extraction (SPE) methods were used for sample preconcentration process.

CHAPTER 2

PESTICIDES AND THEIR PROPERTIES

2.1. Pesticides

Pesticides are natural or synthetic substances used to kill various kinds of animal and plant pests. They are used mainly in agriculture, and also in veterinary, household and hygiene products, and in health protection. The name is derived from the Latin words *pestis* (pestilence, plague) and *caedere* – to kill.

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.

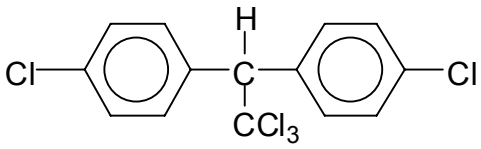


Figure 2.1. Molecular Structure of DDT

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 compound, 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.

Table 2.1. Historical Development of Pesticides

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.
1800's	Many developments occur.
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.
1867	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.
1900's	Insecticides, fungicides and herbicides have all been discovered.
early 1900's	Inorganic substances introduced.
1932	Products to control house hold pests marketed.
1939	The Second World War causes three discoveries: 1. the insecticide DDT. 2. the organophosphorus insecticides. 3. the selective phenoxyacetic herbicides.
1945	After the Second World War, farming intensity intensified production.
1950's	Geigy introduces the carbamates.

2.1.1. Classification of Pesticides

Pesticides can be classified according to their use and chemical structure. 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)

According to their chemical structure, pesticides are classified as inorganic and organic. The inorganic pesticides now constitute only a small part of the pesticides in use. Examples of inorganic pesticides are:

- arsenical pesticides : Paris green [$Cu(CH_3COO)_2 \cdot Cu_3(AsO_3)_2$]
- fluoride insecticides : Cryotile (Na_2AlF_6)
- inorganic herbicides : Borax ($Na_2B_4O_7$)
- inorganic fungicides : Bordeaux mixture ($3Cu(OH)_2 \cdot CuSO_4 \cdot CaSO_4$)

Among the organic pesticides, the following main groups are found: organochlorine pesticides (chlorinated hydrocarbons), organophosphorus pesticides, carbamates, derivatives of phenoxyacetic acid, urea pesticides, derivatives of triazines. Examples of main groups are:

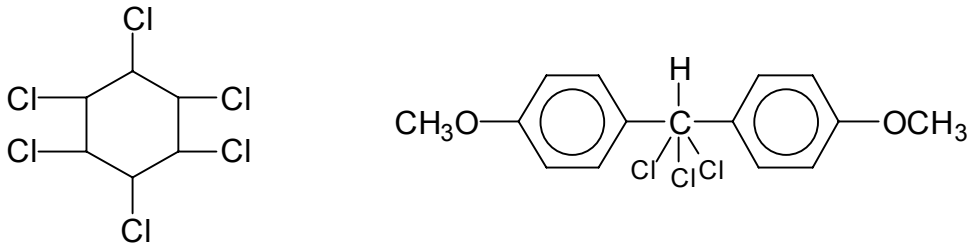
- Organochlorine Pesticides: Hexachlorocyclohexane, DDT, dieldrin, aldrin (hexachloro-hexahydro-dimethano naphtalene), endrin, chlordane, endsulphan (1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylene sulfite), mirex, isobenzene, heptachlor, methoxychlor (1,1,1-trichloro-2,2-bis (p-methoxyphenyl)ethane), pentachlorophenol.
- Organophosphorus Pesticides: Dimethoate, parathion (O, O´-diethyl O´´-nitrophenyl phosphorothioate), malathion (S-(1,2-bis[ethoxycarbonyl]ethyl)-O,O´-dimethyl phosphorodithioate) , dichlorvos (Dimethyl 2,2-dichlorovinyl phosphate), fenthion, chlorfenvinphos, chlorpyrifos, glyphosate (N-(phosphonomethyl)glycine).
- Carbamate Pesticides: Aminocarb, propoxur, carbaryl, aldicarb, dioxacarb, maneb(manganese ethylenebis(dithiocarbamate)).
- Phenoxyacetic acid Herbicides: 2,4-D (2,4-dichlorophenoxyacetic acid), dicamba, 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), MCPA, silvex, 2,3,6-TBA.
- Triazine Herbicides. Simazine, atrazine, propazine.
- Urea Pesticides: Monuron, linion, fenuron, isoproturon, chlorotoluran.
- Pyridinium Herbicides: Diquat, paraquat.

In addition to these main groups there are a lot of individual chemical compounds that are used pesticides, such as trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidin).

2.1.2. Chemical Structure of Pesticides

Pesticides are classified according to their chemical structures. The chemical structures of some important pesticides are given in Figure 2.2. (chlorinated pesticides), 2.3. (organophosphorus pesticides), 2.4. (carbamates), 2.5. (chlorinated phenoxy acid herbicides), and 2.6. (Dinitroaniline Herbicide).

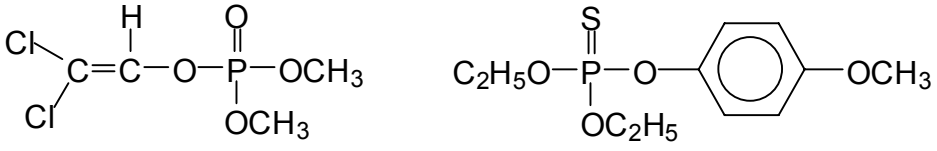
- *Chlorinated Pesticides*



(a) **(b)**

Figure 2.2. (a) Molecular Structure of Hexachlorocyclohexane (HCH)
(b) Molecular Structure of Methoxychlor (DMDT)

- *Organophosphorus Pesticides*



(a) **(b)**

Figure 2.3. (a) Molecular Structure of Dichlorvos (DDVP)
(b) Molecular Structure of Parathion

- *Carbamates*

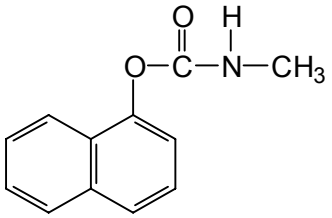


Figure 2.4. Molecular Structure of Carbaryl

- *Chlorinated Phenoxy Acid Herbicides*

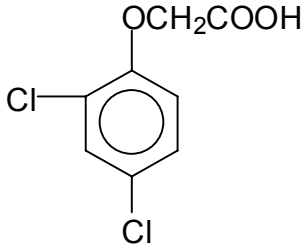


Figure 2.5. Molecular Structure of 2,4-Dichlorophenoxyacetic acid (2,4-D)

- *Dinitroaniline Herbicide*

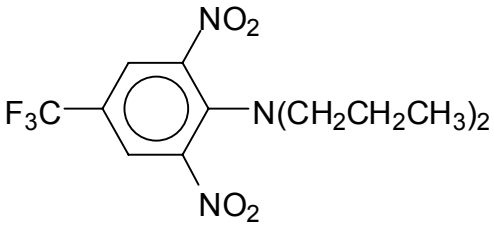


Figure 2.6. Molecular Structure of Trifluralin

2.1.3. 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.

2.1.4. 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.

2.1.5. 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 2.2 [28].

Table 2.2. Solubility of Some Pesticides

		Compound	Solubility, mg/L
INSECTICIDES	Organo-Chlorine	DDT	0.0012
		Aldrin	0.01
		Heptachlor	0.056
		Methoxychlor	0.10
		Dieldrin	0.18
		Endrin	0.23
		Toxaphene	0.30
		Lindane	7.0
	Organo-Phosphorus	Parathion	24.0
		Disulfon	25.0
		Diazinon	40.0
		Chlorfenvinfos	145.0
		Malathion	145.0
		Methyl demeton	330.0
		Dichlorvos	10000.0
		Dimethoate	2500.0
	Carbamates	Carbaryl	40.0
		Carbofuran	700.0
		Aldicarb	6000.0
	Herbicides	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 2.3 [29].

Table 2.3. 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
Dichlone	Diazinon	Chlorfenvinfos	Dieldrin
Dicrotophos	Disulfoton	Chloroxuron	Heptachlor
Endotol	Fenuron	Dimethoate	Isodrin
Endosulfan	MCPA	Diphenamid	Monocrotophos
Fenitrothion	Methoxychlor	Diuron	Benomyl
Malathion	Monuron	Ethion	
Methiocarb	Phorate	Fensulfothion	
Methylparathion	Propham	Linuron	
Parathion	Dichlorvos	Prometion	
Phophamidon		Propazine	
Propoxur		Simazine	
2,4-D		Toxaphene	
Trifluralin			

The persistent pesticides such as DDT, γ-HCH (Hexachlorocyclohexane), dieldrin, endrin and others have only slight solubility in water. However, they usually readily 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 Figure 2.7 [30].

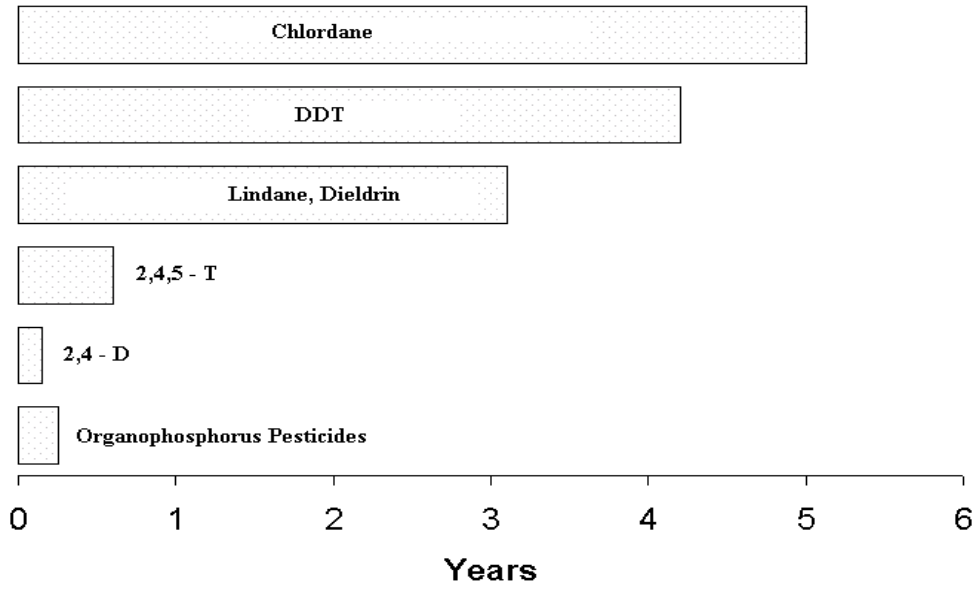


Figure 2.7. The Half-life of Some Pesticides in the Environment

2.1.6. 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.)[30].

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.) [30].

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 either 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 [23].

CHAPTER 3

DICHLORVOS (DDVP) AND TRIFLURALIN PESTICIDES AND THEIR PROPERTIES

3.1. Dichlorvos (DDVP)

Dichlorvos (DDVP) is an organophosphate compound used to control household, public health, and stored product insects. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips, and white flies in greenhouse, outdoor fruit, and vegetable crops. Dichlorvos is used to treat a variety of parasitic worm infections in dogs, livestock, and humans. Dichlorvos can be fed to livestock to control botfly larvae in the manure. It acts against insects both as a contact and a stomach poison. It is used as a fumigant and has been used to make pet collars and pest strips. It is available as an aerosol and soluble concentrate.

3.1.1. General Properties of Dichlorvos (DDVP)

Trade names include Apavap, Benfos, Cekusan, Cypona, Derriban, Derribante, Devikol, Didivane, Duo-Kill, Duravos, Elastrel, Fly-Bate, Fly-Die, Fly-Fighter, Herkol, Marvex, No-Pest, Prentox, Vaponite, Vapona, Verdican, Verdipor, and Verdisol. EPA has classified it as toxicity class I - highly toxic, because it may cause cancer and there is only a small margin of safety for other effects. Products containing dichlorvos must bear the Signal Words DANGER - POISON.

