Summary
Available information from this laboratory indicated that phenol and cadmium are highly toxic compounds to common carp *Cyprinus carpio*. These two compounds alter pituitary gonadotropin release, ovarian cholesterol mobilization and inhibition of steroidogenic enzymes like 3β-HSD and 17β-HSD. The present study investigated: (1) the acute toxicity of phenol and cadmium chloride (CdCl₂) in *C. carpio* *in vivo* and in ovarian follicles *in vitro*; (2) whether these two highly toxic compounds at their physiological safe concentrations can able to affect ovarian steroidogenesis, particularly 17β-estradiol production by the ovarian follicles during vitellogenic stage; (3) whether, the toxicants affect the aromatase activity and P450arom gene expression; and finally (4) to assess the damage caused by oxidative stress at the cellular levels produced by these two toxicants.

Acute toxicity test of phenol and CdCl₂ were determined using Finney’s Probit analysis. In the present observations, it has been noted that the 96 h TLm value for phenol and CdCl₂ are 16.06 mg/L and 316 mg/L respectively. This clearly indicates that fish is less tolerant towards phenol than CdCl₂. This also suggests that phenol is highly toxic than CdCl₂ in this fish. To get a clear idea whether phenol and CdCl₂ have direct effects on the ovarian physiology or they may act indirectly, acute toxicity test was conducted with isolated ovarian follicles *in vitro*. The 16 h LC₅₀ values of phenol and CdCl₂ in ovarian tissues of this fish were shown to be 5.8 µM and 13.8 µM respectively. The LC₅₀ value of phenol was quite low than CdCl₂ which correlates the in *vivo* TLm value. Fifty percent of safe dose of each toxicant was considered as physiological safe dose *in vitro* study. For the present investigations, 2 and 5 µM doses were considered to be safe dose and 1 and 2 µM as physiological safe dose of phenol and CdCl₂ respectively.

For assessing the effect of two toxicants on the production of 17β-estradiol, steroid radioimmunoassay was conducted. To demonstrate the expression of aromatase activity and
P450arom gene expression in ovarian tissues, RT-PCR and real time PCR analysis were done. To understand the effects of toxicants on antioxidant system, activities of oxidative stress parameters like superoxide dismutase and catalase were determined.

Vitellogenic stage fish were exposed to safe doses of phenol and cadmium for 0, 24, 48 and 96 h and serum and ovarian 17β-estradiol levels were estimated. In the *in vitro* study, vitellogenic follicles were incubated with or without LH and a dose- and time-dependent effects of phenol and CdCl₂ on steroid production were examined. Exposure of fish with phenol and CdCl₂ gradually attenuated serum and ovarian 17β - estradiol level with increasing time and maximum inhibition was noticed after 96 h. Administration of phenol and CdCl₂ to the incubation medium significantly inhibited LH induced release of 17β - estradiol *in vitro*. To clarify the mechanism of attenuated production of 17β-estradiol, *in vitro* effect of phenol and CdCl₂ on LH induced P450 aromatase activity (conversion of testosterone to 17β-estradiol) and cytochrome P450arom gene expression were evaluated. Physiological safe dose of phenol significantly inhibited LH-stimulated aromatase activity and P450arom gene expression in ovarian follicles. The present study also demonstrates that exposure of ovarian follicles with phenol and CdCl₂ attenuated LH-stimulated follicular cAMP production. The results indicate that both phenol and CdCl₂ exert inhibitory effect on ovarian steroidogenesis through attenuated ovarian cAMP. The present study further demonstrates that LH-induced stimulation of steroidogenic factor-1 which activate aromatase enzyme was strongly inhibited by phenol and CdCl₂. All these results suggest the endocrine disruption potential of phenol and CdCl₂ in this fish and effect can be mediated via several cellular pathways including inhibition of cellular cAMP content, SF-1 activity, aromatase activity and P450arom gene expression.
Activities of superoxide dismutase (SOD) and catalase were significantly altered in phenol and CdCl$_2$ exposed common carp for 15 days. These changes were both inhibitory and stimulatory indicating that both these toxicants could able to produce oxidative stress or damage in the fish species by generating reactive oxygen species (ROS) in the body. Activities of SOD in serum and ovary decreased gradually from one day of exposure and up to day 4 in comparison to their respective control values. However, after day 4 activities of these enzymes were decrease gradually and significantly up to day 15. This result indicates that immediately after exposure to toxicant fish are not able to activates its protective mechanisms necessary for scavenging O$_2^-$ radical in serum and ovarian tissue but prolong exposure up to 15 days cause activation of such protective mechanism. SOD activities in liver increased immediately after exposure to both phenol and CdCl$_2$ with a maximum at day 4 of exposure after which there was no further change. Results indicate that both phenol and cadmium in fish liver primarily acted as oxidant or producer of free radicals. Immediate increase of the activity of this enzyme showed a good protective mechanism necessary for scavenging O$_2^-$ radical in the liver of this fish. In both the treatment groups both phenol and CdCl$_2$ decrease the catalase activity in serum, liver and ovary up to day 4 of exposure, which then increased gradually and significantly up to day 15. Decreases in activity of catalase in all the tissue after exposure to toxicants shows that immediately after the exposure fish were not able to protect the cell from accumulation of H$_2$O$_2$ but increase activity with prolong exposure with both phenol and CdCl$_2$ indicated their functions to protect the cell against H$_2$O$_2$. The present study indicate that both phenol and CdCl$_2$ induced oxidative stress increased in common carp after 4 to 5 days of exposure of sub-lethal concentration of these two toxicants as evidence by changed SOD and CAT activity. Both SOD and CAT have strong detoxification after few days of adaptation.
Finally findings of the present thesis work suggest that phenol and cadmium chloride can act directly on ovarian follicular cells to diminish 17β-estradiol production by inhibiting cAMP production, aromatase activity, expression of ovarian P450 arom gene, SF-1 activity and antioxidant system. Nevertheless, the present work confirms the previous studies on the diverse toxicity and endocrine disruptive actions of phenol and CdCl₂ in fish and therefore provides further evidence to the control of disposal and use of these toxicants.