ORGAN TOXICITY AND MECHANISMS

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The polycyclic musk 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthaline lacks liver tumor initiating and promoting activity in rats exposed to human-relevant doses

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Abstract 7-Acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthaline (AHTN) is one of the two most widely used fragrances of a group of substances known collectively as the polycyclic musks. In the last few years evidence has been accumulating that AHTN is hepatotoxic when administered at high doses. In the present study the subchronic hepatotoxicity of AHTN administered to rats at doses within the human exposure range was evaluated. For this purpose female and male juvenile Wistar rats were exposed to AHTN (300 µg/kg body weight per day, i.p.) alone or to a single dose of diethylaminoethyl (DEN) (100 mg/kg body weight, i.p.) followed by AHTN (1, 10, 100 or 300 µg/kg body weight per day, i.p.) for 90 days. Thereafter the liver architecture as well as the presence of placental glutathione S-transferase (GST-P)-positive hepatic lesions was assessed. In male animals receiving AHTN alone or in combination with DEN the number of GST-P-positive single hepatocytes was similar to that in untreated rats, while GST-P-positive mini-foci and foci were not observed. In the case of female rats the number of GST-P-positive single hepatocytes and mini-foci in AHTN-treated rats was similar to that in untreated animals, whereas in those animals receiving AHTN either alone or in combination with DEN, GST-P-positive foci could not be detected or were present in a number as similar to that in untreated rats. In conclusion, in the present study it has been shown that AHTN administered over a 90-day period in concentrations similar to those taken up daily by humans does not lead to hepatotoxicity.

Keywords 7-Acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthaline · Hepatotoxicity · Liver tumor initiation · Liver tumor promotion

Introduction

7-Acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthaline (AHTN) and 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[g]-2-benzopyran (HHCB) are the two most widely used fragrances of a group of substances known collectively as the polycyclic musks. According to a survey conducted by the Research Institute for Fragrance Materials (Hackensack, N.J., USA) the fragrance industry has been reported to use about 1,500 tons AHTN and 3,800 tons HHCB per year in the US and Europe. AHTN and HHCB are considered to be essential ingredients in fragrances because of their musky scent, their ability to make a fragrance long-lasting and their positive effect on the balance of the fragrance. They are not only present in fine fragrances but also in cosmetics, soaps and laundry detergents. A number of recent studies show that AHTN and HHCB accumulate in river water and in fish (Eschke et al. 1994, 1995a; Rimmkus and Brunn 1996; Fromme et al. 1999) as well as in human fat and milk (Eschke et al. 1995b; Hahn 1996; Müller et al. 1996; Rimmkus and Brunn 1996; Rimmkus and Wolf 1996; Liebl et al. 2000), which is most probably due to their high lipophilicity and chemical stability.
In the last few years evidence has been accumulating that AHTN is hepatotoxic when administered at high doses. In a first study, Gressel et al. (1980) observed that AHTN enhanced the absolute and relative liver weight of rats treated daily with this polycyclic musk at doses up to 100 mg/kg body weight for 13–26 weeks. In a more recent report Hopkins and Lambert (1996) showed that the liver weights of female rats given 15 mg AHTN/kg body weight per day, and those of male and female rats given 50 mg AHTN/kg body weight per day for 13 weeks were higher than those of the respective control animals. The same authors reported that alkaline phosphatase and alanine aminotransferase activities were enhanced, prothrombin time prolonged and cholesterol, triglyceride and glucose levels decreased in the plasma of AHTN-treated rats; all these biochemical changes are known to occur if the liver is damaged. Abnormally green-colored livers were also observed among male and female rats given 50 mg AHTN/kg body weight per day for 13 weeks, although no histopathological findings were reported in the livers of these animals (Hopkins and Lambert 1996). Steinberg et al. (1999) showed that a single dose of 100 mg AHTN/kg body weight leads to acute hepatic damage in rats, which is characterized by single cell necrosis, inflammation, swelling of liver parenchymal cells, and the presence of cytoplasmic condensations in the hepatocytes, while at the ultrastructural level disorganization of the rough endoplasmic reticulum and mitochondria was evident as well as focal cytolysis.

Although the above-mentioned studies clearly showed that AHTN given acutely or subchronically can lead to hepatotoxicity, the doses used were far above the levels to which humans are exposed. Ford (1998) calculated the total daily exposure of humans to AHTN to be at the most 0.31 mg/kg body weight per day. Taking into account that about 1% of AHTN is absorbed after dermal application in ethanol in humans (Ford et al. 1999), one would estimate the daily systemic exposure to AHTN to be 3.1 µg/kg body weight (0.31 mg/kg per day × 1% absorption). In the present study the hepatotoxicity, including the potential to induce enzyme-altered hepatic foci, of AHTN administered to rats at doses within the human exposure range (1–300 µg/kg body weight per day) for 90 days was evaluated. In this evaluation placental glutathione S-transferase (GST-P) was used for the first time to assess the liver tumor initiating and promoting activity of AHTN.

Materials and methods

Chemicals

Isopropyl myristate, N-nitrosodiethylamine (DEN) and phenobarbital were obtained from Sigma-Aldrich Chemie (Deisenhofen, Germany). AHTN was kindly provided by PFW Aroma Chemicals B.V. (AK Barneveld, Holland) and recrystallized according to a procedure provided by the manufacturer. AHTN used in all the experiments described in this study had a purity of >99%. All other chemicals used were of the highest purity available. For the animal treatments, DEN was dissolved in 0.9% saline, whereas AHTN was formulated in isopropyl myristate.

Animal treatments

The animal experimental protocols were approved by the Animal Welfare Committee of the Land Brandenburg, Germany, before starting the treatments. The rat liver foci bioassay was performed as originally developed by Oesterle and Dern (1983). Briefly, 54 male and 54 female Wistar rats weighing about 60 g (4 weeks old) were purchased from the Tierzucht Schönwalde (Schönwalde, Germany) and 1 week later were allocated to one of nine treatment groups as shown in Fig. 1: Group 1 received no treatment; Group 2 isopropyl myristate (0.1 ml/100 g body weight i.p.), the solvent used to dissolve and dilute AHTN; Group 3 100 mg DEN/kg body weight i.p. at time zero (t = 0); Group 4 100 mg DEN/kg body weight i.p. at t = 0 and thereafter 0.05% phenobarbital in the drinking water for 90 days; Group 5 300 µg AHTN/kg body weight i.p. once daily for 90 days; Group 6 100 mg DEN/kg body weight i.p. at t = 0 and thereafter 1 µg AHTN/kg body weight once daily i.p. for 90 days, Group 7 100 mg DEN/kg body weight i.p. at t = 0 and thereafter 10 µg AHTN/kg body weight i.p. once daily for 90 days, Group 8 100 mg DEN/kg body weight i.p. at t = 0 and thereafter 100 µg AHTN/kg body weight i.p. once daily for 90 days, Group 9 100 mg DEN/kg body weight i.p. at t = 0 and thereafter 300 µg AHTN/kg...