CHAPTER 2

THEORIES

2.1 Ethylbenzene

Ethylbenzene (C_8H_{10}) is a volatile organic compound (VOC) in the 'BTEX' substance group (benzene, toluene, ethylbenzene, and xylene) (Kaoru et al., 2004) which produced by alkylating benzene (C_6H_6) with ethylene (C_2H_4) in an acid-catalyzed chemical reaction (Figure 2.1) (Leigh et al., 2007).

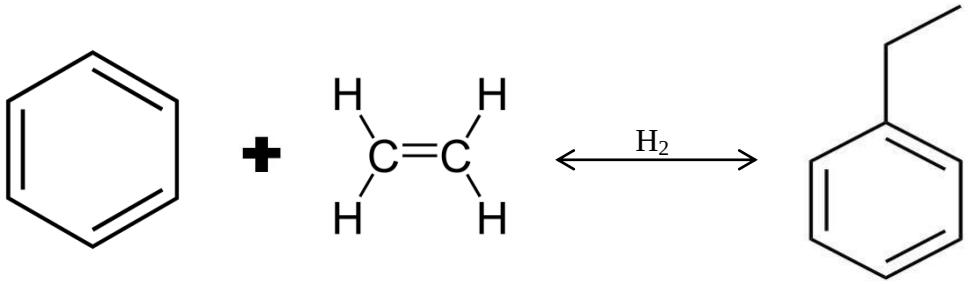


Figure 2.1 Ethylbenzene production

It is classified as a monocyclic aromatic hydrocarbon since it is a compound that contains only carbon and hydrogen atoms (Boonsaner et al., 2011). The properties of ethylbenzene are shown in Table1. Ethylbenzene also occurs at 15 to 20% in the "mixed xylenes" stream isolated at some petroleum refineries for use as a solvent. Ethylbenzene is a colorless liquid that smells similar to gasoline with a sweet aroma, evaporates quickly and is highly flammable. It is naturally found in petroleum, paints, inks, insecticides, carpet glues and tobacco products. It is also present in the environment from human activities. People can be exposed to the pollutant through breathing and from drinking water.

Table 2.1 Properties of ethylbenzene (Chiou *et al*., 1983; Verschueren, 1983: Hodson and Williams, 1988; Howard, 1989; Herman *et al*., 1991; Howard *et al*., 1991; Mackay *et al*., 1992; Budavari, 1996; Lewis, 1996; WHO, 1996 ; ATSDR, 1999; NTP, 2001; HSDB, 2003; RAIS, 2003; OEHHA, 2003)

Property	**Value**
Molecular Weight	106.16
Physical State	Liquid
Melting Point	-94.9 to -95°C
Boiling Point	136.1-136.2°C at 101.3 kPa
Specific Gravity (liquid)	0.866 at 25°C/25°C 0.867 at 20°C/4°C
Specific Gravity (gas; air=1)	3.66

Table 2.1 Properties of ethylbenzene. (cont.) (Chiou *et al*., 1983; Verschueren, 1983: Hodson and Williams, 1988; Howard, 1989; Herman *et al*., 1991; Howard *et al*., 1991; Mackay *et al*., 1992; Budavari, 1996; Lewis, 1996; WHO, 1996 ; ATSDR, 1999; NTP,2001; HSDB, 2003; RAIS, 2003; OEHHA, 2003)

Property	**Value**
Vapour Pressure	0.933 -1.24 at 20°C 1.27 -1.28 at 25°C 1.6 at 30°C
Solubility	Soluble in sulphur dioxide,ether, alcohol and most organic solvents Slightly soluble in chloroform and water
Insoluble	ammonia
Henry's Law Constant	0.00788 - 0.00844 atm.m^3/mol at 25°C
Octanol Water Partitioning Coefficient (log Kow)	3.13-3.15 4.34
Octanol Carbon Partitioning Coefficient (log Koc)	1.98-3.04 2.21-2.22 2.38
Flash Point (closed cup)	21°C
Explosive Limits	1.2% to 6.8%
Autoignition Temperature	432°C
Odour Threshold	2-2.6 mg/m^3
Bioconcentration Factor -Fish -Algae	 15 2.31
Conversion Factors for Vapour (at 25°C and 101.3 kPa)	1 ppm = 4.35 mg/m^3 1 mg/m^3 = 0.23 ppm

2.2 Ethylbenzene Releases to The Environment

The production of ethylbenzene in 1999 was reported by Dewitt at the United States was 6,738,000 metric tons; in Canada were 1,020,000 metric tons and 176,000 metric tons in Mexico (Dewitt, 1999). In Germany, more than 1,200,000 metric tons of ethylbenzene were produced in 2000. The total production volume in the EU (including Germany) can be estimated to be about 5,700,000 tons per year based on data from 13 producers . Ethylbenzene moves easily into the air from water and soil. Ethylbenzene in soil can also contaminate groundwater. If human live in a city or near many factories or heavily traveled highways, you may be exposed to ethylbenzene in the air. United States Toxic Release Inventory (TRI) data indicates that greater than 99% of releases are anticipated to be to the air compartment shown in Table2.2.

Table 2.2 TRI on and off-site reported release of ethylbenzene in United States, 1998, all industries

Emission Rate	Lbs.	Kg	%
Total to Air	8,499,147	3,855,148	99.5
Total to Water	10,408	4,721	0.1
Total to Soil	32,863	14,906	0.4
to Sediment	0	0	0

Alberta Environment has conducted a quantity of air quality monitoring surveys over the past several years in various regions of Alberta (NPRI, 2001). Some of these surveys have reported ambient air concentration of ethylbenzene (Table 2.3). World ethylbenzene demand increased at an average annual rate of 2.9% from 2008 to 2013. Consumption is expected to grow the fastest in the Middle East and China (Figure 2.2) (Chemical Economics Handbook, October 2012).