3.1.2. Physical Properties

- *CAS (Chemical Abstracts Services) Number:* 62-73-7
- *Chemical Name:* 2,2-dichlorovinyl dimethyl phosphate [31]

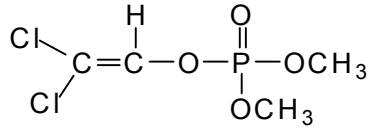


Figure 3.1. Molecular Structure of Dichlorvos (DDVP)

- *Appearance:* Dichlorvos is a colorless to amber liquid with a mild chemical odor [31].
- *Molecular Weight:* 220.98 g/mol
- *Water Solubility:* 10,000 mg/L (estimated) [31]
- *Solubility in Other Solvents:* dichloromethane, v.s[*].; 2-propanol, v.s.; toluene v.s.; ethanol s.[**]; chloroform s.; acetone s.; kerosene s. [31]

  * *v.s:* very soluble, [**]*s:* soluble
- *Melting Point:* Not Available
- *Vapor Pressure:* 290 mPa at 20 ^{0}C [31]
- *Partition Coefficient:* Not Available
- *Adsorption Coefficient:* 30 (estimated) [32]

3.1.3. Toxicological Effects

- **Acute toxicity:** Dichlorvos is highly toxic by inhalation, dermal absorption, and ingestion [33,34]. Because dichlorvos is volatile, inhalation is the most common route of exposure. As with all organophosphates, dichlorvos is readily absorbed through the skin. Acute illness from dichlorvos is limited to the effects of cholinesterase inhibition. Compared to poisoning by other organophosphates, dichlorvos causes a more rapid onset of symptoms, which is often followed by a similarly rapid recovery [33,34]. This occurs because dichlorvos is rapidly metabolized and eliminated from the body. People with reduced lung function, convulsive disorders, liver disorders, or recent exposure to cholinesterase inhibitors will be at increased risk from exposure to dichlorvos. Alcoholic beverages may enhance the toxic effects of dichlorvos. High environmental temperatures or exposure of dichlorvos to light may enhance its toxicity [33,34]. Dichlorvos is mildly irritating to skin [34]. Concentrates of dichlorvos may cause burning sensations, or actual burns [33]. Application of 1.67 mg/kg dichlorvos in rabbits' eyes produced mild redness and swelling, but no injury to the cornea [34]. Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, in coordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, slow heartbeat. Very high doses may result in unconsciousness,

incontinence, and convulsions or fatality. Some organophosphates may cause delayed symptoms beginning 1 to 4 weeks after an acute exposure that may or may not have produced immediate symptoms. In such cases, numbness, tingling, weakness, and cramping may appear in the lower limbs and progress to in coordination and paralysis. Improvement may occur over months or years, but some residual impairment may remain [34]. The oral LD50 for dichlorvos is 61 to 175 mg/kg in mice, 100 to 1090 mg/kg in dogs, 15 mg/kg in chicken, 25 to 80 mg/kg in rats, 157 mg/kg in pigs, and 11 to 12.5 mg/kg in rabbits [31,33,34]. The dermal LD50 for dichlorvos is 70.4 to 250 mg/kg in rats, 206 mg/kg in mice, and 107 mg/kg in rabbits [31,33,34]. The 4-hour LC50 for dichlorvos is greater than 0.2 mg/L in rats [34].

- **Chronic toxicity:** Repeated or prolonged exposure to organophosphates may result in the same effects as acute exposure, including the delayed symptoms. Other effects reported in workers repeatedly exposed include impaired memory and concentration, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, sleepwalking, and drowsiness or insomnia. An influenza like condition with headache, nausea, weakness, loss of appetite, and malaise has also been reported [34]. Repeated, small doses generally have no effect on treated animals. Doses of up to 4 mg/kg of a slow release formulation, given to cows to reduce flies in their feces, had no visibly adverse effects on the cows; but blood tests of these cows indicated cholinesterase inhibition [33]. Feeding studies indicate that a dosage of dichlorvos very much larger than doses which inhibit cholinesterase are needed to produce illness. Rats tolerated dietary doses as high as 62.5 mg/kg/day for 90 days with no visible signs of illness, while a dietary level of 0.25 mg/kg/day for only 4 days produced a reduction in cholinesterase levels [33]. Rats exposed to air concentrations of 0.5 mg/L of dichlorvos over a 5-week period exhibited significantly decreased cholinesterase activity in the plasma, red blood cells, and brain. Dogs fed dietary doses of 1.6 or 12.5 mg/kg/day for 2 years showed decreased red blood cell cholinesterase activity, increased liver weights, and increased liver cell size occurred [35]. Chronic exposure to dichlorvos will cause fluid to build up in the lungs (pulmonary edema). Liver enlargement has occurred in pigs maintained for long periods of time on high doses [33].

Dichlorvos caused adverse liver effects, and lung hemorrhages may occur at high doses in dogs [34]. In male rats, repeated high doses caused abnormalities in the tissues of the lungs, heart, thyroid, liver, and kidneys [34].

- **Reproductive effects:** There is no evidence that dichlorvos affects reproduction. When male and female rats were given a diet containing 5 mg/kg/day dichlorvos just before mating, and through pregnancy and lactation for females, there were no effects on reproduction or on the survival or growth of the offspring, even though severe cholinesterase inhibition occurred in the mothers and significant inhibition occurred in the offspring. The same results were observed in a three-generation study with rats fed dietary levels up to 25 mg/kg/day [33]. Once in the bloodstream, dichlorvos may cross the placenta [34].
- **Teratogenic effects:** There is no evidence that dichlorvos is teratogenic. A dose of 12 mg/kg/day was not teratogenic in rabbits and did not interfere with reproduction in any way. There was no evidence of teratogenicity when rats and rabbits were exposed to air concentrations of up to 6.25 mg/L throughout pregnancy. Dichlorvos was not teratogenic when given orally to rats [33].
- **Mutagenic effects:** Dichlorvos can bind to molecules such as DNA. For this reason, there has been extensive testing of dichlorvos for mutagenicity. Several studies have shown dichlorvos to be a mutagen [35]; for example, dichlorvos is reported positive in the Ames mutagenicity assay and in other tests involving bacterial or animal cell cultures. However, no evidence of mutagenicity has been found in tests performed on live animals. Its lack of mutagenicity in live animals may be due to rapid metabolism and excretion [33].
- **Carcinogenic effects:** Dichlorvos has been classified as a possible human carcinogen because it caused tumors in rats and mice in some studies but not others [36]. When dichlorvos was administered by gavage (stomach tube) to mice for 5 days per week for 103 weeks at doses of 20 mg/kg/day in males and 40 mg/kg/day in females, there was an increased incidence of benign tumors in the lining of the stomach in both sexes. When rats were given doses of 4 or 8 mg/kg/day for 5 days per week for 103 weeks, there was an increased incidence of benign tumors of the pancreas and of leukemia in male rats at both doses. At the highest dose, there was also an increased incidence of benign lung tumors in

males. In female rats, there was an increase in the incidence of benign tumors of the mammary gland [35]. However, no tumors caused by dichlorvos were found in rats fed up to 25 mg/kg/day for 2 years, or in dogs fed up to 11 mg/kg/day for 2 years. No evidence of carcinogenicity was found when rats were exposed to air containing up to 5 mg/L for 23 hours/day for 2 years [36]. A few tumors were found in the esophagus of mice given dichlorvos orally, even though tumors of this kind are normally rare [34]. In sum, current evidence about the carcinogenicity of dichlorvos is inconclusive.

- **Organ toxicity:** Dichlorvos primarily affects the nervous system through cholinesterase inhibition, the blockage of an enzyme required for proper nerve functioning.
- **Fate in humans and animals:** Among organophosphates, dichlorvos is remarkable for its rapid metabolism and excretion by mammals. Exposure of rats to 11 mg/L (250 times the normal exposure) for 4 hours was required before dichlorvos was detectable in the rats [33]. Even then, it was detected only in the kidneys. Following exposure to 50 mg/L, the half-life for dichlorvos in the rat kidney was 13.5 minutes [33]. The reason for this rapid disappearance of dichlorvos is the presence of degrading enzymes in both tissues and blood plasma. When dichlorvos is absorbed after ingestion, it is moved rapidly to the liver where it is rapidly detoxified. Thus poisoning by nonlethal doses of dichlorvos is usually followed by rapid detoxification in the liver and recovery [33]. Rats given oral or dermal doses at the LD50 level either died within 1 hour of dosing or recovered completely [33]. Dichlorvos does not accumulate in body tissues and has not been detected in the milk of cows or rats, even when the animals were given doses high enough to produce symptoms of severe poisoning [33].

3.1.4. Ecological Effects

- **Effects on birds:** Dichlorvos is highly toxic to birds, including ducks and pheasants [31]; the LD50 in wild birds fed dichlorvos is 12 mg/kg.
- **Effects on aquatic organisms:** UV light makes dichlorvos 5 to 150 times more toxic to aquatic life [34]. Grass shrimp are more sensitive to dichlorvos than the

sand shrimp, hermit crab, and mummichog. The LC50 (96-hour) for dichlorvos is 11.6 mg/L in fathead minnow, 0.9 mg/L in bluegill, 5.3 mg/L in mosquito fish, 0.004 mg/L in sand shrimp, 3.7 mg/L in mummichogs, and 1.8 mg/L in American eels. The LC50 (24-hour) for dichlorvos in bluegill sunfish is 1.0 mg/L [35]. Dichlorvos does not significantly bioaccumulate in fish [37].

- **Effects on other organisms:** Dichlorvos is toxic to bees [31].

3.1.5. Environmental Fate

- **Breakdown in soil and groundwater:** Dichlorvos has low persistence in soil. Half-lives of 7 days were measured on clay, sandy clay, and loose sandy soil [32,37]. In soil, dichlorvos is subject to hydrolysis and biodegradation. Volatilization from moist soils is expected to be slow. The pH of the media determines the rate of breakdown [37]. Breakdown is rapid in alkaline soils and water, but it is slow in acidic media. For instance, at pH 9.1 the half-life of dichlorvos is about 4.5 hours. At pH 1 (very acidic), the half-life is 50 hours [37]. Dichlorvos does not adsorb to soil particles and it is likely to contaminate groundwater [32,37]. When spilled on soil, dichlorvos leached into the ground with 18 to 20% penetrating to a depth of 12 inches within 5 days [37].
- **Breakdown in water:** In water, dichlorvos remains in solution and does not adsorb to sediments. It degrades primarily by hydrolysis, with a half-life of approximately 4 days in lakes and rivers. This half-life will vary from 20 to 80 hours between pH 4 and pH 9. Hydrolysis is slow at pH 4 and rapid at pH 9 [34,37]. Biodegradation may occur under acidic conditions, which slow hydrolysis, or where populations of acclimated microorganisms exist, as in polluted waters. Volatilization from water is slow. It has been estimated at 57 days from river water and over 400 days from ponds [37].
- **Breakdown in vegetation:** Except for cucumbers, roses, and some chrysanthemums, plants tolerate dichlorvos very well [34].

3.2. Trifluralin

Trifluralin is a selective, pre-emergence dinitroaniline herbicide used to control many annual grasses and broadleaf weeds in a large variety of tree fruit, nut, vegetable, and grain crops, including soybeans, sunflowers, cotton, and alfalfa. Pre-emergence herbicides are applied before weed seedlings sprout. Trifluralin should be incorporated into the soil by mechanical means within 24 hours of application. Granular formulations may be incorporated by overhead irrigation. Trifluralin is available in granular and emulsifiable concentrate formulations. The technical material is approximately 96% pure and the emulsifiable concentrate is about 45% pure.

3.2.1. General Properties of Trifluralin

Trade names include Crisalin, Elancolan, Flurene SE, Ipersan, L-36352, M.T.F., Su Seguro Carpidor, TR-10, Trefanocide, Treficon, Treflan, Tri-4, Trifluralina 600, Triflurex Trim, and Trust. The compound may be found in formulations with other herbicides. Products containing trifluralin bear the Signal Words CAUTION or WARNING, depending on the type of formulation. This compound is a General Use Pesticide (GUP) in toxicity class III - slightly toxic. N-nitrosamine contaminant levels in trifluralin are required to be below 0.5 ppm, a level which EPA believes will result in no toxic effects.

