Chapter-I

INTRODUCTION
The ever increasing demand for more food, fiber and timber has necessitated the use of increasing quantities of pesticides. Though pesticides are used mostly in the terrestrial environment, their residues are carried to the aquatic environment either in rain or through run-off and sediment. Besides the pesticides, residues of a host of industrial organic chemicals are also carried into the aquatic environment. It is now very well recognized that the aquatic environment is the ultimate sink for all man made chemicals, and any chemical used on an industrial scale would ultimately find its way into the aquatic environment. Of the large number of chemicals in daily use, many are known to be contaminants of the aquatic environment. It is also a known fact that the environmental pollutants pose a greater problem to the aquatic organisms than to those in other compartments of this planet. As a consequence, the evaluation of the hazard of a chemical to the aquatic organisms has been made mandatory.

For every new chemical that is to be registered, the producer or the intending user has to furnish data on the acute toxicity of that chemical to a species of fish, *Daphnia* and an algae, the species for base-level aquatic toxicity testing. At higher levels, other types of tests like chronic toxicity, residue accumulation and other tests may also be needed. The regulatory agencies (US Environmental Protection Agency, the Environment Canada, etc.) may call for similar information of the chemicals already being used to reassess the risk posed by them. Acute toxicity test with fish has been chosen as one of the mandatory aquatic toxicity tests because it is not only a time- and cost- efficient test, but is also legally and scientifically defensible and reproducible to a large extent.

Of late, in India there has been a tremendous increase in the use of pesticides and industrial chemicals. Phenol and its derivatives are common substances present in industrial waste waters i.e chemical, petrochemical, paint, textile, etc. and in non-specific pesticides, herbicides, bactericides and fungicides
(Hori et al., 2008; Avilez et al., 2008). The contamination of water bodies with phenol is a serious problem in terms of environmental considerations due to its high toxicity.

Phenols and phenolic substances are aromatic hydroxy compounds classified as monohydric (with one hydroxy group e.g., phenol, cresols [methylphenols], xylenols [dimethylphenols]), dihydric (with two hydroxy groups e.g., catechols [o-dihydroxybenzenes], resorcinols [m-dihydroxybenzenes]) or polyhydric (with three or more hydroxy groups e.g., pyrogallol [1,2,3-trihydroxybenzene]), depending on the number of hydroxyl groups attached to the aromatic benzene ring. Phenols and particularly chlorophenols are toxic and particularly carcinogenic, and can affect the taste and odor of drinking water with concentrations as low as a few µg l⁻¹. As a consequence, both the USEPA (1977) and EU (2001) have included some phenols, mainly chlorophenols and nitrophenols in their lists of priority pollutants.

The structures of eleven phenols considered priority pollutants are shown in Fig.1. EU Directive 2455/2001/EC sets a maximum concentration of 0.5 µg l⁻¹ in drinking water and their individual concentration should not exceed 0.1 µg l⁻¹. Phenol is essentially a benzene molecule in which one hydrogen atom is replaced by a hydroxyl group. The chemical formula for phenol is C₆H₅OH and its CAS (Chemical Abstract Service) number is 108-95-2. It is colorless to light pink crystalline solid at room temperature and ambient pressure although it readily absorbs moisture and liquefies (ECB, 2006). It is water soluble and a weak acid. The physical and chemical properties of phenol are given in table 1.1.
Fig. 1.1. Structures of eleven phenolic compounds considered priority pollutants.
<table>
<thead>
<tr>
<th>1</th>
<th>CAS Registry Number</th>
<th>108-95-2</th>
<th>ATSDR, (2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Synonym(s)</td>
<td>benzenol, hydroxybenzene, phenyl alcohol, phenyl hydrate, phenyl hydroxide, oxybenzene, monophenol</td>
<td>ATSDR, (2008)</td>
</tr>
<tr>
<td>3</td>
<td>Registered trade name(s)</td>
<td>carbolic acid, phenic acid phenic alcohol</td>
<td>ATSDR, (2008)</td>
</tr>
<tr>
<td>5</td>
<td>Molecular weight</td>
<td>94.11</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>6</td>
<td>M.Pt, °C</td>
<td>40.9</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>7</td>
<td>B.Pt, °C</td>
<td>181.8</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>8</td>
<td>Vapour pressure, at 25°C</td>
<td>0.3513</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>9</td>
<td>Density, at 45°C</td>
<td>1.0545</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>10</td>
<td>Flash point (open cup)</td>
<td>85°C</td>
<td>ATSDR, (2008)</td>
</tr>
<tr>
<td>11</td>
<td>Solubility</td>
<td>1 g /15 ml water acetone</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>13</td>
<td>Log K&lt;sub&gt;ow&lt;/sub&gt;</td>
<td>1.46</td>
<td>Budavari, (2001)</td>
</tr>
<tr>
<td>14</td>
<td>Odor threshold</td>
<td>0.047 ppm (0.18 mg/m³) – 100% response 0.006 ppm (0.02 mg/m³) – sensitive</td>
<td>Budavari, (2001)</td>
</tr>
<tr>
<td>15</td>
<td>Conversion factors</td>
<td>1 ppm (v/v) = mg/m³ x 0.260 1 mg/m³ = ppm (v/v) x 3.85</td>
<td>Budavari, (2001)</td>
</tr>
<tr>
<td>16</td>
<td>Color / Form</td>
<td>colorless, acicular crystals</td>
<td>Budavari, (2001)</td>
</tr>
<tr>
<td>17</td>
<td>Odor</td>
<td>sweet acrid odor</td>
<td>MERCK, (2006)</td>
</tr>
<tr>
<td>18</td>
<td>Taste</td>
<td>sharp burning taste</td>
<td>MERCK, (2006)</td>
</tr>
<tr>
<td>19</td>
<td>Critical temperature</td>
<td>694.2 K</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>21</td>
<td>Dissociation constant, pKa</td>
<td>9.89 @ 20°C</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>22</td>
<td>Heat of combustion (25°C)</td>
<td>-3053.5 kJ/mole</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>23</td>
<td>Heat of vaporization</td>
<td>57.82 kJ/mole</td>
<td>Lide, (2005)</td>
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<tr>
<td>24</td>
<td>pH (aqueous solution)</td>
<td>6.0</td>
<td>Budavari, (2001)</td>
</tr>
<tr>
<td>25</td>
<td>Surface tension at 50°C</td>
<td>38.20</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>26</td>
<td>Vapor density</td>
<td>3.24</td>
<td>Lide, (2005)</td>
</tr>
<tr>
<td>28</td>
<td>Chemical formula</td>
<td><img src="image" alt="Chemical structure of phenol" /></td>
<td></td>
</tr>
</tbody>
</table>
Production, import and export