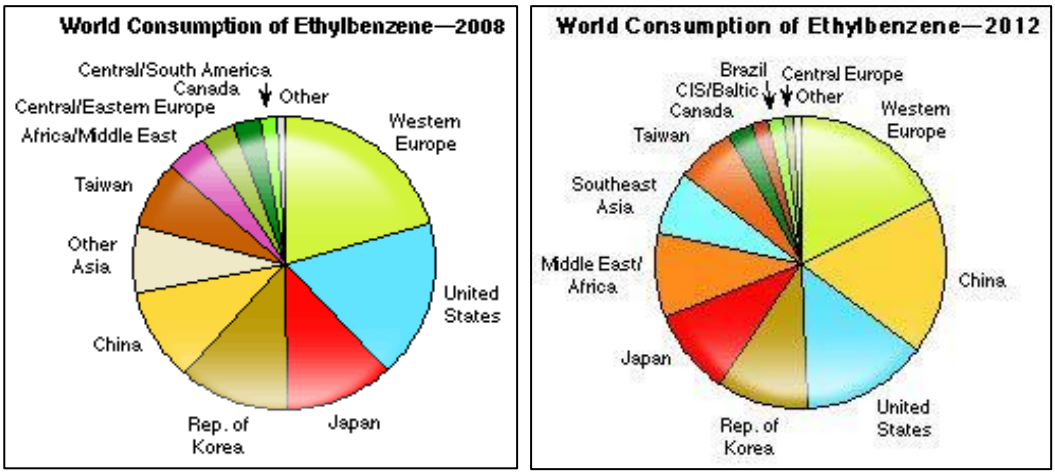


Figure 2.2 World consumption of ethylbenzene (Chemical Economics Handbook, October 2012

Table 2.3 Total On-site Releases (ton/year) of ethylbenzene in Alberta (NPRI, 2001)

Facility Name	City	Total Releases (tones/year)			
		Air	Land	Water	Tatal
Syncrude Canada Ltd. – Mildred Lake Plant Site	Fort McMurray	70.09	0	0	70.09
Suncor Energy Inc.-Suncor Energy Inc. Oil Sands	Fort McMurray	41.28	0	0	41.28
Shell Chemical Canada Ltd.-Scotford Chemical Plant	Fort Saskatchewan	16.79	0	0	16.79
Chevron Canada Resources-Kaybob South#3 Gas Plant	Fox Creek	11.13	0	0	11.13
Shell Canada Products-Shell Scotford Refinery	Fort Saskatchewan	3.08	1.29	0	4.37
Petro-Canada- Edmonton Refinery	Edmonton	1.16	1.88	0	3.04
Imperial Oil-Strathcona Refinery	Edmonton	1.92	0.33	0	2.26
Novagas Canada Limited Partnership-Harmattan Gas Plant	Olds	1.29	0	0	1.29
Conoco Canada Limited Partnership-Harmatten Gas Plant	Edson	1.11	0	0	1.11
Paramount Resources Limited- Kaybob Gas Plant	Fox Creek	0.98	0	0	0.98

2.3 Health Effect of Ethylbenzene

Ethylbenzene is readily absorbed following inhalation, oral, and dermal exposures, distributed throughout the body, and excreted primarily through urine. There are two different metabolic pathways for ethylbenzene with the primary pathway being the α-oxidation of ethylbenzene to 1-phenylethanol, mainly as the R-enantiomer. The pattern of urinary metabolite excretion varies with different mammalian species. In humans, ethylbenzene is excreted in the urine as mandelic acid and phenylgloxylic acids except rats and rabbits excrete hippuric acid and phenaceturic acid as the main metabolites.

Ethylbenzene can induce liver enzymes and hence its own metabolism as well as the metabolism of other substances. Low order of ethylbenzene has an acute toxicity by the oral, dermal or inhalation routes of exposure. Studies in rabbits indicate that ethylbenzene is irritating to the skin and eyes. There are numerous repeat dose studies available in a variety of species these include rats, mice, rabbits, guinea pig and rhesus monkeys (Table 2.4).

Table 2.4 Acute toxicity, Irritation, and Sensitization of ethylbenzene **(**Wolf et al., 1956; Smyth et al., 1962; Opdycke,1975**)**

Acute oral	Rat	LD50 = 3.5 to 4.7 g/kg (5.4 ml/kg)
Acute inhalation	Rat	4 hr LC50 = 17.4 mg/l (4000 ppm)
Acute dermal	Rabbit	LD50 = 15.4 g/kg
Dermal irritation	Rabbit	Irritating
Eye irritation	Rabbit	Irritating

In a 13 weeks inhalation, effect of male and female rats on doses >250 ppm of ethylbenzene was found in mild body weight or organ weight such as kidney, liver and lung. In chronic toxicity/carcinogenicity studies of both rats and mice to ethylbenzene via inhalation to 0, 75, 250 or 750 ppm for 104 weeks found that kidney rats was the target organ of toxicity, with renal tubular hyperplasia noted in both males and females at the 750 ppm level only. In liver and lung mice were the principal target organs of toxicity. In male mice exposed at 750 ppm lung toxicity on alveolar epithelial metaplasia and occur liver toxicity such as hepatocellular syncitial alteration, hypertrophy and mild necrosis as a result to accompany by increased follicular cell hyperplasia in the thyroid were reported no adverse effect in male mice was observed when exposed to ethylbenzene at 250 ppm.

2.4 Ethylbenzene Degradation

2.4.1 Ethylbenzene Degradation in Air

Ethylbenzene undergoes atmospheric transformations through reaction with photochemically generated hydroxyl radicals, and atomic oxygen (Grovenstein and Mosher 1970; Herron and Huie 1973). The prominent degradation pathway for ethylbenzene in the atmosphere occurs via reaction with hydroxyl radicals and nitrate radicals with the other degradation mechanisms being of only minor importance. The rate constant for the vapor phase reaction of ethylbenzene with photochemically generated hydroxyl radicals (Kwok and Atkinson 1994). Atmospheric degradation occurs more rapidly during summer months as opposed to winter since the concentration of hydroxyl radicals in the atmosphere peaks during summer (Ravishankara et al. 1978; Singh et al. 1981), and is also faster under photochemical smog conditions (Dilling et al. 1977). Oxidation by-products from the reaction with hydroxyl radicals and nitrogen oxides include ethylphenols, benzaldehyde, acetophenone, and m- and p-nitroethylbenzene (Hoshino et al. 1978). The major degradation pathways for ethylbenzene in the atmosphere are summarized in Figure 2.3.