3.2.2. Physical Properties

- *CAS (Chemical Abstracts Services) Number:* 1582-09-8
- *Chemical Name:* α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine [31]

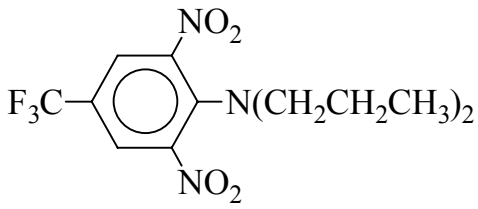


Figure 3.2 Molecular Structure of Trifluralin

- *Appearance:* Trifluralin is an odorless, yellow-orange crystalline solid [31].
- *Molecular Weight:* 335.50 g/mol

- *Water Solubility:* <1 mg/L at 27 ^{0}C [31]
- *Solubility in Other Solvents:* s.** in organic solvents such as acetone dichloromethane and xylene [31]

  **s: soluble
- *Melting Point:* 48.5-49 ^{0}C [31]
- *Vapor Pressure:* 13.7 mPa at 25 ^{0}C [31]
- *Partition Coefficient:* 5.0719 at pH 7 and 25 ^{0}C [31]
- *Adsorption Coefficient:* 8000 [38]

3.2.3. Toxicological Effects

- **Acute toxicity:** Pure trifluralin is practically nontoxic to test animals by oral, dermal, or inhalation routes of exposure [39]. The oral LD50 for technical trifluralin in rats is greater than 10,000 mg/kg, in mice is greater than 5000 mg/kg, and in dogs, rabbits, and chickens, is greater than 2000 mg/kg. However, certain formulated products that contain trifluralin may be more toxic than the technical material itself. For example, the oral LD50 for Treflan TR-10 in rats is greater than 500 mg/kg. The dermal LD50 for technical trifluralin in rabbits is greater than 2000 mg/kg. The 1-hour inhalation LC50 for technical trifluralin in rats is greater than 2.8 mg/L [40]. Nausea and severe gastrointestinal discomfort may occur after eating trifluralin. Trifluralin does not cause skin irritation. When applied to the eyes of rabbits, trifluralin produced slight irritation, which cleared within 7 days [41]. Skin sensitization (allergies) may occur in some individuals. Inhalation may cause irritation of the lining of the mouth, throat, or lungs [41].
- **Chronic toxicity:** Prolonged or repeated skin contact with trifluralin may cause allergic dermatitis [41]. The administration of 25 mg/kg/day to dogs for 2 years resulted in no observed toxicity [40]. In another study of beagle dogs, toxic effects were observed at 18.75 mg/kg/day. These included decreased red blood cell counts and increases in methemoglobin, total serum lipids, triglycerides, and cholesterol [42]. Trifluralin has been shown to cause liver and kidney damage in other studies of chronic oral exposure in animals [43].
- **Reproductive effects:** The reproductive capacity of rats fed dietary concentrations of trifluralin as high as 10 mg/kg/day was unimpaired through four successive generations. Trifluralin administered to pregnant rabbits at doses

as high as 100 mg/kg/day, and to rats at doses as high as 225 mg/kg/day, produced no adverse effect on either the mothers or offspring [40]. Loss of appetite and weight loss followed by miscarriages were observed when pregnant rabbits were fed high doses of 224 or 500 mg/kg/day. Fetal weight decreased and there was an increase in the number of fetal runts at the 500-mg/kg/day dosage [41]. It is unlikely effects on reproduction will be produced in humans at expected exposure levels.

- **Teratogenic effects:** No abnormalities were observed the offspring of rats fed doses as high as 10 mg/kg/day for four generations [40]. Studies in the rat and rabbit show no evidence that trifluralin is teratogenic. The highest doses tested in these studies were 1000 mg/kg/day in rats and 500 mg/kg/day in rabbits [39]. Trifluralin does not appear to be teratogenic.
- **Mutagenic effects:** No evidence of mutagenicity was observed when trifluralin was tested in live animals, and in assays using bacterial and mammalian cell cultures [39].
- **Carcinogenic effects:** In a 2-year study of rats fed 325 mg/kg/day, the highest dose tested, malignant tumors developed in the kidneys, bladder, and thyroid [39]. However, more data are needed to characterize its carcinogenicity.
- **Organ toxicity:** Liver, kidney, and thyroid damage appear to be the main toxic effects in chronic animal studies [43].
- **Fate in humans and animals:** Trifluralin is not readily absorbed into the bloodstream from the gastrointestinal tract; 80% of single oral doses administered to rats and dogs was excreted in the feces [41].

3.2.4. Ecological Effects

- **Effects on birds:** Trifluralin is practically nontoxic to birds [44]. The LD50 in bobwhite quail is greater than 2000 mg/kg, as it is in female mallards and pheasants [44]. These values are for the technical product.
- **Effects on aquatic organisms:** Trifluralin is very highly toxic to fish and other aquatic organisms. The 96-hour LC50 is 0.02 to 0.06 mg/L in rainbow trout, and 0.05 to 0.07 mg/L in bluegill sunfish [45]. The 96-hour LC50 in channel catfish is approximately 1.4 to 3.4 mg/L [45]. Variables such as temperature, pH, life

stage, or size may affect the toxicity of the compound. Trifluralin is highly toxic to Daphnia, a species of small freshwater crustacean, with a 48-hour LC50 of 0.5 to 0.6 mg/L [46]. The compound shows a moderate tendency to accumulate in aquatic organisms.

- **Effects on other organisms:** At exposure levels well above permissible application rates (100 mg/kg), trifluralin has been shown to be toxic to earthworms. However, permitted application rates will result in soil residues of approximately 1 ppm trifluralin, a level that had no adverse effects on earthworms [46]. It is nontoxic to bees [31].

3.2.5. Environmental Fate

- **Breakdown in soil and groundwater:** Trifluralin is of moderate to high persistence in the soil environment, depending on conditions. Trifluralin is subject to degradation by soil microorganisms. Trifluralin remaining on the soil surface after application may be decomposed by UV light or may volatilize. Reported half-lives of trifluralin in the soil vary from 45 to 60 days [38] to 6 to 8 months [31]. After 6 months to 1 year, 80 to 90% of its activity will be gone [41]. It is strongly adsorbed on soils and nearly insoluble in water [38]. Because adsorption is highest in soils high in organic matter or clay content and adsorbed herbicide is inactive, higher application rates may be required for effective weed control on such soils [40,41]. Trifluralin has been detected in nearly 1% of the 5590 wells tested. However, it has been detected at very low concentrations, typically ranging from 0.002 μg/L to 15 μg/L [41].
- **Breakdown in water:** Trifluralin is nearly insoluble in water [31]. It will probably be found adsorbed to soil sediments and particulates in the water column.
- **Breakdown in vegetation:** Trifluralin inhibits the growth of roots and shoots when it is absorbed by newly germinated weed seedlings [40]. Trifluralin residues in crop plants will occur only in root tissues, which are in direct contact with contaminated soil. Trifluralin is not translocated into the leaves, seeds, or fruit of most plants. On most crops, trifluralin applied to the leaves has no effect, but on certain crops, such as tobacco and summer squash, leaf distortion may occur [40].

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 [47], 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 [48]) 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.

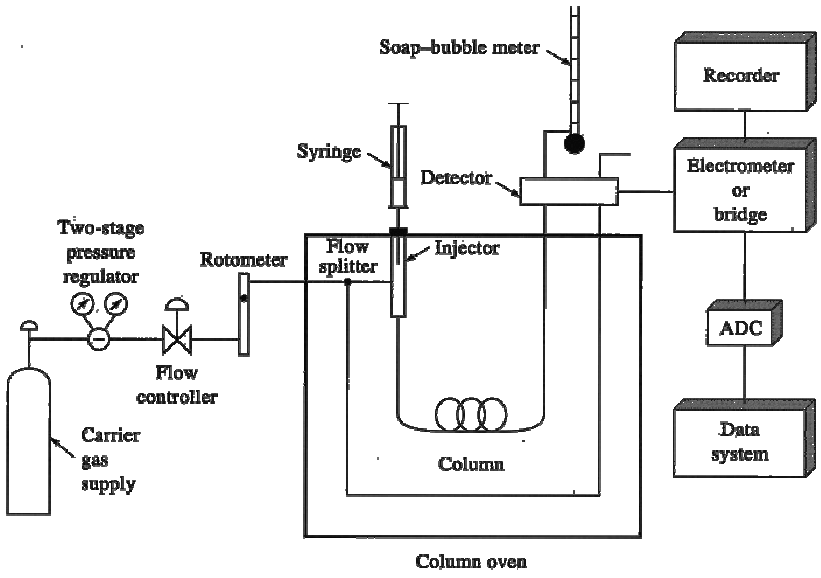


Figure 4.1. Schematic of a Gas Chromatograph

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 [49].

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 [48].

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.

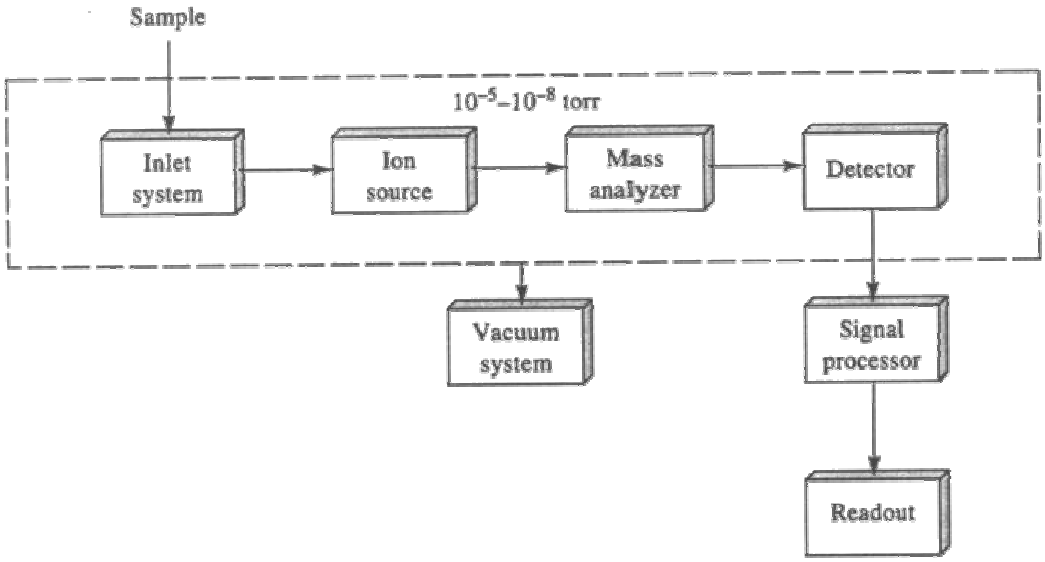


Figure 4.2. Components of a Mass Spectrometer

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 [50].

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 [48]). 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.

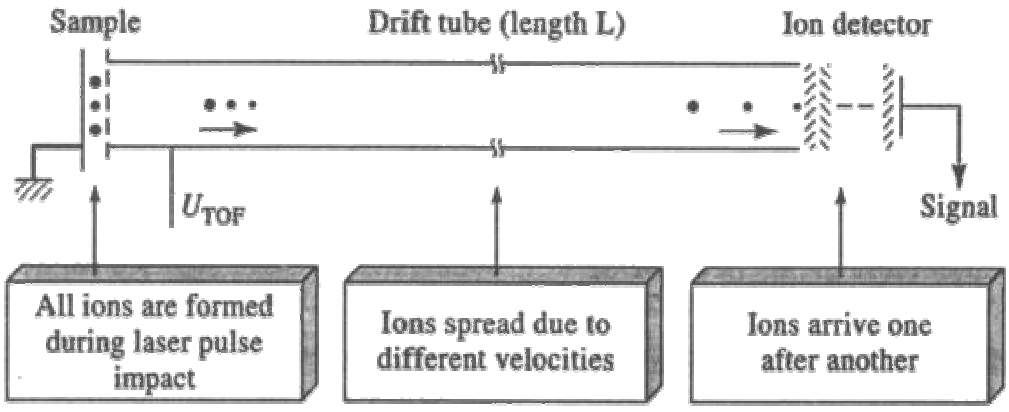


Figure 4.3. A Time-of-flight Mass Spectrometer

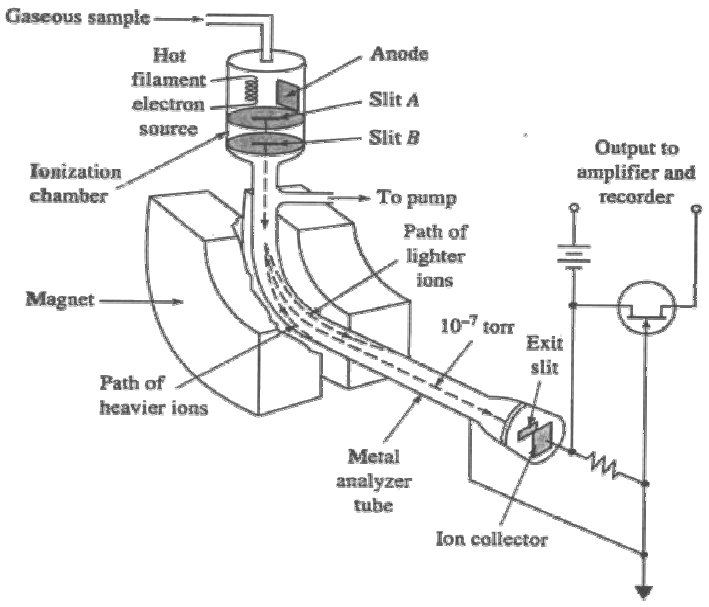


Figure 4.4. A Magnetic Mass Spectrometer

The most popular mass analyzer is the quadrupole as shown in Figure 4.5 [48], 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 [51].

