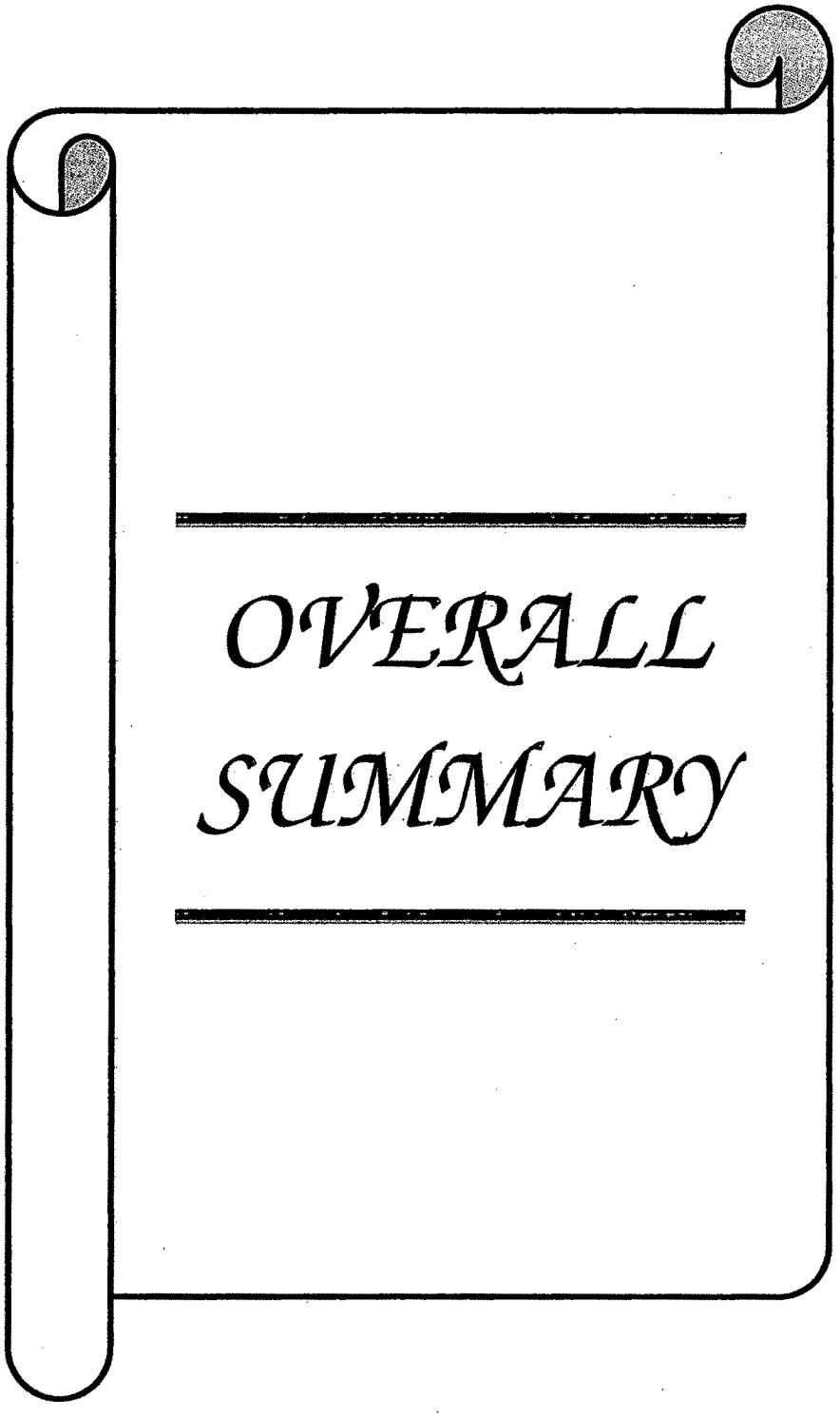
OVERALL
SUMMARY

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Pesticides and heavy metals as food contaminants have raised serious threat to human and other organisms due to pollution boom during the last 4-5 decades. Pesticides in food are predominantly residues from their application on different steps/stages of growth and processing of agricultural products, whereas, heavy metals contaminate food at various stages along the food production line, starting from agricultural lands to food processing. Due to large scale use of these two kinds of chemicals, their common existence everywhere throughout food chain is inherent. Toxicity of chemicals is influenced by number of factors. The presence of two or more chemicals at a given time leads to various interactions depending on the environment i.e., both inside the living organisms and also outside the biotic environment. Most of the interactions are so complex and obscure, that they remain unidentified. The organophosphorus pesticides form chelating complexes with heavy metals. The chance of humans being simultaneously get exposed to these two different classes of chemicals is higher in recent days.

Chlorpyrifos being an organophosphorus compound exhibits its toxicity mainly by inhibiting cholinesterases. Lead is a well known developmental neurotoxicant and systemic toxicant. However, no work has been published on their combined action when they are simultaneously challenged to test systems. Human beings are exposed to chemicals normally through single lower/higher dose level (e.g., accidental contamination/poisoning) or repeated exposures relatively at lower dose levels (e.g., as residues in food). Hence, the present study was designed to evaluate combination effects of chlorpyrifos and lead on neurobehavioral aspects, biochemical parameters

and at cellular level after single and nonlethal high dose levels and repeated dose low level of dietary exposure.

Single Dose study

Single dose oral gavage study was performed using 7 groups of animals, each comprising 5 animals per group at two different dose levels as chlorpyrifos and lead alone and their combination. Neurobehavioral studies on days 1 (i.e., after 2-3 hours of exposure), 2 and 14 and blood and serum analysis (on days 3 and 15) were evaluated. To correlate behavioral and biochemical parameters or other changes with cellular changes, histopathological evaluations (after sacrifice) were made.