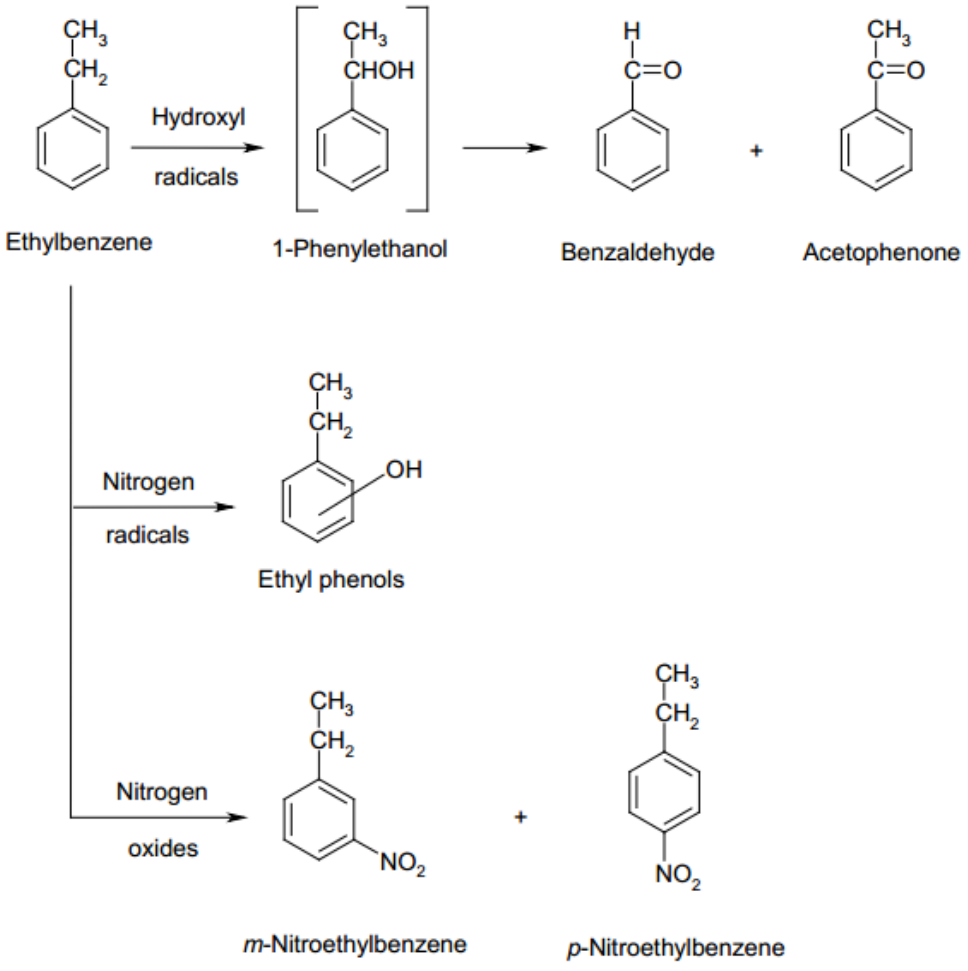


Figure 2.3 Major Degradation Pathways of ethylbenzene in the Atmosphere(Hoshino et al. 1978)

Experiments conducted with various hydrocarbons on the formation of photochemical aerosols or the haze associated with smog revealed that aromatics such as ethylbenzene produced only low yields of aerosol when compared with more reactive compounds such as alkenes (O'Brien et al. 1975). The photoreactivity of ethylbenzene is intermediate relative to other atmospheric hydrocarbons, and it is less reactive than gasoline, toluene, and alkenes such as propene (Yanagihara et al. 1977).

2.4.2 Biodegradation of Ethylbenzene

Bioremediation is an alternative that offers the possibility to break or render harmless various contaminants using natural biological activity. It uses relatively low-cost, low technology techniques, which generally have a high public acceptance and can often be carried out on site. By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. Although the methodologies employed are not technically complex, considerable experience and dexterity may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result.

The control and optimization of bioremediation processes is a complex system of many factors. These factors include the existence of a microbial population capable of degrading the pollutants the availability of contaminants to the microbial population the environment factors such as type of soil, temperature, and pH, the presence of oxygen or other electron acceptors, and nutrients.

Because bioremediation seems to be a good alternative to conventional clean-up technologies, especially in the United States, rapidly increasing. Bioremediation has been used at a number of sites worldwide, including Europe, with varying degrees of success. Techniques are improving as greater knowledge and experience are gained, and there is no doubt that bioremediation has great potential for dealing with certain types of site contamination. Unfortunately, the principles, techniques, advantages, and disadvantages of bioremediation are not widely known or understood, especially among those who will have to deal directly with bioremediation proposals, such as site owners and regulators.

2.4.2.1 Phytoremediation

Phytoremediation is an emerging technology that utilizes plants and then the associated rhizosphere microorganisms to remove, transform, or contain toxic chemicals located in soils, sediments, ground water, surface water, and even the atmosphere. Currently, phytoremediation is used for treating many classes of contaminants including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radionuclides, and landfill leachates. (Sridhar S., Victor F., (2002)). Phytoremediation techniques have five types, classified based on the contaminant fate: phytoextraction, phytotransformation, phytostabilization, phytodegradation, rhizofiltration, even if a combination of these can be found in nature.

2.4.2.2 Phytoextraction or Phytoaccumulation

Phytoextraction or phytoaccumulation is the process used by the plants to accumulate contaminants into the roots and aboveground shoots or leaf. This technique saves tremendous remediation cost by accumulating low levels of contaminants from a widespread area. Unlike the degradation mechanisms, this process produces a mass of plants and contaminants (usually metals) that can be transported for disposal or recycling.

