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SUB-CHRONIC INHALATION TOXICITY OF 1,2,4-TRIMETHYLBENZENE (PSEUDOCUMENE) IN RATS

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Abstract. Toxic effects of exposure to 1,2,4-trimethylbenzene (pseudocumene) in the condition of sub-chronic inhalation experiment were examined. Rats were exposed to vapours of pseudocumene at concentrations of 123 mg/m^3, 492 mg/m^3 and 1230 mg/m^3, 6 h/day, 5 days/week for 3 months. After 3 months of inhalation exposure animals were necropsied. Blood samples were obtained and selected organs were weighted and prepared for histological examinations.

Sub-chronic inhalation exposure to pseudocumene resulted in an overall low degree of systemic toxicity. There were no changes in body weight gain, food consumption and absolute and relative organ weights. Slightly higher activity of sorbitol dehydrogenase was observed in male rats exposed to all concentrations applied. Some disturbances in hematological parameters characterised by decrease in red and increase in white blood cells were observed in male rats exposed to high concentration of 1230 mg/m^3. The pulmonary lesions observed in male and female rats were statistically significant at mid and high concentrations of pseudocumene.

INTRODUCTION

Pseudocumene (1,2,4-trimethylbenzene, CAS No. 95-65-6), one of the trimethylbenzene isomers, is produced mostly during catalytic reforming of petroleum, and it enters the composition of many commonly used commercial solvent mixture like Solvesso 100 (Exxon Chemical Belgium), Shellsol A (Shell Netherland Chemie B.V.), Jolasol (J.L.C. Chemie, Austria) and Farbasol (Polifarb-Cieszyn S.A., Poland).

Trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) make 44% of the whole Farbasol mixture, and pseudocumene is in a large majority among trimethylbenzenes (~30%) (19).

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Trimethylbenzenes are present in gasoline and in white spirit. They are used in paint and varnish formulations, paint thinners, printing inks and pesticide formulations. They also serve as intermediates in dye and plastic industries.

A large number of studies on the neurotoxic and irritating effects of the exposure to different methyl (toluene) and dimethylbenzene (xylenes) derivatives have been reported (7,8,11). However, toxicological data on trimethylbenzenes are scarce. The ACGIH TLV-TWA concentration of 25 ppm (123 mg/m^3) (1) and the Polish MAC value of 100 mg/m^3 (ca. 20 ppm) (15) for trimethylbenzene isomers have been established using incomplete, unreliable and obsolete data (3,4). Symptoms and signs of impairment of the respiratory and nervous systems have been observed in 27 industrial workers exposed for a number of years to a paint thinner containing alkylbenzenes (pseudocumene – 50%, mesitylene – 30%; hemimellitene, 1-methyl--2-ethylbenzene and 1-methyl-4-ethylbenzene: percentages not specified). The hematopoietic effects were also observed, but they were probably due to benzene contaminations of the thinner. The levels of hydrocarbon vapours in the atmosphere ranged between 49 and 295 mg/m^3 (10–60 ppm) (3,4).

In condition of acute inhalation exposure, pseudocumene caused concentration-dependent irritating effects in mice and neurotoxic effects in rats (13,14).

The established pseudocumene concentration of 2844 mg/m^3 (14), depressing the respiratory rate in mice to 50% (RD_{50}), may indicate a lower MAC value than that binding at present (15).

Bearing in mind three very active methyl groups in trimethylbenzene isomer molecules, the extensive use of pseudocumene, and limited data regarding the toxicity of the chemical, the toxicological studies were undertaken to assess sub-chronic toxic effects of pseudocumene in rats.

MATERIALS AND METHODS

Chemicals

Pseudocumene (1,2,4-trimethylbenzene) purum > 97% was supplied by Fluka.

