Chapter 5

Summary and Conclusion
The current practice in the risk assessment of pesticide residues in food is generally based upon data from studies on single compounds. Even though the consumer is often exposed to more than one pesticide via the food. The knowledge of adverse health effects of exposure to combination of pesticides is limited. When pesticides are ingested together, toxic effect observed may differ quantitatively and/or qualitatively from those observed following exposure to the single pesticide. Three basic modes of joint action or interaction of combinations of chemicals have been identified: simple similar action, simple dissimilar action and true interaction. Simple action covers the situation where each chemical in the mixture acts in the same way by the same mechanism(s) and the individual chemicals differ only in their potencies. Simple dissimilar action means that each chemical in the mixture exerts individual effects but does not modulate the effects of the other chemicals in the mixture. In the last situation, interaction can result in a stronger effect (synergism) or a weaker effect (antagonism, inhibition).

In the present study a total of 250 samples of five food commodities vegetables, fruits, cereals, spices and milk (The selected fruits were apple, orange, mango, guava and papaya; vegetables were cabbage, cauliflower, tomato, okra and brinjal; cereals were wheat, rice, and pulses; spices were turmeric, coriander, chilli, cumin seed, black paper, small cardamom, big cardamom, fenugreek, aniseed, caraway, dry-ginger, mace, nutmeg, cinnamon, bay-leaf and clove and milk. The samples were collected in polythene bags, transported to the laboratory in cold condition, and analyzed as soon as possible or stored at 4°C till the analysis). 10 samples of each were collected from local market basket samples of Lucknow, city, Uttar Pradesh, India. Forty eight multi-pesticides like: OCs- α-HCH, β-HCH, α-HCH, δ –HCH, Dicofol, Aldrin, o,p-DDE, p,p-DDE, o,p-DDD, p,p-DDD, p,p-DDT, α-endosulfan, β-endosulfan, SPs-fenpropathrin, λ-cyhalothrin, permethrin-l, permethrin-
II, β-cyfluthrin-I, β-cyfluthrin-II α-cypermethrine, fenvalerate-I, fenvalerate-II, deltamethrine, OPs-dichlorvos, phorate, phorate sulfone, phorate sulfoxide, dimethoate, diazinon, methyl parathion, chlorpyriphos methyl, fenitrothione, malathion, chlorpyriphos, chlorofenvinfos, profenofos, ethion, edifenphos, anilophos, phosalone, H-atrazine, dimethachlor, atrazin, fluchloralin, dimethachlor, alachlor, pendimethalin, butachlor and hexaconazole were analysed using QuEChERS method. The pesticide residues were found in 35.2 % of all analyzed samples. The trend of detected pesticides in food commodities were 48% of vegetables, 34% of fruits, 34% of cereals, 37.5 % of spices and 0% of milk.

The most frequently detected pesticide were dicofol, malathion and cypermethrin and showed the residual level above MRL (PFA,1954) also in 17% samples. In vegetables like; brinjal, endosulfan (0.680 mg/kg) and cypermethrin (0.528 mg/kg), cabbage; malathion (3.554 mg/kg), in cauliflower; dicofol (2.310 mg/kg) recorded was above MRL (PFA, 1954). In fruits OPs were frequently detected above MRL, in mango, guava and papaya; malathion detected above MRL in range of (0.228-3.120 mg/kg) Table 18. In cereals cypermethrin was detected above MRL (0.630 mg/kg) in wheat; in rice malathion was above MRL (4.504 mg/kg) and in pulses dicofol was above MRL (3.010 mg/kg), malathion was also in higher level but its MRL value was not available in PFA, 1954 list (Table 19). In spices like coriander, malathion was above MRL (3.554 mg/kg), chilli; dicofol (2.310 mg/kg) and in aniseed malathion (0.258 mg/kg) and anilophos (0.042 mg/kg) were found above MRL. However in recent times, pre dominance of cypermethrin residues in fruits and vegetables were also reported by several workers in their respective studies area indicating their excessive use.
The result of present investigation further support the findings of the studies carried out in India and abroad.

Malathion, cypermethrin and dicofol were found above from their respective MRL in most of the samples. Real world exposure to pesticides normally occurs through lower level single or repeated exposure, as residues in food products. Hence, 90 days oral residual toxicity studies on mixture of these three pesticides namely dicofol, malathion and cypermethrin was conducted in rat model and lowest exposure dose was selected with reference to their respective MRLs to find out the synergistic effects of mixture of these chemical pesticides at lowest doses.

Results, shows those 90 days oral exposure of high dose combination of dicofol, malathion and cypermethrin to male rats has produced significant toxic effects. It is interesting to know that there was significant decrease in the body weight of high dose exposed rats together with reduced food consumption and increased liver weight. The weight gain in animals serves as index of growth rate. The reduced food consumption and increased liver weight of high dose exposed animals seems to be due to toxic potential of combination of dicofol, malathion and cypermethrin. The significant increase in weight of liver was, however, found to be associated with concomitant increase in the activity of GOT and GPT in serum. It is important to note that the elevated activity of serum GOT and GPT recorded in the study may be due to liver damage. This has been confirmed by hepatocellular damage in the combination of higher dose treated animals. Our present knowledge on liver changes induced by combination of dicofol, malathion and cypermethrin is both limited and equivocal. In contrast from the previous finding of others, the food consumption is significantly decreased at higher dose levels. The reduced effects on the body weight of the high dose treated rats may not be clearly treatment related and could be attributed to food palatability problems in animals. However, increased level of blood glucose may also
be the indication of mild combination of dicofol, malathion and cypermethrin induced changes in carbohydrate metabolism. No significant changes in cholesterol indicated that combination dose of has not attributed in fat metabolism. Interestingly the elevated levels of blood urea nitrogen together with tubular changes in the kidney and its increased weight at higher dose exposed rats have also indicated its nephrotoxic effects.