2.4.2.3 Phytotransformation or Phytodegradation

Phytotransformation or phytodegradation refers to the uptake of organic contaminants from soil, sediments, or water and, subsequently, their transformation to more stable, less toxic, or less mobile form. Metal chromium can be reduced from hexavalent to trivalent chromium, which is a less mobile and noncarcinogenic form.

2.4.2.4 Phytostabilization

Phytostabilization is a technique in which plants reduce the mobility and migration of contaminated soil. Leachable constituents are adsorbed and bound into the plant structure so that they form a stable mass of plant from which the contaminants will not reenter the environment.

2.4.2.5 Phytodegradation or Rhizodegradation

Phytodegradation or rhizodegradation is the breakdown of contaminants through the activity existing in the rhizosphere. This activity is due to the presence of proteins and enzymes produced by the plants or by soil organisms such as bacteria, yeast, and fungi. Rhizodegradation is a symbiotic relationship that has evolved between plants and microbes. Plants provide nutrients necessary for the microbes to thrive, while microbes provide a healthier soil environment.

Phytoremediation is a technique by which plants and the associated rhizosphere microorganisms are utilized to remove, transform, or contain toxic chemicals located in soils, sediments, groundwater, surface water, and the atmosphere. Phytostimulation involves the stimulation of the microorganisms in the location by using plants that have been tested for the destruction of PAH, BTEX, and other petroleum hydrocarbons. Advantages and disadvantages of phytoremediation over traditional technologies such as pump and treat of contaminated groundwater and soil excavation and above-ground treatment are shown in table 2.5.

Table 2.5 Advantages and disadvantages of phytoremediation

Advantages	Disadvantages
Relatively low cost Easily implemented and maintained Several mechanisms for removal Environmentally friendly Aesthetically pleasing Reduces landfilled wastes Harvestable plant material	Longer remediation times Climate dependent Results are variable Effects to food web might be unknown Ultimate contaminant fates might be unknown

2.5 Plant Species in Research

2.5.1 *Zamioculcas zamiffolia*

In 2013, *Zamioculcas zamiifolia* has the highest potential to reduce the concentration of BTEX from contaminated indoor air by 75% of ethylbenzene was removed by stomata and 25% of ethylbenzene was removed by cuticles (Sriprapat and Thiravetyan, 2013).

Figure 2.4 *Zamioculcas zamiifolia*

Zamioculcas (Common name "Zanzibar Gem") is a genus of flowering plant in the family Araceae, containing the single species *Zamioculcas zamiifolia*. It is a tropical perennial plant native to eastern Africa, from Kenya south to northeastern South Africa. Dutch nurseries started wide-scale commercial propagation of the plant around 1996.

It is an herbaceous plant growing to 45-60 cm tall, from a stout underground, succulent rhizome.It is normally evergreen, but becomes deciduous drinking drought, surviving drought due to the large potato-like rhizome that stores water until rainfall resumes.The leaf are pinnate, 40-60 cm long, with 6-8 pairs of leaflets 7-15 cm long; they are smooth, shiny, and dark green. The flowers are produced in a small bright yellow to brown or bronze spadix 5-7 cm long, partly hidden among the leaf bases; flowering is from midsummer to early autumn. (Catherine and Horwood (2007)).

2.5.2 *Sansevieria trifasciata* hort. ex Prain cv. Hahnii Green

Figure 2.5 *Sansevieria trifasciata*

Sansevieria trifasciata is an ornamental plant in the Dracaenaceae family and common name is Snake Plant or Mother-in-Laws. *Sansevieria trifasciata* is classified as plants, grasses and herbaceous plants that occur naturally in the season. Is native to the arid tropical climates such as South Africa, India, Sardar Cascardi. Arabian Peninsula (www.woldplantcenter.com). The trunk is the head or rhizomes in the soil. Leaf is a long rod and flat or sharp edges smooth thick it is slightly bend. A width of about 4-7 cm. and height of 30-60 cm. *Sansevieria trifasciata* colorful leaf are pale green to dark green (http://meen5540133.blogspot.com/p/3.html).

2.5.3 *Sansevieria kirkii* var. pulchra Coppertone

Figure 2.6 *Sansevieria kirkii*

Sansevieria kirkii var. pulchra "Coppertone" is really different in that it keeps its copper color regardless of watering and even in humid climates. Lack of light will turn it a grayish color, also very attractive. A deep green, almost black is overlaid with copper color and the edges are in copper also. The stout leaves, 1-3 to a growth, is very thick and rigid, spreading horizontally and can grow 2.5-6' long and 2-3" wide (Annie's Magic Garden**).**