Phenol has been obtained by distillation from petroleum and synthesis by oxidation of cumene or toluene and by vapor-phase hydrolysis of chlorobenzene (ATSDR, 2008). In 2004, nearly 98% of U.S. phenol production was based on oxidation of cumene except at one company that used toluene oxidation and a few companies that distilled phenol from petroleum (CMR, 2005). In 2004, the total annual capacity of phenol production approached 6.6 billion pounds (CMR, 2005). According to the National Trade Data Bank (USITC, 2008), exports of phenol were 503 million kg (USITC, 2008). The major importer of phenol from the United States was Canada, with an import value of 117 million kg during 2007. The total amount of phenol imported to the United States was 1.3 million kg in 2005. The largest exporter of phenol to the United States was South Africa, which exported 1 million kg of phenol (USITC, 2008).

Natural sources

Phenol is produced naturally and synthesized as a manufactured chemical. Naturally, it is a constituent of coal tar and creosote, decomposing organic material, human and animal wastes, and as a compound found in many non-foods and foods. For example, salicylic acid is a natural phenolic compound found in willow bark. Salicylic acid is also synthesized from phenol as an intermediate in the industrial production of aspirin. Phenol is formed during forest fires (ECB, 2006) and by the atmospheric degradation of benzene in the presence of light (Lovely, 2000). In addition, phenol is produced by the body and excreted as a metabolic product independent of external exposure or intake.
Anthropogenic sources

The most commonly used production method for phenol, on a worldwide scale, is from cumene. The emission factor of phenol into air during this process has been reported to be 0.16 g phenol emitted per kg phenol produced (ATSDR, 2008). Phenol is the basic feedstock from which a number of commercially important materials are made, including phenolic resins, bisphenol-A, caprolactam, alkyl phenols, as well as chlorophenols such as pentachlorophenol. The most important phenol emissions result from the use of phenolic resins which are used as a binding material in insulation materials, chipboard and triplex, paints and casting sand foundries. Emissions are approximately proportional to the concentration of free phenol, which is present as a monomer in these materials (1-5%) (ECB, 2006). In addition, phenol may be released as a result of thermal decomposition of the resins.

Phenols are also released through automobile exhaust, fireplaces, cigarette smoke (Smith et al., 2002) and gases from incinerators (Ryu et al., 2006). While these do not release directly into water, transfer to water systems may occur, as 1.3–15 µg l\(^{-1}\) via precipitation (Michalowicz and Duda, 2007). Another potential source of phenol is the atmospheric degradation of benzene under the influence of light (Lovely, 2000).