The following are the findings:

Neurobehavioral Studies

Neurobehavioral studies performed reveal more pronounced effects of chlorpyrifos on neurobehavioral parameters after 24 hours of treatment at higher dose level i.e., chlorpyrifos -50 mg/kg body weight/day plus lead acetate-1000 mg/kg body weight/day when compared with chlorpyrifos 50 mg/kg body weight /day alone. Groups treated with chlorpyrifos alone at 50 mg/kg body weight /day revealed overt signs of cholinergic stimulation immediately after 2-3 hours of dosing followed by recovery within 2 – 3 days. In combination group (chlorpyrifos -50 mg/kg body weight/day plus lead acetate-1000 mg/kg body weight), symptoms of cholinergic over stimulation were noticed after 24 hours of exposure with more severity and higher incidences.

Hematology

Hematological parameters revealed slight anemic effects in lead treated groups.

Biochemical Analysis

Biochemical parameters of serum revealed increased inhibition of serum cholinesterase at a combination dose level of 50 mg/kg chlorpyrifos plus 1000 mg/kg lead than chlorpyrifos alone at 50 mg/kg dose level after 3 days of exposure. After 15 days, animals treated with 50 mg/kg chlorpyrifos plus 1000 mg/kg lead reveal incomplete recovery of RBC cholinesterase as compared to chlorpyrifos alone treated group. Increased serum electrolyte content was noticed due to lead treatment.

Histopathology

Histopathological examination of nervous system and other visceral organs did not reveal any treatment related changes except for some lead induced degenerative changes in thymus.

Repeated Dose Study

The repeated dose study was carried out for a period of 90-days at two different dose levels of chlorpyrifos and lead and their combination i.e., chlorpyrifos -1 ppm, lead acetate - 50 ppm, chlorpyrifos -1 ppm + lead acetate 50 ppm, chlorpyrifos -10 ppm, lead acetate -500 ppm and chlorpyrifos -10 ppm + lead acetate - 500 ppm through dietary exposure with concurrent control. To study reversibility and/or persistence of any effects additional 4 more groups were included in the study and designated as recovery group animals. The recovery group animals were kept post-treatment for a period of 28-days. Neurobehavioral and biochemical parameters were evaluated at the end of weeks 4, 13 and 17 (for recovery

animals). Histopatholigical examination was performed for nervous system and required systemic organs.