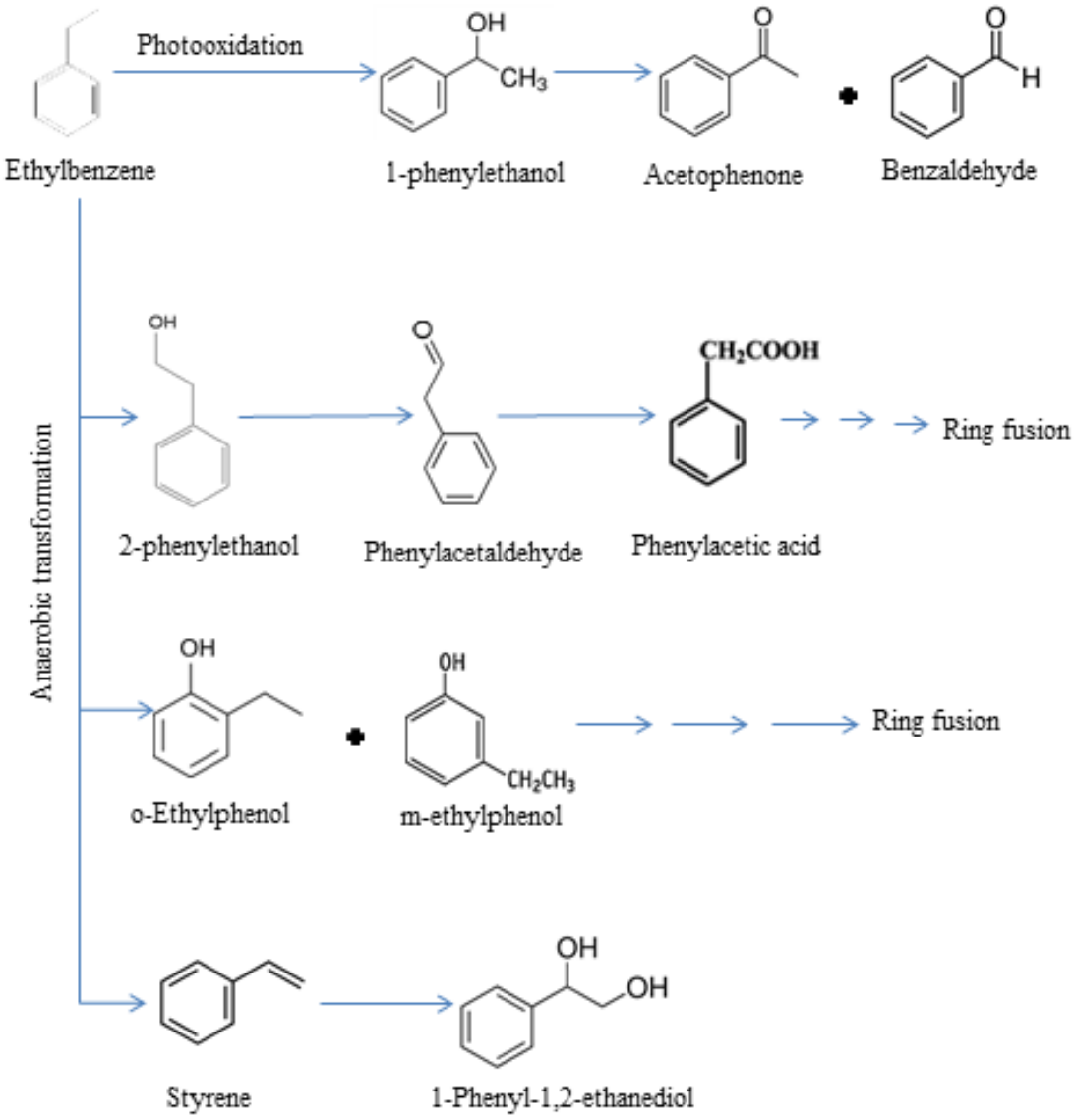
2.6 Soil Microorganisms degradation

Biodegradation of ethylbenzene by aerobic soil microbes has been reported by various researchers. Burback and Perry (1993) reported than Mycobacterium vaccae can catabolize a number of major groundwater pollutants, including ethylbenzene. 50 ppm of ethylbenzene was degraded about 80% of the added ethylbenzene and product 4-ethylphenol and small amount of 1-phenylethanol.

The common soil microorganism *Pseudomonas putida* is able to utilize ethylbenzene as a sole source of carbon and energy (Fukuda et al. 1989; Gibson et al. 1973). In some instances, co-oxidation or cometabolism was observed such as *Nocardia sp.* degraded ethylbenzene in the presence of other compounds that are more readily metabolized by the microorganism (Jamison et al. 1970; Van der Linden and Thijsse 1965). Yadav and Reddy (1993) reported that the white-rot fungus *Phanerochaete chrysosporium* efficiently degraded ethylbenzene as well as benzene, toluene and xylenes when these chemicals were added either individually or as a composite mixture. Chen and Taylor (1995) reported that two thermophilic bacterial strains, *Thermus aquaticus* and *an unidentified Thermus sp.* degraded ethylbenzene (in a mixture with other BTEX chemicals) by 18% after 45 days of incubation at 70 °C and by 32% after 45 days of incubation at 60 °C, respectively.

The microorganism uses of hydrocarbons as their carbon and/or energy sources and degrade the hydrocarbons to carbon dioxide and water. Since the crude oil contains simple aromatic, and polyaromatic hydrocarbons (PAHs), its biodegradation involves the interaction of many different microorganisms. The common hydrocarbon degrading organisms in the marine environment are *Pseudomonas*, *Acinetobacter, Nocardia*, *Vibro*, and *Achromobacter* (Floodgate, 1984; Salleh et al., 2003).

Straight chain alkanes are easily and rapidly degraded by several microorganisms, including *Acinetobacter* sp., *Actinomycetes, Arthrobacter, Bacillus sp., Candida sp., Micrococcus sp., Planococcus, Pseudomonas sp., Calcoaceticus*, and *Streptomyces* (Surzhko et al., 1995). *Pseudomonas* sp., *Ralstonia* sp., *Rhodococcus* sp, and *Sphingomonas* sp. are some of the microorganisms that are known to oxidative degrade monoaromatics like benzene, toluene, ethylbenzene and xylenes (BTEX) (Lee and Lee, 2001; Parales et al., 2000). Toluene aerobically degrades more rapidly than other BTEX compounds in a wide variety of strains such as *Pseudomonas putida* mt-2, *P. mendocina*, and *R. picketti* PKO1, either through the formation of substituent groups on the benzene ring or on the methyl group. The products could be cresols, benzyl alcohol, or dihyrol. A *Pseudomonas* sp. oxidizes xylenes at the methyl group, similar to the degradation of toluene, forming several intermediates. The major degradation pathways for ethylbenzene are summarized in Figure 2.7 (Burback and Perry 1993; Ehrhardt and Petrick 1984; Van der Linden and Thijsse 1965).

