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RESPIRATORY IRRITATIVE EFFECTS OF TRIMETHYLBENZENES: AN EXPERIMENTAL ANIMAL STUDY

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Abstract. Sensory respiratory irritation effects of trimethylbenzene isomers (TMBs) (hemimellitene, mesitylene and pseudocumene) in male Balb/C mice were investigated in conditions of acute exposure and in male Wistar rats in conditions of repeated 90-day inhalation exposure to pseudocumene.

The pseudocumene, mesitylene and hemimellitene concentrations depressing the respiratory rate to 50% (RD_{50}) were 578, 519, 541 ppm, respectively.

Inhalation exposure to pseudocumene for 90 days increased the total number of cell macrophages, polymorphonuclear leukocytes and lymphocytes number at all three test concentrations compared with the controls. Total protein lactate dehydrogenase (LDH) and acid phosphatase activity in bronchoalveolar lavage (BAL) were significantly increased in all exposed groups.

Based on the effects observed in the respiratory tract, the threshold limit value of at least 10 ppm should be considered for the occupational exposure to trimethylbenzene isomers.

INTRODUCTION

Trimethylbenzene isomers (TBMs) are usually produced during catalytic reforming of petroleum and they are components of many popular commercial solvent mixtures like Solvesso 100 (Exxon Chemical, Belgium), Shellsol A (Shell Netherland Chemie B.V.), Jolasol (J.L.C Chemie, Austria) and Farbasol (Petrochemia Płocka S.A., Poland). Trimethylbenzene isomers are one of the substantial constituents of these solvent mixtures. They are present in gasoline, and white spirit. They are used in paint and varnish formulations, paint thinners, printing inks and pesticide formulations. They olso serve as intermediates in dye and plastics 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 (13,14,16,17). However, toxicological data on trimethylbenzene derivatives are scarce. The ACGIH TLV-TWA concentration of 25 ppm (5) and the Polish MAC value of 100 mg/m^3 (ca. 20 ppm) (26) for trimethylbenzene isomers

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(pseudocumene, mesitylene and hemimellitene) have been established using incomplete, unreliable and obsolete data (7,8). Symptoms and signs of impairment of the respiratory and the nervous systems were observed in 27 industrial workers exposed for a number of years to a paint thinner containing various 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 49–295 mg/m^3 (10–60 ppm) (7,8).

Bearing in mind three very active methyl groups in trimethylbenzene isomer molecules and the extensive use of TMBs, the present study was aimed at evaluating their irritating effects on the respiratory tract in the condition of acute inhalation. Pseudocumene, one of the TMBs, present in most of commercial solvent mixtures at highest concentrations (28) was selected for further study in a 90-day experiment.

MATERIALS AND METHODS

Chemicals

Hemimellitene (1,2,3-trimethylbenzene) 90–95% (GC), mesitylene (1,3,5-trimethylbenzene) puriss ~ 99% (GC), pseudocumene (1,2,4-trimethylbenzene) purum ⩾ 97% (GC), were supplied by Fluka Co.

Conversion factors for trimethylbenzene isomers:

1 ppm ≈ 4.92 mg/m^3

1 mg/m^3 ≈ 0.20 ppm

Animals

Male Wistar rats of IMP:DAK outbred stock, weighing 250–300 g, and Balb/C male mice, weighing 25–30 g, were used. Animals were housed in stainless steel wire mesh cages. Standard rat chow (Murigran) and water were provided *ad libitum*. Animal rooms were maintained at 22–25°C with a light-dark cycle of 12/12 h (lights on at 6:00 AM). Animals were acclimated for 1 week prior to the experiment.

Inhalation exposure

Animals were exposed to vapours of trimethylbenzene isomers in a dynamic inhalation chamber (1.3 m^3 volume, 12 to 15 air changes per hour). Vapours were generated by heating of 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 flame-ionization detector using 5 m metal column with 10% OV-17 on 80/100 mesh WHD chromosorb as a stationary phase at 150°C column temperature.

In a subchronic experiment, groups of 10 male rats were exposed (6 hours/day, 5 days/week for 90 days) to pseudocumene at 25 ppm, 100 ppm, 250 ppm and were accompanied by sham-exposed control group.