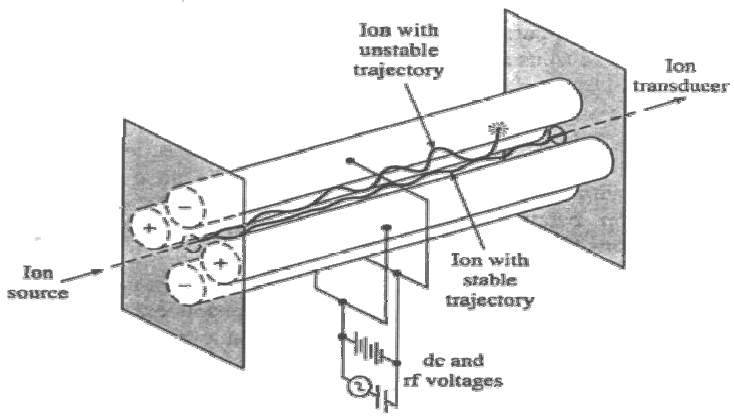


Figure 4.5. A Quadrupole Mass Spectrometer

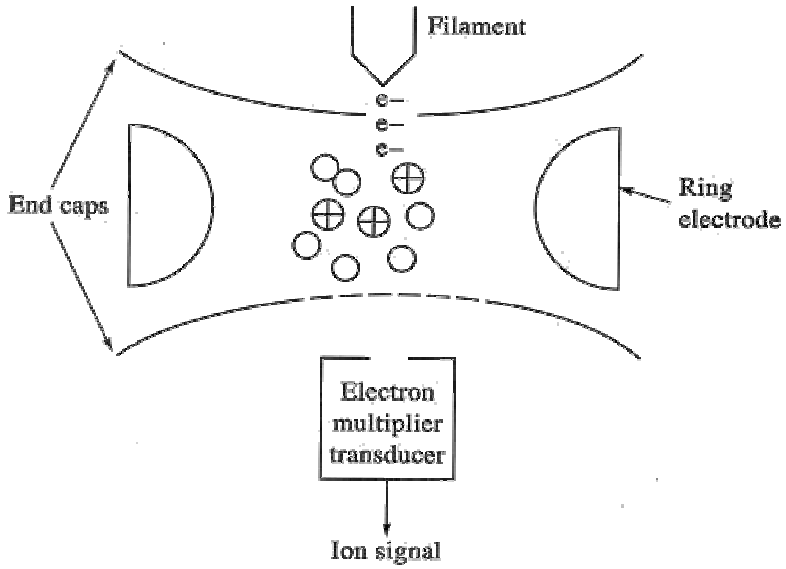


Figure 4.6. Ion Trap Mass Spectrometer

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.

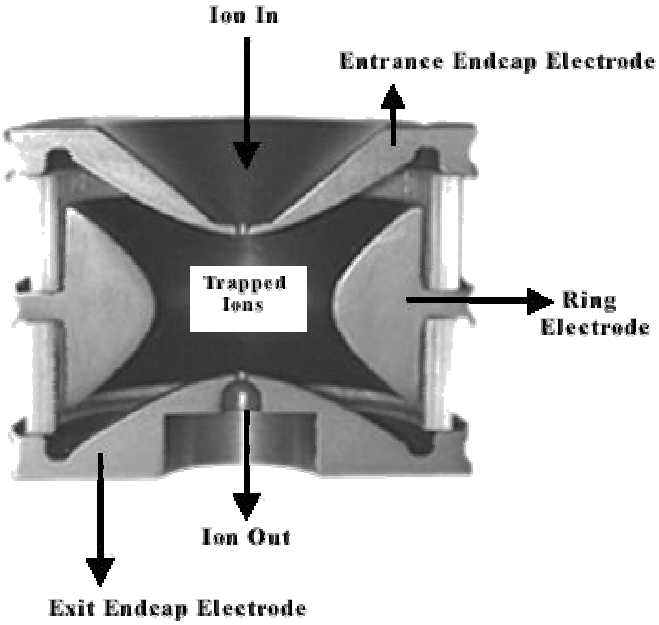


Figure 4.7. A Schematic Diagram of an Ion Trap Mass Spectrometer

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;
  1. Ion formation and matrix ion ejection,
  2. Parent ion isolation,
  3. Product ion formation,
  4. Product ion mass scanning.

  The utility of the MS-MS technique derives from the following;

  1. optimally filling an ion trap with the selected parent ion,
  2. obtaining qualitative structural information about the sample by forming the product ion spectrum,
  3. 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 [52,53], 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 [54]. 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 Dichlorvos (DDVP) and Trifluralin 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 each compound were prepared from 1000 mg/L stock standard solutions. From these 10 mg/L standard pesticide solutions, a mixed solution containing 1 mg/L of each pesticide was prepared. From this mixed solution, nine calibration solutions (from 0.025 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 20°C/min to 280°C (kept 2.50 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 2 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 June and October 2002 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 4,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 2 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 2 µl [GC–MS system under MS–MS, and SIS modes]

5.6. Analysis of Water Samples Using Liquid-Liquid Extraction (LLE) Preconcentration Method

In this preconcentration process, 500 ml of spiked or real sample water was extracted with 20 ml of dichloromethane. Obtained extract was evaporated to dryness with gentle N_2 stream, redissolved in 500 µl of dichloromethane and then 25 µl of pentachloronitrobenzene was added as an internal standard before injection to the GC-MS system.

The analytical procedure can be summarized as follows:

500 ml of water sample put into separation funnel

Add 20 ml dichloromethane and shake about 10 minutes

Take the dichloromethane phase into tube

Evaporate solvent under Nitrogen gas stream
Redissolve in exactly 500µl dichloromethane
Add Pentachloronitrobenzene (I.S.) (25µl)

Inject 2 µl [GC–MS system under MS–MS, and SIS modes]

CHAPTER 6

RESULTS AND DISCUSSION

6.1. Method Comparison

In this study, two different methods were used for identification and quantification of the two target pesticides.

First method was GC-MS full scan mode. This mode was used for identification of the two pesticides. Standard pesticide mixture solutions were injected under full scan mode. Total ion GC-MS chromatogram (Figure 6.1.) and mass spectra of each pesticide were obtained (Figure 6.2. and 6.3.).

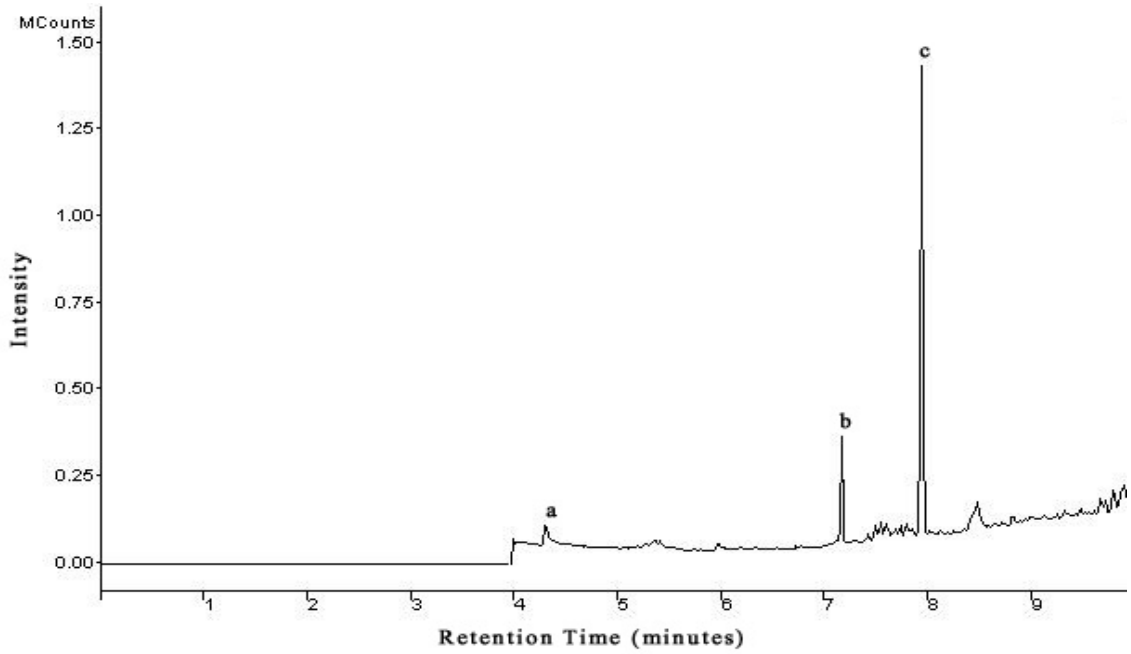


Figure 6.1. Total Ion GC-MS Chromatogram of Standard Pesticide Mixture Solution (1 mg/L); a = Dichlorvos (DDVP); b = Trifluralin; c = Pentachloronitro benzene (Internal Standard)

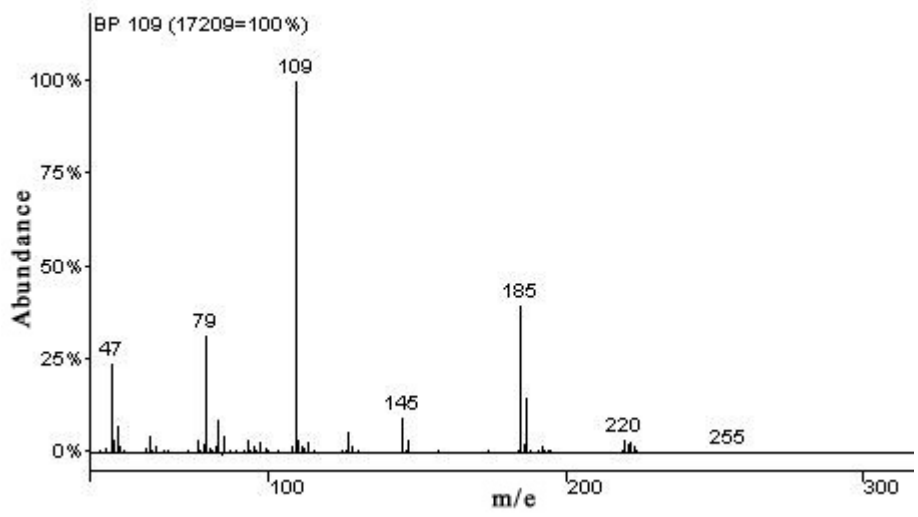
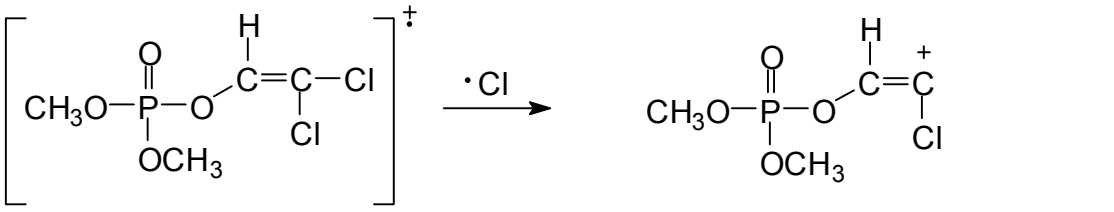
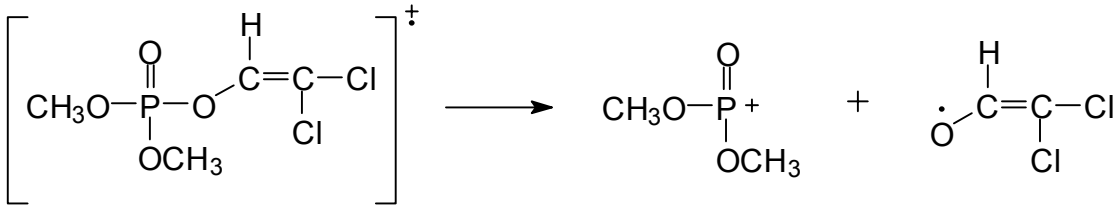


Figure 6.2. Mass Spectrum of Dichlorvos (DDVP)

In the mass spectrum of Dichlorvos (Figure 6.2.), two important peaks were examined to compare with reference mass spectrum of dichlorvos from pesticides library. These two peaks (109 and 185) are most probably formed by the following bond cleavages.



