Data part 4: Toxicology – Human health

DACOs: 4.1, 4.2.1, 4.2.2, 4.2.3, 4.2.4, 4.2.5, 4.2.6

AEF Global Inc. has previously requested a waiver for the entire data part 4 (Toxicology - Human health) in the original registration.

Since the registration of Cyclone PLUS was granted by PMRA, AEF Global obtained tox studies results for the end-product Cyclone PLUS and would like to modify the label warning based on these results.

See the following summaries for the tox studies:

CYCLONE PLUS, ACUTE ORAL TOXICITY (UDP) IN RATS, OCSPP 870.1100, STUDY ID 25980-22, STILLMEADOW, 2023

Test substance Cyclone PLUS was evaluated for acute oral toxicity potential in female albino rats when administered as a gavage dose at 5000 mg/kg. The study was terminated following the stopping rules of UDP procedure. No mortality occurred during the study. There were no clinical signs of toxicity during the study. Animals exhibited weekly weight gain, except for one that lost weight between Days 0 and 7. Gross necropsy conducted at Day 14 terminal sacrifice revealed no observable abnormalities. The test substance acute oral LD50 is determined to be greater than 5000 mg/kg.

CYCLONE PLUS, ACUTE DERMAL TOXICITY (UDP) IN RATS, OCSPP 870.1200, STUDY ID 25981-22, STILLMEADOW, 2023

Test substance Cyclone PLUS was evaluated for dermal toxicity potential and relative skin irritancy when a single undiluted dose of 5050 mg/kg was applied to the intact skin of 5/sex albino rats for 24 hours. No mortality occurred during the study. There were no clinical signs of toxicity or signs of exposure-area skin irritation at any time throughout the study. Animals exhibited weekly weight gain during the study. Gross necropsy conducted at Day 14 study termination revealed no observable abnormalities. The test substance dermal LD50 is determined to be greater than 5050 mg/kg.

CYCLONE PLUS, ACUTE INHALATION TOXICITY IN RATS, OCSPP 870.1300, STUDY ID 25982-22, STILLMEADOW, 2023

Test substance Cyclone PLUS was evaluated for acute inhalation toxicity potential in albino rats. Five males and five females were exposed for four hours to an aerosol generated from undiluted liquid test substance at 5.10 mg/L. There was no mortality during the study. There were no

clinical signs of toxicity observed during the study. All animals lost weight between Days 0 and 1; all males and most females had weight gain at following weigh points during the study. Gross necropsy at Day 14 terminal sacrifice revealed no observable abnormalities except for dark spot on lungs in three animals. The test substance acute inhalation LC50 is determined to be greater than 5.10 mg/L.

CYCLONE PLUS, ACUTE EYE IRRITATION IN RABBITS, OCSPP 870.2400, STUDY ID 25983-22, STILLMEADOW, 2023

An acute eye irritation study was conducted on three albino rabbits using test substance Cyclone PLUS. Undiluted test substance (0.1 mL) was placed into the conjunctival sac of the right eye of each animal selected for testing. All treated eyes were washed with room temperature deionized (DI) water for one minute after recording the 24-hour observation. The number of animals testing positive (according to Legend A) for each parameter over number of animals observed is presented below.

	Time After Treatment Hours			
	1	24	48	72
Cornea				
Opacity	0/3	0/3	0/3	0/3
Iritis	0/3	0/3	0/3	0/3
Conjunctivae				
Redness	0/3	0/3	0/3	0/3
Chemosis	0/3	0/3	0/3	0/3

There were no positive effects exhibited in any eyes after treatment. Therefore, Cyclone PLUS is assigned Toxicity Category IV. Per Legend B, the test substance is rated minimally irritating.

CYCLONE PLUS, ACUTE SKIN IRRITATION IN RABBITS, OCSPP 870.2500, STUDY ID 25984-22, STILLMEADOW, 2023

An acute skin irritation study was conducted on three albino rabbits using test substance Cyclone PLUS. There was one intact test site per animal. Each test site was treated with 0.5 mL of undiluted test substance and wrapped with semi-permeable dressing. Test substance was maintained in contact with the skin for 4 hours. Observations for skin irritation and defects were made at ~1, 24, 48 and 72 hours after unwrapping. Irritation scores derived from respective erythema and edema scores through 72-hour observations for each animal are presented below.

	Erythema Hours after Unwrap				**Edema** Hours after Unwrap				Irritation Scores
	1	24	48	72	1	24	48	72	
545-F	1	1	0	0	0	0	0	0	0.50
547-F	0	0	0	0	0	0	0	0	0.00
549-F	1	1	0	0	0	0	0	0	0.50
							Primary Irritation Index (PII) =		0.3

Based on 0.3 PII, Cyclone PLUS is rated slightly irritating. Based only on scores at 72-hour observations, the test substance is assigned Toxicity Category IV.

CYCLONE PLUS, SKIN SENSITIZATION IN GUINEA PIGS, OCSPP 870.2600, STUDY ID 25985-22, STILLMEADOW, 2023

A skin sensitization study was conducted on 30 short-haired albino guinea pigs to determine if test substance Cyclone PLUS produced a sensitizing reaction. Animals were assigned to each of two groups, designated Naive control (10) and Test (20). Naive control group animals remained untreated during induction phase of the study. Test group animals were treated with 0.4 mL of undiluted test substance (selected from range-finding). Test animals were treated once weekly for three weeks, for a total of three inductions. After a two-week rest period, all animals (both groups) were challenged at a virgin test site with an application of 0.4 mL of undiluted test substance.

The test substance produced no reaction (0% positives) in Test animals and no reaction (0% positives) in Naive control animals after the challenge treatment. Therefore, Cyclone PLUS is not a skin sensitizer in guinea pigs.

Summary of the toxicity category:

ACUTE ORAL TOXICITY (UDP) IN RATS	The test substance acute oral LD50 is determined to be greater than 5000 mg/kg. Toxicity Category IV.
ACUTE DERMAL TOXICITY (UDP) IN RATS	The test substance dermal LD50 is determined to be greater than 5050 mg/kg. Toxicity Category IV.
ACUTE INHALATION TOXICITY IN RATS	The test substance acute inhalation LC50 is determined to be greater than 5.10 mg/L. Toxicity Category IV.
ACUTE EYE IRRITATION IN RABBITS	Cyclone PLUS is assigned Toxicity Category IV. The test substance is rated minimally irritating.
ACUTE SKIN IRRITATION IN RABBITS	Cyclone PLUS is rated slightly irritating. Based only on scores at 72-hour observations, the test substance is assigned Toxicity Category IV.
SKIN SENSITIZATION IN GUINEA PIGS	Cyclone PLUS is not a skin sensitizer in guinea pigs.

Conclusion: Based on these tox studies, AEF Global would like to remove then warning DANGER – EYE AND SKIN IRRITANT from the label.