Uses

The two major uses of phenol in 2004 were the production of bisphenol-A (48%) and the production of phenolic resins (25%) (CMR, 2005). The largest use of bisphenol-A is as an intermediate in the production of epoxy resins. Phenol-formaldehyde resins comprise over 95% of the market. Other major uses of phenol include the production of caprolactam (11%), aniline (2%), alkylphenols (4%), xylenols (4%) and miscellaneous uses (6%) (CMR, 2005).
Phenolic compounds, their parent compounds and examples of environmental sources are listed in Table 1.2 (Wolff et al., 2007). Phenol is also used as a slimicide (a chemical toxic to bacteria and fungi characteristic of aqueous slimes) and as a general disinfectant in solution or mixed with slaked lime for toilets, stables, cesspools, floors, drains and other areas (MERCK, 2006; ATSDR, 2008). Phenol has antiseptic properties, and was used by Sir Joseph Lister (1827-1912) in his pioneering technique of antiseptic surgery, though the skin irritation caused by continual exposure to phenol eventually led to the substitution of aseptic (germ-free) techniques in surgery. Lister decided that the wounds themselves had to be thoroughly cleaned. He then covered the wounds with a piece of rag or lint covered in carbolic acid (Lister, 1867). It is also the active ingredient in some oral analgesics such as Chloraseptic spray as well as Carmex. Phenol was also the main ingredient of the Carbolic Smoke Ball, a device marketed in London in the 19th century as protecting the user against influenza and other ailments. The medical uses of phenol today include: incorporation into disinfectants, antiseptics, lotions, salves and ointments (EC, 2006).

Minor uses of phenol include: manufacture of paint and varnish removers, lacquers, paints, rubber, ink, illuminating gases, tanning dyes, perfumes, soaps and toys (EC, 2006). The occurrence of phenol in the environment also stems from the production and use of numerous pesticides, in particular phenoxy herbicides like 2,4-dichlorophenoxyacetic acid (2,4-D) or 4-chloro-2-methylphenoxyacetic acid (MPCA) and also phenolic biocides like pentachlorophenol (PCP), dinoseb or diarylether pesticides (Budavari, 2001).
<table>
<thead>
<tr>
<th>S. No</th>
<th>Chemical name or common synonym</th>
<th>Abbreviation</th>
<th>Parent Compound, if applicable</th>
<th>Additives, commercial and personal product exposure sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bisphenol A</td>
<td>BPA</td>
<td></td>
<td>Polycarbonate containers and coatings (cans, cups), dental sealant</td>
</tr>
<tr>
<td>2</td>
<td>Benzophenone-3(2-hydroxy-4-methoxy-benzophenone),(oxy benzene)</td>
<td>BP3</td>
<td></td>
<td>Sunscreen agent, photostabilizer for synthetic resins</td>
</tr>
<tr>
<td>3</td>
<td>2,4-Dichlorophenol and trichlorophenols (chlorinated phenols)</td>
<td>2,4-DCP, 2,4,5-TCP, 2,4,6-TCP</td>
<td>Phenoxy- and other derivatives (2,4,5 and 2,4,6 TCP are metabolites of Hexachlorobenzene and Hexachlorocyclohexane)</td>
<td>Herbicides (organochlorine pesticides)</td>
</tr>
<tr>
<td>4</td>
<td>2,5-Dichlorophenol</td>
<td>2,5-DCP</td>
<td>4-dichlorobenzene (metabolite of p-DCB)</td>
<td>Mothballs</td>
</tr>
<tr>
<td>5</td>
<td>ortho-Phenylphenol</td>
<td>o-PP</td>
<td></td>
<td>Fungicide</td>
</tr>
<tr>
<td>6</td>
<td>4-tert-Octylphenol</td>
<td>4-t-OP</td>
<td></td>
<td>Detergent surfactant</td>
</tr>
<tr>
<td>7</td>
<td>Triclosan [5-chloro-2 (2,4-dichlorophenoxy) phenol]</td>
<td>TRCS</td>
<td></td>
<td>Microbicide in home cleaning and personal care products</td>
</tr>
</tbody>
</table>
### Abiotic degradation

Phenol may react in air with hydroxyl and NO$_3^-$ radicals, and undergo other photochemical reactions to form dihydroxy-benzenes, nitrophenols and ring cleavage products (Harrison et al., 2005). The half-life for phenol in air was found to be 4-5 h under photochemically reactive conditions in a smog chamber (Spicer et al., 1985); this is in good agreement with the estimated half-life of phenol in air of 5 h based on its estimated reaction rate with hydroxyl radicals (RIVM, 1986). Howard (1989) reported an estimated half-life of 15 h for the reaction of phenol with hydroxyl radicals in air. The reaction of phenol with nitrate radicals during the night may be a significant removal process; a half-life of 15 min has been estimated at an atmospheric concentration of 2x10$^8$ nitrate radicals per cm$^3$ (Howard, 1989). Phenol absorbs light in the region of 290-330 nm and therefore could photolyse (Howard, 1989). Phenols generally react in sunlit natural water via reaction with photochemically produced hydroxyl and peroxy radicals; typical half-lives were reported to be 100 and 19.2 h, respectively (Howard, 1989). Phenol was found to be oxidized in water in the presence of oxygen and sunlight to form carbon dioxide under experimental conditions (Mahvi et al., 2007). It was reported to react with nitrate ions in dilute aqueous solutions to form dihydroxybenzenes, nitrophenols, nitrosophenol and nitroquinone, presumably by a radical mechanism involving hydroxyl and phenoxy radicals (Harrison et al., 2005). Phenol has been found to react with nitrous acid in wastewater to form cyanide (Adachi and Okano, 2003), and to form chlorophenols in chlorinated drinking-water (Ge et al., 2006) and p-benzoquinone in the presence of chlorine dioxide (Ganiev et al., 2004).