The respiratory rate was measured in Balb/C male mice weighing 25–30 g by the use of the whole-body plethysmographic method (27). Each animal was placed in

a body plethysmograph attached to a small (2.3 dm^3) dynamic inhalation chamber. A Stattham pressure transducer was attached to each plethysmograph. The respiratory pattern was recorded by the use of a Beckman polyphysiograph. The respiratory rate was recorded continuously 10 minutes before the exposure to solvent, during 6 minutes of exposure and 6 minutes after termination of the exposure.

Each exposed group consisted of 810 male mice. The maximum decrease in respiratory rate, observed in the second minute of exposure was used for calculation of RD_{50} value with the aid of PROBAN software (19).

Bronchoalveolar lavage. Twenty four hours after termination of subchronic (13-week) inhalation exposure to pseudocumene rats were anaesthetized by an i.p. injection of pentobarbital sodium of 60 mg/kg body weight, and their lungs were lavaged with 2·5 ml of 0.9% NaCl solution. The lavage fluid was collected and centrifuged at 400 g for 10 min at 4°C. Smears were prepared from BAL cells, stained according to May-Grunwald and Giemsa method, and differential cell counts were obtained by using a light microscope.

For cell viability evaluation, the trypan blue exclusion was used. Total protein concentration (22), mucoprotein concentration (15), lactate dehydrogenase (LDH) activity (9), and acid phosphatase activity (12) in the BAL supernatant were determined.

Statistical analysis

The concentrations depressing the respiratory rate in mice to 50% (RD_{50}) were calculated from least squares regression lines of concentration-effect relationship (19). Kruskall-Wallis test (30) was applied for evaluation of total protein and mucoprotein levels and the activity of enzymes in the BAL fluid.

RESULTS

The examined trimethylbenzene isomers showed irritating effect on the respiratory tract and caused concentration-dependent decrease in the mice respiratory rate (Figs 1, 2). The maximum decrease in the respiratory rate was always observed between first or second minute of exposure (Fig 1). The concentration depressing the respiratory rate in mice to 50% (RD_{50}) with its 95% confidence intervals were: 541 ppm (273–828 ppm), 519 ppm (285–780 ppm) and 578 ppm (311–793 ppm) for hemimellitene, mesitylene and pseudocumene, respectively.

All rats exposed to pseudocumene for 90 days survived the experiment. Clinical observations were of no toxicological relevance. No significant differences in final body weigt were observed in rats exposed to pseudocumene when compared with controls. The results of the differential cell count in BAL and cell viability are shown in Table 1. Inhalation exposure to pseudocumene for 90 days increased the total number of cells at all three test concentrations in comparison with the controls. It caused statistically significant increase in macrophage, polymorphonuclear leucocyte and lymphocyte numbers as compared with BAL obtained from control rats. A slight effect on cells viability was found in all groups exposed to pseudocumene for 90 days.