Animals

Male and female rats of outbred Imp:WIST from the Nofer Institute of Occupational Medicine animal husbandry were used. They were identified by ear number and allowed to acclimatise to laboratory conditions for two weeks prior to initiation of the study. Test animals were 2–2.5 months old at the start of inhalation. The mean initial body weight was 213 ± 20 g and 160 ± 11 g, respectively for male and female rats. They were housed in polypropylene cages with wire-mesh covers, five animals in each, and maintained under 12 h light/12 h dark cycle, lighting on from 6 a.m. to 6 p.m. Food (Murigran pelleted rodent chow, Fodder Plant, Motycz, Poland) and water were available *ad libitum* in their home cages. Relative humidity and temperature were maintained at 50–65% and 20.5–22.5°C, respectively. Animals were randomised and assigned to the experimental groups. Each of the four experimental groups was composed of 10 male and 10 female rats except for that of high-exposure concentration (20 males and 20 females). The control group was sham exposed and the others were exposed to pseudocumene at

concentrations of 123 mg/m^3 ≈ 25 ppm (low concentration), 492 mg/m^3 ≈ 100 ppm (mid concentration), and 1230 mg/m^3 ≈ 250 ppm (high concentration). Animals were exposed 6 h/day, 5 days/week, 3 months.

Ten male and ten female rats exposed to pseudocumene at concentration of 1230 mg/m^3 were observed and examined during two additional weeks after termination of exposure. All rats were deprived of food and water during the 6-h exposure periods.

Animals were observed two times daily for overt signs of toxicity. Body weights were recorded prior to the first inhalation exposure and weekly thereafter during the test period. Food consumption was measured weekly.

Inhalation exposure

Animals were exposed to vapours of pseudocumene in a dynamic inhalation chamber (1.3 m^3 volume); 16 changes per hour. Vapours of pseudocumene were generated by heating liquid solvent in washers. The desired concentrations of vapours were obtained by diluting them in the air. Concentrations of solvent vapours in the exposure chamber were measured every 30 min by a Hewlett-Packard gas chromatograph with a flame ionization detector using 40 m capillary HP-5 column (0.53 mm × 0.33 μm) at 100°C column temperature.

Hematology

Hematological parameters in the tail blood were evaluated prior to the beginning of the study and 1 week before termination of the experiment. Erythrocyte count, hemoglobin concentration, hematocrit, leucocyte count, platelet count and clotting time were conducted in the animal control and exposure groups.

Clinical pathology determinations

Blood clinical laboratory studies were conducted 18 h after termination of a 3-month inhalation exposure. Animals were deprived of food 24 h prior, than anaesthetized in light ether anaesthesia, exanguined from the femoral artery and vein, and subjected to gross necropsy. Blood samples were collected for serum chemistry determinations. These were made for aspartate aminotransferase (AspAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), gamma glutamyltransferase (GGT), bilirubin, total cholesterol, glucose, total protein, albumin, creatinine, urea and electrolytes – calcium, phosphorous, sodium, potassium, chloride.

Beckman Clinical System 700 autoanalyser, Ciba-Corning 614 analyser and standard test combination kits were used for determinations.

Histopathology

The necropsy was performed in all animals. Organ weights were obtained for the lungs, liver, spleen, kidneys, adrenals, heart and gonads (ovaries, testes). For the histopathological investigation brain, nose, larynx, trachea, thymus, lungs, heart,

liver, spleen, kidney, adrenals, thyroid gland, pancreas, gonads, urinary bladder, stomach, duodenum, small and large intestines, and salivary glands were preserved in neutral formalin. The lungs, removed in toto, were perfused with 10 neutral buffered formalin via trachea at a pressure of 20 cm H_2O.

The tissue were embedded in paraffin-wax, sectioned at 5 μm, and stained with hematoxylin and eosin. The changes in lungs reflecting the degree of proliferation of peribronchial lymphatic tissue, lymphoepitelium in bronchi mucosa, interstitial lymphocytic infiltration, macrophage infiltration and inflammatory processes,were graded using an arbitrary scale of 0–3 or 0–4 (0 = normal status, 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked) (17).

Statistical analysis

Except for body weight data, other parameters from the groups treated were compared statistically with those of the control group by performing the following tests: Bartlett's test of homogeneity of variance was used to find out whether the groups had equivalent variances at the $p < 0.01$ level (20). If the variances were not significantly different, the groups were compared by employing a standard one-way analysis of variance (ANOVA) (21). If significant differences among the means were indicated, Dunnett's test was used to determine which treatment groups differed fom controls (21). If the groups did not have equivalent variances at the $p < 0.01$ level, then the Kruskal-Wallis test was used to assess differences in group means. If the means were different, Dunn's summed rank test was used to determine which treatment group differed significantly from controls (9). Body weight data for each sex were analyzed by one-way classification analysis of covariance (ANCOVA), using pre-exposure (day 0) weights as the covariate. For comparison of histopathological data, Fisher's exact test was used. Graduated morphological changes in lungs were analysed by the Kruskal-Wallis test in Dunn's modification (6). The trend analysis was used to evaluate the relation between the extent of changes in hematological and clinical chemistry parameters; morphological changes in lungs; and the exposure level (12).

