Oral (Dietary) 90-Day Repeat Dose Toxicity Study in the Rat (Haas et al., 2010)

Study Design

EC No. 272-234-3 (B1145) was offered ad libitum to male and females Crl:CD(SD) rats (10 animals per sex per dose) via the basal diet for a minimum of 90 consecutive days at dosage levels of 0, 125, 250, 500, and 1000 mg/kg/day. Following 91 or 92 days of test diet administration, all animals were euthanized and subjected to a complete necropsy. Selected tissues were examined microscopically from all animals in the control and 1000 mg/kg/day groups. In addition, gross lesions were examined from all animals in the 125, 250, and 500 mg/kg/day groups. This study was designed to meet or exceed the OECD 408 guideline. Note that no evaluation of semen parameters or estrous cyclicity occurred.

EC No. 272-234-3 contains approximately 6.7 wt% residual tetrapropenyl phenol. The converted approximate equivalent residual concentration TPP present in each dose level of 0, 125, 250, 500, and 1000 mg/kg/day is (0), (8.4), (16.7), (33.5), and (67) mg/kg/day, respectively. Where presented, TPP dose appears in parenthesis to distinguish TPP dose from test article dose.

Summary of No Observed Adverse Effect Level

Gender	**NOAEL (mg/kg bw/day)**
Male / Female	1000 (67 mg TPP/kg bw/day)

Results

Clinical Observations

- There were no treatment related deaths
- No observations were attributed to test substance by the study director

Body Weight:

Lower mean body weight gains were noted for the 250, 500, and 1000 mg/kg/day group males and females as early as study week 0 to 1. For some weeks, body weight gains were statistically significant from control group.

- Males
    - Decreased, but not statistically significant, cumulative body weight gain at 13 weeks:
        - 1000 mg/kg/day test material (67 mg/kg/day TPP)
            - 311 ± 39.2 g vs. 361 ± 62.3 g; - 13.9 % compared to control
        - 500 mg/kg/day test material (33.5 mg/kg/day TPP)
            - 331 ± 41.8 g vs. 361 ± 62.3 g; - 8.3 % compared to control
        - 250 mg/kg/day test material (16.7 mg/kg/day TPP)
            - 340 ± 39.2 g vs. 361 ± 62.3 g; - 5.8 % compared to control
    - Decreased, but not statistically significant, absolute body weight at 13 weeks:
        - 1000 mg/kg/day test material (67 mg/kg/day TPP)
            - 500 ± 43.0 g vs. 550 ± 54.5 g; - 9.1 % compared to control

        - 500 mg/kg/day test material (33.5 mg/kg/day TPP)
            - 519 ± 45.6 g vs. 550 ± 54.5 g; - 5.6 % compared to control
        - 250 mg/kg/day test material (16.7 mg/kg/day TPP)
            - 529 ± 34.6 g vs. 550 ± 54.5 g; - 3.8 % compared to control

- Females
    - Decreased cumulative body weight gain at 13 weeks:
        - 1000 mg/kg/day test material (67 mg/kg/day TPP)
            - 125 ± 17.7 g vs. 153 ± 24.4 g; - 18.3% compared to control
        - 500 mg/kg/day test material (33.5 mg/kg/day TPP)
            - 131 ± 20.0 g vs. 153 ± 24.4 g; - 14.3% compared to control
        - 250 mg/kg/day test material (16.7 mg/kg/day TPP)
            - 138 ± 15.8 g vs. 153 ± 24.4 g; - 9.8 % compared to control (not statistically significant)
    - Decreased, but not statistically significant, absolute body weight gain at 13 weeks:
        - 1000 mg/kg/day test material (67 mg/kg/day TPP)
            - 272 ± 17.6 g vs. 297 ± 29.9 g; - 8.4% compared to control
        - 500 mg/kg/day test material (33.5 mg/kg/day TPP)
            - 277 ± 19.7 g vs. 297 ± 29.9 g; - 6.7% compared to control
        - 250 mg/kg/day test material (16.7 mg/kg/day TPP)
            - 284 ± 21.9 g vs. 297 ± 29.9 g; - 4.4% compared to control

    Note: At the end of the study the difference between the absolute mean body weights and the control group did not exceed 10%. Therefore, the study director considered the body weight effects to be non-adverse.

Food Consumption

Food consumption in both sexes at all dose levels was unaffected by test substance administration. There were no statistically significant differences when the control and test substance-treated groups were compared.

