

5-tert-Butyl-2,4,6-trinitro-m-xylol**(CAS-NR.: 81-15-2)****Genotoxicity:**

There is a series of results from standard genotoxicity tests:

Ames-Test/S. typh. TA 97, 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	negative (+/- 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]
UDS-Assay in vitro/rat hepatocytes	negative (- S9)	[7,10]
UDS-Assay ex vivo/F 344-rat liver (500; 1500; 5000 mg/kg bw gavage)	negative	[7,11]

On the basis of these negative results there is no suspicion for a genotoxic action of musk xylene.

Carcinogenicity:

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:

There is no multigeneration study available with musk xylene.

In a 90-day study on rats with dermal applications of max. 240 mg/kg bw/day (occlusive) there were no toxic effects seen in the reproductive organs [14].

Likewise, in a chronic feeding study with musk xylene on mice with maximum dosage of ca. 200 mg/kg bw/day for 80 weeks there were also no effects seen in the reproductive organs [12].

In a peri/postnatal study on rats with exposure of the pups in utero and during lactation and subsequent mating there were no effects observed on reproductive performance (see next chapter for details of the study) [15].

Reproductive Toxicity/Development:

There has been performed a peri/postnatal study on 28 pregnant CD-rats per group with daily doses of musk xylene via gavage: 0 (control); 2.5; 7.5 and 25 mg/kg bw, respectively starting from gestational day 14 until weaning of offspring on day 21 post partum. From the litters 24 males and 24 females per group were retained to maturity and assessed for behavioural changes and for reproductive capability. The only exposure of F1 generation to musk xylene was in utero during the perinatal phase or through any transfer in the milk of the lactating dams.

Signs of maternal toxicity were only present in the 25 mg/kg bw-group and consisted of a slightly (but statistically significant [$P < 0.05$] only on days 7 and 14 post partum) decreased body weight gain without concomitant reductions in absolute mean body weight.

Offspring from females of the 25 mg/kg bw-group had a slightly (but not statistically significant) lower body weight from day 4 post partum. In males this lower body weight and decreased body weight gain (each ca. 6 % lower versus controls) persisted through the F1 generation. There were however no effects on sexual maturation, post weaning behavioural capability or subsequent reproductive performance.

At all dose levels musk xylene was excreted via the milk of the lactating dams. Musk xylene was present in the body fat of the offsprings on day 15 post partum and to a lesser extent also at weaning. The levels of musk xylene in milk and fat were generally proportional to the doses applied. There were no significant differences between the musk xylene level in milk on day 7 and 14 of lactation. This means that there was no apparent accumulation of musk xylene in milk over the time.

These results show that daily gavage applications of 25 mg/kg bw of musk xylene lead to slight toxic effects on the dams and their offspring (reduced bw gain) but without apparent adverse effects during further development of the offsprings [15].

In a dosage range-finding study groups of 8 pregnant Sprague-Dawley rats received by gavage 0, 60, 200, 600 or 2000 mg musk xylene/kg bw/day on days 7 through 17 of gestation. Musk xylene was administered in corn oil. On day 20 of gestation the rats were sacrificed. Tremors occurred in all treatment groups while urine stained abdominal fur occurred in groups treated with 60, 600 and 2000 mg/kg bw. Dried red or red perioral substance occurred at 200 mg/kg bw. Chromodacryorrhea, dried red or red perioral substance and red substance on forepaws occurred at 600 and 2000 mg/kg bw. Rats at 200, 600 and 2000 mg/kg bw showed dose relatedly reduced body weight gains and food consumption. No caesarian sectioning or litter parameters were affected by administration up to 2000 mg/kg bw. No gross external fetal alterations, malformations or variations were observed [16].

In an oral developmental toxicity study groups of 25 Sprague-Dawley rats received by gavage 0, 20, 60 or 200 mg musk xylene/kg bw/day during day 7 through 17 of gestation. Musk xylene was administered in corn oil. On day 20 of gestation the rats were sacrificed. A significant number of rats (12-25) at 200 mg/kg bw and a few (1-3) at 60 mg/kg bw had tremors, chromorhinorrhea and urine stained abdominal fur. These signs were first observed after the initial dose and were not observed after the fourth dosage. Body weight gain was significantly and dose relatedly decreased during the treatment period in rats at 60 and 200 mg/kg bw. Absolute and relative feed consumption values were significantly reduced for the entire dosage period in the 60 and 200 mg/kg bw body weight groups. Reproduction and litter parameters were unaffected by musk xylene administration. Extra thoracic ribs and increased ossification of hyoid sites and forepaw phalanges (both significant) were observed in the 200 mg/kg group. The NOAEL for maternal toxicity in this study can be established at 20 mg/kg bw/day and the NOAEL for developmental toxicity at 60 mg/kg bw/day [17,18].