RESULTS

During 3 months of exposure, mean concentrations of pseudocumene vapours were 129 ± 18 mg/m^3, 492 ± 62 mg/m^3, 1207 ± 76 mg/m^3. There were no deaths during the course of the study. Clinical observations were of no toxicological relevance. When compared with controls no significant differences in food consumption and body weight gain were observed in all rats exposed to pseudocumene (data not shown).

The differences in some absolute and relative organ weights between exposed and control animals of both sexes (Table 1) were observed; changes were not concentration-dependent and seemed to be accidental of no toxicological relevance.

After termination of a 3-month exposure, a decrease in red blood cells and an increase in white blood cells was noted in male rats. The trend analysis showed

Table 1. Effect of a 3-month exposure to pseudocumene on organ and terminal body weight

Parameters	Control (n = 10)	Pseudocumene (mg/m^3)		
		123 (n = 10)	492 (n = 10)	1230 (n = 10)
Males				
Terminal body weight (g)	368 ± 22[a]	390 ± 26	399 ± 22	389 ± 29
Absolute organ weight (g)				
Lungs	1.78 ± 0.28	1.83 ± 0.25	2.93* ± 0.26	1.78 ± 0.36
Liver	10.27 ± 1.82	11.43 ± 1.05	10.78 ± 1.33	10.86 ± 2.04
Spleen	0.68 ± 0.08	0.85* ± 0.19	0.79 ± 0.09	0.72 ± 0.08
Kidney	2.06 ± 0.13	2.24 ± 0.15	2.14 ± 0.15	2.18 ± 0.16
Adrenals	0.048 ± 0.007	0.046 ± 0.005	0.054 ± 0.011	0.047 ± 0.005
Testes	3.72 ± 0.35	3.90 ± 0.38	4.03 ± 0.27	3.87 ± 0.24
Heart	0.90 ± 0.04	0.94 ± 0.06	0.94 ± 0.08	0.96 ± 0.07
Relative organ weight (g)				
Lungs	0.496 ± 0.056	0.475 ± 0.056	0.586 ± 0.115	0.477 ± 0.080
Liver	2.869 ± 0.456	2.894 ± 0.427	2.990 ± 0.465	2.901 ± 0.479
Spleen	0.189 ± 0.011	0.220 ± 0.041	0.210 ± 0.018	0.200 ± 0.018
Kidney	0.588 ± 0.029	0.585 ± 0.022	0.587 ± 0.065	0.586 ± 0.040
Adrenals	0.011 ± 0.003	0.010 ± 0.000	0.022 ± 0.024	0.011 ± 0.003
Testes	1.041 ± 0.076	1.020 ± 0.079	1.067 ± 0.102	1.039 ± 0.077
Heart	0.252 ± 0.013	0.239 ± 0.020	0.249 ± 0.014	0.258 ± 0.020
Females				
Terminal body weight (g)	243 ± 16	243 ± 19	230 ± 14	229 ± 21
Absolute organ weight (g)				
Lungs	1.29 ± 0.18	1.32 ± 0.12	1.25 ± 0.13	1.23 ± 0.11
Liver	6.48 ± 1.02	6.54 ± 0.69	5.81 ± 0.83	6.72 ± 1.34
Spleen	0.59 ± 0.08	0.61 ± 0.11	0.49* ± 0.06	0.52 ± 0.08
Kidney	1.55 ± 0.12	1.50 ± 0.14	1.38* ± 0.11	1.44 ± 0.19
Adrenals	0.065 ± 0.007	0.070 ± 0.008	0.066 ± 0.010	0.061 ± 0.013
Ovaries	0.09 ± 0.02	0.09 ± 0.01	0.09 ± 0.27	0.09 ± 0.02
Heart	0.66 ± 0.07	0.64 ± 0.05	0.61 ± 0.07	0.63 ± 0.06
Relative organ weight (g)				
Lungs	0.555 ± 0.058	0.581 ± 0.040	0.596 ± 0.051	0.569 ± 0.053
Liver	2.770 ± 0.222	2.881 ± 0.309	2.758 ± 0.223	3.078 ± 0.434
Spleen	0.255 ± 0.025	0.266 ± 0.031	0.237 ± 0.036	0.241 ± 0.033
Kidney	0.667 ± 0.030	0.661 ± 0.047	0.660 ± 0.042	0.662 ± 0.036
Adrenals	0.028 ± 0.006	0.031 ± 0.006	0.032 ± 0.006	0.029 ± 0.006
Ovaries	0.043 ± 0.008	0.041 ± 0.006	0.045 ± 0.013	0.047 ± 0.009
Heart	0.284 ± 0.023	0.283 ± 0.025	0.291 ± 0.025	0.289 ± 0.015