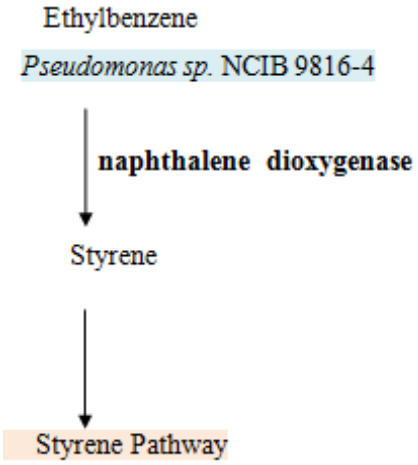


Sorce: Burback and Perry 1993; Ehrhardt and Petrick 1984;

Van der Linden and Thijsse 1965

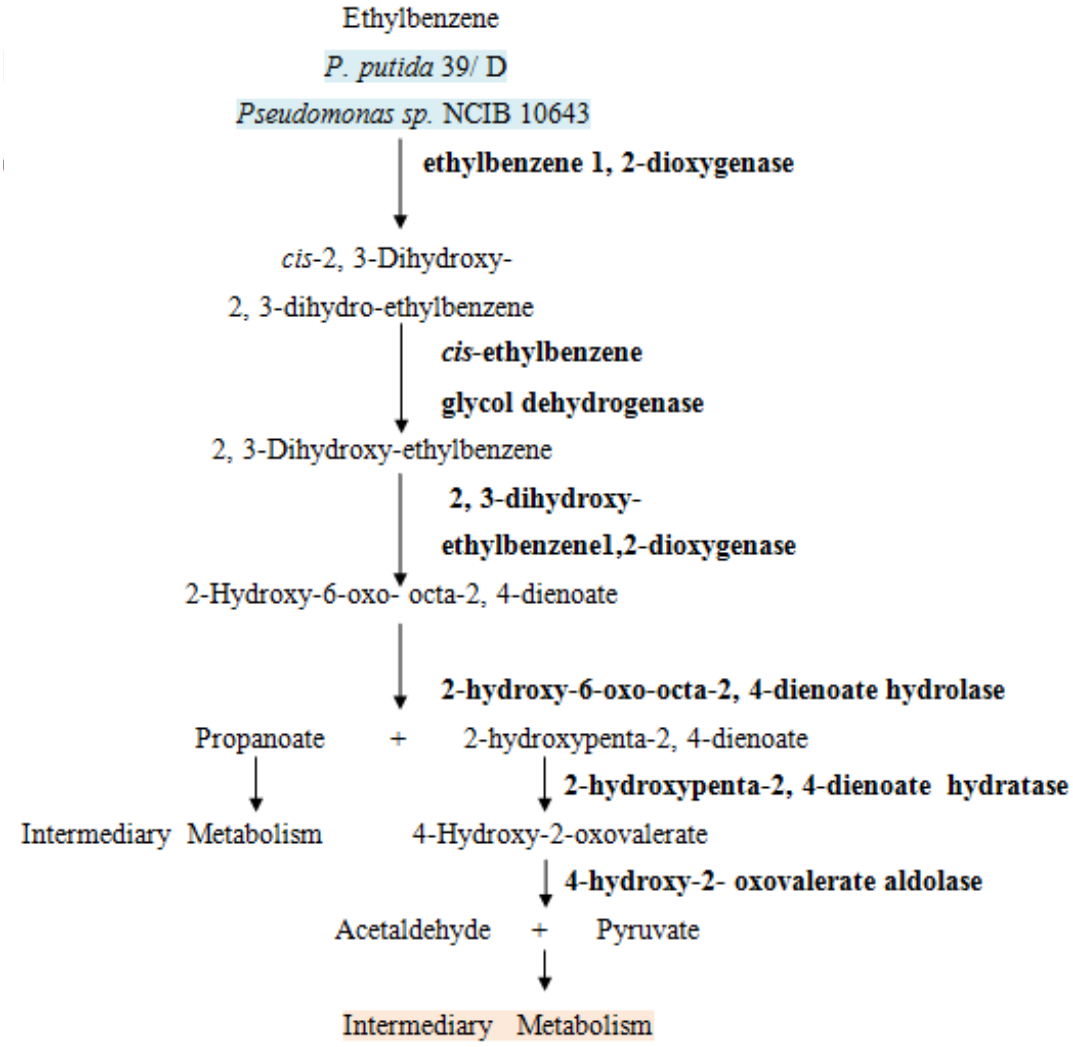
Figure 2. 7 Major pathways of ethylbenzene degradation in water, sediment, and soil by microorganisms.

Ethylbenzene degradation pathway by microorganisms was contributed by Ryan Mc Leish, University of Minnesota (Figure 2.8-2.19). *Pseudomonas sp.* NCIB 9816-4 is reported by Lee & Gibson (1996) that napthalene dioxygenase show highly relaxed substrate specificity and is capable of aerobically degrading ethylbenzene to styrene and 2-hydroxy acetophenone (Figure 2.8 and 2.10). This degradation is initiated by a dioxygenation of the aromatic ring and leading to an extradiol ring cleavage. Information concerning the further degradation of 2-hydroxyacetophenone is not available, though 4-hydroxyacetophenone can undergo aerobic biodegradation. Naphthalene dioxygenase has many other catalytic abilities, which are documented in a table of the reactions of naphthalene 1, 2-Dioxygenase. *Pseudomonas sp*. strain NCIB 10643 has been shown to utilize ethylbenzene and degrade too many chemical (Figure 2.9).



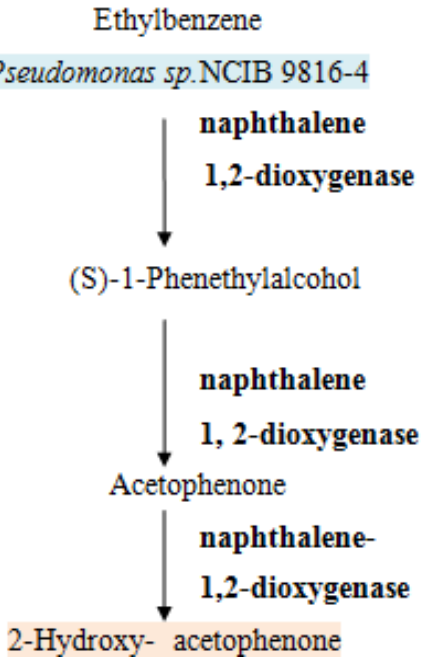
Source: Lee & Gibson (1996)

Figure 2.8 Ethylbenzene degradation by *Pseudomonas sp*. NCIB 9816-4



Source: Lee & Gibson (1996)

Figure 2.9 Ethylbenzene degradation by *P.putida 39/D* and *Pseudomonas sp*. NCIB 10643



Source: Lee & Gibson (1996)

Figure 2.10 Ethylbenzene degradation by *Pseudomonas sp.* NCIB 9816-4

Oxygenases are classified as dioxygenases and monooxygenases according to the number of atoms of oxygen that are transferred to a carbon compound in the catalyzed reaction. In dioxygenase reactions, both oxygen atoms are incorporated into one or two carbon compounds. Examples of dioxygenases in plant cells are lipoxygenase which catalyzes the addition of two atoms of oxygen to unsaturated fatty acids and prolyl hydroxylase which was the enzyme that converts proline to the less common amino acid hydroxyproline.

