SCIENTIFIC COMMITTEE ON HEALTH AND ENVIRONMENTAL RISKS

SCHER

Opinion on

Classification of Musk ketone

Adopted by the SCHER
during the 9th plenary of 27 January 2006
1. BACKGROUND

Musk ketone (CAS n. 81-14-1) was recommended by the relevant Commission Expert Working Group (CMR WG) for the 29th Adaptation to Technical Progress (ATP) proposal for classification, among others, as “carcinogenic category 3”.

The recommendation for “carcinogenic category 3” was obtained by reading-across from musk xylene (which is classified as such since the 29th ATP), since there are no test data on carcinogenicity on musk ketone itself. Physico-chemical properties are quite comparable for both substances and both musk ketone and musk xylene are phenobarbital-like inducers of liver enzymes (CYP 450) in both rats and mice.

The expert group for risk assessments under Regulation 793/93 on existing substances agreed, when considering the draft risk assessment of musk ketone, to “carcinogenic category 3”. The CSTEE on 8 January 2004 concluded that “the data available on [the carcinogenicity of] musk xylene can be used for the risk characterisation” of musk ketone and that further testing is not needed (EC 2004a, 2004b).

2. TERMS OF REFERENCE

The SCHER is asked to assess if the data available on the carcinogenicity of musk xylene can be used for the hazard assessment and therefore the classification of musk ketone.

3. OPINION

3.1 Introduction

Both musk ketone and musk xylene are synthetic fragrances with wide application in cosmetics and therefore considerable potential for human exposures. The toxicology of both compounds has recently been summarized in detailed risk assessment reports by EU Chemicals Bureau (EU 2003a, b). General aspects of toxicity which may be relevant for classification will therefore not be repeated here.

Musk xylene has been classified as “category 3 carcinogen”, based on a 80 week oral carcinogenicity study in mice and absence of genotoxicity. The classification of musk xylene as “category 3 carcinogen” is considered as a borderline case since an increase in liver tumours in the highly sensitive B6C3F1 mouse is considered of little relevance for human hazard assessment.

Musk ketone has similar physico-chemical properties as musk xylene, but a carcinogenicity study is not available. Based on the structural similarities and the enzyme-inducing properties of both musk xylene and musk ketone in rodent liver, it is proposed to classify musk ketone as “category 3 carcinogen” based on read across.

3.2 Available data for evaluation

To assess carcinogenicity, groups of male and female B6C3F1-mice were fed diets containing musk xylene (0, 750 and 1500 mg/kg diet, approx. doses of 0, 70-125 and 141-228 mg/kg bw for males and 0, 80 – 143 and 166 – 259 mg/kg bw for females) for 80 weeks. Both groups of mice fed diets containing musk xylene showed statistically significant increases in the incidences of
Musk ketone

liver adenomas (9/49, 19/50 and 20/47 in males and 1/46, 14/50 and 13/49 in females for the control, low- and high-dose groups, respectively). Moreover, in males both musk xylene groups showed statistically significant increases in the incidences of liver carcinomas (2/49, 8/50 and 13/47). No details on non-neoplastic compound-related liver lesions were reported. However, a dose-finding study using a 17 week administration of musk xylene in the diet (0, 0.0375, 0.075, 0.15, 0.3, and 0.6 % in diet, estimated daily doses of 0, 54, 107, 214, 429, and 857 mg/kg bw) reported slightly increased absolute and relative liver weights and enlargement and irregularity of liver cells in both males and females receiving daily dose of 214 mg/kg bw (the lowest dose in this study not associated with mortality). In addition, musk xylene caused a statistically significant increase of adenomas of Harder’s gland in male mice only (control 2/49, low-dose group 9/50 and high-dose group 10/47) (EU 2003).

For musk ketone, a carcinogenicity study is not available (EU 2003b).

Both musk xylene and musk ketone have been tested for genotoxicity in many in vitro and in vivo systems. Overall, both compounds are not genotoxic as concluded in the risk assessment reports (EU 2003a, b).

To assess possible modes of action for the tumourigenicity of musk xylene to mouse liver and the relevance of the liver tumours induced by musk xylene for human hazard assessment, a number of mechanistic studies were performed in rats and mice. These mechanistic studies focused on induction of xenobiotic metabolizing cytochrome P450 by musk xylene and musk ketone in the liver (Lehman-McKeeman et al. 1995, 1997a, b, c, Stuard et al. 1997).

