

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Preliminary findings from Phases 2A and B of the toxicology study

Stage 2A

1. Three groups of three female CD-1 mice were administered with 1mL of extract giving rise to the atypical response, 1mL of extract negative for atypical responses or 1mL of the vehicle (1% [w/v] aqueous Tween 60) by ip injection. Clinical signs (Irwin screen) were recorded post-administration. Macroscopic examination of decedents and mice killed at term (24 hours) was conducted on necropsy. Major organs and tissues (brain, heart, kidney, liver, lungs, spleen and gastrointestinal tract) were preserved for histopathological examination.

Findings of stage 2A

2. The clinical signs observed in all three treatment groups are shown in table 1. All three animals treated with extract giving rise to the atypical response showed severe symptoms within 5 minutes and died within 13-15 minutes of administration. There were no consistent macroscopic findings on necropsy in any of the treatment groups.

Stage 2B

3. Three groups of three female CD-1 mice were administered with 1mL of extract giving rise to the atypical response, 1mL of extract negative for atypical responses or 1mL of the vehicle (1% [w/v] aqueous Tween 60) by oral gavage. Clinical signs (Irwin screen) were recorded post-administration. Macroscopic examination of decedents and mice killed at term (24 hours) was conducted on necropsy. Major organs and tissues (brain, heart, kidney, liver, lungs, spleen and gastrointestinal tract) were preserved for histopathological examination.

Findings of stage 2B

4. The clinical signs observed in the group treated with extract giving rise to the atypical response are shown in table 2. All three animals showed a range of clinical symptoms within 2-3 minutes of administration, which continued for 60 minutes with the animals appearing normal after that time. No clinical signs were observed in the animals treated with the extract negative for atypical responses or the vehicle. There were no consistent macroscopic findings on necropsy in any of the treatment groups.

Table 1 Total number of female mice exhibiting signs following intraperitoneal administration of vehicle or cockle extract.

Experimental phase: 28-29 January 2004	Group 1: Vehicle			
	Total number of animals exhibiting signs (n=3)			
Approximate observation time-point (min)	0-60	90	150	180-300
Occasional abdominal spasm	3	1	-	-
Ptosis	-	-	1	
No abnormalities detected	-	2	2	3
Comments: No further signs were seen during the 24-hour observation period.				

Experimental phase 28-29 January 2004	Group 2: cockle extract (atypical)			
	Total number of animals exhibiting signs (n=3)			
Approximate observation time point (min)	0-5	13-15	N/A	N/A
Respiratory distress	3	-	-	-
Agonal kicking of hind limbs	3	-	-	-
Dead	-	3	-	-
Comments: None				

Experimental phase 28-29 January 2004	Group 3: cockle extract (negative)			
	Total number of animals exhibiting signs (n=3)			
Approximate observation time point (min)	0-5	90	150-300	N/A
Subdued	3	-	-	-
Abnormal dispersion within the home cage	3	-	-	-
Occasional abdominal spasm	3	-	-	-
Abdominal respiration	3	-	-	-
Ptosis	3	1	-	-
Abnormal gait	3	-	-	-
Decreased locomotor activity	-	2	-	-
Slight apathy	-	2	-	-
Slightly decreased transfer arousal	-	2	-	-
Slightly decreased spatial locomotion	-	1	-	-
No abnormalities detected	-	1	3	-
Comments: No further signs were seen during the 24-hour observation period.				

Table 2 Total number of female mice exhibiting signs following oral administration of cockle extract (atypical)

Experimental phase: 29-30 January 2004	Total number of animals exhibiting signs (n=3)				
Observation time point (min)	15	30	60	150	300
Slightly decreased locomotor activity	-	2	-	-	-
Moderately decreased locomotor activity	3	1	-	-	-
Moderately decreased alertness	3	-	-	-	-
Slight apathy	3	2	3	-	-
Moderate apathy	-	1	-	-	-
Slightly decreased transfer arousal	1	3	-	-	-
Moderately decreased transfer arousal	1	-	-	-	-
Substantially decreased transfer arousal	1	-	-	-	-
Slightly decreased spatial locomotion	1	3	-	-	-
Spatial locomotion – no movement	2	-	-	-	-
Slightly decreased startle	3	3	-	-	-
Slight piloerection	3	3	-	-	-
Slightly decreased touch escape	3	3	-	-	-
Moderately decreased grip strength	1	-	-	-	-
Slight hypothermia	2	1	-	-	-
Slightly decreased body tone	3	1	1	-	-
Slightly decreased fearfulness	3	-	-	-	-
Slight tremor	1	1	-	-	-
Slight lacrimation	-	1	-	-	-
Slight irritability	-	-	1	-	-
90° tail elevation (due to pro-convulsant stance)	1	1	-	-	-
Splayed legs on transfer to the arena	1	-	-	-	-
Flattened posture	3	1	-	-	-
No abnormalities detected	-	-	-	3	3
Comments:					
At 2-3 minutes post dose all animals exhibited apathy, decreased respiration, flattened posture, splayed hind legs and remained motionless until stimulated.					
At 60 minutes post-dose, although all animals displayed substantial apathy, upon stimulation they appeared normal.					
No further signs were seen during the 24-hour observation period.					