[a] Mean ± SD

* Significantly different from the control ($p < 0.05$)

that changes were concentration-dependent ($p = 0.0004$ and 0.0019, respectively). As compared to controls changes were statistically significant in the male group exposed to pseudocumene at concentration of 1230 mg/m^3.

Two weeks after termination of exposure, red blood cells counts were comparable to those observed just after termination of exposure, whereas white blood cells counts decreased and were similar to that observed in the control group (Table 2).

Table 2. Effect of a 3-month exposure to pseudocumene on hematological parameters

Parameters	Control	Pseudocumene (mg/m^3)				Jonckheere's trend test
		123	492	1230	1230[a]	
Hematocrit (%)						
Male	49.9±1.9	50.4±2.0	50.0±1.9	50.6±1.5	50.1±1.1	p = 0.2993
Female	46.0±1.6	46.6±2.7	47.0±2.7	46.5±4.1	45.8±1.3	p = 0.2336
Hemoglobin (g/dl)						
Male	15.1±1.1	15.6±0.9	15.4±0.9	15.4±0.6	16.0±1.0	p = 0.2112
Female	14.5±0.9	13.8±1.3	14.4±0.9	14.2±0.9	14.9±0.9	p = 0.3461
Red blood cells (x10^6/mm^3)						
Male	9.98±1.68	9.84±1.82	8.50±1.11	7.70**±1.38	7.61±1.6	p = 0.0004
Female	8.22±1.16	7.93±2.04	8.51±1.13	7.71±1.58	6.99±1.8	p = 0.1891
White blood cells (x10^3/mm^3)						
Male	8.68±2.89	8.92±3.44	8.30±1.84	15.89**±5.74	7.11±2.1	p = 0.0019
Female	7.50±1.31	6.76±2.95	9.55±4.48	9.83±3.74	7.11±2.4	p = 0.0307
Differential variable						
Rod neutrophil (%)						
Male	0.0±0.0	0.4±0.5	0.2±0.4	0.9±1.5	0.7±0.8	p = 0.0586
Female	1.4±1.6	0.5±0.7	0.4±0.5	0.4±0.9	0.5±0.7	p = 0.3270
Segmented neutrophil (%)						
Male	24.1±9.2	19.7±6.5	20.7±7.7	18.9±10.8	29.4±6.4	p = 0.0730
Female	22.8±6.5	15.5±7.9	20.7±7.5	17.4±9.3	20.5±9.5	p = 0.1868
Eosinophil (%)						
Male	1.2±1.7	1.2±1.0	0.4±0.6	1.7±1.4	1.5±1.5	p = 0.2950
Female	1.2±0.6	1.6±1.6	1.1±1.7	1.2±2.1	2.0±1.7	p = 0.1051
Lymphocyte (%)						
Male	73.5±10.3	76.2±7.1	76.3±8.5	75.8±16.0	65.4±8.9	p = 0.1297
Female	73.2±7.9	79.4±8.4	75.5±7.4	78.8±11.6	74.1±9.5	p = 0.2140
Monocyte (%)						
Male	1.1±1.3	2.5±2.1	2.3±2.2	1.8±2.5	2.7±2.5	p = 0.3818
Female	1.2±1.3	2.6±2.8	1.3±1.7	1.5±0.8	1.5±1.4	p = 0.4156
Lymphoblast (%)						
Male	0.0±0.0	0.0±0.0	0.0±0.0	0.8±1.3	0.3±0.9	p = 0.1387
Female	0.0±0.0	0.1±0.3	0.5±1.5	0.7±1.1	0.8±1.3	p = 0.1361
Myelocyte (%)						
Male	0.0±0.0	0.0±0.0	0.2±0.4	0.0±0.0	0.0±0.0	p = 0.4046
Female	0.0±0.0	0.0±0.0	0.5±1.5	0.1±0.3	0.1±0.3	p = 0.3189
Erytroblast (%)						
Male	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	p = 0.5000
Female	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	p = 0.5000
Reticulocyte (%)						
Male	3.1±2.3	2.3±1.4	2.8±2.1	3.1±2.5	6.4±3.2	p = 0.4900
Female	3.5±2.6	1.7±2.0	1.8±0.9	1.0*±0.6	5.8±3.6	p = 0.0137
Blood platelet (x10^3/mm^3)						
Male	294±46	293±73	359±46	335±80	386±70	p = 0.0741
Female	306±34	234*±50	303±48	325±57	349±77	p = 0.1542
Clotting time (s)						
Male	43±19	41±17	37±13	33±7	56±21	p = 0.1457
Female	30±10	23±4	19**±5	22*±7	48±19	p = 0.0034

