

4'-tert-Butyl-2',6'-dimethyl-3',5'-dinitroacetophenon**(CAS-NR.: 81-14-1)****Genotoxicity:**

Musk ketone was negative in several in vitro tests (bacterial gene mutation tests, SOS chromotests, a mammalian gene mutation test, micronucleus test and SCEs in mammalian cells, and an UDS test). A chromosomal aberration test in mammalian cells in vitro provided an equivocal result, but as an in vivo mouse micronucleus test was negative, it can be concluded that musk ketone is not an in vivo mutagen.

Ames-Test/S. typh. TA97, 98, 100, 102, 1535, 1537, 1538	negative (+/- S9)	[1,2,3,4]
SOS Chromotest/E.coli PQ37	negative (+/- S9)	[3,4,5]
Mouse-Lymphoma-Assay/L5178Y	negative (+/- S9)	[6,7]
CA-Assay/CHO cells	inconclusive (+/- S9)	[7,8]
SCE-Assay/human lymphocytes	negative (+/- S9)	[5]
Micronucleus Assay in vitro/human lymphocytes	negative (- S9)	[9]
Micronucleus Assay in vitro/human Hep G2 cells	negative (- S9)	[9,10]
UDS-Assay in vitro/rat hepatocytes	negative (- S9)	[7,11]
Micronucleus Assay in vivo/mouse (250; 500; 1000 mg/kg KGW i.p.)	negative	[12,13]

Carcinogenicity:

There are no carcinogenicity data for musk ketone.

The analogous substance musk xylene Musk xylene has been tested for carcinogenicity in B6C3F1-mice by dietary administration in one experiment with a duration of 80 weeks plus 10 weeks post observational period [12]. Both dose levels tested (0.075 and 0.15 %) resulted in statistically significantly increased incidences of hepatocellular adenomas in both sexes and of hepatocellular carcinomas in males. The incidence of Harderian gland adenomas was also statistically significantly increased in males at both dose levels. Some other tumours, like lung adenomas in both sexes and lymphomas and Harderian gland adenomas in females, occurred in greater number in the treated groups but the differences with control incidences were not statistically significant. The lowest dose tested, 0.075%, equivalent to 70-125 mg/kg bw/day in male mice and 80-143 mg/kg bw/day in female mice, is an effect dose.

Tumor Type	Control		Hist. Contr.*		100 mg/kg		200 mg/kg	
	m	f	m	f	m	f	m	f
Liver adenoma	18 %	2 %	16.3 %	1.9 %	38 %	28 %	43 %	27 %
Liver carcinoma	4 %	0 %	46.9 %	11.3 %	16 %	2 %	28 %	4 %
Liver adenoma+carcinoma	22 %	2 %	63.2 %	13.2 %	54 %	30 %	70 %	31 %
Harderian gland adenoma**	4 %	7 %	2.5 %	2.8 %	18 %	6 %	21 %	10 %
Harderian gland carcinoma**	2 %	0 %	0.4 %	0.0 %	2 %	0 %	0 %	0 %

*) Tamano et al. [13] (Control incidences for animals sacrificed between weeks 79 to 104 of experiment)

***) Tumors of the Harderian gland appeared in historical controls after an experimental duration of 105 weeks only

In consideration of the historical control data obtained with the same mouse strain and delivered from the same supplier in Japan [13] it is quite evident that the incidences of hepatocellular carcinomas of animals of both dose groups lie within the frame of the historical data especially if the age of the animals at sacrifice is considered. In this study the surviving animals were already sacrificed after 90 weeks. The increased incidences for Harderian gland adenomas in the actual control group as well as in the two dose groups are somewhat strange since this type of tumour appeared in the historical controls only after 105 weeks duration of experiment. Even more notable is the comparably high incidence for Harderian gland carcinomas (2 %) in control males and in low dose males (i.e 1 male affected in each of these groups) in light of the very low historical control incidence (0.4 %; 1 from 244 males affected) and the normally late appearance of this tumor type in the terminal phase of study [13].

Reproductive Toxicity/Fertility:

With respect to fertility no multi-generation reproductive toxicity study was available for either route.

In the 90-day dermal toxicity study with rats, musk ketone caused no effects on the reproductive organs [16].