### Biodegradation

Biodegradation is used to describe the complete mineralization of the starting compound to simpler ones like CO$_2$, H$_2$O, NO$_3^-$ and other inorganic...
compounds (Atlas and Bartha, 1998). The term has been proposed for describing the ultimate degradation and recycling of an organic molecule to its mineral constituents. Degradation of phenol occurs as a result of the activity of a large number of microorganisms including bacteria and fungi. Bacterial species include: *Bacillus spp.*, *Pseudomonas spp.*, *Acinetobacter spp.*, *Achromobacter spp.* etc. *Fusarium spp.*, *Phanerochaete chrysosporium*, *Coriolus versicolor* etc are also proved to be efficient fungal groups in phenol biodegradation. Bacteria play a major role in the degradation of phenol in soil, sediment and water.

Phenol may be converted by bacteria under anaerobic conditions to carbon dioxide (Boll and Fuchs, 2005) or methane (Ke et al., 2008) and under aerobic conditions to carbon dioxide (Agarry et al., 2008). Benzoate, catechol, cis-cis-muconate, β-ketoadipate, succinate and acetate have all been identified as intermediates in the biodegradation of phenol (Agarry et al., 2008). Some of the carbon derived from the degradation of phenol may be incorporated into the bacterial biomass. However, repeated phenol exposure may result in acclimation (the promotion of strains capable of utilizing phenol as food) (Nair et al., 2008).

Several external factors can limit the rate of biodegradation of organic compounds. These factors may include: temperature, pH, oxygen content and availability, substrate concentration and physical properties of contaminants. Each of these factors should be optimized for the selected organism for the maximum degradation of the organic compound of choice. The optimization of the substrate concentration in phenol biodegradation is particularly important since it inhibits the growth of the organism at higher concentrations (Nair et al., 2008).

**Effects on laboratory mammals and *in vitro* test systems**

Phenol has moderate acute toxicity for mammals. Oral LD$_{50}$ values in rodents range from 300 to 600 mg phenol/kg body weight. Dermal LD$_{50}$ values for
rats and rabbits range from 670 to 1400 mg/kg body weight, respectively, and the 8-h LC$_{50}$ for rats by inhalation is more than 900 mg phenol/m$^3$. Clinical symptoms after acute exposure are neuromuscular hyper excitability and severe convulsions, necrosis of skin and mucous membranes of the throat, and effects on lungs, nerve fibers, kidneys, liver, and the pupil response to light (ATSDR, 2008).

Solutions of phenol are corrosive to skin and eyes. Phenol vapours can irritate the respiratory tract. There is evidence that phenol is not a skin sensitiser. The most important effects reported in short-term animal studies were neurotoxicity (Veronesi et al., 1986), liver and kidney damage, respiratory effects and growth retardation. Toxic effects in rat kidney have been reported to occur at oral dose levels of 40 mg/kg/day or more. Liver toxicity was evident in rats administered at least 100 mg/kg/day (Dosemeci et al., 1991).

There are no adequate studies on the reproductive toxicity of phenol. Phenol has been identified as a developmental toxicant in studies with rats and mice. In two multiple dose rat studies, NOAEL values of 40 mg/kg/day (the lowest-observed-adverse-effect level (LOAEL) was 53 mg/kg/day) and 60 mg/kg/day (the LOAEL was 120 mg/kg/day) have been reported. In the mouse, the NOAEL was 140 mg/kg/day (the LOAEL was 280 mg/kg/day) (ATSDR, 2008).

The majority of bacterial mutagenicity tests have given negative results. Mutations, chromosomal damage and DNA effects have been observed in mammalian cells in vitro. Phenol has no effect on intercellular communication (measured as metabolic cooperation) in cultured mammalian cells. Induction of micro-nuclei in bone marrow cells of mice has been observed in some studies. No micronuclei were observed in mice studies at lower doses (EPA, 2002).

Two carcinogenicity studies have been conducted with male and female rats
and mice receiving phenol in their drinking water. Malignancies (e.g., C-cell thyroid carcinoma, leukaemia) were only seen in low-dose male rats. No adequate dermal or inhalation carcinogenicity studies have been conducted. Two-stage carcinogenicity studies have shown that phenol, applied repeatedly to mouse skin, has promoting activity (ECB, 2006).

**Effects on organisms in the environment**

**Acute effects**

A number of short-term studies were available for freshwater organisms. The lowest reported study was an 8-day EC$_{50}$ (growth) of 7.5 mg l$^{-1}$ in the green alga *Selenastrum capricornutum* (Beaubien *et al*., 1986). Shorter exposure periods result in far higher effect concentrations (lower toxicity) with 96 h EC$_{50}$s (growth) of 370 and 61.1 mg l$^{-1}$ reported in *Chlorella vulgaris* and *Selenastrum capricornutum*, respectively (Shigeoka *et al*., 1988; Laurent *et al*., 1992). Crustaceans appear to be more sensitive than algae. Acute toxicity for *Daphnia magna* includes a 96 h LC$_{50}$ of 27.9 mg l$^{-1}$ phenol (Tisler and Zagor-Koncan, 2003). Other species of crustacean appear to be less sensitive with a 96 h LC$_{50}$ of 69 mg l$^{-1}$ reported for *Gammarus pulex* (Green *et al*., 1985). Other 48-h values for phenol include a LOEL (reproduction) of 0.19 mg l$^{-1}$ for the rotifer *Brachionus calyciflorus* (Snell and Moffat, 1992) and an LC$_{50}$ of 3.1 mg l$^{-1}$ for *Ceriodaphnia dubia* (Oris *et al*., 1991).