The findings from the repeated dose dietary exposure are:

Neurobehavioral Studies

Neurobehavioral studies performed reveal treatment related behavioral signs at the end of 4 weeks of exposure but very much comparable with controls at the end of 13 weeks of exposure.

Hematology

Hematological parameters revealed slight anemic effects at the end of 4 and 13 weeks of exposure in lead treated animals. The reduction was observed in males at the end of 13 weeks of exposure, whereas, in females, the reduction was noticed at the end of week 4. Variations in clotting time of blood were noticed in lead and chlorpyrifos treated as singly or in combination.

Biochemical Analysis

Biochemical parameters of serum revealed increased sodium and chloride concentration at the end of 4 weeks of exposure and comparable levels at the end of 13 weeks of exposure in lead treated animals. Decrease in calcium content was noticed at end of 4 and 13 weeks of exposure in lead treated groups. Glucose level was increased at the end of 13 weeks of exposure in chlorpyrifos (G5 males), lead plus chlorpyrifos treated males (G4 and G7) and after 4 weeks of recovery period in higher dose of lead plus chlorpyrifos treated group (G7R males and females). Decrease in activity levels of ALT and AST was noticed due to lead treatment. Serum and RBC cholinesterases were reduced at the end of 4 and 13 weeks of exposure in rats treated with chlorpyrifos alone and in those exposed to a combination of lead and

chlorpyrifos, at higher dose level. The RBC cholinesterase did not return to normal level completely at the end of even 4 weeks of recovery period in lead plus chlorpyrifos treated groups.

Histopathology

Histopathological examination of studied organs of systemic and nervous system reveal lead induced degenerative changes in thymus at higher dose level. No other treatment related changes were noticed in any other groups.

CONCLUSIONS

The results from the single dose oral gavage study reveal, increased inhibition of both serum and RBC cholinesterases to a great extent for longer duration and thereby, cause severe cholinergic symptoms in rats exposed to a combination of chlorpyrifos and lead acetate. The severity and persistence of cholinergic excitotoxicity for longer duration observed in lead plus chlorpyrifos treated animals suggest increased cholinergic toxicity with chlorpyrifos in presence of lead. This gives validity for the potentiating role of lead on chlorpyrifos excitotoxicity of chlopyrifos. Hence, exposure to combination of chlorpyrifos and lead simultaneously is more dangerous than to an exposure of either alone. As chlorpyrifos and lead are extensively used in and around the home environment, there is every likelihood of children being exposed to them inadvertently. Hence, this study on comparatively young animals will be potentially more relevant for physicians/scientists to decipher more about variability/mechanism of action that could arise from accidental poisoning by these agents. No interactive effects are noticed at cellular level.

The results from repeated dietary exposure to chlorpyrifos and lead acetate combination at dose levels of 10 ppm of chlorpyrifos (i.e., equivalent to 1 mg/kg body weight/day) and 500 ppm of lead acetate (i.e., equivalent to 45 mg/kg body weight/day) in Wistar rats for a period of 13 weeks revealed some behavioral changes such as cholinergic symptoms and decrease in rearing counts before week 5. The occurrence of these changes before week 5 and absence of these findings at week 13 suggest that lead plays no effect on the neurobehavioral parameters with upto 10 ppm of chlorpyrifos (i.e., less than or equal to 1.0 mg/kg body weight/day) and 500 ppm of lead acetate (i.e., equivalent to 45 mg/kg body weight/day). However, further higher complex neurobehavioral tests for cognitive functions might be necessary before declaring the validating the conclusion about negative effects of CPF plus lead acetate on nervous system at the presently employed dose levels.