m/e=220 m/e=185



m/e=220 m/e=109

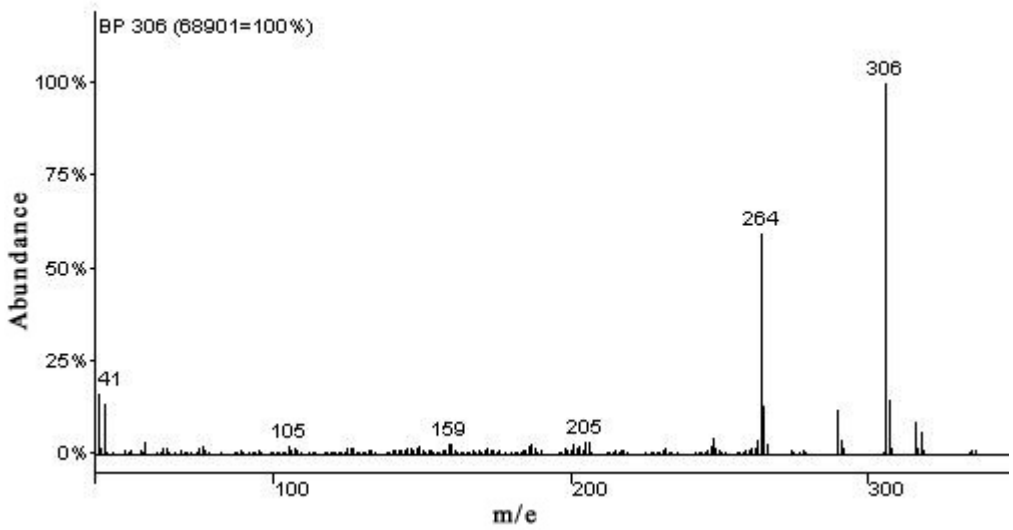
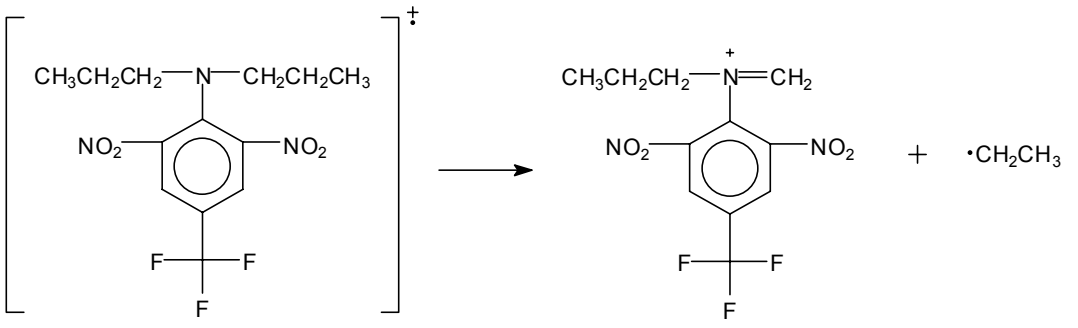
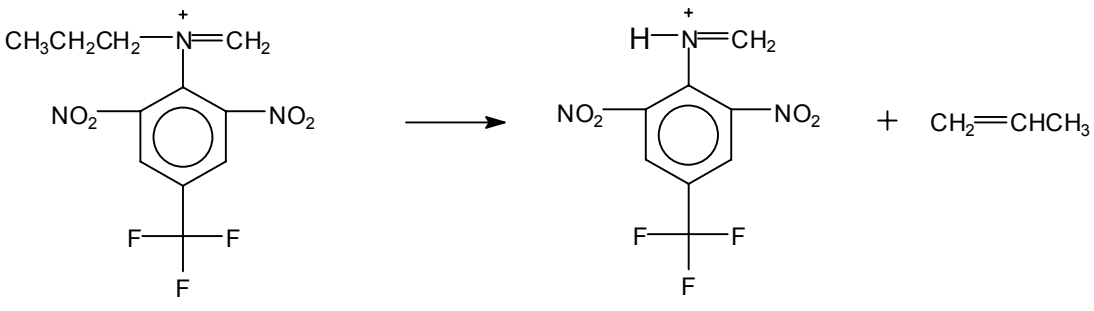


Figure 6.3. Mass Spectrum of Trifluralin

In the mass spectrum of trifluralin (Figure 6.3.), two important peaks were examined to compare with reference mass spectrum of trifluralin from pesticides library. These two peaks (264 and 306) are most probably formed by the following bond cleavages.



m/e =335 m/e =306



m/e =306 m/e =264

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 of limited sensitivity and therefore, for target pesticide analysis, MS-MS mode was preferred. The MS-MS parameters are shown in Table 6.1.

Table 6.1. MS-MS Parameters

Pesticides	Activation Time (min)	m/e Range	Major Fragment Ion (m/e)	Excitation Amplitude (V)	Excitation Storage Level (m/e)
Dichlorvos	4.00 – 5.25	50 – 300	185	57.0	66.0
Trifluralin	5.25 – 7.75	50 – 400	306	45.0	75.0

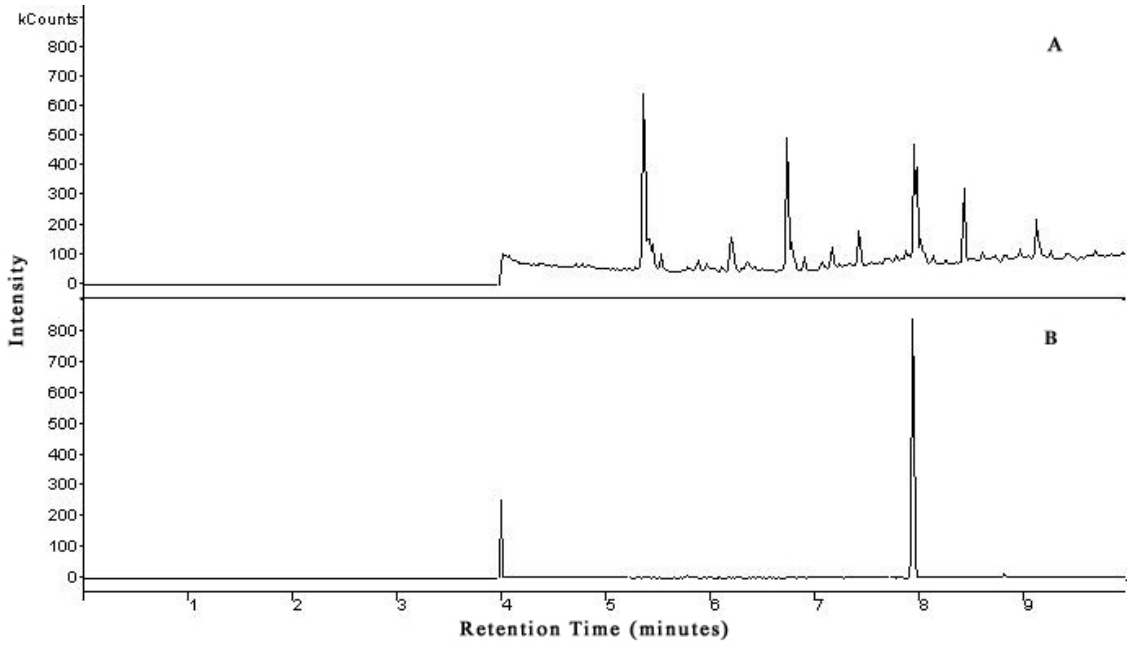


Figure 6.4. Chromatogram A obtained with GC-MS mode, chromatogram B obtained with GC-MS-MS mode after SPE step of 500 ml of water sample

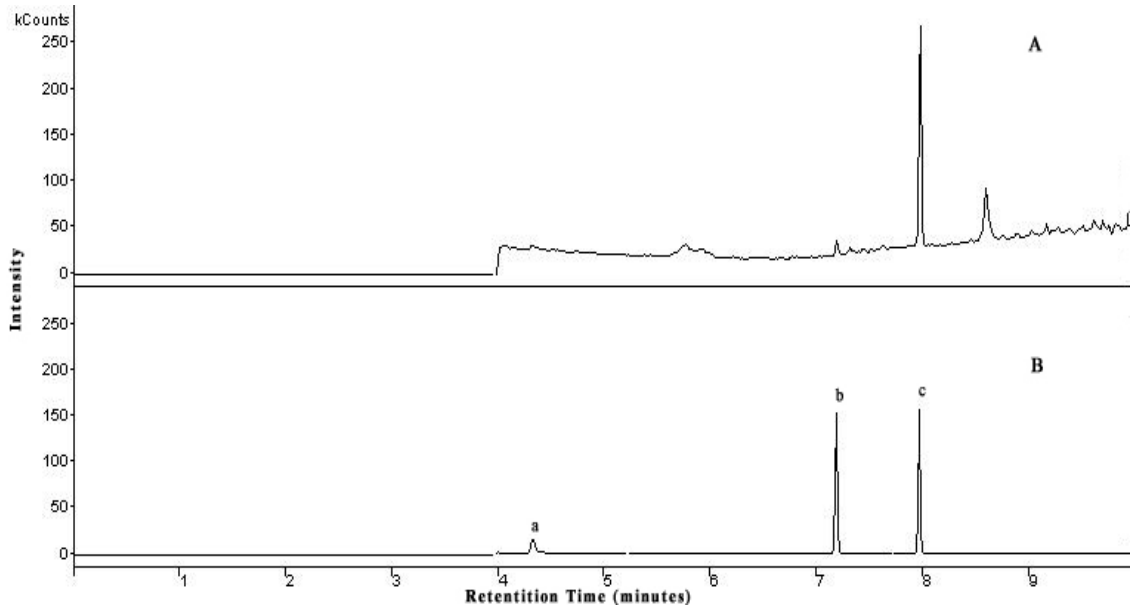


Figure 6.5. Chromatogram A obtained with GC-MS mode, Chromatogram B obtained with GC-MS-MS mode 0.5 mg/L of pesticides standard solution. [a= Dichlorvos(DDVP); b = Trifluralin; c = Pentachloronitrobenzene (Internal Standard)]

Figure 6.4.B and 6.5.B show that 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. And also, the tandem mass technique allows highly specific MS analyses, with possibility of directly analyzing complex environmental samples without extensive clean-up steps.

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

6.2. Calibration Results

The instrument calibration for GC-MS-MS was performed by injecting standard solutions of each pesticide at levels ranging from 0.025 to 5 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 6.6. and 6.7. Good linearity of the response was found for Trifluralin and Dichlorvos at concentration belonging to cited interval, with determination coefficients (or correlation coefficient) higher than 0.994. The calibration plots for dichlorvos and trifluralin are shown in Figure 6.8. to 6.10.

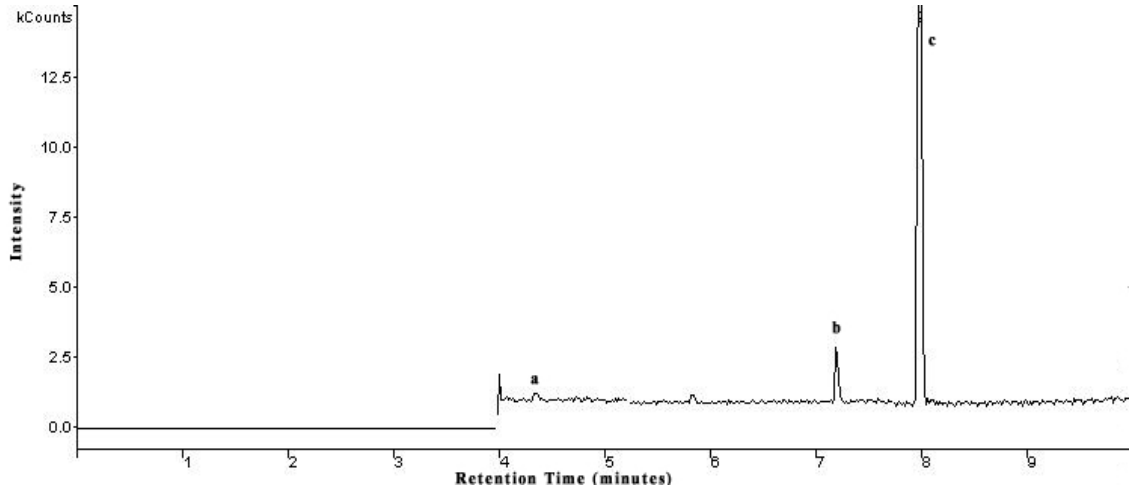


Figure 6.6. Chromatogram obtained with GC-MS-MS mode 0.025 mg/L of pesticides standard solution.