[a] Parallel group, 14 days after termination of exposure. Mean values±SD for 10 rats
Statistically significant difference as compared to the control * $p < 0.05$, ** $p < 0.01$

Table 3. Effect of a 3-month exposure to pseudocumene on clinical chemistry values

Parameters	Control	Pseudocumene (mg/m^3) 123	492	1230	Jonckheere's trend test
Aspartate aminotransferase (U/dl)					
Male	138.7±20.6	141.3±21.0	134.5±27.0	138.4±35.0	p = 0.2223
Female	139.4±16.6	136.7±27.1	145.5±22.7	141.4±15.6	p = 0.2118
Alanine aminotransferase (U/dl)					
Male	51.7±5.9	48.3±7.8	49.7±9.1	46.8±5.1	p = 0.0637
Female	49.8±6.3	51.4±8.2	50.4±9.0	55.1±9.5	p = 0.1844
Alkaline phosphatase (U/dl)					
Male	80.4±12.0	86.2±22.0	84.9±21.0	90.5±19.0	p = 0.1518
Female	41.2±7.8	37.2±6.8	39.8±11.0	49.8±15.5	p = 0.1740
Sorbitol dehydrogenase (U/dl)					
Male	6.6±1.4	8.1**±0.8	7.8*±1.0	8.0**±1.1	p = 0.0083
Female	5.9±1.5	7.3±1.7	7.1±1.8	7.0±1.6	p = 0.0637
γ-glutamyltransferase (μU/ml)					
Male	0.22±0.44	0.20±0.42	0.20±0.42	0.20±0.42	p = 0.4700
Female	0.20±0.42	0.30±0.48	0.10±0.32	0.44±0.53	p = 0.2821
Bilirubin (mg/dl)					
Male	1.027±0.193	0.974±0.338	1.016±0.289	0.932±0.175	p = 0.2594
Female	0.745±0.342	0.690±0.396	0.743±0.248	0.642±0.257	p = 0.3092
Total cholesterol (mg/dl)					
Male	63.6±13.0	69.1±12.0	72.4±14.9	70.6±19.5	p = 0.0920
Female	64.5±11.9	65.7±12.8	64.1±10.8	62.5±7.6	p = 0.4775
Glucose (mg/dl)					
Male	141.9±23.9	163.8±29.7	157.9±23.2	162.2±28.9	p = 0.0876
Female	118.2±28.8	138.8±38.5	104.5±23.8	129.9±39.7	p = 0.4838
Total protein (g)					
Male	5.43±1.00	5.47±1.39	5.34±1.29	5.82±1.49	p = 0.3242
Female	6.91±0.53	7.44±0.89	7.08±0.35	6.94±0.64	p = 0.4036
Albumin (g)					
Male	3.25±0.60	3.45±0.56	3.41±0.83	3.53±0.66	p = 0.2279
Female	3.42±0.24	3.46±0.27	3.61±0.26	3.42±0.15	p = 0.2408
Creatinine (mg/dl)					
Male	0.506±0.099	0.437±0.138	0.510±0.150	0.490±0.178	p = 0.3982
Female	0.655±0.135	0.553±0.104	0.629±0.153	0.577±0.133	p = 0.1641
Urea (mg/dl)					
Male	54.2±8.6	48.8±8.3	47.6±3.4	49.0±8.7	p = 0.1145
Female	52.7±7.8	49.6±6.7	52.8±10.5	52.2±11.8	p = 0.4718
Calcium (mg/dl)					
Male	10.4±0.5	10.8±0.5	10.7±0.8	10.8±0.7	p = 0.2449
Female	10,5±0.6	10.8±0.8	10.6±0.5	10.8±0.6	p = 0.3011
Phosphorous (mg/dl)					
Male	6.27±0.49	6.50±0.57	6.49±0.61	6.46±0.78	p = 0.1580
Female	4.75±0.54	5.05±0.70	5.34±0.74	4.90±1.01	p = 0.4050
Sodium (mmol/l)					
Male	139.0±1.4	139.3±1.3	139.6±1.4	139.0±1.4	p = 0.4950
Female	137.9±1.7	138.0±1.8	137.8±2.5	138.2±2.2	p = 0.3628
Potasium (mmol/l)					
Male	4.87±0.36	4.97±0.34	4.97±0.25	4.83±0.40	p = 0.2907
Female	4.54±0.22	4.39±0.61	4.51±0.26	4.46±0.25	p = 0.4108
Chloride (mmol/l)					
Male	106.6±1.2	106.1±1.7	106.3±1.5	106.7±1.2	p = 0.4353
Female	104.9±2.0	105.5±1.3	105.9±1.6	106,4±1.8	p = 0.0601