The biochemical parameters studied for systemic toxicity after repeated dietary exposure, reveal cholinesterases (serum and RBC) and serum glucose level observed in recovery group of animals treated with 10 ppm chlorpyrifos plus 500 ppm lead acetate (Group 7R) to be not comparable with the recovery animals exposed to 10 ppm chlorpyrifos alone upto 28 days of post-treatment. This suggests long lasting and/or persistent effects of chlorpyrifos in presence of lead. The chemical intake data suggests that, as low as 1.0 mg/kg body weight/day (10 ppm) chlorpyrifos plus 45.0 mg/kg body weight/day (500 ppm) lead acetate causes persistent long lasting effects on cholinesterases and other parameters like serum glucose. Hence, it can be concluded that repeated exposure to low levels of lead along with chlorpyrifos can cause inhibition of cholinesterases for protracted periods and that this observed effect is other than potentiating, synergistic or additive effect of the two. Increased level of glucose in the recovery group of

animals treated with 10 ppm chlorpyrifos plus 500 ppm lead acetate at the end of 28 days of recovery also suggests, lead plus chlorpyrifos combination to cause prolonged changes in blood glucose level and which can be considered as an indication of systemic toxicity. As lead and chlorpyrifos are more common contaminants in food commodities as residues, this observation will be very much useful in hazard/risk assessment of mixtures of chemicals particularly for regulatory bodies such as US EPA, ATSDR etc to extrapolate to human beings.

Since cholinesterase plays an important role in neuronal architecture of brain and other normal functioning of nervous system, and hence, the long lasting or persistence effects of CPF along with and lead may result in impaired cognitive functions of brain. Therefore, future attempts should bee made on neurobehavioral changes and/or neurotoxicity studies to evaluate effects on developing brain i.e., behavioral teratology and reproduction toxicity studies.

The declared reference dose (RfD) for chlorpyrifos by U.S. EPA was 3 µg/kg/day (Yano *et al*, 2000). Reference dose is a dose level at or below which daily aggregate exposure over a life time will not pose appreciable risk of deleterious effects to human health. With increased frequency and/or magnitude of exposure to chemical exceeding the RfD, the probability of adverse effects in human population could also increase. Earlier (February 21, 1986), the EPA's Health Effects Division's (HED) RfD/Peer Review Committee established a Reference Dose (RfD) of 0.003 mg/kg/day for chlorpyrifos based on the NOAEL of 0.03 mg/kg/day in a study on humans and an Uncertainty Factor of 10 for intra-species variation. Later (HED, 2000), due to increased susceptibility and sensitivity of infants and children

to chlorpyrifos, upped the uncertainty factor(s) to 100 to include and account for inter-species (10X) extrapolation and intra-species (10X) variation.

A draft prepared by Wagner (1999) on toxicological evaluations of chlorpyrifos at the end of a Joint meeting of the FAO Panel of Experts on pesticide residues in food and the environment and the WHO Core Assessment Group, in Rome, suggests acceptable daily intake (ADI) for humans to be 0-0.01 mg/kg/day and an acute reference dose of 0.1 mg/kg/day. This is based on a NOAEL (No Observed Adverse Effect Level) of 0.1 mg/kg/day in humans exposed to chlorpyrifos for 9 days, with a 10-fold safety factor and supported by studies in rats and dogs. These data indicate variation in levels of ADI. Variation in dose levels of ADI/RfD indicates its toxic potential and cautions for its appearance in food as residues.

The U.S. CDC (Centre for Disease Control) limits for lead is 10 µg/dL, however, ATSDR (2005) concludes no clear threshold for neurotoxic effects of lead in children. As it is reported that lead is a well known developmental neurotoxicant and more hazardous to children/immature animals than adults, neurotoxic and systemic effects of a combination of chlorpyrifos and lead acetate also should be considered in preweanling/neonatal animals. As lead and chlorpyrifos are more hazardous to immature animals than adults, the present study has provided inciting base to work more on combination effects of these two chemicals on cognitive functions.

The present repeated dietary exposure study declares no major adverse effects on nervous system based on major neurobehavioral tests and on systemic organs at cellular level upto dose levels of 10 ppm of chlorpyrifos (i.e., equivalent to 1.0 mg/kg body weight/day) and 500 ppm of lead acetate

(i.e., equivalent to 45.0 mg/kg body weight/day). Biochemical evaluations reveal long lasting effects of lead and chlorpyrifos combination on cholinetreases and serum glucose. Hence, higher level behavioral tests for cognitive functions and electron microscopy of nervous system will be more helpful to gauge further information on their interactive effects.