[a= Dichlorvos(DDVP); b = Trifluralin; c = Pentachloronitrobenzene (Internal Standard)]

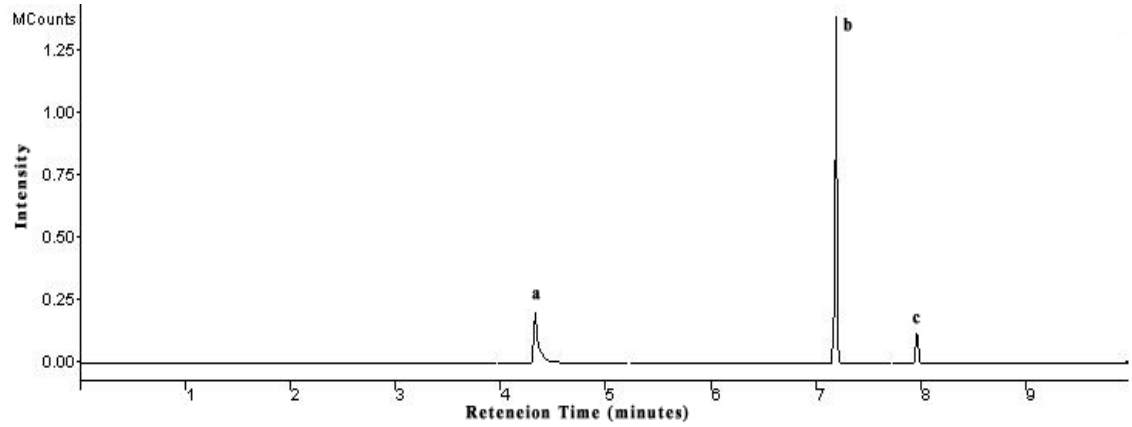


Figure 6.7. Chromatogram obtained with GC-MS-MS mode 5 mg/L of pesticides standard solution.

[a= Dichlorvos(DDVP); b = Trifluralin; c = Pentachloronitrobenzene (Internal Standard)]

Table 6.2. 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)
Trifluralin	7.17- 7.21	306	264	0.025 - 0.500	0.997	8.5	0.8	0.0008	2.7	0.0027
Trifluralin	7.17- 7.21	306	264	0.500 – 5.000	0.994	5.8	0.8	0.0008	2.7	0.0027
Dichlorvos	4.31-4.38	185	93	0.025 - 0.500	0.999	11.4	10.5	0.0105	35.0	0.0350
Dichlorvos	4.31-4.38	185	93	0.500 – 5.000	0.998	10.3	10.5	0.0105	35.0	0.0350

[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).

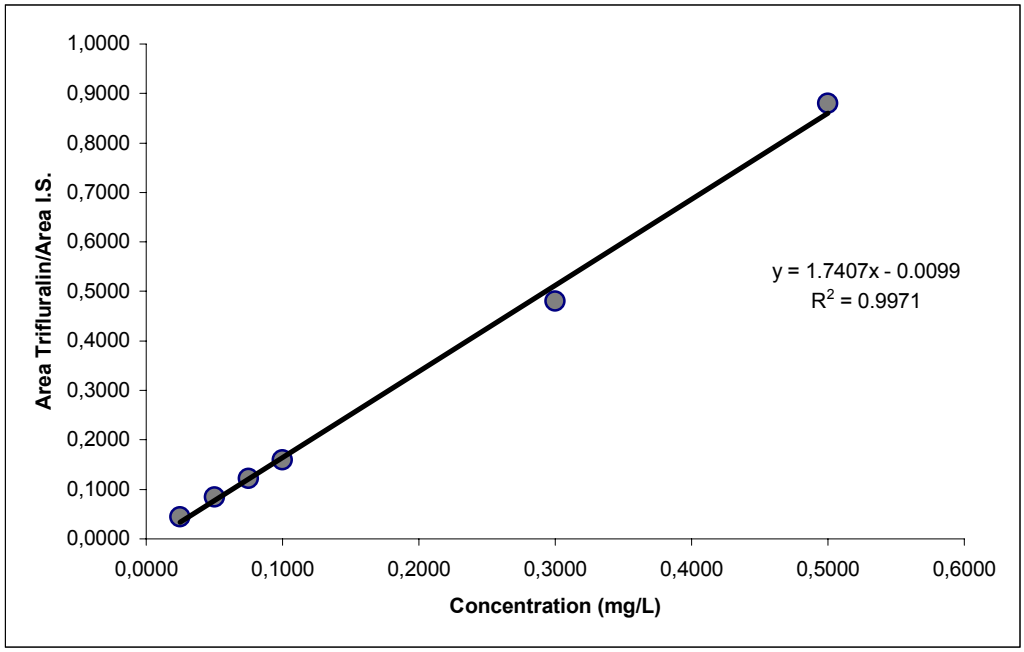


Figure 6.8. Calibration Plot for Trifluralin for Concentration Range of 0.025 mg/L - 0.5 mg/L

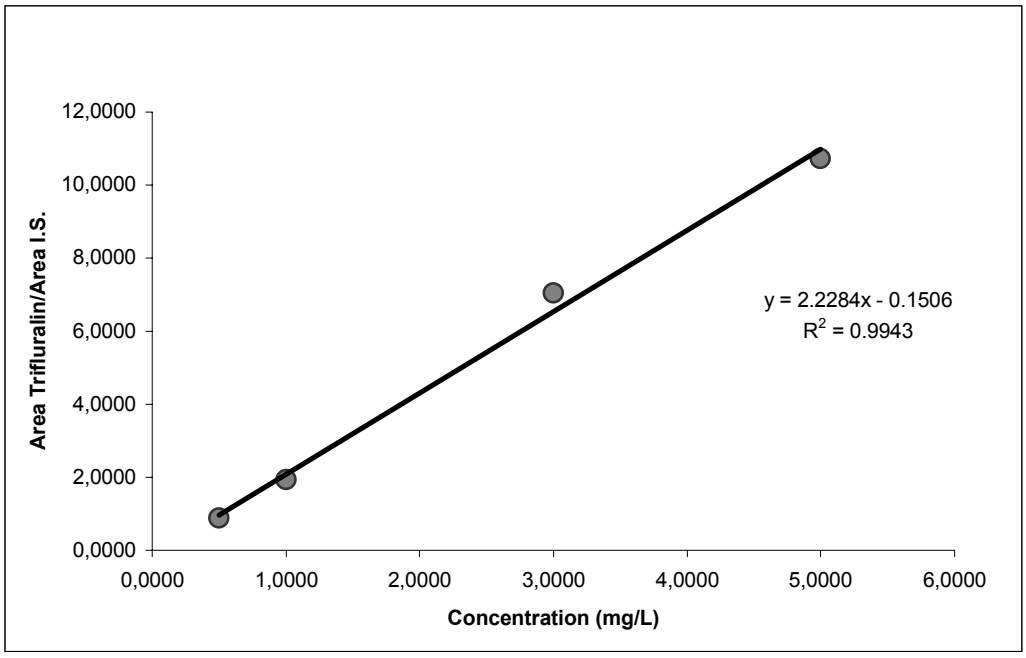


Figure 6.9. Calibration Plot for Trifluralin for Concentration Range of 0.5 mg/L - 5 mg/L

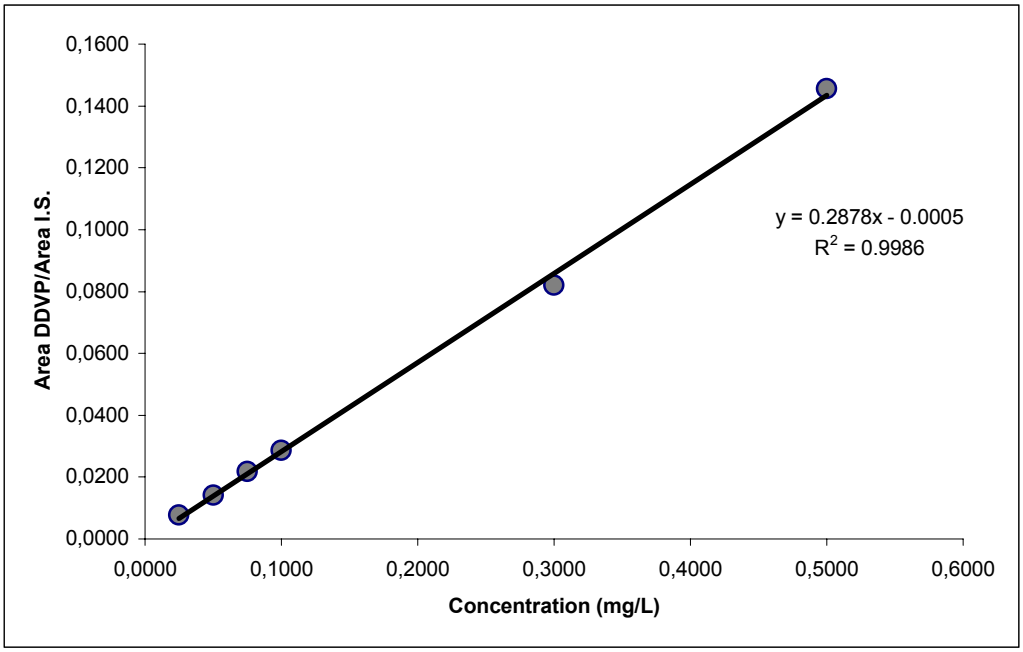


Figure 6.10. Calibration Plot for DDVP for Concentration Range of 0.025 mg/L - 0.5 mg/L

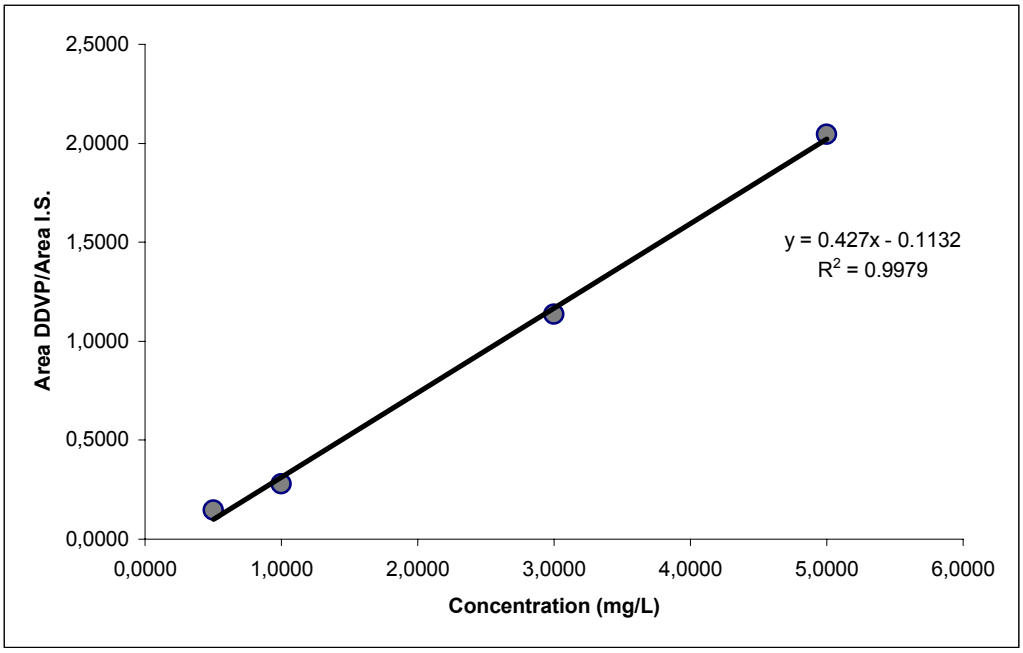


Figure 6.11. Calibration Plot for DDVP for Concentration Range of 0.5 mg/L - 5 mg/L

6.3. Liquid-Liquid Extraction (LLE)

In this process, 500 ml of pesticide-free water spiked with 0.2 µg/L of each target pesticide were used to study the extraction efficiency of the analytes. Good recoveries were obtained (90.8% for Trifluralin and 86.0% for Dichlorvos (DDVP)). This process was also repeated for 250 ml and 1000 ml of spiked water samples. The results obtained are in Table 6.3.

Table 6.3. Recoveries of Liquid-Liquid Extraction of Pesticide Spiked in Pesticide-free Water at Different Sample Volumes*

Pesticides	Volumes					
	250 ml		500 ml		1000 ml	
	Recovery %	RSD %	Recovery %	RSD %	Recovery %	RSD %
Trifluralin	111.2	5.8	90.8	10.3	28.6	13.9
Dichlorvos	103.3	10.2	86.0	6.3	20.4	13.2

*The values are means of four determinations

Recoveries were good enough using volumes ≤ 500ml of sample. A volume of 500 ml was chosen as optimum volume of sample to use. This volume is also the most used volume in literature [27, 55].