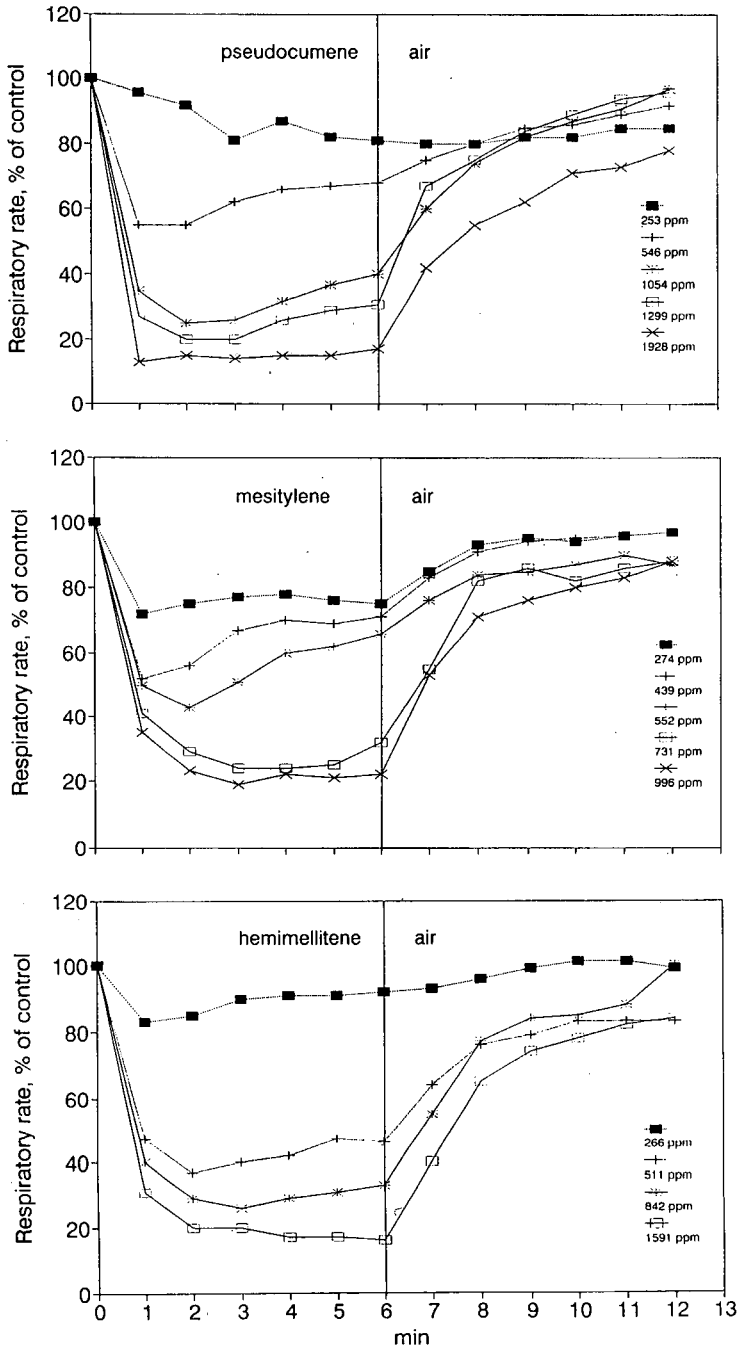

Fig. 1. Time-response relationship for the effect of trimethylbenzene isomers on the respiratory rate in mice. Each point represents the mean value in 8-10 mice. After termination of a 6 min exposure, a recovery of the respiratory rate was observed.

