

**2,4,5-Trimethylanilin**  
**(CAS-Nr.: 137-17-7)**  
**und sein Hydrochlorid**  
**(CAS-Nr.: 21436-97-5)**

**Preamble:**

This document is mainly based on the criteria document of the German MAK Commission dating from 1993 [a] and the IARC Monograph [b]. All numerical citations refer to the citations in the document of the German MAK Commission [a].

2,4,5-trimethylaniline and its hydrochloride are inducers of methaemoglobinemia; they furthermore lead to damages in liver and lung. 2,4,5-trimethylaniline is a metabolite of the red dye Ponceau 3R [c].

**Genotoxicity:**

2,4,5-trimethylaniline has proved to be mutagenic in the Ames-Test after metabolic activation as well as in a wing spot test in *Drosophila* and in a mutation test on rat fibroblasts in culture (Table 1). A DNA damaging activity of 2,4,5-trimethylaniline could be shown neither in V79 cells in vitro (Table 1) nor in rat liver in vivo (Table 2).

Table 1: Results of genotoxicity tests in vitro

Assay/Species	Concentr./Dose	S9	Results	References
Ames-test/S.typh. TA98, 100, 1537	max. 203 µg/pl.	+	positive	4
Ames-test/S.typh. TA98, 100, 1537	max. 203 µg/pl.	-	negative	
Ames-test/S.typh. TA98	max. 338 µg/pl.	+	positive	5
Ames-test/S.typh. TA100	max. 1082 µg/pl.	+	positive	
Ames-test/S.typh. TA100	max. 100 µg/pl.	+	positive	6
Ames-test/S.typh. TA100	100 µg/pl.	+	positive	c
Wing spot test/ <i>Drosophila</i>	max. 2700 µg/ml	-	positive	5
SLRL-test/ <i>Drosophila</i>	300 ppm feeding	-	negative	7
SLRL-test/ <i>Drosophila</i>	2000 ppm injection	-	negative	7
6-TG resistance test/rat fibroblasts	max. 100 mg/ml	-	positive	5
Alkaline elution/V79 cells	max. 405 µg/ml	+	negative	4
SLRL: sex-linked recessive lethal assay				6-TG: 6-thioguanine

**Table 2: Results of genotoxicity tests in vivo**

Assay/Species	Dose	Results	References
Alkaline elution/female rat liver	max. 988 mg/kg bw p.o.	negative	d

**Carcinogenicity:**

In chronic carcinogenicity studies on rats (CD, F 344) and mice (CD-1, B6C3F<sub>1</sub>) with application in the feed 2,4,5-trimethylaniline hydrochloride proved to be tumorigenic in both species. Localisations of the tumors are mainly the liver and to a lesser extent also the lung. The substance is hepatotoxic leading to liver hyperplasia and to neoplastic liver nodules.

Author:	Weisburger et al. 1978 [2]
Test Substance:	2,4,5-trimethylaniline hydrochloride (purity 97-99 %)
Species:	male Charles River CD rats
Animals per group:	25
Application:	with the feed
Dose:	0 (untreated control) 1000 and 2000 mg/kg diet for 18 months
Treating time:	18 months; sacrifice after 24 months
Toxicity:	no data
Tumors:	increased incidence in subcutaneous fibromas/fibrosarcomas and in liver tumors and elevated rate of multiple tumors

	Pooled Control	Sim.-Control	1000	2000	ppm
Fibromas/fibrosarc.	18/111 (16 %)	4/22 (18 %)	6/17 (35 %)*	1/25 (4 %)**	
Liver tumors	2/111 (2 %)	2/22 (9 %)	3/17 (18 %)*	2/25 (8 %)	
Multiple tumors	14/111 (13 %)	1/22 (5 %)	6/17 (35 %)*	5/25 (20 %)	
*) P < 0,025			**) Lipoma		

Author:	NCI 1979 [3]
Test Substance:	2,4,5-trimethylaniline (purity not given; 1 impurity found by GLC)
Species:	male and female F 344 rats
Animals per group:	50 per sex      Control: 20 per sex
Application:	with the feed
Dose:	0 (control diet) 200 and 800 mg/kg diet
Treating time:	101 weeks; followed by sacrifice
Toxicity:	delayed body weight gain
Tumors:	increased tumor incidences in liver and lung

	Control	200	800	ppm
<b>Male Rats:</b>				
Neoplastic liver nodules	1/19 (5 %)	3/50 (6 %)	11/50 (22 %)	
Liver carcinoma	0/19 (0 %)	3/50 (6 %)	11/50 (22 %)*	
Neopl. nod.+carcinoma	1/19 (5 %)	6/50 (12 %)	20/50 (40 %)**	
Bile duct carcinoma	0/19 (0 %)	0/50 (0 %)	4/50 (8 %)	
Lung carcinoma	1/20 (5 %)	0/49 (0 %)	2/50 (4 %)	
Lung adenoma	0/20 (0 %)	0/49 (0 %)	5/50 (10 %)	
Lung adenoma+carcinoma	1/20 (5 %)	0/49 (0 %)	7/50 (14 %)	
<b>Female Rats:</b>				
Neoplastic liver nodules	0/20 (0 %)	12/49 (24 %)	20/50 (40 %)	
Liver carcinoma	0/20 (0 %)	0/49 (0 %)	9/50 (18 %)**	
Neopl. nod.+carcinoma	0/20 (0 %)	12/49 (24 %)**	27/50 (54 %)**	
Bile duct carcinoma	0/20 (0 %)	0/49 (0 %)	1/50 (2 %)	
Lung carcinoma	0/20 (0 %)	2/43 (5 %)	2/50 (4 %)	
Lung adenoma	0/20 (0 %)	1/43 (2 %)	9/50 (18 %)	
Lung adenoma+carcinoma	0/20 (0 %)	3/43 (7 %)	11/50 (22 %)**	
*) P = 0.020	**) P = 0.004	**) P = 0.039	**) P = 0.010	
*****) P < 0.001	*****) P = 0.017			