In a range-finding teratogenicity study groups of 8 pregnant Sprague-Dawley rats received by gavage 0, 60, 200, 600 or 2000 mg musk ketone (in corn oil)/kg bw/day during days 7-17 of gestation. At day 20 of gestation the animals were sacrificed. Observations after caesarean sectioning showed decreases in litter sizes and live fetuses and increases in early and late resorptions and percent resorbed conceptuses at 200 mg/kg bw and higher [18].

In the main developmental toxicity study 0, 15, 45 and 150 mg musk ketone (in corn oil)/kg bw/day were administered to groups of 25 pregnant Sprague-Dawley rats by gavage during days 7 through 17 of gestation. At day 20 of gestation the animals were sacrificed. No abortions, premature deliveries or deaths occurred during the study. Pregnancy incidences were comparable in all four groups. Increased post-implantation loss (evident as significant increases in litter averages for total and early resorptions, a slight tendency for increased late resorptions and percentage of resorbed conceptuses per litter, and increased numbers of dams with any resorptions or with all conceptuses dead or resorbed) were observed at 150 mg/kg bw. Two

dams from this group showed litters consisting of only resorbed conceptuses. There were no dead fetuses. The NOAEL for maternal toxicity can be established at 15 mg/kg bw/day, while the NOAEL for reproductive toxicity can be established at 45 mg/kg bw/day [19,20].

In a peri/postnatal gavage study on rats no effect on reproductive performance was reported in pups that were exposed to musk ketone in utero and during lactation [17].

Reproductive Toxicity/Development:

In a range-finding study groups of 8 pregnant Sprague-Dawley rats received by gavage 0, 60, 200, 600 or 2000 mg musk ketone (in corn oil)/kg bw/day during days 7-17 of gestation. At day 20 of gestation the animals were sacrificed. Two rats at 2000 mg/kg bw and 3 rats at 600 mg/kg bw were found dead during the study, due to maternal toxicity. Examination of the uterus of these animals showed conceptuses that appeared normal for the developmental age. Treatment-related clinical signs (including urine-stained abdominal fur, excessive salivation, alopecia, ungroomed coat, cold to touch, emaciation, red perioral and perivaginal substance, chromodacryorrhea, chromorhinorrhea and/or decreased motor activity) were observed at 200 mg/kg bw and higher. At 200 mg/kg bw a treatment related increase in the occurrence of tremors was seen, but only at gestation days 7 through 9. In all treatment groups reduced body weight gain and food consumption occurred. Observations after caesarean sectioning showed decreases in fetal body weights at 200 mg/kg bw and higher. No gross external fetal alterations were observed [18].

Based on the results of the range-finding study dosages of 0, 15, 45 and 150 mg musk ketone (in corn oil)/kg bw/day were selected for the main developmental toxicity study, which was performed according to current guidelines. These dosages were administered to groups of 25 pregnant Sprague-Dawley rats by gavage during days 7 through 17 of gestation. At day 20 of gestation the animals were sacrificed. No abortions, premature deliveries or deaths occurred during the study. Dose-related increased incidences of dried faeces and perioral substance occurred in the 45 and 150 mg/kg bw groups. In the highest dose group also urine-stained abdominal fur, excessive salivation, dehydration, red substance on forepaws and tremors occurred in significantly increased numbers. One or two rats in the highest dose group showed also chromorhinorrhea or chromodacryorrhea. Effects were first observed on gestation days 13 and 7 in the 45 and 150 mg/kg bw groups, respectively. Body weight gain and food consumption were dose-relatedly and statistically significantly reduced in the 45 and 150 mg/kg groups for the entire dosage period, with a rebound in these parameters on gestation days 18-20. Significant weight loss occurred at 150 mg/kg during days 7-10 of gestation. There were no dead fetuses. Significantly reduced fetal body weight was seen at 150 mg/kg bw. There was no indication for teratogenicity. The NOAEL for maternal toxicity can be established at 15 mg/kg bw/day, while the NOAEL for developmental toxicity can be established at 45 mg/kg bw/day [19,20].