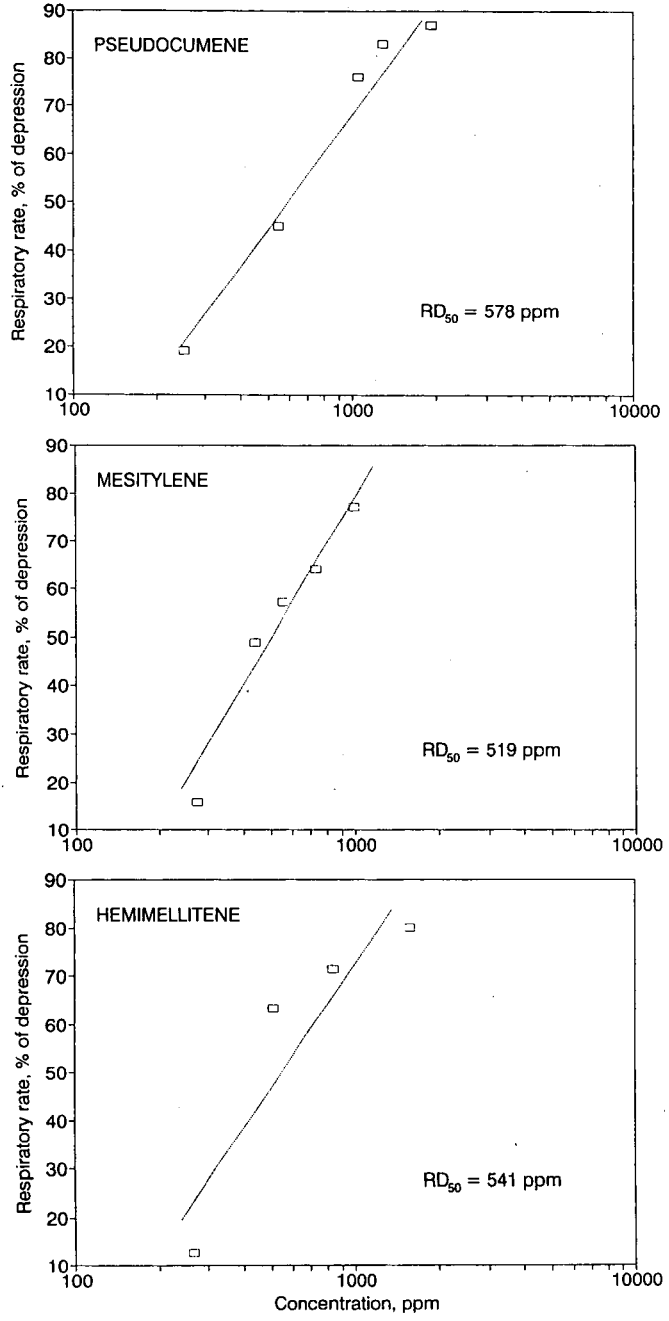

Fig. 2. Respiratory rate of mice exposed to trimethylbenzene isomers. Each point represents mean value of separate measurements in 8-10 mice. The decrease in the respiratory rate observed in the 1st minute of exposure was taken into consideration. The regression line was determined by the least squares procedure.

Table 1. Total number, percentage and the viability of the cells obtained by bronchoalveolar lavage from lungs of rats exposed to pseudocumene for 90 days

	Control (n = 6)	Pseudocumene		
		25 ppm (n = 6)	100 ppm (n = 7)	250 ppm (n = 6)
Body weight (g)	411 ± 28*	383 ± 25	409 ± 56	416 ± 27
Total cells ($10^6/cm^3$)	1.93 ± 0.79	5.82 ± 1.32***	5.96 ± 2.80**	4.45 ± 1.58*
macrophages ($10^6/cm^3$)	1.83 ± 0.03	3.78 ± 0.8	4.95 ± 0.2**	3.96 ± 0.3**
polymorphonuclear leucocytes ($10^6/cm^3$)	0.04 ± 0.02	1.54 ± 0.7	0.52 ± 0.6	0.21 ± 0.3
lymphocytes ($10^6/cm^3$)	0.06 ± 0.01	0.5 ± 0.2	0.5 ± 0.4	0.2 ± 0.1
Cell viability (%)	98.0 ± 1.7	95.5 ± 1.6	95.3 ± 3.5	95.3 ± 3.1

*, **, *** Significantly different from control at $p < 0.05$; $p < 0.01$ and $p < 0.001$, respectively.
Results expressed as a mean ± SD.

The activities of enzymes and total protein and mucoprotein contents of lavage fluid are shown in Table 2. The total protein level, LDH and the acid phosphatase activity were significantly increased in all exposed groups.

Table 2. Total protein and mucoprotein levels and the activity of enzymes in the BAL fluid of rats exposed to pseudocumene for 90 days

	Control (n = 6)	Pseudocumene			Jonckheere test
		25 ppm (n = 6)	100 ppm (n = 7)	250 ppm (n = 6)	
Total protein (mg/ml)	0.19 ± 0.04	0.26 ± 0.07*	0.26 ± 0.06*	0.24 ± 0.08	p = 0.0577
Mucoproteins (mg/ml)	0.16 ± 0.03	0.14 ± 0.02*	0.13 ± 0.02	0.12 ± 0.02	p = 0.3949
Lactate dehydrogenase (mU/ml)	34.2 ± 8.52	92.5 ± 37.2***	61.3 ± 22.9*	53.8 ± 28.6	p = 0.2805
Acid phosphatase (mU/ml)	0.87 ± 0.20	1.28 ± 0.37*	1.52 ± 0.42*	1.26 ± 0.22*	p = 0.0164

Statistically significant difference as compared to controls * $p \pm 0.05$, *** $p \geq 0.001$.