Hydroxyproline is an important component of the cell wall protein extension. The synthesis of hydroxyproline from proline differs from the synthesis of all other amino acids in that the reaction occurs after the proline has been incorporated into protein, and it is therefore a posttranslational modification reaction. Prolyl hydroxylase is localized in the endoplasmic reticulum, suggesting that most proteins containing hydroxyproline are found in the secretory pathway.

Monooxygenases add one of the atoms in molecular oxygen to a carbon compound. The other oxygen atom is converted into water. Monooxygenases are sometimes referred to as mixed-function oxidases because of their ability to catalyze simultaneously both the oxygenation reaction and the oxidase reaction. The monooxygenase reaction also requires a reduced substrate (NADH or NADPH) as an electron donor, according to the following equation:

$$A + O_2 + BH_2 \rightarrow AO + H_2O + B$$

Where A represents an organic compound and B represents the electron donor.

An important monooxygenase in plants is the family of heme proteins collectively called cytochrome P450 which catalyzes the hydroxylation of cinnamic acid to p-coumaric acid. In monooxygenases, the oxygen is first activated by being combined with the iron atom of the heme group by NADPH serves as the electron donor. The mixed-function oxidase system is localized on the endoplasmic reticulum and is capable of oxidizing a variety of substrates, including mono- and diterpenes and fatty acids.

Oxygenases play an important role in the degradation/detoxification of pollutants in the environment. Mono- or di-oxygenases with a broad substrate specificity, including ammonia monooxygenase (AMO), (Hyman et al.,1988; Arciero et al., 1989; Rasche et al., 1991; Keener and Arp,1994; Chang et al., 2002; Shi et al., 2004; Sayavedra-Soto et al.,2010), propane monooxygenase (PMO) (Wackett et al., 1989; Steffan et al., 1997; Sharp et al., 2005; Vainberg et al., 2006) and biphenyl dioxygenases (Furukawa et al., 2004; Fritsche and Hofrichter,2006; Haritash and Kaushik, 2009; Robrock et al., 2011) have shown the ability to cometabolize a wide range of aliphatic and/or aromatic compounds. Accordingly, it is possible that other oxygenase-expressing bacteria can degrade ethylbenzene via singlemetabolic reactions or co-metabolic reactions.

2.7 Epiphytic Bacteria

Aerial plant parts harbor hundreds of species of bacteria, yeast, and fungi. Bacteria are largely the most numerous colonists often being found at more of 10^7 cells/cm^2 of leaf surface (Lindow S. E. and Leveau J., 2002). When one considers that a large fraction of the earth's surface is covered with plants that leaf surfaces often represent a substantial multiple of the soil surface area and that leaf and flowers often have complex topographical features on which colonization can occur. The potential population size of microbial associates of plants is really impressive. The aerial habitat influenced by plants is termed the phyllosphere and inhabitants are called epiphytes (Lindow S. E. and Leveau J., 2002).

Epiphytic bacteria are bacteria that are capable of living or multiplying on plant surfaces (Hirano and Upper, 1991; Leben, 1965). In contrast to those that are considered endophytic bacteria, the epiphytes can be removed from leaf by washing (Leben, 1965), or killed by UV irradiation or chemical surface sterilization (Henis and Bashan, 1986). All the bacteria associated with leaf have been referred to as "phyllobacteria" (Beattie and Lindow, 1999) in parallel to the term, "rhizobacteria" that has been used to refer to all the root-associated bacteria.

Members of all plant phyla are colonized by microbial epiphytes with more than 85 different species of microorganisms in 37 genera recovered from the phyllosphere of rye, olive, sugar beet, and wheat despite the hostile environment of the leaf surface (Morris et al., 1997; Yang et al., 2001). The most common bacterial such as *Pseudomonas fluorescens*, *P. corrugata*, *P. tolaasii*, *P. paucimobilis*, *X. campestris*, and *Enterobacter cloacae,* species also found on plant surfaces (Tanprasert and Reed, 1998).

2.8Chemical metabolite in plant

2.8.1 1-Phenylethanol

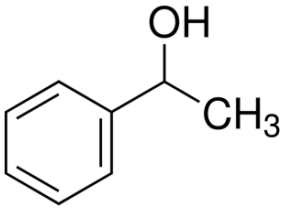


Table 2.6 Physical property of 1-phenylethanol (Hans-Georg Elias, 2009; Paula Bruice Yurkanis, 2013

Synonyms	α-methylbenzyl, Phenylmethylcarbinol
Chemical Formula	$C_8H_{10}O$
Molecular Weight	122.17 g/mol
Density	1.01g/cm^3
Melting point	20ºC
Boiling point	204ºC
Solubility	Slightly soluble in water (20g/l at 20ºC) Soluble in most organic solvents
Refractive index	1.527 (20ºC)

1-phenylethanol is a chemical compound from the group of alcohols and structural isomer to 2-phenylethanol. 1-phenylethanol can be obtained by reduction of acetophenone or by the oxidation of ethylbenzene are recovered (Hazardous Substances Data Bank, 2013). 1-phenylethanol is a little volatile, highly non-flammable, colorless liquid with a faint flowery smell that is slightly soluble in water ((Hazardous Substances Data Bank, 2013 and *CAS-No. 98-85-1,* 2013) with an acidic solution of sodium dichromate reacts to benzoic acid (Paula Bruice Yurkanis, 2013). 1-phenyl ethanol is used as high boilers in coating materials (*CAS-No. 98-85-1,* 2013). It is also used for production of styrene used (Hans-Georg Elias, 2009).