Peri/postnatal toxicity study

Musk ketone (in corn oil) was administered by gavage at dosages of 0, 2.5, 7.5 or 25 mg/kg bw/day to groups of 28 time-mated Charles River CD rats from day 14 of pregnancy (end of organogenesis) through to weaning on day 21 post partum (Makin and Bottomley, 1997). The females were allowed to litter and rear their young to weaning (litters were standardised to 8 pups on day 6 post partum). From all offspring the age at which certain developmental stages were attained was determined by examining surface righting reflex, startle reflex, air righting reflex and pupil reflex. From the litters, selected offspring were retained (24 males and 24 females per group) to maturity and assessed for behavioural changes (in motor-coordination and balance, activity, and avoidance) and reproductive capability (by mating on a one male to one female basis, and following the pregnant animals through gestation, parturition and allowing the pups to grow to weaning). The only exposure the F₁ generation had to the test substance was in utero during the perinatal phase or through any transfer in the milk of the lactating dams.

A statistically significantly lower body weight gain was noted for dams at 25 mg/kg bw during the first two days of treatment (74.6% of that of controls). Due to this, lower absolute body weights were apparent in these dams from mid-pregnancy onwards and became slightly more pronounced during lactation. During lactation food intake was slightly but statistically significantly lower at 25 mg/kg bw (90% of controls). In this group mean pup weight was lower at birth (4.8%) and through to weaning (11.8%). Linked with this lower pup weight was a slightly later day of attainment for surface and air righting in these pups compared with controls and a later day of attainment of sexual maturation, although there was no effect on reproductive performance. Lower body weight gains during the pre-mating and mating phases were seen in F₁ males from F₀ dams receiving 7.5 and 25 mg/kg bw (6.5-7.6% and 11.5-12.9%, respectively).

F₁ pups were exposed at levels in the mothers milk of up to 20900 µg musk ketone/l throughout the entire nursing period (3 weeks). These exposures caused no direct effect on performance in specific behavioural tests or on reproductive capacity in maturity.

Concentrations of musk ketone measured in adipose tissue of excess F₁ pups killed on day 6 or day 22 post partum showed no sex-related differences. For the samples on day 6, although the fat concentration increased with dose, there was evidence that the concentration of musk ketone in the fat was not proportional to the dose. For the samples on day 22 a proportionality of the musk ketone concentration in adipose tissue to the dose level could not be excluded [17].

The NOAEL for maternal toxicity is 7.5 mg/kg bw/day. The NOAEL for peri/postnatal toxicity in this study can be established at 2.5 mg/kg bw/day. It is recognised that the biological significance of the only effect seen at 7.5 mg/kg bw (a marginal, but statistically significant decrease in body weight gain in F₁ males during a period in which the F₁ males no longer were exposed to musk ketone via mothers milk) is unclear. However, as the cause cannot be deduced from the parameters investigated (possibilities are exposure via the milk, reduced milk production by the

dams, reduced maternal care), and the same, but even stronger, effect is seen at the next higher dose of 25 mg/kg bw, it cannot be excluded that the effect is biologically relevant and related to musk ketone treatment of the F₀ dams. Therefore, the NOAEL is (conservatively) set at 2.5 mg/kg bw/day.

Enzyme Induction:

Treatment of male B6C3F1-mice with musk ketone in doses of 5 – 500 mg/kg bw/day for 7 days led to an increased liver weight, centrilobular hepatocellular hypertrophy. At the highest dose there was a marked induction of cytochrome CYP 2B and a weak induction of CYP 1A and Cyp 3A [21].

Musk ketone (1 x 200 mg/kg bw oral) led to a slight inhibition (20 %) of the enzyme induction of cytochrome P 450 isoenzyme CYP 2B in mice pretreated with phenobarbital.

An equimolar dosage of musk xylene led to a CYP 2B-inhibition of 90 % due to the generation of a p-amino-metabolite of musk xylene. These results point to some principal differences in metabolism of these two musk derivatives [21].

Musk ketone was tested in female A/J mice at a gavage dose of 20 mg (ca. 1000 mg/kg bw) once every 2 days for a total of 3 doses for its ability to induce glutathione S-transferase (GST) activity in liver, lung, forestomach, and small and large intestinal mucosa. Compared with controls, that were given the vehicle cottonseed oil alone, musk ketone increased GST enzyme activity in liver, small intestinal mucosa and colon (not statistically significant in the latter), but not in lung and forestomach. Musk ketone also elevated the glutathione level (as measured by acid-soluble sulfhydryl) in the small intestine mucosa, but decreased it in the other tissues [22].

