

typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538.

3. *Reproductive and developmental toxicity.* No reproductive or developmental toxicity studies on *cis*-3-hexen-1-ol were identified in the scientific literature. Given the metabolism of *cis*-3-hexen-1-ol to endogenous substrates of fatty acid oxidation pathways, or to readily excreted carboxylic acids, it is not expected to be a reproductive toxicant. A teratogenicity study has been conducted on 4-pentenoic acid, a substance similar to the potential carboxylic acid metabolites of *cis*-3-hexen-1-ol. In this study, 2 groups of 15 female NMRI mice were mated with males for a period of 2 hours, and, on day 8 of gestation, were administered, by subcutaneous injection, the sodium salt of 4-pentenoic acid as a single 600 mg/kg body weight dose. Implantation sites were counted and each live fetus was weighed and examined for neural tube defects and any other visceral or skeletal abnormalities. The study authors concluded that 4-pentenoic acid had no effect on embryo survival, the number of live fetuses, fetal weight, or on the incidence of neural tube defects. There were no reports of effects of 4-pentenoic acid on the incidence rates of other visceral or skeletal abnormalities.

Summaries of two reproductive toxicity studies on higher chain length saturated primary alcohols also demonstrated lack of toxicity. In these 1-generation reproductive toxicity studies, dodecan-1-ol or octadecan-1-ol was administered in the diet to groups of male and female rats for 14-days prior to mating and to pregnant females for an additional 3 weeks. The maximum dietary dose tested for both compounds was 2,000 mg/kg body weight/day. There were no reported effects of treatment with either alcohol on the reproductive and developmental parameters measured.

4. *Subchronic toxicity.* *Cis*-3-Hexen-1-ol has been evaluated for subchronic toxicity in a 98-day drinking water study in rats. In this study, groups of 15 male and 15 female weanling SPF-derived CFE rats, housed 5 to a cage, were allowed to consume ad libitum drinking water containing either 0, 310, 1,250, or 5,000 ppm *cis*-3-hexen-1-ol. Due to volatilization loss, fresh solutions were prepared every 2 days. Body weights, and food and water consumption were measured weekly. During the sixth week of study, blood was collected from the tail vein of eight rats of each sex from the control, 1,250, and 5,000 ppm dose groups. At study termination, blood was collected from the aorta of all animals. Hematological

parameters measured included: hemoglobin concentration, hematocrit value, erythrocyte and reticulocyte counts, and total and differential leukocyte counts. At study termination, serum was analyzed for the concentration of urea and for the activities of glutamic-oxalacetic and glutamic-pyruvic transaminases. Urine was collected from eight rats of each sex from each dose group during the sixth week of study. Urine samples were also collected from 12 rats of each group during the last week of study. Urine was analyzed for pH, presence of microscopic constituents, and for bile, blood, and glucose content. Kidney function was further evaluated through measurement of the volume and specific gravity of urine produced during: (i) A 6-hour period of water deprivation, (ii) the first 2 hours after loading with a water dose of 25 milliliter (ml)/kg body weight, and (iii) a 4-hour period starting 16 hours after water loading. Following termination with barbiturate, all rats were necropsied, and all major tissues grossly observed.

The brain, pituitary gland, thyroid gland, heart, liver, spleen, kidneys, adrenals, and gonads were weighed. Tissues from the high-dose and control rats were subject to histopathological examination.

Treatment with *cis*-3-hexen-1-ol had no effect on mortality, clinical signs, body weight gains, or on food consumption. In males treated at 5,000 ppm there was tendency to decreased water consumption, likely as a result of the reduced palatability of the solution. In addition, in the high-dose males, relative kidney weights were increased and the urine collected during the first 2 hours following water loading more concentrated (i.e., had a higher specific gravity) in comparison to the controls. In high-dose females, transitory anemia was observed with reduced hemoglobin concentration during week 6 of the study. The no observed adverse effect level (NOAEL) was considered by the study authors to be 1,250 ppm in the drinking water. This concentration was stated to equate to a *cis*-3-hexen-1-ol intake of approximately 120 to 150 mg/kg body weight/day.

5. *Chronic toxicity.* *Cis*-3-Hexen-1-ol has not been tested in a chronic toxicity or in an oncogenicity study in rodents. Based on a lack of structural alerts for genotoxicity, and given its biotransformation to endogenous substrates that participate in fatty acid metabolism in conjunction with the lack of target organ toxicity or of effects potentially considered preneoplastic (e.g., hyperplasia) in the 98-day drinking water study, *cis*-3-hexen-1-ol is

not expected to possess carcinogenic properties. In addition, *cis*-3-hexen-1-ol is a linear unsaturated alcohol unrelated to the branched chain saturated alcohol, 2-ethylhexanol, for which there exists some evidence of hepatotoxic and neoplastic potential in rodents as a result of the cascade of events associated with the induction of peroxisome proliferation.

6. *Animal metabolism.* The metabolism of *cis*-3-hexen-1-ol in mammalian systems has not been specifically investigated. One study on the saturated homologue of *cis*-3-hexen-1-ol, *n*-hexanol, demonstrated that following an oral dose of 8 millimol (mmol)/kg body weight (816 mg/kg body weight) to rabbits, the main metabolites (90%) were those associated with oxidation to the corresponding aldehyde and acid, with further α -oxidation to carbon dioxide and water. Direct conjugation of *n*-hexanol with glucuronide was reportedly a minor (10%) metabolic pathway.

Primary aliphatic alcohols attached to either linear, branched, or unsaturated alkyl chains (i.e., as is the case with *cis*-3-hexen-1-ol) are efficiently oxidized to the corresponding aldehyde by NAD⁺/NADH-dependent alcohol dehydrogenase and then to the carboxylic acid by aldehyde dehydrogenase. As a result, *cis*-3-hexen-1-ol would be expected to be efficiently oxidized to the corresponding aldehyde and acid. The unsaturated carboxylic acids that result from the oxidative metabolism of linear unsaturated primary alcohols (e.g., *cis*-3-hexen-1-ol) are known to participate in normal fatty acid metabolism.

7. *Metabolite toxicology.* The metabolism of *cis*-3-hexen-1-ol has not been studied in mammalian species. Potential metabolites include 3-hexanal, hexenoic acid, hexanoic acid and lower homologues produced through α -oxidation. The Joint Expert Committee on Food Additives has determined that at expected *per capita* intakes in the United States, that *cis*-3-hexenal and *cis*-3-hexenoic acid pose no safety concern. Dietary intakes were based on the use of the substances as flavoring agents.

8. *Endocrine disruption.* In the 98-day drinking water study on *cis*-3-hexen-1-ol in rats, there were no effects on endocrine or reproductive tissues. There was no evidence of any toxic effect that could be interpreted to indicated hormone-disrupting activity.

C. Aggregate Exposure

1. *Dietary exposure.* Chronic dietary exposure to *cis*-3-hexen-1-ol has occurred for centuries due its natural presence in many foodstuffs and due to