Statistically significant difference as compared to the control * $p < 0.05$, ** $p < 0.01$

Table 4. Pulmonary lesions observed in male and female rats exposed to pseudocumene for 3 months at concentrations of 0, 123, 492, 1230 mg/m^3

Changes		Pseudocumene (mg/m^3)				Statistical analysis	
		0	123	492	1230		
		1	2	3	4	Comparison with controls	Jonckheere's trend test
Proliferation of peribronchial lymphatic tissue (0-4)[a]	m	16.0[b]	15.6	30.6	17.4	1–3*	J = 1.11; p = 0.13
	f	19.4	21.7	21.2	17.5	–	J = −0.33; p = 0.36
Formation of lymphoepithelium in bronchi (0-4)	m	18.1	15.6	27.9	18.2	–	J = 0.76; p = 0.22
	f	18.3	20.1	25.1	16.1	–	J = −0.05; p = 0.48
Bronchitis and bronchopneumonia (0-4)	m	19.0	18.3	26.1	16.5	–	J = 0.012; p = 0.49
	f	19.0	22.9	19.0	19.0	–	J = −0.22; p = 0.48
Interstitial lymphocytic infiltrations (0-3)	m	14.8	18.4	26.9	19.4	1–3*	J = 1.16; p = 0.12
	f	15.8	14.5	21.5	29.2	1–4*	J = 2.92; p = 0.0017
Alveolar macrophages (0-3)	m	14.1	14.8	24.1	26.4	1–4*	J = 2.84; p = 0.002
	f	19.7	14.9	16.6	29.8	–	J = 1.8; p = 0.03
Cumulative score of all individuals	m	13.9	15.1	29.1	21.3	1–3**	J = 2.04; p = 0.02
	f	16.8	15.3	21.3	27.3	–	J = 2.5; p = 0.01

[a] Grading system (0-4, 0-3), see 'Methods'

[b] Results presented as ranges of the Kruskal-Wallis test

* Significant at $p < 0.05$

** Significant at $p < 0.01$

It was also observed that reticulocyte counts decreased in females from all groups treated; at concentration of 1230 mg/m^3 the changes were significantly different from the control. Clotting time decreased in males and females of treated groups; in females exposed to pseudocumene at concentrations of 492 and 1230 mg/m^3, the changes were statistically significant. Two weeks after termination of exposure in males and females of parallel groups exposed to pseudocumene at concentration of 1230 mg/m^3, a twofold increase in both reticulocyte count and clotting time was found as compared to controls.