Author: Weisburger et al. 1978 [2]  
 Test Substance: 2,4,5-trimethylaniline hydrochloride (purity 97-99 %)  
 Species: male and female CD-1 mice  
 Animals per group: 25 per sex  
 Application: with the feed  
 Dose: 0 (untreated control)  
 1000 and 2000 mg/kg diet for 18 months  
 Treating time: 18 months; sacrifice after 24 months  
 Toxicity: no data  
 Tumors: increased incidence in tumors of liver, lung and vessels and elevated rate of multiple tumors

	Pooled Control	Sim.-Control	1000	2000	ppm
<b>Male Mice:</b>					
Liver tumors	7/99 (7 %)	3/18 (17 %)	9/14 (64 %)*	19/21 (90 %)*	
Lung tumors	24/99 (24 %)	5/18 (28 %)	11/14 (79 %)*	10/21 (48 %)*	
Vascular tumors	5/99 (5 %)	0/18 (0 %)	3/14 (21 %)	3/21 (14 %)	
Multiple tumors	14/99 (14 %)	6/18 (33 %)	9/14 (64 %)*	14/21 (67 %)*	
<b>Female Mice:</b>					
Liver tumors	1/102 (1 %)	0/20 (0 %)	6/15 (40 %)*	14/22 (64 %)*	
Lung tumors	32/102 (32 %)	6/20 (30 %)	11/15 (73 %)*	12/22 (55 %)*	
Vascular tumors	9/102 (9 %)	0/20 (0 %)	3/15 (20 %)	3/22 (14 %)	
Multiple tumors	21/102 (21 %)	6/20 (30 %)	9/15 (60 %)*	12/22 (55 %)*	
*) P < 0,025					

Author: NCI 1979 [3]  
 Test Substance: 2,4,5-trimethylaniline (purity not given; 1 impurity found by GLC)  
 Species: male and female B6C3F1 mice  
 Animals per group: 50 per sex Control: 20 per sex  
 Application: with the feed  
 Dose: 0 (control diet)  
 50 and 100 mg/kg diet  
 Treating time: 101 weeks; followed by sacrifice  
 Toxicity: slightly delayed body weight gain in males  
 Tumors: increased tumor incidences in liver

	Control	50	100	ppm
<b>Male Mice:</b>				
Hyperplastic liver nodules	1/20 (5 %)	3/50 (6 %)	7/50 (14 %)	
Liver carcinoma	5/20 (25 %)	26/50 (52 %)*	27/50 (54 %)**	
<b>Female Mice:</b>				
Hyperplastic liver nodules	0/20 (0 %)	4/49 (8 %)	13/50 (26 %)	
Liver carcinoma	0/20 (0 %)	18/49 (37 %)**	40/50 (80 %)**	
*) P = 0.035	***) P = 0.025	***) P = 0.001	****) P < 0.001	

### Summary:

#### Genotoxicity:

Since the only available in vivo genotoxicity test, a rat liver DNA-damage assay after oral application of 988 mg/kg bw to female rats using alkaline elution for detection, yielded a negative result, according to the EU classification criteria no classification of 2,4,5-trimethylaniline and its hydrochloride is warranted (M:-).

#### Carcinogenicity:

The chronic oral application of 2,4,5-trimethylaniline has led to the development of carcinoma in liver and lung in rats and mice. The liver tumors are presumably a consequence of the hepatotoxicity of the substance since DNA damage in the liver could not be demonstrated in female rats after acute oral application of the substance. Besides the liver tumors the substance led to the development of lung tumors in F 344 rats and CD-1 mice. No lung tumors were detected in CD-rats (higher dosage but only 18 months treatment) and in B6C3F<sub>1</sub> mice (dosage much lower than for CD-1 mice). Taking together the development of tumors in 2 species and the mutagenic potential in vitro 2,4,5-trimethylaniline should according to the EU-classification criteria be classified as Carc. Cat. 2 (C:2).

Reproductive Toxicity/Fertility:

Due to the lack of data the substance can not be classified (R<sub>F</sub>: -).

Reproductive Toxicity/Developmental Effects:

Due to the lack of data the substance can not be classified (R<sub>E</sub>: -).

**References:**

- [1] Greim, H. (Hrsg.): Occupational Toxicants. Critical Data Evaluation for MAK Values and Classification of Carcinogens. Vol. 4, 335-340 (1992)
- [2] WHO-IARC: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 27, 177-188 (1982)
- [3] Hartman, C.P., Andrews, A.W., Chung, K.-T.: Production of a mutagen from Ponceau 3R by a human intestinal anaerobe. Infection and Immunity 23, 686-689 (1979) Kitchin, K.T., Brown, J.L.: Dose-response relationship for rat liver DNA damage caused by 49 rodent carcinogens. Toxicology 88, 31-49 (1994).

**Further data concerning other toxicological endpoints:**

Acute toxicity: hydrochloride LD50 p.o. rat 1585 mg/kg bw [1]

due to methaemoglobinemia upgraded classification: T, R 23/24/25

Irritation potential: no data

- [1] Lindstrom, H.V. et al.: The toxicity and metabolism of mesidine and pseudo-cumidine in rats. J. Pharmacol. Exp. Ther. 167, 223-234 (1969)

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