The 96 h LC$_{50}$ for the fathead minnows (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*) was 27.3 and 13 mg l$^{-1}$ respectively (Broderius *et al*., 1995; Tisler and Zagor-Koncan, 2003). For the embryo-larval stage of the leopard frog (*Rana pipiens*), the 9- and 5-d LC$_{50}$s were 0.04 and 0.05 mg l$^{-1}$, respectively (Birge *et al*., 1980). For the amphibian *Bufo arenarum*, LC$_{50}$, LC$_{99}$, NOEC, TC$_{50}$ and TI$_{50}$ values were 183.70, 250, 60, 113 mg l$^{-1}$ and 1.62.
respectively, at 96 h of treatment (Paisio et al., 2009). Teratogenic effects more frequently produced by phenol in this amphibian were: axial flexure, persistent yolk plug and different abnormalities which caused death of blastulae. Moreover, other malformations were registered, such as irregular form, acephalism, edema, axial shortening and underdevelopment of gills, among others.

For short-term data on marine species, only E(L)C_{50} data for one alga species and four crustacean species were available. The tests indicate low sensitivity under short-term exposures to phenol, with a 4-day EC_{50} (growth rate) of 149 mg l^{-1} for the red alga Gracilaria tenuistipitata (Haglund et al., 1996). Marine crustaceans appear to be far more sensitive to phenol. If subjected to osmotic stress, juveniles of the saltwater crustacean Archaeomysis kokuboi seem to be more sensitive to phenol exposure than the most sensitive freshwater organisms (Kim and Chin, 1995). The 96 h LC_{50} of Archaeomysis kokuboi was reported to range from 260 to 710 µg l^{-1}, depending on salinity (16–32 ppt). Law and Yeo (1997) investigated the toxicity of phenol to eggs, larvae and post-larvae of Macrobrachium rosenbergii. The 12 day EC_{50} value for egg hatchability was 0.96 mg l^{-1} phenol; embryonic development was completely inhibited at 5 and 10 mg l^{-1}, and there were statistically significant differences in hatching rate at phenol concentrations as low as 0.05 mg l^{-1}. The acute toxicity of phenol to larvae and post-larvae, expressed as 48 h LC_{50} values, ranged from 11.83 mg l^{-1} for stage 2 larvae to 29.92 mg l^{-1} for post-larvae PL_{42}. The 96 h LC_{50} values were similar, ranging from 16.67 to 23.04 mg l^{-1} for PL_{21} to PL_{42}. In 42 day exposures to 0.23, 2.30 and 6.80 mg l^{-1} phenol, the growth rate of post-larvae was inhibited by 11.76, 29.41 and 47.06% respectively.

**Chronic effects**

For freshwater organisms the lowest reliable data were several EC_{10} (population growth) values for the green alga Selenastrum capricornutum
(Van der Heever and Grobbelaar, 1996). The lowest value obtained was a 24 h EC$_{10}$ of 329 µg l$^{-1}$. Corresponding values of 495 and 969 µg l$^{-1}$ were reported at 48 and 72 h respectively. These data were generated in a static system with measured exposure concentrations. Crustaceans appear to be of similar sensitivity to algae. The lowest reliable crustacean study reported a 16-day EC$_{10}$ of 460 µg l$^{-1}$ for the growth of *Daphnia magna* (Deneer *et al*., 1988). Other species of crustacean exhibit similar sensitivity with a 192 h NOEC of 840 µg l$^{-1}$ reported in *Ceriodaphnia dubia* (Cowgill and Milazzo, 1991). Chronic effects of phenol (16 weeks exposure) were investigated on some physiological parameters of *Oreochromis niloticus* exposed to three sublethal concentrations of 0.7, 1.4 and 2.8 mg l$^{-1}$ (Gad and Saad, 2008). Decrease in serum tri-iodothyronine (T3) and thyroxin (T4) hormones and an increase in number of micronuclei production (genotoxic potential) was observed. Also decrease in growth performance and accumulation of phenol in fish tissues (liver, muscle and gills) were detected.

