Evaluation of a Dietary 90-day Sub-chronic Toxicity Study on β-Caryophyllene

An enhanced OECD 408 dietary 90-day study was conducted on test material, β-Caryophyllene, a widely used flavor and fragrance ingredient at about 11 metric tons per annum worldwide, and as a flavoring agent at about 0.1 ppm in food. Male rats were fed diets containing 0, 3500, 7000, and 21000 ppm of β-Caryophyllene. The estrous cycling and sperm analyses were also conducted. There was a significant reduction in bodyweight, bodyweight gain and food consumption among high dose group animals, attributed to a decrease in palatability of test material. There were no alterations in clinical chemistry and hematological parameters attributed to test material administration. There was an increase in liver weights among high dose group males. An increase in relative liver weights and the incidence of centrilobular hypotrophy without any pathological changes were reported among animals in the mid- and high-dose groups and were considered to be adaptive responses. Microscopic findings included a dose-dependent increase in the incidence and severity of α-2-globulin related renal tubule nephropathy among treated males, which is considered a male ratspecific effect not relevant to humans. There were no test material-related effects involving the testes, epididymides and accessory sex glands among treated males. Similarly, there were no test material-related effects involving the female reproductive tract in animals administered β-Caryophyllene. Thus the NOAEL for males and females was determined to be 7000 and 14000 ppm respectively, based on decreased bodyweight, bodyweight gain and food consumption among high-dose group animals.

Abstract

Introduction

β-Caryophyllene is used as a flavoring ingredient at a volume of about 100 metric tons per annum worldwide, and as a flavoring agent at about 11 metric tons per annum (IFRA, 2011; IOFI, 2013). β-Caryophyllene is a colorless oily liquid which has a woody, spicy, dry and ten descriptions include the word "clove-like." The maximum daily human systemic exposure via the dermal route from the use of multiple cosmetic products containing β-Caryophyllene has been calculated to be 0.003 ppm per kg/day for a 60 kg high-end user of these products (IFRA, 2004). This study included no dose or reproductive toxicity data available on β-Caryophyllene. Hence a 90-day repeated dose toxicity study with detailed male and female reproductive organ analysis was conducted.

Methods

A GLP/OECD 408 study was conducted on groups of 10 Cr:Sprague-Dawley® CD® IGS rats/sex/dose group. Males were maintained ondiets containing 0, 3500, 7000, or 21000 ppm of β-Caryophyllene calculated to provide an average daily intake of 0, 222, 456, or 1367 mg/kg/day, respectively, for 90 days. Female rats were maintained on diets containing 0, 3500, 14000, or 28000 ppm of β-caryophyllene, calculated to provide an average daily intake of 0, 263, 1032, or 4278 mg/kg/day, respectively, for 90 days. The stability, homogeneity and concentration were analyzed to confirm that target concentrations were achieved in diet for all intake levels. The following parameters were recorded for control and treated animals: ophthalmological alterations, clinical signs, bodyweights, food consumption, hematological alterations, urinalysis, clinical chemistry and gross necropsy. Hematological evaluation on selected organs and tissues were performed on all study animals. All rats were monitored twice during the study over fourteen days, once during the middle (Weeks 6-7) and again at the end of the study (Weeks 12-13). Male reproductive parameters evaluated included, sperm motility, epididymal sperm count, homogenization-resistant sperm (HR3) count, or sperm morphological development.

Results

Figure 1-2: Statistically significant reductions in bodyweight, bodyweight gain and food consumption were reported among high dose males due to the decrease in test substance palatability. There was no significant difference in bodyweight, bodyweight gain or food consumption among low dose dose group males. * (P<0.05) as compared to controls.

Figure 3-4: Statistically significant reductions in bodyweight, bodyweight gain and food consumption were reported among high dose females due to the decrease in test substance palatability. There was no significant difference in bodyweight, bodyweight gain or food consumption among low dose dose group females. * (P<0.05) as compared to controls.

Figure 5-6: Statistically significant dietary dose-dependent decreased absolute heart weight and absolute spleen weight were reported among high dose males. Absolute heart weights was decreased in high dose males; however this difference was not dietary dose-dependent. * (P<0.05) as compared to controls. ** (P<0.01) as compared to controls.

Figure 7-8: Statistically significant dietary dose-dependent increase in absolute liver weights were reported among middle and high dose females. Absolute spleen and liver weights were decreased among high dose females. * (P<0.05) as compared to controls. ** (P<0.01) as compared to controls.

Conclusions

• Results from the homogeneity, stability, and concentration analyses of the test diets indicate that β-caryophyllene was homogeneously distributed, stable and was considered to reach target concentrations in the diet for all intake levels.
• Statistically significant dose-dependent reductions in bodyweight, bodyweight gain and food consumption in males and females at the highest dietary level during the study were attributed to the decrease in palatability.
• Statistically significant changes in hematology, clinical chemistry, coagulation and urinalysis parameters in treated rats were within the range of historical control values.
• Microscopic findings in male rats included enlarged kidneys in a single male and enlarged liver in a single female rat at the highest dietary level.
• Microscopic liver changes were characterized by centrilobular to midzonal distributed histopathologic findings.
• Microscopic findings of nephropathy and tubular cytoplasmic droplets in the male kidneys at all dietary levels and increased relative kidney weights in males at the highest dietary level were observed. These findings were considered as a globulin nephropathy a male ratspecific effect and not considered to be of concern for human health.
• There were no macroscopic or microscopic test substance-related effects involving the male and female reproductive systems evaluated.
• Thus the NOAEL for males and females was determined to be 7000 and 14000 ppm respectively, based on decreased bodyweight, bodyweight gain and food consumption among high dose group animals.

Acknowledgement

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References


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Figures:

Figure 1: Mean Weekly Bodyweights (Males)

Figure 2: Mean Weekly Bodyweights (Females)

Figure 3: Mean Weekly Bodyweights (Males)

Figure 4: Mean Weekly Bodyweights (Females)

Figure 5: Absolute Organ Weights (Males)

Figure 6: Absolute Organ Weights (Females)

Figure 7: Absolute Organ Weights (Males)

Figure 8: Absolute Organ Weights (Females)

Figure 9: Relative Organ Weights (Males)

Figure 10: Relative Organ Weights (Females)

Figure 11: Relative Organ Weights (Males)