Among the various clinical chemistry values measured an increase in sorbitol dehydrogenase was observed only. Changes were statistically significant in all males of exposed groups (Table 3).

Microscopic examinations of the upper respiratory tract (mucosa of bronchi and trachea) of rats did not reveal significant changes related to pseudocumene exposure at concentrations of 123, 492 and 1230 mg/m^3. In comparison with the controls in the lungs of male and female rats, exposed to the mid and high concentrations of pseudocumene, an increased number of animals with peribronchial, lung parenchymal and perivascular lymphocytic infiltrations was observed. In some rats, the bronchial epithelium with proliferation of peribronchial lymphatic tissue focally lost its cuboidal character forming lymphoepithelium. Table 4 gives the tabulated grading of pulmonary lesions. Intensity of peribronchial lymphatic tissue proliferation, chronic bronchitis and marked lung parenchymal interstitial lymphocyte infiltrations occurred in male rats exposed to pseudocumene at the mid concentration, and in females exposed at the high concentration (Table 4).

The trend analysis revealed that the number of alveolar macrophages was concentration-dependent in male and female rats ($p = 0.002$ and 0.03, respectively). The cumulative score and intensity of changes in the lower respiratory tract was related to the exposure level. Based on the analysis of the trend commutative score of all pathological changes index in male and female rats ($p = 0.02$ and 0.01, respectively) it is concluded that pseudocumene revealed relatively low toxic effect on the respiratory system of rats at concentration of 492 mg/m^3.

In the other examined organs and tissues of all male and female rats exposed to pseudocumene, no significant changes which could be related to the exposure level, were detected.

DISCUSSION

Sub-chronic inhalation exposure to pseudocumene resulted in an overall low degree of systemic toxicity. For some parameters tested (body weight, food consumption and absolute and relative organ weights) the high concentration of 1230 mg/m^3 was the no-observed-effect-level (NOEL) for both sexes.

Slightly higher activity of sorbitol dehydrogenase was observed in male rats exposed to pseudocumene at all concentrations applied. Sorbitol dehydrogenase activity seems to be higher in female rats, but changes were not statistically significant. Sorbitol dehydrogenase activity was found to be the most sensitive biochemical marker of the liver damage also in the case of other hepatotoxic chemical compounds (16).

For clinical chemistry parameters like sorbitol dehydrogenase activity, the concentration of 123 mg/m^3 was the lowest-observed-effect-level (LOEL) in males and NOEL in females.

A statistically significant decrease in red, and an increase in white blood cell counts observed in male rats at pseudocumene concentration of 1230 mg/m^3 were similar to that observed in mice as a result of exposure to other solvents (5,10). Several factors may be responsible for this observation, including deficiency in vitamin B_{12}, folic acid, protein as well as hepatic diseases or chronic inflammation (18). Thus, the increased white blood cell counts, decreased red blood cell counts, and increased sorbitol dehydrogenase activity observed in male rats could be the result of hepatotoxic pseudocumene activity. However, microscopic examinations of the liver did not show any changes, which could be related to pseudocumene exposure.

The decreased erythrocyte counts and an increase in white blood cells may result from inflammation caused by exposure to pseudocumene which indicates the pulmonary lesions, and quite strong acute respiratory irritative effect of pseudocumene observed earlier (14). In condition of sub-chronic inhalation exposure to pseudocumene, inflammatory processes in the respiratory tract seem to be possible since the increased susceptibility to respiratory bacterial infections in mice exposed to toluene has already been reported (2).

For hematological parameters the concentration of 1230 mg/m^3 was LOEL in males and NOEL in females.

The microscopically observed pathological changes in the lower respiratory system indicate relatively low toxic effect of pseudocumene at concentrations of 492 mg/m^3 and 1230 mg/m^3. The pseudocumene concentration of 123 mg/m^3 was established as NOEL in male and female rats.

Our sub-chronic studies performed on rats reveal that pseudocumene, like other solvents, may affect blood, however, the effect is not specific and it is not a primary target tissue following pseudocumene exposure. Similar to other solvents, pseudocumene exerts mainly neurotoxic (13) and irritative effects on the respiratory system (14).

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