Acute Health Effects

1. This material causes severe eye damage.
2. Repeated exposure can cause contact dermatitis which is characterized by redness, swelling and blistering.
3. Skin contact with the material may damage the health of the individual; systemic effects may result following absorption.

2.8.2 4-Ethylphenol

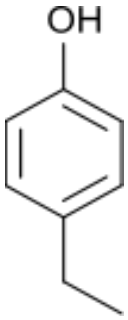


Table 2.7 Physical property of 4-ethylphenol

Synonyms	*p*-Ethylphenol 1-Ethyl-4-hydroxybenzene 1-Hydroxy-4-ethylbenzene 4-hydroxyphenlethane
Chemical Formula	$C_8H_{10}O$
Molecular Weight	122.167 g/mol
Melting point	42-45ºC
Boiling point	218ºC

4-Ethylphenol is a phenolic compound which industry produced from the precursor *p*-coumaric acid. However, it can degrade from ethylbenzene by microorganisms such as *Aromatoleum aromaticum* EbN1 and this microorganism can utilize 4-ethylphenol under anoxic conditions (Wohlbrand et al., 2008) to other chemical (Figure 2.11).

Acute Health Effects

1. Harmful if ingested or inhaled. Minimize exposure to this material. Severe overexposure can result in injury or death.
2. Irritating to eyes and skin on contact. Inhalation causes irritation of the lungs and respiratory system. Inflammation of the
3. Eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, occasionally, blistering.

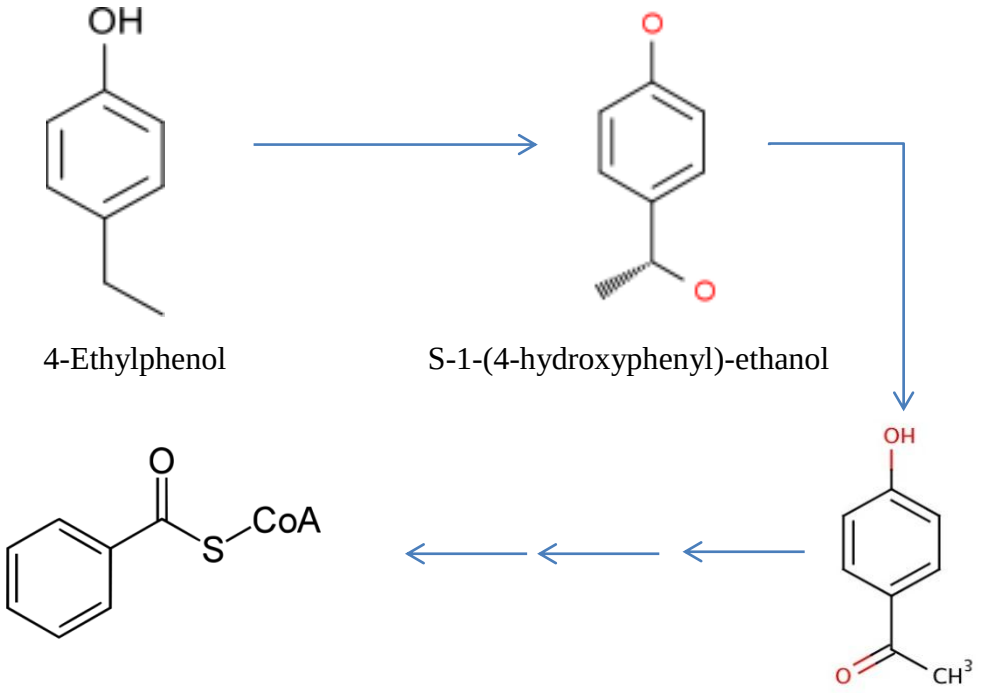


Figure 2.11 4-ethylphenol degradation pathway by *Aromatoleum aromaticum* EbN1 (Wohlbrand et al., 2008)

2.8.3 Acetophenone

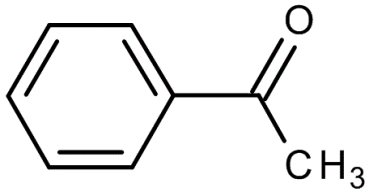


Table 2.8 Physical property of 4-ethylphenol (Hardo Siegel and Manfred Eggersdorfer, 2012)

Synonyms	Phenyl methyl ketone
Chemical Formula	$C_8H_{10}O$
Molecular Weight	120.15g/mol
Density	1.028g/cm^3
Melting point	19-20ºC
Boiling point	202ºC
Solubility	Slightly soluble in water (5.5g/l at 25ºC, 12.2g/l at 80ºC) Soluble in most organic solvents
Flash point	77ºC

Acetophenone is the organic compound with the formula $C_6H_5C(O)CH_3$. It is the simplest aromatic ketone. This colourless, viscous liquid is a precursor to useful resins and fragrances (Hardo Siegel and Manfred Eggersdorfer, 2012). Acetophenone can be obtained by a variety of methods. In industry, acetophenone is recovered as a by-product of the oxidation of ethylbenzene, which mainly gives ethylbenzene hydroperoxide for use in the production of propylene oxide (Hardo Siegel and Manfred Eggersdorfer, 2012).

Acute Health Effects

1. Acute exposure of humans to acetophenone vapor may produce skin irritation and transient corneal injury. (U.S. Environmental Protection Agency, 1987 and U.S. Department of Health and Human Services, 1993)
2. Acute oral exposure has been observed to cause hypnotic or sedative effects, hematological effects, and a weakened pulse in humans. (Sittig M., 1985 and U.S. Department of Health and Human Services, 1993)
3. Tests involving acute exposure of rats, mice, and rabbits have demonstrated acetophenone to have moderate acute toxicity from oral or dermal exposure. (U.S. Department of Health and Human Services, 1993)