6.4. 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 0.2 µg/L of each target pesticide were used to study the extraction efficiency of the analytes.

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

The effect of three different pH values were tested; pH of pesticide free water was adjusted to 2.0, 4.0 and 6.0 by adding hydrochloric acid and NaOH before the preconcentration step. Good recoveries were obtained for Dichlorvos and Trifluralin at pH 4 (as shown in Figure 6.12.). Recovery results are shown in Table 6.4.

Table 6.4. Effect of pH on Recoveries in the Solid Phase Extraction Process

Pesticides	pH		
	2	4	6
	Recovery %	Recovery %	Recovery %
Trifluralin	98.7	107.5	98.0
Dichlorvos	40.7	63.0	31.0

Recoveries of the Dichlorvos and Trifluralin for solid phase extraction were 63.0 (±5.7)% and 107.5 (±4.5)% in water samples spiked with 200 ng/L pesticides at pH 4.

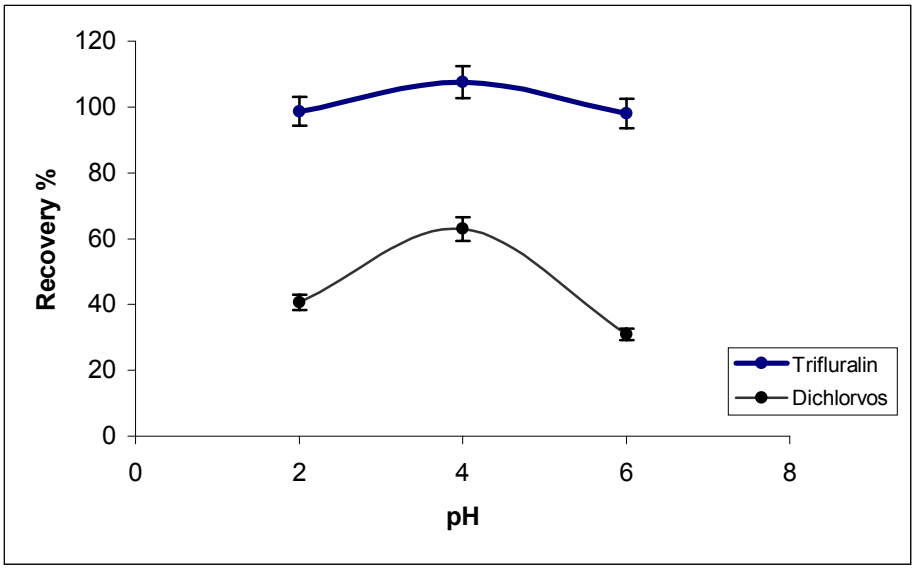


Figure 6.12. Effect of pH on The Recovery of Target Pesticides

Another parameter tested was the addition of salt (NaCl) at four different concentrations, 5, 10, 15 and 20 g/L. The results as figured in Table 6.5. 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 6.5. Effect of Salt (NaCl) on Recoveries in the Solid Phase Extraction Process*

Pesticides	Salt (NaCl) g/L			
	5	10	15	20
	Recovery %	Recovery %	Recovery %	Recovery %
Trifluralin	87.7	107.5	77.3	79.7
Dichlorvos	30.7	63.0	30.7	40.0

* These values were obtained at pH 4

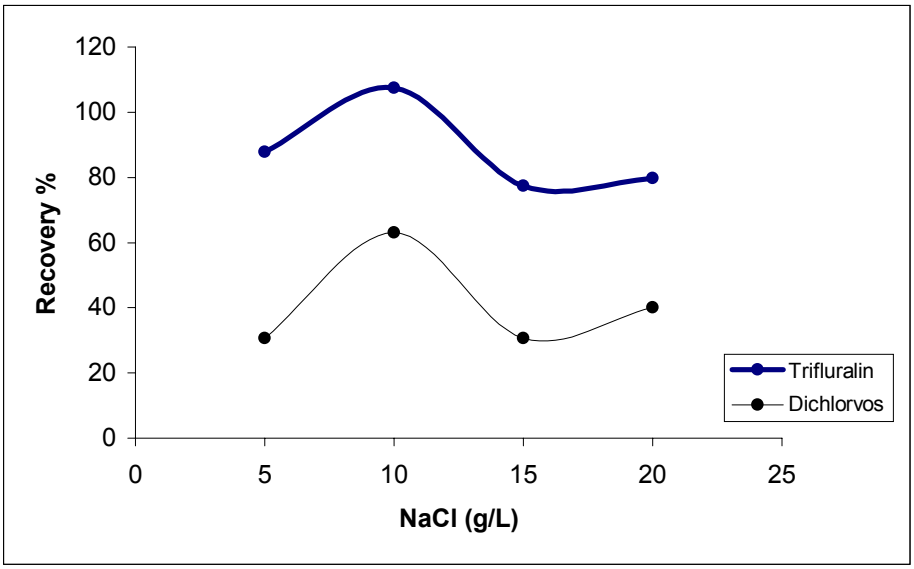


Figure 6.13. Effect of Salt Addition on The Recovery of Target Pesticides

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 6.6 recoveries for each pesticide obtained with GC-MS-MS is shown.

Table 6.6. Recoveries of Solid Phase Extraction of Pesticides at Different Sample Volumes*

Pesticides	Volumes					
	250 ml		500 ml		1000 ml	
	Recovery %	RSD %	Recovery %	RSD %	Recovery %	RSD %
Trifluralin	99.0	11.3	98.7	4.1	87.3	9.7
Dichlorvos	66.3	11.3	40.7	8.6	23.3	2.5

* The values are mean values of four determinations obtained at pH 2

As seen from Table 6.6, when the extraction volumes were increased, recoveries of pesticides decreased. Optimum a volume of 500 ml was chosen for further studies.

6.5. Real Sample Analysis

Analyzed water samples were collected between 01 June to 30 September 2002 by İZSU. Solid Phase Extraction and Liquid-Liquid Extraction methods were used to analyze all the water samples. Obtained results are below the detection limit for each pesticide. A typical chromatogram obtained with a real sample from Tahtalı Dam Water is shown in Figure 6.14.B.

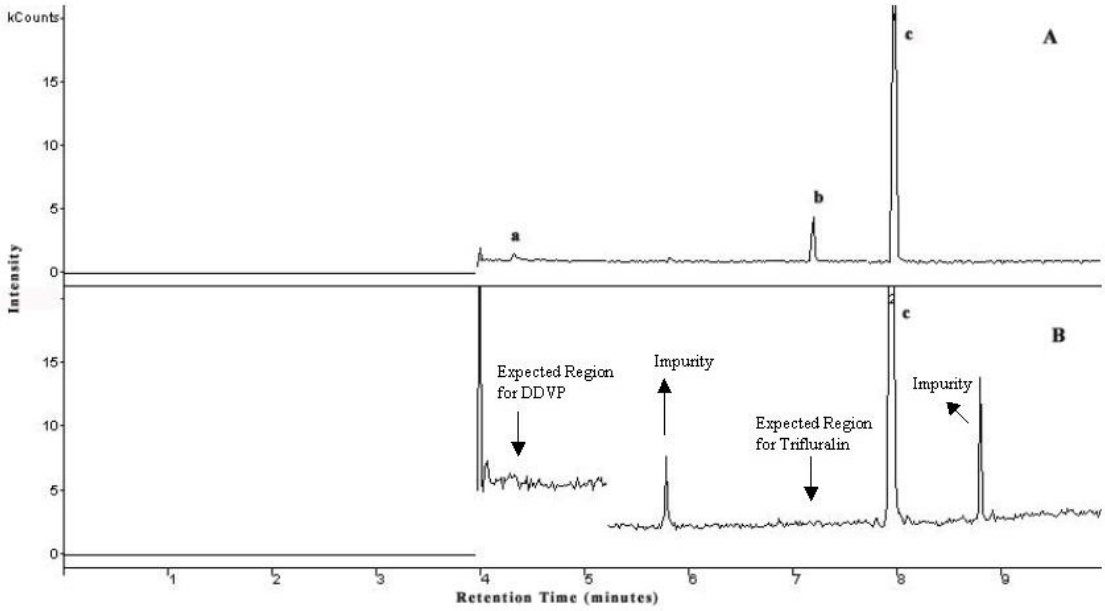


Figure 6.14. Chromatogram A obtained with GC-MS-MS mode 0.025 mg/L of standard pesticide solution, Chromatogram B obtained with GC-MS-MS mode after SPE step of 500 ml of water sample

[a= Dichlorvos(DDVP); b = Trifluralin; c = Pentachloronitrobenzene (Internal Standard)]

In Figure 6.14, chromatogram B was obtained with GC-MS-MS mode from real water sample after SPE whereas chromatogram A was obtained from 0.025 mg/L

standard pesticides solution. Comparison of these two chromatograms and analysis of water samples collected between 01 June to 30 September 2002 shown that Dichlorvos (DDVP) and Trifluralin pesticides are not present at detectable levels in Tahtalı Dam Water.

CHAPTER 7

CONCLUSION

1. The above studied pesticides could be analyzed both with GC and GC-MS. However during this research study GC-(IT)-MS instrument was used for analysis. Comparison of two techniques are as follows:

Advantages of the GC	**Disadvantages of GC**
a. Detection limits for a certain compound can be lowered with a specific detector.	a. Since sample detection relies on retention time, one cannot be completely sure of the sample analyzed. b. Retention time can change and give positive errors for the same compound but for different analysis.
Advantages of the GC- (IT)-MS	**Disadvantages of the GC- (IT)-MS**
a. Compounds can be completely identified by their mass spectra. b. Different compounds can be analyzed with the same ion source. c. Detection limit can be lowered by using tandem MS and SIS. d. Analysis of samples can be straightforward even in solutions with a big matrix.	a. In some cases sensitivity of the instrument cannot be as high as a specific GC-Detector.

Therefore GC- (IT)-MS was found to be a suitable technique for analyzing trace amounts of the studied pesticides.

2. Following conclusions are deduced from the analysis results

a. Both dichlorvos and trifluralin are in negligible amounts in Tahtalı Dam Water. Soil and sediment analysis can complement our study. However, although these two pesticides are widely used in Tahtalı Dam Basin, they degrade reasonably fast and the probability of finding them in water, soil or sediment seems low.
b. Analysis interval was planned for at least one year, but due to some unavoided reasons (organizing water sample supplies with İZSU, MS going out of order and time spent for servicing, time spent for missing chemicals and Spe disks), samples between 01 June 2002 to 30 September 2002 intervals could be analyzed. We are planning to continue the analysis for at least one year or may be two years.
c. Solid Phase Extraction (SPE) and Liquid-Liquid Extraction (LLE) were both used for the extraction of studied pesticides and gave results in the same range. However, spe will be used in future studies, because it performs better separation especially for samples with big matrix effects.
d. Solid Phase Extraction (SPE) is also preferable for environmental reasons because amount of polluting extraction solutions are minimized.

7.1. Future Proposed Research

In order to follow the changes in concentration levels if any, this study is aimed to be continued in 2003 especially in spring and autumn seasons.

If some changes in concentration are detected, these pesticides should be analyzed for two or more years to get more significant results and to form a mathematical model.

Analysis of other pesticides are also planned.

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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 :	4.00 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 :	4.00 Min.
Segment End Time :	5.25 Min.
Segment Low Mass :	50 m/z
Segment High Mass :	300 m/z
Ionization Mode :	EI/AGC
Ion Preparation Technique :	MS/MS
Target TIC :	5000 counts
Prescan Ionization Time :	1500 microseconds

Background Mass : 50 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 : 185.0 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 : 66.0 m/z
- Excitation Amplitude : 57.00 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 : 5.25 Min.

Segment End Time : 7.75 Min.

