ACUTE ORAL TOXICITY TO TERRESTRIAL INVERTEBRATES (HONEY BEE)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks field: The 3M production lot number was 217. The test substance is a white powder. Sample was stored AT 16-20^{o}C prior to testing. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OECD Guideline 213, EPPO Guideline 170
Test type: Acute Oral
GLP: Yes
Year Completed: 2001
Species: *Apis mellifera L.*
Analytical monitoring: None – nominal concentrations
Test honey bee source: Obtained from colony number 32 belonging to the Central Science Laboratory (CSL), Sand Hutton, York, UK, National Bee Unit.
Test honey bee age at study initiation: Young adult
Test honey bee type: Worker honey bees, free of acarine, nosema and amoeba.
Varroacide treatment: None within the 4 weeks prior to test initiation.

Test conditions

Humidity: 65% $\pm$ 5%
Temperature: 25 $\pm$ 2^{o}C
Lighting: Conducted in darkness

Stock and test solutions preparation:

Test substance: Initial stock solution prepared in analytical grade acetone to a final concentration of 47.8 µg PFOS/µL (nominal concentration). Final test concentrations prepared from dilutions of this solution with 50% w/v sucrose. Resulting acetone concentration was 5%.
Reference toxicant: Primary stock solution of dimethoate was prepared in deionized water containing 1 g/L Triton X-100 to a final concentration of 3.0 µg/µL. Secondary stock solutions were made

by diluting the primary stock solution in deionized water containing 1 g/L Triton X-100. Final test concentrations prepared from dilutions of these solutions with 50% w/v sucrose.

Stability of the test chemical solution: A dispersion test was carried out on an 86 µg PFOS/µL acetone solution before the toxicity study was performed. The homogeneity of the mixture was assessed after 2 hours. The test item formed a clear solution on mixing; after 2 hours at room temperature, slight sediment was noted. For the toxicity test, all solutions were re-mixed prior to use. The contract laboratory considered the solutions of the test doses to be homogenous for the purpose of administration.

Exposure vessels: Clean, well-ventilated, inverted petri dishes, measuring approximately 9 cm in diameter.

Feeding: During the first four hours of the test, bees provided with 50% w/v aqueous sucrose solutions containing the appropriate PFOS dose. After 4-hours, dosed sucrose removed, and bees provided with 50% w/v aqueous sucrose solutions, continuously available through the end of the exposure period.

Number of replicates: Three

Number of bees per replicate: Ten

Negative control: 50% w/v sucrose

Solvent control: 50% w/v sucrose plus 5% acetone

Reference substance: Dimethoate

Reference substance control: Triton X-100

Number of concentrations: five plus a negative and a solvent control

Dose administration: The bees were anaesthetized with carbon dioxide immediately before dosing and gently tipped out onto filter paper and counted into the petri dish cage (drones were discarded). Each group of 10 bees was offered 0.2 mL of a given test concentration or control solution. The dose was measured into a small, pre-weighed, glass feeder within the cage using a variable volume pipette. This volume of solution is equivalent to 20 µL per bee.

Dose frequency: Once, for 4 hours of exposure

Dose calculation: Feeders were weighed after removal from the cages to determine the dose consumed per bee.

Element basis: Mortality

RESULTS

Nominal concentrations: Negative control (sucrose only), acetone + sucrose control, 0.205, 0.450, 0.991, 2.17, 4.78 µg/bee

Element value and 95% confidence interval :

24-hour LD_{50} = 0.72 (0.60 – 0.85) µg/bee

48-hour LD_{50} = 0.46 (0.32 – 0.55) µg/bee

72-hour LD_{50} = 0.40 (0.33 – 0.48) µg/bee

72-hour NOEL = 0.21 µg/bee

All element values based on nominal concentrations

Statistical Evaluation: Probit mortality plotted against the logarithm of dose using the contract laboratory Probit 1 package. A least-squares regression (Finney 1971) was fitted to these. The NOELs were estimated using Student's t-test ($p<0.05$)

Biological observations: There was significant mortality at all doses above a mean intake of 0.21 µg/bee with a steep dose response between mean intakes of 0.45 and 2.2 µg/bee.

Cumulative percent mortality:

Nominal Test Conc., µg/bee	4-hours	24-hours	48-hours	72-hours
Negative Control	0	0	0	0
Solvent Control	0	3.3	3.3	3.3
0.205	0	0	6.7	10
0.450	0	20	50	60
0.991	0	70	93	97
2.17	6.7	100	100	100
4.78	30	100	100	100

Sub-lethal Effects – Percent Knockdown (K) or Stumbling (S):

Nominal Test Conc., µg/bee	4-hours	24-hours	48-hours	72-hours
Negative Control	0	0	0	0
Solvent Control	0	0	0	0
0.205	0	0	0	0
0.450	0	3.3 (S)	3.3 (K)	3.3 (K)
0.991	0	0	0	0
2.17	0	0	0	0
4.78	10 (K)	0	0	0

Control response: satisfactory

Reference toxicant response: satisfactory - dimethoate 72-hour LD_{50} = 0.11 µg/bee

CONCLUSIONS

The potassium perfluorooctanesulfonate 72-hour oral LD_{50} for the honey bee was determined to be 0.40 µg/bee with a 95% confidence interval of 0.33 – 0.48. The 72-hour no observed effect level was 0.21 µg/bee. The dose response was steep between a mean uptake of 0.45 and 2.2 µg/bee.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 1

REFERENCES

This study was conducted at Central Science Laboratory, Sand Hutton, York, UK, under contract by Wildlife International, Ltd, Easton, MD at the request of the 3M Company, Lab Request Number U2723, 2001.

OTHER

Last changed: 5/1/01