Enzyme induction may contribute to liver tumours formation in rodents and has been postulated to be involved in liver tumours induction by Phenobarbital. Phenobarbital is hepatocarcinogenic in rodents and the effects on the liver observed with musk xylene have been stated to resemble those seen in rodents administered phenobarbital. Therefore, liver tumours induced by musk xylene may also be a consequence of enzyme induction.

Oral administration of nitromusks (0, 1, 5, 10, 20, 50, 100, and 200 mg/kg bw for musk xylene and 0, 5, 10, 20, 50, 100, 200, and 500 mg/kg bw for musk ketone, daily for seven days) to mice induced similar changes in relative organ weight, increased total P450 activities, caused proliferation of the endoplasmatic reticulum in hepatocytes, and increased microsomal protein content in liver. Both musk xylene and musk ketone produced high levels of cytochrome P450 2B1 mRNA and CYP 2B1 protein. Therefore, both nitro musks are inducers of cytochrome P450 2B1. However, musk xylene effects on the liver cytochrome P450 activities are different from those of musk ketone (Lehman-McKeeman et al. 1995, 1997a, b).

While both musk xylene and musk ketone induce CYP 2B gene expression, the induced cytochrome P450 2B protein is present in an inactivated form after musk xylene administration resulting in a much lower CYP 2B1 associated catalytic activity. In contrast, high levels of active cytochrome P450 2B are present after administration of musk ketone. Studies have shown that a reduction product formed from musk xylene by the intestinal microflora, most probably 4-amino-2,6-dinitro-t-butylyxylene, and administration of synthetic 4-amino-2,6-dinitro-t-butylyxylene cause a suicide-inhibition of CYP 2B1. Inactivation of cytochrome P450 2B1 by this mechanism results in a feed-back-loop-induction of CYP 2B1 biosynthesis producing high levels of CYP 2B1 in rodent liver. The feed-back-loop induction mechanism is relevant to liver effects of musk xylene since treatment with antibiotics (which blocks reduction of musk xylene by intestinal microflora to
the corresponding amine) abolished general liver effects of musk xylene and enzyme induction in mice (Lehman-McKeeman et al. 1997c).

Due to its chemical structure, musk ketone cannot be reduced to an enzyme inhibiting p-amino metabolite and therefore induces, but does not inactivate CYP 2B enzymes in mice. Both musk xylene and musk ketone also seem to be weak inducers of cytochrome P450 1A in mouse liver resulting in a three to five fold increase in P450 1A protein concentrations resp. activity after the highest doses.

In rats, musk xylene administration also causes an increased formation of catalytically inactive cytochrome P450 2B, presumably by a mode-of-action identical to that for musk xylene in mice. As in mice, musk ketone administration to rats also resulted in induction of catalytically active cytochrome P450 2B. However, in addition, musk ketone was a more potent inducer of CYP 1A1 and 1A2 in rats as compared to musk xylene. The mode-of-action of musk xylene in both mice and rats therefore seems to be identical, while some species differences in the pattern of cytochrome P450 induction by musk ketone are observed (Lehman-McKeeman et al. 1999).

The role of enzyme induction in the development of liver tumours by musk xylene in mice and in the toxicity of repeated administration of musk ketone is not well defined. There are similarities of the effects of both musk xylene and musk ketone to effects of phenobarbital, which also induces liver tumours in rodents by a non-genotoxic mode-of-action and is also an inducer of cytochrome P450 2B. Musk xylene has been classified as a “category 3” carcinogen based on the observation of liver tumours induction in a very sensitive strain of mice with high spontaneous frequency of liver tumours formation and similarities in effects on cytochromes P450 in rodent liver. Assuming that the induction of cytochrome P450 2B is a relevant mode-of-action for liver tumours induction by musk xylene, read across based on “enzyme induction” and structural and physico-chemical properties may be sufficient as a basis for classification of musk ketone as a “category 3 carcinogen” since musk ketone is also an inducer of this enzyme. More detailed information on the mechanisms of enzyme induction by musk ketone is not available.

Since the classification scheme requires animal data on tumorigenicity, musk ketone cannot be formally classified as a “category 3” carcinogen since these animal data are not available. However, based on the comparison of the effects of musk ketone with those of musk xylene and phenobarbital with regard to enzyme induction a classification of musk ketone as a “category 3” carcinogen may be supported.

The SCHER recommends defining the role and importance of the tumour promoting activity of a chemical as a criterion for classification into categories of carcinogenicity. After this evaluation, the classification of musk xylene as a “category 3” carcinogen should be revisited. Moreover, the classification of musk xylene and musk ketone as “category 3 carcinogen” has to be considered as borderline as stated in the RARs.

4. REFERENCES


5. ACKNOWLEDGEMENTS

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