Segment Low Mass : 50 m/z

Segment High Mass : 400 m/z

Ionization Mode : EI/AGC

Ion Preparation Technique : MS/MS

Target TIC : 5000 counts

Prescan Ionization Time : 100 microseconds

Background Mass : 50 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 : 306.0 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 : 75.0 m/z

Excitation Amplitude : 45.00 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

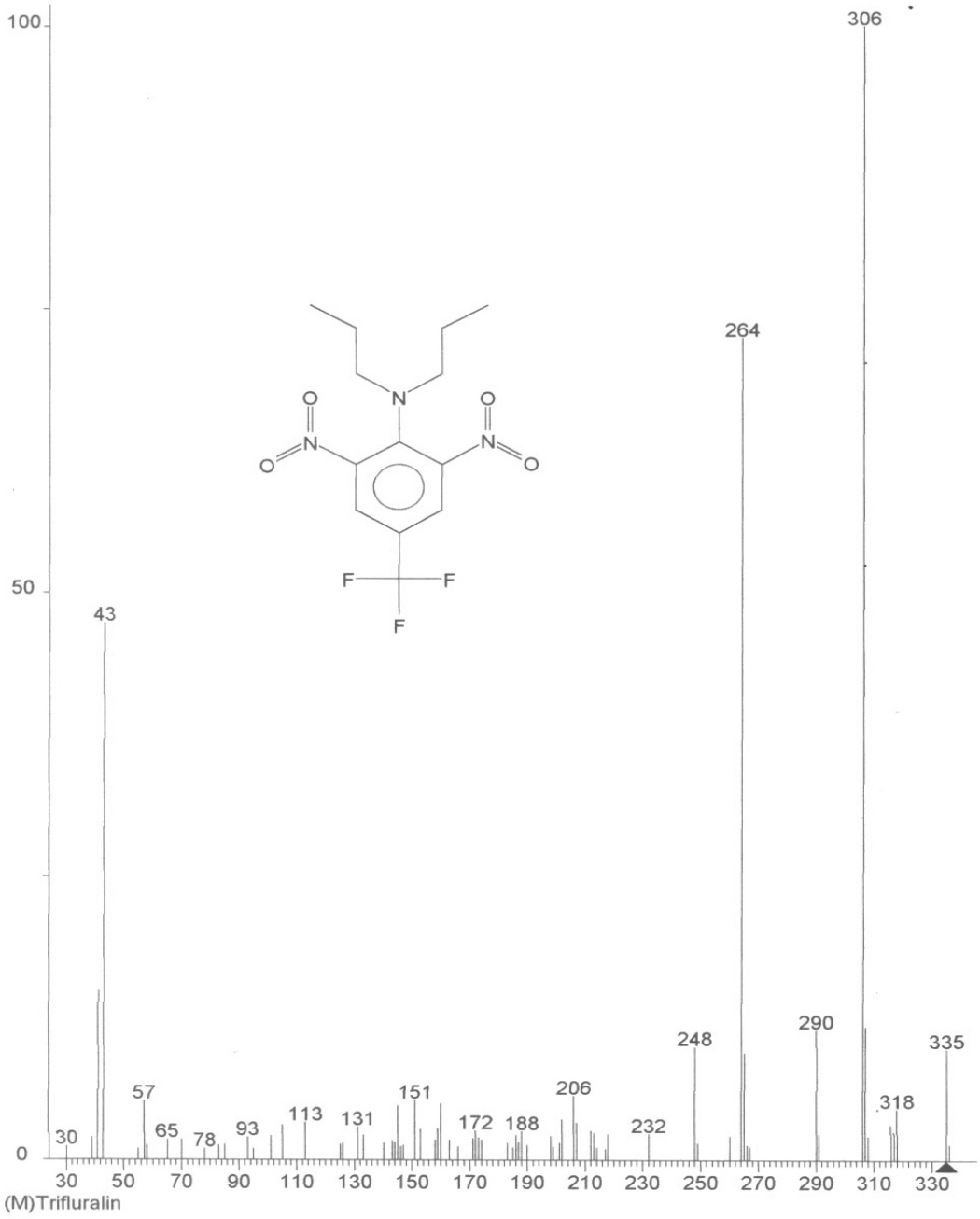

Figure B.1. Mass Spectrum of Trifluralin (from NIST Pesticides Library)

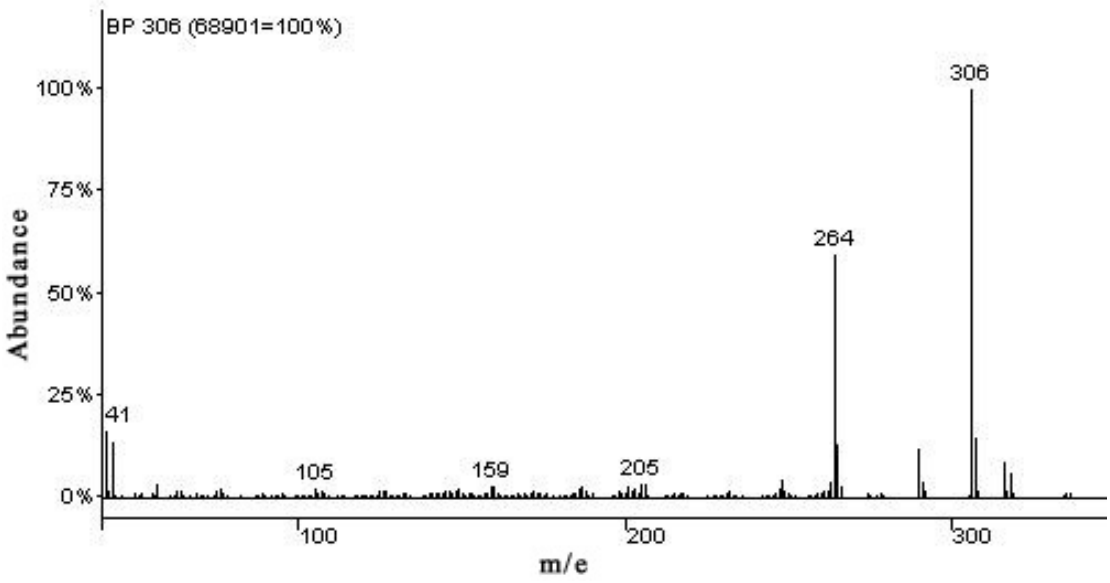

Figure B.2. Mass Spectrum of Trifluralin

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

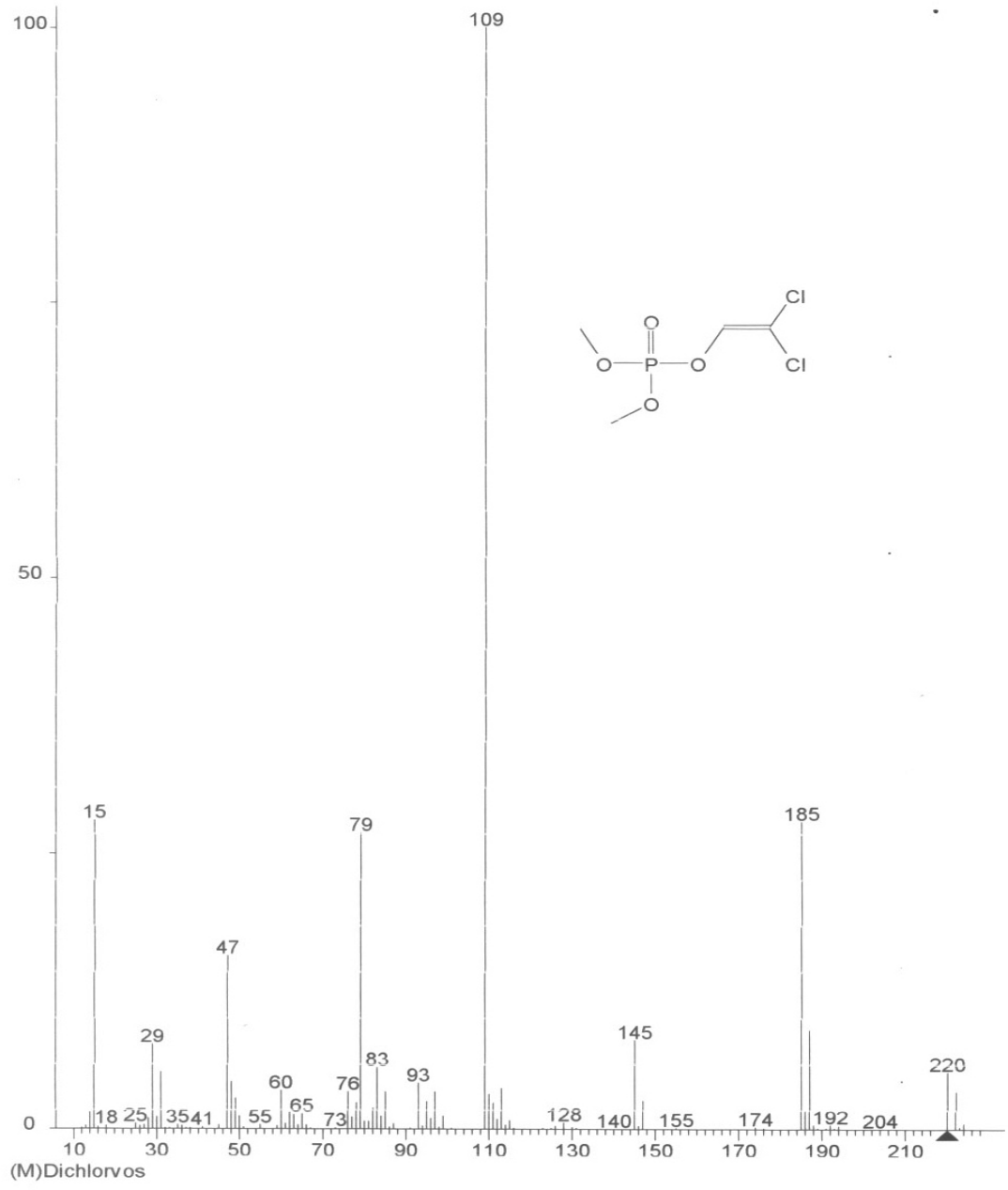

Figure B.3. Mass Spectrum of Dichlorvos (DDVP) (from NIST Pesticides Library)

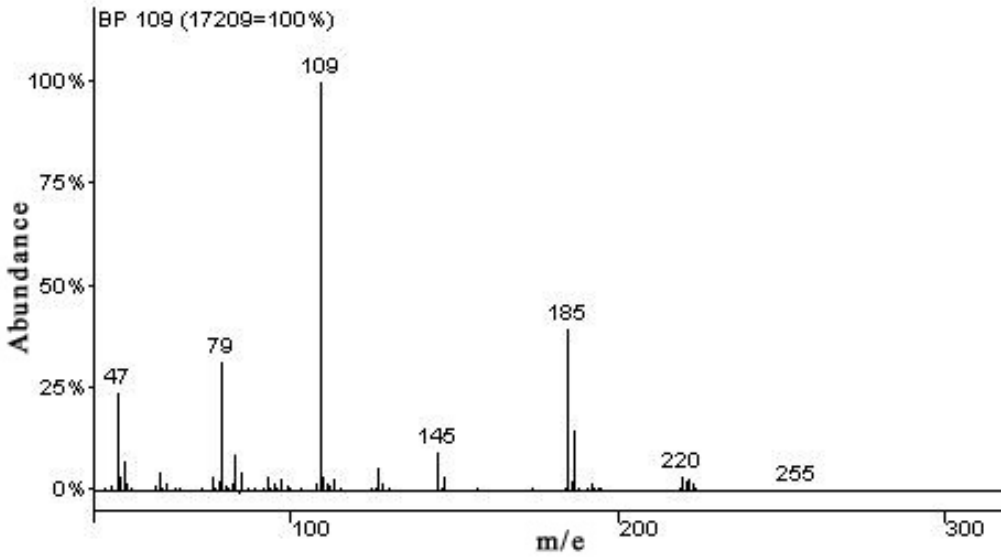

Figure B.4. Mass Spectrum of Dichlorvos (DDVP)

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

APPENDIX C

GENERAL INFORMATION ABOUT TAHTALI DAM

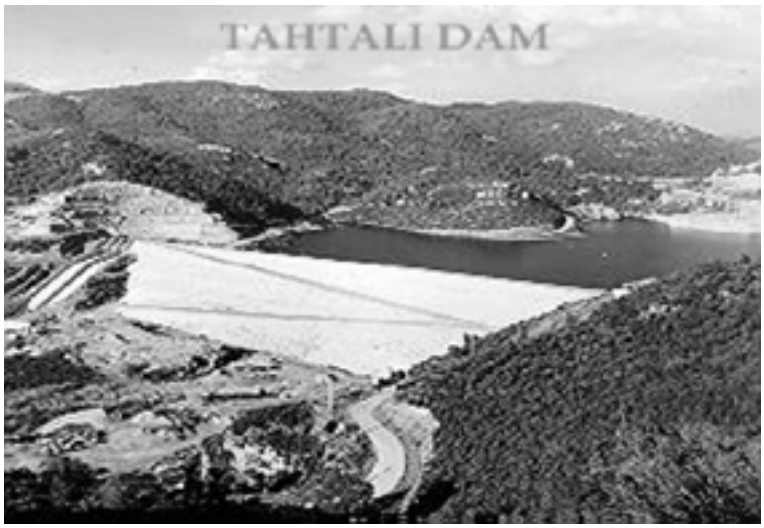

Figure C.1. General View Of Tahtalı Dam

- 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.