For saltwater organisms exposed to phenol the lowest available long-term data point for microalgae was a 120 h NOEC of 13 mg l$^{-1}$ for the diatom *Skeletonema costatum* (Cowgill *et al*., 1989). In addition, data were available for saltwater macroalgae that indicate higher sensitivity, with a 14-day MATC (reproduction) of <7.8 mg l$^{-1}$ in the red alga *Champia parvula* (Thursby *et al*., 1985). Saltwater crustaceans appear to be more sensitive than algae, with a 6-day NOEC of 1 mg l$^{-1}$ for changes in the behavior of the barnacle *Balanus amphitrite* (Wu *et al*., 1997). Additional crustacean data were available for the shrimp, *Penaeus japonicus*, with a 1-year EC$_{10}$ of 52 mg l$^{-1}$ (Qixing, 1999). As with the freshwater environment, fish appear to be the most sensitive organisms to long-term exposure to phenol. An 8-day NOEC (mortality) of 500 µg l$^{-1}$ was reported in the grey mullet (*Mugil auratus*) (Krajnovic-Ozretic and Ozretic, 1988).
Kinetics and metabolism

Phenol is readily absorbed by all routes of exposure. After absorption, the substance is rapidly distributed to all tissues. The liver, the lung, and the gastrointestinal mucosa are the most important sites of phenol metabolism. The relative role played by these tissues depends on route of administration and dose. *In vivo* and *in vitro* studies have demonstrated covalent binding of phenol to tissue and plasma proteins. Some phenol metabolites also bind to proteins. Urinary excretion is the major route of phenol elimination in animals and humans. The rate of urinary excretion varies with dose, route of administration, and species. A minor part is excreted in the faeces and expired air. Figure 1.2 shows the general metabolic pathways that transform phenol prior to its excretion in the urine. Three different enzymes systems catalyze the reactions that transform phenol. Cytosolic phenol sulfotransferases catalyze the transfer of inorganic sulfur from the activated 3' phosphoadenosine-5' phosphosulfate donor molecule to the hydroxyl group on phenol. Microsomal membrane-located uridine diphosphate (UDP) glucuronosyltransferases catalyze the transfer of an activated glucuronic acid molecule to the hydroxyl moiety of phenol to form an O-glucuronide conjugate. Cytochrome P4502E1, also microsomally located, catalyzes the hydroxylation of phenol to form hydroquinone (and to a much lesser extent, catechol), which is then acted upon by the phase II enzymes (Gut *et al.*, 1996; Powley and Carlson 2001; Buxton, 2006). Hydroquinone can, in turn, form conjugates, undergo peroxidation to form benzoquinone, or undergo further oxidation to form trihydroxybenzene. All three enzyme systems that metabolize phenol are found in multiple tissues and there is competition among them not only for phenol, but also for subsequent oxidative products, like hydroquinone. As a consequence, the relative amount of the products formed can vary based on species, dose and route of administration. Cytochromes other than CYP2E1 also seem to be involved in the metabolism of phenol as demonstrated by Powley and Carlson (2001) in experiments utilizing
Figure 1.2. Metabolism of Phenol

Biphenyl polymers

\[ \text{Biphenol}^* \]

\[ \text{peroxidase} \]

\[ \text{Diphenyquinone}^* \]

\[ \text{Glutathione conjugate} \]

\[ \text{peroxidase} \]

\[ \text{Glucuronide conjugate} \]

\[ \text{Sulfate conjugate} \]

\[ \text{CYP2E1} \]

\[ \text{Phenol} \]

\[ \text{UGT} \]

\[ \text{Sulfate and glucuronide conjugates} \]

\[ \text{PST} \]

\[ \text{Catechol} \]

\[ \text{Hydroquinone} \]

\[ \text{PST} \]

\[ \text{Sulfate and glucuronide conjugation} \]

\[ \text{UGT} \]

\[ \text{PST} \]

\[ \text{CYP2E1} \]

\[ \text{Trihydroxybenzene}^* \]

\[ \text{Sulfate and glucuronide conjugates} \]

\[ \text{UGT} \]

\[ \text{Benzoquinone}^* \]

\[ \text{Glutathione conjugate} \]

\[ \text{peroxidase} \]

PST = phenol sulfotransferase; UGT = UDP-dependent glucuronosyl transferase

*Indicates metabolites identified in vitro only

Source: EPA 2002
chemical inhibitors of CYP2E1, CYP2B and CYP2F2 knockout mice. The investigators found that CYP2E1 was responsible for only approximately 50% of phenol metabolism in liver, suggesting the participation of other cytochromes. Experiments in pulmonary microsomes showed that both CYP2E1 and CYP2F2 played important roles in the metabolism of phenol.

Four principal metabolites have been identified in mammals: two phenol and two hydroquinone conjugates of sulfate and glucuronide (Kenyon et al., 1995; Hoffmann et al. 1999). In fish Oncorhynchus mykiss, hydroquinone and catechol metabolites were reported after exposure to phenol (Solem et al., 2003). Phenyl sulphate, phenyl glucuronide and quinol sulphate were reported in gold fish, Carassius auratus (Nagel, 1983) and zebrafish, Brachydanio rerio (Kasokat et al., 1987). In frog, Rana temporaria Phenyl sulphate, phenyl glucuronide, quinol sulphate, catechol sulphate, resorcinol and catechol were reported (Beyer and Frank, 1985). The relative amounts of glucuronide and sulfate conjugates vary with dose and animal species. A shift from sulfation to glucuronidation was observed after increasing the phenol dose.