Hematology:

- Males
    - 1000 mg/kg/day (67 mg/kg/day TPP) vs. control:
        - Increased platelets (1221± 109.4 vs. 1032 ± 122.9 thousand/ µL, +18.3 % compared to control)
        - Increased activated partial thromboplastin time (APTT) (23.2 ± 3.64 s vs. 20.6 ± 1.51 s, +12.6 % compared to control)
    - 500 mg/kg/day (33.5 mg/kg/day TPP) dose group vs controls:
        - Increased platelets (1204 ± 150.4 vs. 1032 ± 122.9 thousand/ µL, +16.7 % compared to control)

- Females
    - No statistically significant findings noted at any dose

Note: the study director did not consider observed hematologic findings to be evidence of test substance effect because all observations were within the laboratory's historic range of values.

Serum Chemistry:

- Males
    - No statistically significant findings noted at any dose
- Females
    - 1000 mg/kg/day (67 mg/kg/day TPP) dose group vs. controls:
        - Serum triglyceride decreased (49 ± 7.7 vs. 62 ± 13/9 mg/dl, -21% compared to control)
    - 500 mg/kg/day (33.5 mg/kg/day TPP) dose group vs. controls:
        - Serum triglyceride decreased (50 ± 8.9 vs. 62 ± 13/9 mg/dl, -19.4% compared to control)
    - 250 mg/kg/day (16.7 mg/kg/day TPP) dose group vs. controls:
        - No statistically significant findings noted
    - 125 mg/kg/day (8.4 mg/kg/day TPP) dose group vs. controls:
        - Serum calcium decreased (10.5 ± 0.14 vs. 10.9 ± 0.46 mg/dl, -3.7 % compared to control)
        - Glucose increased (118 ± 7.1 vs. 105 ± 9.6 mg/dl, +12.4 % compared to controls)

Note: There were a limited number of statistically significant differences in serum chemistry. The study director did not consider these findings to be biologically significant considering individual variability and overlap with control population values.

Organ Weights

- Males treated at 1000 mg/kg/day (67 mg/kg/day TPP):
    - Liver
        - Increased mean weight relative to final body weight (3.046 ± 0.1718 vs. 2.783 ± 0.1396, + 9.5% treated compared to control)
    - Kidney
        - Increased mean weight relative to final body weight (0.841 ± 0.053 vs. 0.749 ± 0.0545, +12.3% treated compared to control)
    - Accessory sex organs –
        - Decreased mean relative seminal vesicle/coagulating gland weight (1.58 ± 0.142 vs. 1.84 ± 0.0302, –14.1% treated compared to control)
- Females treated at 1000 mg/kg/day (67 mg/kg/day TPP):
    - Liver
        - Increased mean weight relative to final body weight compared to controls (3.073 ± 0.1699 vs. 2.812 ± 0.0613, +9.3% treated compared to control).

Note: Although the decreased absolute body weight observed in the 1000 mg/kg/day treatment group was not sufficient to achieve statistical significance, the study director indicated that the decrease contributed to a limited number of statistically significant differences in organ weights relative to terminal body weight. Specifically, the study director attributed the findings in relative liver weights in both sexes and male kidney weights to the decrease in absolute body weight. The study director did not consider these organ findings a direct effect of the test substance in the absence of correlating findings in serum chemistry or histopathology.

Separately, the absolute mean weight of the seminal vesicle/coagulating gland was reduced compared to controls in the 1000 mg/kg/day (67 mg/kg/day TPP) treatment group. The study director noted that only a single organ weight fell outside historic control values and the specific finding was likely due to loss of fluid in handling rather than a test substance effect.

Histological Changes

No test-substance related microscopic findings were noted for either sex. The study director noted that any finding observed was consistent with typical background lesions for the strain and age of rat on test. Therefore, all findings were considered spontaneous or incidental in nature. There were no statistically significant differences when the control and test substance-treated groups were compared.

Discussion and Conclusions

The no-observed-effect level (NOEL) for dietary administration of EC No.272-234-3 (B1145) to rat was 125 mg/kg/day (8.4 mg/kg/day TPP) based on lower body weights and body weight gains at dose levels of 250 mg/kg/day (16.7 mg/kg/day TPP) and higher. However, all effects observed throughout the study were considered non-adverse by the study director. Therefore, the no-observed-adverse-effect (NOAEL) level was considered to be 1000 mg/kg/day (67 mg/kg/day TPP).