DISCUSSION AND CONCLUSIONS

It has been found that TMBs produce local irritation of the respiratory tract and affect the central nervous system (10). However, the concentrations causing such effects have not been specified. No irritation or effects on the central nervous system were seen in volunteers exposed to hemimellitene, mesitylene or pseudocumene vapours at concentration of 25 ppm for 2 hours (18).

Sensory irritation of trimethylbenzene isomers, a probable effect of stimulation of trigeminal nerve endings in the nasal mucosa, was quantified by measurements of the respiratory rate in mice. It is suggested that the depression of the respiratory rate in mice correlates well with the extent of eye and respiratory irritation in man (3,25).

The concentration that depressed the respiratory rate in mice to 50% (RD_{50}) amounted to 519 – 578 ppm and was about 8 times lower than that of toluene (RD_{50} – 4750 ppm) and 4 times lower than that of xylene (2440 ppm), established in similar experimental conditions (21). Similar RD_{50} values were reported by other authors: 5300 ppm in Swiss/Webster mice (11) and 3373 ppm in Swiss OF_1 mice (24) for toluene; 1467 ppm for o-xylene and 1326 ppm for p-xylene isomers in Swiss OF_1 mice (24). From these data correlation between the potency of the irritating effect and the number of methyl groups in methylated benzene (toluene, xylene, trimethylbenzenes) seems to be very likely and agrees with the belief that methylated substances are characterized by stronger biological activity.

Our results show that trimethylbenzenes are potent respiratory sensory irritants, which may act by stimulation of trigeminal nerve endings in the nasal mucosa. It is suggested that sensory irritation may occur through activation of so called sensory irritant receptor (1,23,24). Activation of the receptor can be only due to physical adsorption of the agonist or physical adsorption and chemical reaction with different binding sites of the receptor. The latter is more efficient and this type of reaction is characteristic of potent irritants. A model for the receptor protein has been proposed with two main sites for benzene moieties and thiol group (3,4).

Irritation, considered as a pathophysiological response to chemical noxa, caused by stimulation of specific nerve endings, involves typical signs of inflammatory reaction. Subchronic, a 90-day exposure to pseudocumene caused significant alterations in BAL cells and in components of BAL fluid. Statistically significant increase in the number of inflammatory (macrophage, polymorphonuclear leucocyte and lymphocyte) cells and their activation evidenced by the increase in LDH and acid phosphatase activity and the increase in total protein content (increased permeability of the alveolar-capillary barrier) point to the inflammatory nature of the response in the respiratory tract. However, the observed changes were not concentration-dependent, showing no progression of effects even if exposure was 10 times higher. Therefore, adaptation to respiratory irritation effect of pseudocumene may be taken into consideration.

Occupational exposure limits (OELs) based on the prevention of sensory irritation between 0.01 RD_{50} and 0.1 RD_{50} were proposed (6). Later on Alarie (2) argued that taking a single value instead of a whole range of values is better to predict OEL from the test system. A good correlation (correlation coefficient: 0.92) between the logarithm of RD_{50} and the logarithm of ACGIH TLV for 40 chemicals was reported. This is not surprising since it has been established that 60 – 70% of TLV values and of the OSHA Toxic Substances list are based on irritation, mostly sensory irritation. Bearing in mind this assumption, the MAC value for TMBs as based on RD_{50} value, should be between 10 and 30 ppm. Irritation effects observed in the BAL at the lowest pseudocumene concentration used, namely 25 ppm, indicated that a lower value of 10 ppm should be rather taken into consideration.

Occupational exposure limits for most of the organic solvents are predominantly based on the protection against their neurotoxic effects. It has been shown that the neurotoxic effects caused by TMBs were more pronounced than that of toluene and xylenes, both after the acute exposure and subchronic exposures (20). In subchronic experiment with pseudocumene, LOEL, based on disturbed locomotor activity and pain sensitivity in males, was 100 ppm and NOEL 25 ppm.

On the basis of the effects observed in the respiratory and central nervous systems, the threshold limit value of at least 10 ppm should be considered in the occupational exposure to trimethylbenzene isomers.

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