**Bioconcentration and bioaccumulation**

The bioconcentration factors of phenol in various types of aquatic organisms are in general very low (< 1-10), although some higher values (up to 2200) have also been reported. Phenol, therefore, is not expected to bioaccumulate significantly (Howard, 1989). Bioaccumulation is not expected to be significant because of the low log Kows (0.5-2.5) of phenols (Verschueren, 1983; Tisler and Zagorc-Koncan, 2003).
Indian studies

Earlier the toxicity and effects of phenol to the Indian fishes have been reported by several authors. Verma et al. (1980) reported the toxicity of phenol to *Notopterus notopterus* (Asiatic knife fish), *Saccobranchus fossilis* (stinging catfish) and *Colisa fasciatus* (giant gourami). The 96 h LC$_{50}$ values for the three fish were 12.53, 39.40 and 32.70 mg l$^{-1}$ respectively. For the guppy *Lebistes reticulatus* the 24, 48, 72 and 96 h LC$_{50}$ of 83, 64, 50 and 48 mg l$^{-1}$ were reported (Gupta et al. 1982). Razani et al. (1986a; 1986b) studying the sensitive developmental stages of zebrafish, *Brachydanio rerio* reported a 24 h LC$_{50}$ of 28 ppm and NOEC, LOEC and MATC levels of 2.2, 4.9 and 3.28 ppm phenol respectively. Toxicity and behavioral changes to nine species of freshwater fish were also reported by Kondaiah and Murty (1994). The LC$_{50}$ values ranged from 8.1 mg l$^{-1}$ to 30.6 mg l$^{-1}$.

Enzymatic changes in the tissues of fish *Notopterus notopterus* exposed to phenolic compounds (phenol, 2,4-dinitrophenol and pentachlorophenol) which include either of inhibition in phosphatases activity (Verma et al., 1980) or stimulation in transferases activity (Gupta et al., 1983; Gupta and Dalela, 1985) were reported. Depletion in carbohydrates of the Indian catfish *Heteropneustes fossilis* (Gupta and Srivastava, 1984) and alteration in carbohydrate, protein and lipid metabolism in fish *Cyprinus carpio* (Sannadurgappa et al., 2006) and *Oreochromis mossambicus* (Sannadurgappa et al., 2007) were also reported after exposure to phenol.

Verma et al. (1984) reported chronic toxicity of phenol to fish *Cirrhinus mrigala*. Starting with 2 day old larvae for an exposure period of 60 days, a MATC (maximum allowable toxicant concentration) range of 77-94 µg l$^{-1}$ and a NOEC (no observed effect concentration) of 77 µg l$^{-1}$ were reported. This MATC is supported by an MATC of 110-130 for biomass gain of common carp *Cyprinus carpio* (Verma et al., 1981).
Chronic effects which include reduction in growth, development (at 2.58 and 4.11 mg l\(^{-1}\)) fecundity and maturity index (at 1.26 mg l\(^{-1}\)) as a result of 90 days exposure to phenol for the fish *Oreochromis mossambicus* (Saha et al., 1999) and lower mean wet weight gain, specific growth rate, food conversion efficiency and protein digestibility (at 5 and 10 mg l\(^{-1}\)) as a result of 28 days exposure for the fish *Labeo rohita* (Rajasekharan Nair and Sheriff, 1998) were also available.

**Significance of the Present Study**

Biological monitoring is one of the ways developed to effectively monitor the quality of the environment at chemically and biologically desirable level. As the fishes constitute one of the important sources of protein, a thorough understanding of the effect of xenobiotics on fishes would be useful for fish conservation and development of the fisheries. The usual measure of the environmental effect of any pollutant on an animal is mortality; however, other effects, which are more delicate and indicative of any physiological changes, may be ultimately detrimental to the population survival. The impairment of behavioral and physiological functioning could result in a gradual reduction in the adaptive capacity of species, leading to a decrease in their survival ability and population level. Even though, little alteration of any one process may increase the stress, the organism is less able to cope at every stage of life.

Phenol and its compounds are ubiquitous water pollutants which come to the natural water resources from the effluents of chemical industries such as coal refineries, phenol manufacturing, pharmaceuticals and industries of resin, paint, dyeing, textile, leather, petrochemical and pulp mill (Loh et al., 2000; Avilez et al., 2008). They are commonly used in agriculture as non-specific pesticides, herbicides, bactericides and fungicides. From industrial and agricultural operations, these compounds find their way into the natural water resources (Hori et al., 2008). In the aquatic environment these compounds are deleterious to life.
Despite the active metabolism and detoxification of phenol in fish (Solem et al., 2003) sublethal and lethal concentrations of phenol affect the oxygen uptake rate (Kanabur and Sannadurgappa, 2001), metabolism (Hori et al., 2006; Avilez et al., 2008), growth and development (Saha et al., 1999), histological processes (Abdel-Hameid, 2007) and reproductive potential of fish (Mukherjee et al., 1990; Saha et al., 1999). They can also give an unacceptable taint to water and fish, especially chlorophenols which are formed from the chlorination of phenols.