Rats were treated with i.p.-applications of 10; 20; 40 mg musk ketone/kg bw/day for 5 days, followed by preparation of hepatocellular postmitochondrial fraction (S9_M) and SOS-Chromo-test in vitro with aflatoxin B₁, benzo(a)pyrene (B(a)P) and 2-aminoanthracene (2AA), respectively in presence of S9_M. Pretreatment of animals with > 10 mg/kg bw/day musk ketone led to a significant increase of the genotoxic action of aflatoxin B₁, B(a)P and 2AA. This result gives evidence for an enzyme induction of the cytochrome P 450-isoenzymes CYP 1A1 and CYP 1A2 by musk ketone [3,23].

Conclusions:

Genotoxicity:

Musk ketone was negative in several in vitro tests (bacterial gene mutation tests, SOS chromotests, a mammalian gene mutation test, micronucleus test and SCEs in mammalian cells, and an UDS test). A chromosomal aberration test in mammalian cells in vitro provided an equivocal result, but as an in vivo mouse micronucleus test was negative, it can be concluded that musk ketone is not an in vivo mutagen. Therefore no classification for mutagenicity is proposed (M: -).

Carcinogenicity:

There are no data from carcinogenicity tests with musk ketone.

For the analogous substance musk xylene it is difficult to deduce the carcinogenic risk from the available data. This because:

- only one species has been tested, i.e. the B6C3F1 mouse;
- this strain of mice is particularly prone to develop certain types of tumours, especially liver tumours;
- the incidences for hepatocellular carcinomas in dosed males and females fits well to the historical control incidences for this mice strain especially when comparing the data for animals of comparable age;
- the incidences of Harderian gland tumors in control and dose groups don't fit at all to the historical control data from this mouse strain and are therefore of unclear relevance;
- the mechanism behind the tumour development is not entirely understood, although it is clear that musk xylene has no genotoxic potential and that enzyme induction plays an important role in the development of the liver tumours observed.

Keeping in mind these arguments, the fact that there are no data for musk ketone, and the differences concerning the profiles of enzyme induction in the liver of these two substances no classification of musk ketone is proposed (C: -).

Reproductive Toxicity/Fertility:

The available data from subchronic studies on rats (dermal) and mice (feeding) and from a chronic feeding study on mice as well as the data from a peri/postnatal gavage study in rats give no indication for a possible impairment of fertility in the absence of maternal toxicity. Therefore no classification is proposed (R_F: -).

Reproductive Toxicity/Development:

The available data obtained from the peri/postnatal toxicity study indicate that musk ketone needs not to be classified for reproductive toxicity (R_E: -).

Given the marginal effects elicited in the offspring in that study and the fact that these effects are of uncertain biological significance, there is also no need to label musk ketone with R64 ("May cause harm to breast fed babies").

References:

- [1] McConville M. (1980). Testing for mutagenic activity of GIV 72-6000 with *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98. Unpublished report of Inveresk Research International, Edinburgh, Scotland. IRI project no. 703821, report no. 1826.
- [2] Zeiger E., Anderson B., Haworth S., Lawlor T. and Mortelmans K. (1988). *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Mol. Mutagen.*, 11, Suppl. 12, 1-158.
- [3] Mersch-Sundermann V., Rheinhardt A. and Emig M. (1996). Untersuchungen zur Muta-genität, Genotoxizität und Kogenotoxizität umweltrelevanter Nitromoschusverbindungen. *Zbl. Hyg.*, 198, 429-442.
- [4] Emig M., Reinhardt A. and Mersch-Sundermann V. (1996). A comparative study of five nitro musk compounds for genotoxicity in the SOS chromotest and *Salmonella* mutagenicity. *Toxicol. Letters*, 85, 151-156.
- [5] Kevekordes S., Grahl K., Zaulig A. and Dunkelberg H. (1996). Nitro musk compounds. Genotoxic activity. Genotoxicity testing of nitro musks with the SOS-chromotest and the sister-chromatid exchange test. *Environ. Sci.& Pollut. Res.*, 3, 189-192.
- [6] Bigger C.A.H. and Clarke J.J. (1993). L5178Y TK+/- mouse lymphoma mutagenesis assay. Unpublished report of Microbiological Associates, Inc. Rockville, Maryland, USA. Laboratory study number TD338.701.
- [7] Api A.M., E.A. Pfitzer and San R.H.C. (1996). An evaluation of genotoxicity tests with musk ketone. *Food Chem. Toxicol.*, 34, 633-638.
- [8] Putman D.L., Curry P.T. and Schadly E.H. (1994). Chromosome aberrations in Chinese hamster ovary (CHO) cells using multiple harvest times. Unpublished report of Microbiological Associates, Inc., Rockville, Maryland, USA. Laboratory study number TD338.337035.
- [9] Kevekordes S., Zaulig A. and Dunkelberg H. (1997). Genotoxicity of nitro musks in the micronucleus test with human lymphocytes in vitro and the human hepatoma cell line Hep G2. *Toxicol. Letters*, 91, 13-17.
- [10] Mersch-Sundermann V., Schneider H., Freywald C., Jenter C., Parzefall W. and Knasmüller S. (2001). Musk ketone enhances benzo(a)pyrene induced mutagenicity in human derived Hep G2 cells. *Mutat. Res.*, 495, 89-96.
- [12] Gudi R. (1996). Micronucleus cytogenetic assay in mice. Unpublished report of Microbiological Associates, Inc., Rockville, Maryland, USA. Laboratory study number G96AQ68.122.
- [13] Api A.M. and Gudi R. (2000). An in vivo mouse micronucleus assay on musk ketone. *Mutat. Res.*, 464, 263-267.
- [14] Maekawa A., Matsushima Y., Onodera H., Shibutani M., Ogasawara H., Kodama Y., Kurokawa Y. and Hayashi Y. (1990). Long-term toxicity/carcinogenicity of the musk xylol in B6C3F1 mice. *Food Chem. Toxicol.*, 28, 581-586.

- [15] Tamano S., Hagiwara A., Shibata M.-A., Kurata Y., Fukushima S. and Ito N. (1988). Spontaneous tumors in aging (C57BL/6N X C3H/HeN)_F₁ (B6C3F₁) mice. *Toxicol. Pathol.* 16, 321-326.
- [16] Ford R.A., Api A.M. and Newberne P.M. (1990). 90-Day dermal toxicity study and neurotoxicity evaluation of nitromusks in the albino rat. *Food Chem. Toxicol.*, 28, 55-61.
- [17] Makin A. and Bottomley S.M. (1997). Musk ketone - Study for effects on peri- and postnatal development including maternal function in the rat (gavage administration). Unpublished report of Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, UK. Report no. RIF 39/961581.
- [18] Parker R.M. (1997). Oral (gavage) dosage-range developmental toxicity study of musk ketone in rats. Unpublished report, Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Protocol number 1318-003P.
- [19] Christian M.S., Hoberman A.M. and Parker R.M. (1997). Oral (gavage) developmental toxicity study of musk ketone in rats. Report to Research Institute for Fragrance Materials. Unpublished report of Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Protocol 1318-003.
- [20] Christian M.S., Parker R.M., Hoberman A.M., Diener R.M. and Api A.M. (1999). Developmental toxicity studies of four fragrances in rats. *Toxicol. Letters*, 111, 169-174.
- [21] Stuard S.B., Caudill D. and Lehman-McKeeman L.D. (1997). Characterization of the effects of musk ketone on mouse hepatic cytochrome P450 enzymes. *Fund. Appl. Toxicol.*, 40, 264-271.
- [22] Zheng G.-Q., Kenney P.M. and Lam L.K.T. (1992). Isolation and biological evaluation of potential cancer chemopreventive agents from ambrette musk residue. *J. Pharm. Sci.*, 81, 950-953.
- [23] Mersch-Sundermann V., Emig M. and Rheinhardt A. (1996). Nitro musks are cogenotoxicants by inducing toxifying enzymes in the rat. *Mutat. Res.*, 356, 237-245.