Significant decrease in spontaneous locomotor activity in the rats treated with the high dose of combination of dicofol, malathion and cypermethrin has indicated the accumulation of cypermethrin in the brain. Some author has reported the accumulation of cypermethrin in mouse brain following direct intra peritoneal administration. However, Brunet has also reported that malathion and cypermethrin is highly absorbed in human intestinal cell suggesting its potential effects. These neurobehavioral deficits may reflect dysfunction at multiple anatomical areas in central nervous system. The pathological changes in brain seen in the present study suggest that multiple brain region abnormalities may be involved in changes of spontaneous locomotor activity. Earlier studies have shown that concentration of pesticides in brain generally correlate with the severity of toxicity and symptoms of neurotoxicity which were found to increase with the pesticide concentration in brain. Necrosed purkinje cells and loss of granules in the granular layer of cerebellum have also provided support to the neurobehavioral effects indicating accumulation of malathion and cypermethrin in the brain.

The results of the genotoxicity revealed that combination of dicofol, malathion and cypermethrin causes significant induction of CAs and MN in a higher (20+16+8 mg/kg/b.wt./d) mixture dose of dicofol, malathion and cypermethrin has been reported to have genotoxic effects. In this study, we report for the first time the genotoxic potential of dicofol, malathion and cypermethrin in wistar rat. The
frequency of CAs is a sensitive cytogenetic assay for detecting exposure to mutagens and carcinogens. The dicofol, malathion and cypermethrin induced CAs recorded in the present study may be due to early changes that either caused an increase in induced DNA damage or interfered with their repair mechanism. Micronuclei induction is considered to be another sensitive biological indicator of genotoxicity.

The formation of MN is related to the loss of acentric chromatin fragments and/or whole chromosomes. Our results suggest that the increase in the frequency of MN in the combination of higher dose dicofol, malathion and cypermethrin and cypermethrin individual (8 mg/kg/b.wt/d) exposed animals may be the consequences of mitotic spindle break down. However, another possibility suggests that combination of dicofol, malathion and cypermethrin exposure may also cause an aneugenic mode of action because it inhibits cell division and the mitotic spindle apparatus.

The decrease activity of SOD, GPx, CAT and GSH content together with increase LPO may be attributed to induce free radicals in middle and high combined dose of dicofol, malathion and cypermethrin treated rats. The toxicity of many xenobiotics is associated with the production of free radicals which are not only toxic themselves, but may also implicate in the pathophysiology of many diseases.
Conclusion

The research work embodied in this thesis may be concluded as below-

The validated QuEChERS method applied in the present study fulfills the established criteria for sensitivity and confident identification and quantification of organochlorines (OCs), organophosphate (OPs), synthetic pyrethroids (SPs) and herbicide (H) pesticides at low level in food commodities. Results of validation revealed that the recovery is in the range of 70-95 % with RSD < 15% and limit of quantification (LOQ) 0.003-0.035mg/kg for various pesticides in multipesticide residue analysis by this method.

The results of the study conducted on measuring the level of pesticide residues in various food commodities revealed that most frequent encountered pesticides were dicofol, malathion and cypermethrin (Individually or combination). These pesticides were found above maximum residual limit (MRL) in many samples, hence the consumer of Lucknow city India, commonly exposed to dicofol, malathion and cypermethrin through dietary intake.

The toxicity study was conducted for these pesticides in rat model. In view of parameters such as development of signs of intoxication and mortality, organ body weight ratio, hematology, morphology, histopathology, enzymatic changes, chromosomal aberration and neurobehavioral examination of experimental male rats (90 days oral exposure) it may be concluded that dicofol, malathion and cypermethrin individual 5, 4, 2 mg/kg/b.wt./day and in
combination 5+4+2 mg/kg/b.wt/day produced no desinerable effects, therefore this dose may be considered as no observed adverse effect level (NOAEL), whereas higher doses increasingly produced toxic effects.

At middle and higher combination doses of these three pesticides induced decrease in food consumption and body weight of rat, lesion in brain, multiple anatomical area in central nervous system, results in decrease spontaneous locomotor activity (SLA). Significant decrease in the activity of SOD, GPx, CAT and GSH content together with increase LPO may be attributed to induce free radicals in treated male rats. DNA damage or interference with DNA repair mechanism as indicated by formation of micronuclei suggests the genotoxic effects. The synergistic effect observed here might be due to the combined impact of these pesticides, simultaneously acting in different ways magnifying their efficacy, or an additive interaction of these pesticides acting by the same mechanism and at the same target.

In order to determine underlying molecular mechanism of toxicity we performed in-silico study, result of which indicate that MMP8, MMP3 Oxidosqualene cyclase, Myeloperoxidase, hCBG, cAMP, Acetylcholinesterase Glycolate oxidase, TGF proteins may be direct target of pesticide induced toxicity. Hence, 10, 8, 4mg/kg/b.wt/day their combination 10+8+4 mg/kg/day dose may be considered as lowest observed adverse effect level (LOAEL) to male rats. It is also concluded that dicofol, malathion and cypermethrin are not toxic individually at MRL level doses. However, these can impart deleterious effects if ingested in combination.