In the study area, both agricultural and aquaculture practices are in competence on an industrial scale. The importance of acute toxicity study is to prevent a massive kill of the fish by the contamination with the toxicant in nearby water bodies and the overall objective in taking up of such a work is to assess the damage done. The fish *Catla catla, Labeo rohita, Cirrhinus mrigala, Ctenopharyngodon idella* and *Channa punctatus* are traditionally known fish for their excellence, with an inviting appearance, firm flesh texture and a tongue tingling taste. The information regarding the toxicity and effect of phenol to these fishes are scarce. A detailed investigation of the effect of phenol on freshwater fishes would help to assess the damage caused and also to verify the non-toxic consumption of the fish. Hence, the present study is undertaken to determine the toxicity of phenol to the fish *Catla catla, Labeo rohita, Cirrhinus mrigala, Ctenopharyngodon idella* and *Channa punctatus*. The other aspects include studies on respiratory, biochemical and histopathological changes and bioaccumulation in the fish *Labeo rohita*. 
Morphological description of the fish

The following five species of freshwater fish are selected for the present study (Plate I):

- *Catla catla* (Hamilton)
- *Labeo rohita* (Hamilton)
- *Cirrhinus mrigala* (Hamilton)
- *Ctenopharyngodon idella* (Valenciennes)
- *Channa punctatus* (Bloch)

The systematic position of the test species is given in table 1.4. The above fish could be identified in the field with the help of the following morphological characters. A brief morphological description of the species is included.

*Catla catla* (Hamilton) - *Catla catla* is one of the renowned and the fastest growing of the Indian major carps. It is non-predatory and its feeding is restricted to the surface and mid waters. This species is identified by the presence of very deep body, enormous head, upturned mouth and conspicuously large scales.

*Labeo rohita* (Hamilton) - This graceful riverine fish is one of the major carps of India. It is a bottom feeder and prefers to feed on plant matter including decaying vegetation. This species can be identified by the presence of an inferior mouth, thick and fringed lips with a distinct inner fold to each lip and a pair of small maxillary barbels concealed in lateral groove.

*Cirrhinus mrigala* (Hamilton) - *Cirrhinus mrigala* is also a member of the Indian major carps. It is a bottom feeder and feeds on the detritus and small invertebrate organisms. This species can be identified by the presence of linear body, terminal mouth, indistinct lower lip and a single pair of rostrals.
*Ctenopharyngodon idella* (Valenciennes) - It is commonly called as grass carp since it feeds selectively on the submerged aquatic vegetation in the ponds. It is an exotic fish to Indian waters. Identifying features are elongated body, broad and anteriorly depressed head, terminal mouth and a horizontal rostral fold covering the base of upper lip.

*Channa punctatus* (Bloch) - *Channa punctatus* is commonly called as spotted murrel. It is an air breathing fish since it possesses accessory respiratory organs, which permit the fish to live in waters with low dissolved oxygen like swamps and derelict water bodies. This species feeds on insect larvae and small sized minor carps and lives near the bottom of the pond. This fish has elongated body, small head, large mouth, and rounded caudal fin with black spots on the body.
Table 1.4. SYSTEMATIC POSITION OF THE TEST SPECIES

<table>
<thead>
<tr>
<th>Species</th>
<th>Phylum</th>
<th>Subphylum</th>
<th>Grade</th>
<th>Class</th>
<th>Subclass</th>
<th>Order</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Catla catla</em></td>
<td>Phylum : Chordata</td>
<td>Subphylum : Vertebrata</td>
<td>Grade : Pisces</td>
<td>Class : Osteichthyes</td>
<td>Subclass : Actinopterygii</td>
<td>Order : Cypriniformes</td>
<td>Family : Cyprinidae</td>
</tr>
<tr>
<td><em>Cirrhinus mrigala</em></td>
<td>Phylum : Chordata</td>
<td>Subphylum : Vertebrata</td>
<td>Grade : Pisces</td>
<td>Class : Osteichthyes</td>
<td>Subclass : Actinopterygii</td>
<td>Order : Cypriniformes</td>
<td>Family : Cyprinidae</td>
</tr>
<tr>
<td><em>Ctenopharyngodon idella</em></td>
<td>Phylum : Chordata</td>
<td>Subphylum : Vertebrata</td>
<td>Grade : Pisces</td>
<td>Class : Osteichthyes</td>
<td>Subclass : Actinopterygii</td>
<td>Order : Cypriniformes</td>
<td>Family : Cyprinidae</td>
</tr>
<tr>
<td><em>Channa punctatus</em></td>
<td>Phylum : Chordata</td>
<td>Subphylum : Vertebrata</td>
<td>Grade : Pisces</td>
<td>Class : Osteichthyes</td>
<td>Subclass : Actinopterygii</td>
<td>Order : Channiformes</td>
<td>Family : Channidae</td>
</tr>
</tbody>
</table>
See Plate I