SUMMARY

Acute neurotoxicity of the insecticide combination (Cypermethrin 4% + Profenofos 40%) has been evaluated at 25, 75 and 225 mg/kg body weight doses, whereas, systemic toxicity and developmental neurotoxicity potential has been evaluated at 6, 15 and 38 mg/kg body weight doses for studying end points in the present investigation in male and female Wistar rats through means of a battery of toxicological parameters. The overall findings of the investigation are as follows:

Acute Neurotoxicity Screening Battery Tests

Clinical Observations

No treatment related mortalities were observed in acute neurotoxicity screening battery study. However, severe dose dependent and test article related neurological symptoms *viz.*, tremors, salivation, writhing, hyperactivity to sound/touch, abnormal gait and ataxia were observed in 75 and 225 mg/kg body weight dose groups immediately after dosing. Serum cholinesterase levels were significantly reduced in males treated at 25, 75 and 225 mg/kg body weight and in females treated at 75 and 225 mg/kg body weight dose level as compared to respective controls. However, test article did not cause any change in corresponding body weight and food consumption.

Neurobehavioural Observations

Males treated at 225 mg/kg body weight and females treated at 75 and 225 mg/kg body weight revealed neurological symptoms such as abnormal posture, vertical jumping, writhing, clonic and tonic convulsions in home cage. Handling reactivity and ease of removal from home cage was very difficult in most of the males and females treated at 225 mg/kg body weight dose level, in addition, lacrimation, salivation and piloerection were observed in animals from 225 mg/kg body weight dose groups. In the open field observations, animals of both sex treated at 225

mg/kg body weight dose group revealed slight to severely abnormal gait, slightly to totally impaired mobility, low to very low arousal and tonic/clonic movements.

Sensory reactivity measurements: Approach response was slight or energetic in almost all the animals from control and test article treated groups, whereas, majority of animals from 75 and 225 mg/kg body weight revealed exaggerated response to touch and click stimulus. No constriction of pupil was observed in majority of animals from 225 mg/kg body weight dose group. All the males from 225 mg/kg landed on side in air righting reflex test, whereas, 2 out of 5 females of the same dose group landed on side and rest of the females landed on back. Majority of males and females from 225 mg/kg body weight dose group did not respond to tail pinch and few responded slightly to tail pinch.

Hind limb grip strength was significantly reduced, whereas, hind limb foot splay was significantly increased in males and females treated at 225 mg/kg body weight dose level on day 1 of dosing. However, complete recovery from such abnormalities was apparent on day 7. Reduced motor activity was observed in males and females treated 225 mg/kg body weight dose groups as compared to control group on day-1.

Acute effects of test article over-exposure regressed over several days. Functional observational battery tests performed on day 7 and 14 in treatment groups did not reveal any abnormal behaviour. Which clearly indicates the recovery from the test article induced effects.

However, no histopathological lesions that were related to treatment with test article were noticed in any of the treatment groups.

Repeated Dose 90 Days Oral Toxicity Study

In repeated dose 90 days oral toxicity study, no treatment related mortality or clinical signs/symptoms were observed. Further, no dose related changes in body weight and food consumption were noticed in any of the dose groups.

Hematology Parameters

No test article related variations were observed in any of the hematology parameters studied at the end of 90 days of treatment period.

Clinical Chemistry Parameters

Glucose level was significantly increased in 15 and 38 mg/kg body weight dose group females, whereas, no such variations were observed in male counterparts. In both males and females treated 38 mg/kg body weight dose level ALT values were significantly increased. Whereas, total bilirubin level was significantly increased only in males treated at 38 mg/kg body weight dose level. Increased creatinine was observed in males and females treated 38 mg/kg body weight dose and increased total protein and albumin were observed in males treated at 6, 15 and 38 mg/kg body weight dose levels. Though a certain increase in total protein and albumin was noticed in females of treated groups the variation was statistically insignificant when compared to controls.

Serum cholinesterase was reduced in males treated at 38 mg/kg body weight dose group and females treated at 6, 15 and 38 mg/kg body weight dose levels.

Functional observational Battery Tests

No treatment related variations were observed in any of the functional parameters studied during the study.

Developmental Neurotoxicity Study

In the present investigation, test article did not exert any adverse effect on estrous cycle and gestation period of females treated for more than 90 days. Further, body weights and food consumption of test article treated dams were unaffected during gestation and lactation periods.

Administration of test article did not affect male and female fertility indices as well as mating index of test article treated group animals.

No variation was observed in the gestation index, mean duration of gestation and lactation indices in test article treated group animals. However, lactation was reduced; in addition, survival index and mean litter size (both male and female pups) were also significantly reduced in treated groups. Percent pup mortality significantly increased during lactation period at 38 mg/kg body weight dose group.

Organ Weights

No variations in the organ weights of parent males and females treated with the insecticide combination were established. However, terminal observations of pups revealed increased absolute weight of liver in 38 mg/kg dose group male pups and 15 mg/kg and 38 mg/kg body weight dose group female pups. Even relative weights (organ to body weight ratio) as well as relative brain weights of both male and female pups at 38 mg/kg body weight were significantly increased.

Absolute, relative and organ to brain weight ratio of spleen in 38 mg/kg body weight dose group male pups were significantly increased.

Relative weight of kidneys in 38 mg/kg body weight dose group pups of both sex were significantly increased.

Gross Pathology

Parents

The gross pathology of the animals treated at 38 mg/kg body weight dose group showed varying degrees of different lesions in liver (hyperemic, congestion, consolidation, mottling, pin point hemorrhages and petechial hemorrhages), spleen (diffused white foci, congestion, atrophy and enlarged), kidneys (pallor and congestion), lungs (diffused pneumonic foci, edematous and hemorrhages), Uterus (thickened wall with abscess formation of pyometra and hydrometra) and intestine (hyperemic).

Pups

The gross pathology of the pups from 38 mg/kg body weight dose group showed varying degrees of different lesions in liver (congestion, molting, consolidation and

mottling), spleen (enlarged and hyperemic), kidneys (pallor and congestion), lungs (sub plural hemorrhages, diffused pneumonic foci and hemorrhage).

Histopathology

Parents

Histopathology of organs from control and test article treated groups revealed lesions in **liver** (congestion, minimal fatty changes centribular hypertrophy, multi focal degeneration and necrotic foci), **kidney** (cortical tubular dilatation with flattened epithelium, congestion and multifocal regenerating tubules), **nerve** (demyelination, vacuolation and axonal degeneration), **brain** (inflammatory cell infiltration and gliosis), **ovary** (atretic follicle), and **thymus** (atrophy and epithelisation).

Pups

Microscopic examination of different organs from test article treated group pups showed lesions in **brain** (multiple pale acellular areas, gliosis, demyelination), **liver** (cystic tubules, extramedullary hematopoisis, sinusoidal dilation, vacoulation and congestion, sinusoidal dilation), **thymus** (atrophy characterized by epithiolisation), **kidney** (tubular degeneration with flattened epithelium) and **spleen** (extramedullary hematopoisis, megalokaryocytosis).

Taking into overall consideration of overall incidence of path morphological changes in various parameters of male and female animal belong to different treated groups, it appears to be more prominent in 38 mg/kg body weight dose group when compared to control group.

The prominent changes observed in histopathology of kidney, liver, ovary, brain and peripheral nerve in parental animals and similar findings in even pups of test article treated group i.e., 38 mg/kg body weight indicates that combination of cypermethrin and profenofos could cause effect on general health in addition to cholinesterase inhibition.