Female and male Long Evans rats (5-6 weeks old) were fed musk xylene through their diet at dose levels of 0, 1, 10, 33, 100 or 1000 mg musk xylene/kg feed (corresponding to 0, 0.07-0.08, 0.7-0.8, 2-3, 7-8 or 70-80 mg/kg bw/day) for at least 10 weeks before pregnancy. Females were mated with males exposed to matching musk xylene diets. Young animals were examined one day before birth (GD22), at postnatal day 1 within 12 h after birth (PN1) or at postnatal day 14 (PN14). For GD22 and PN1 offspring liver samples of males and females were pooled within one litter. Administration of musk xylene continued throughout pregnancy up to postnatal day 14, where applicable. F1 offspring was examined for effects on liver weights, liver crude microsomal protein levels, MROD and EROD activities and liver microsomal contents of CYP1A, 2B and 3A proteins.

In PN14 offspring, liver weights in the offspring of dams exposed to musk xylene tended to be lower than liver weights of control offspring, but effects were neither sex- nor dose-related and did not reach statistical significance. No effects were seen on liver crude microsomal protein levels. In PN14 offspring, EROD and MROD activities were enhanced by 1.6 and 1.8 fold, respectively, at a maternal exposure level of 33 mg/kg feed and by 3 and 3.7-fold at 100 mg/kg feed (1000 mg/kg feed not studied). No induction of EROD was seen at 10 mg/kg feed (MROD not studied). There was no differences in sensitivity with respect to sex. Immunoblotting and densitometry confirmed induction of CYP1A, CYP2B and CYP3A proteins in the 100 and 1000 mg/kg feed dose groups.

In PN1-offspring born to dams exposed to 100 mg/kg feed before and during pregnancy EROD activity was enhanced by 3 to 7 fold, as compared to PN1 offspring from control dams. MROD activity was also increased. It was stated that in GD22-offspring of dams exposed to 100 mg/kg feed, neither EROD nor MROD activities could be detected, but data on these pups or on comparable control animals were not provided [19].

Cross-fostering study

Female and male Long Evans rats (5-6 weeks old) were fed musk xylene through their diet at dose levels of 0 and 100 mg musk xylene/kg feed (corresponding to 0 and 7-8 mg musk xylene/kg bw/day) for at least 10 weeks after which females were mated with males exposed to matching musk xylene diets. Offspring was exchanged between the various maternal exposure groups within 12 hours after birth, to reach the following study design:

	pre-natally control	pre-natally exposed
Post-natally control	group 1	group 3
Post-natally exposed	group 2	group 4

F1 offspring was examined at PN14 for effects on MROD and EROD activities. As compared to group 1 offspring, in the pups of both group 2 and 4, but not in those of group 3, the enzyme activities were enhanced to a similar degree (ca. 2.5-fold for MROD and ca. 2-fold for EROD). In combination with the observation that no EROD or MROD activities could be detected in GD22 offspring, the results of the cross-fostering study indicate that the observed difference in enzymatic activities was largely due to postnatal exposure.

The NOAELs for maternal toxicity and peri/postnatal toxicity in this study can be established at 7.5 mg/kg bw/day. It is recognised that the effects seen at 25 mg/kg bw in both dams and pups were only marginal and, in general, not statistically significant [19].

Enzyme Induction:

Musk xylene (1 x 200 mg/kg bw oral) led to a marked inhibition (90 %) of the enzyme induction of cytochrome P 450 isoenzyme CYP 2B in mice pretreated with phenobarbital due to the generation of a p-amino-metabolite of musk xylene. Equimolar dosages of musk ketone led to an CYP 2B-inhibition of 20 % only, pointing to some principal differences in metabolism of these two musk derivatives [20].

Rats were treated with i.p.-applications of 10; 20; 40 mg Musk xylene/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 xylene led to a significant increase of the genotoxic action of aflatoxin B₁ and 2AA, but not of B(a)P. This result gives evidence for an enzyme induction of the cytochrome P 450-isoenzyme CYP 1A2 by musk xylene [3,21].

Conclusions:

Genotoxicity:

On the basis of the negative results in standard assays in vitro and in an UDS assay ex vivo there is no suspicion for a genotoxic action of musk xylene. Therefore no classification is proposed (M: -).

Carcinogenicity:

- It is difficult to deduce the carcinogenic risk of musk xylene 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 and due to the questionable relevance of an increased incidence of hepatocellular adenomas in treated male and female mice and in light of the rather unclear relevance of an increased incidence of Harderian gland adenomas mainly in male mice classification of musk xylene is proposed (C: 3).

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. Therefore no classification is proposed (R_F: -).

Reproductive Toxicity/Development:

The available data obtained from two oral developmental studies on rats and from the peri/postnatal gavage study on rats indicate that musk xylene needs not to be classified for developmental 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 xylene with R64 ("May cause harm to breast fed babies").

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