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TOXIC EFFECTS OF ACUTE INHALATION EXPOSURE TO 1,2,4,5-TETRAMETHYLBENZENE (DURENE) IN EXPERIMENTAL ANIMALS

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Abstract. Neurotoxic and sensory respiratory irritation effects of 1,2,4,5-tetramethylbenzene (durene) in male rats and male Balb/C mice were investigated in the condition of acute inhalation exposure. Rotarod performance and pain sensitivity behaviour were tested in rats exposed to durene at concentrations of 880, 1100 and 1280 mg/m^3 immediately after termination of a four-hour exposure. The respiratory rate was measured in mice by the whole body pletysmographic method in a 6-min exposure to various concentrations of durene.

Exposure to durene resulted in concentration-dependent decrease in pain sensitivity in rats and depression of respiratory rate in mice.

At applied durene concentrations, no statistically significant disturbances in rotarod performance behaviour were observed. The concentration depressing the respiratory rate to 50% (RD_{50}) was 838 mg/m^3. As based on RD_{50} value, MAC of 25 mg/m^3 is suggested for durene.

INTRODUCTION

Tetramethylbenzene isomers (prenitene, isodurene, durene) enter into the composition of many commonly used commercial solvent mixtures like Solvesso 150 (Exxon Chemical Belgium) or Shellsol AB (Shell Netherland Chemie B.V.) (7). Among other constituents, tetramethylbenzene isomers may reach 10% of these solvent mixtures.

For industrial hygiene the additivity rule of health effects has often been used to cope with the problem of combined exposure to solvents (8). In order to apply this principle the MAC values of each mixture constituents are necessary.

As the MAC value for tetramethylbenzene isomers has not as yet been established, the objective of the present study was to evaluate the neurotoxic and irritating effects on the respiratory tract in the condition of acute inhalation.

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MATERIALS AND METHODS

Male Wistar rats of IMP:DAK stock outbred body weight 250–300g and Balb/C male mice weighing 25–30 g were used. Animals were housed in wire mesh stainless steel cages. LSK lab chow and water were provided *ad libitum*. Animal rooms were maintained at 22–25°C with a 12-hr light-dark cycle (lights on 6:00 AM). Animals were acclimated for 1 week prior to the experiment. Rats were exposed to durene at concentrations of 880, 1100 and 1280 mg/m^3 for 4 hours. Animals were exposed to vapours of durene in a dynamic inhalation chamber (volume of 1.3 m^3, 12 to 15 air changes per hour). Vapours of durene were generated by heating to 90°C. The desired concentrations of vapours were obtained by diluting them in the air. Concentration of solvent vapours in the exposure chamber were measured every 30 min with a gas chromatograph Hewlett-Packard with flame-ionization detector, using 30 m HP-1 column at temperature of 100°C.

Durene 98% (GC) was supplied by Aldrich.

Rotarod performance was tested according to the principle described by Kaplan and Murphy (10). The rotarod apparatus used consisted of a 8-cm diameter wooden rod rotating at 12 rpm and suspended horizontally 20 cm above the floor which was constructed of metal bars connected to a power source of 80 V and 2 mA. The ability of rats to remain on the rotating rod for 2 min was taken as an index of normal neuromuscular function. Before the experiment, the animals were trained and only those rats which could perform normally on the rotarod for at least 10 consecutive days were used in the experiment. Rotarod performance was tested before exposure and immediately after termination of exposure to several concentrations of durene and in sham exposed control animals for four hours. Each group consisted of 10 rats.

Hot plate behaviour was tested immediately after termination of exposure. The hot-plate test was used to measure the level of analgesia (6). The rat was placed on the hot-plate within the plastic enclosure and after the occurrence of the expected response - licking the foot, or after 60 sec, the animal was removed. The latency of the paw-lick response was measured at plate temperature of 54.5°C. Each group consisted of 10 rats.

The respiratory rate was measured in Balb/C male mice weighing 25–30 g using the plethysmographic method (18). Each animal was placed in a body plethysmograph attached to a small dynamic inhalation chamber (volume of 2.3 dm^3). A Stattham pressure transducer was attached to each plethysmograph. The respiratory pattern was recorded by a Beckman polyphysiograph. The respiratory rate was recorded continuously before the exposure to solvent, during 6 min of exposure and 6 min after termination of exposure. Each exposure group consisted of 8–10 mice. For calculation of RD_{50} value, the maximum decrease in respiratory rate, observed in the third minute of exposure, was used.

Exposure concentrations of durene are expressed in mg/m^3

conversion factors: 1 mg/m^3 = 0.179 ppm,
1 ppm = 5.583 mg/m^3.

STATISTICS

The concentration depressing the respiratory rate in mice to 50% (RD_{50}) was calculated from the least squares regression lines of concentration-effect relationship (9). The Kruskall-Wallis test (19) was applied for evaluating the decrease in sensitivity to pain.

RESULTS

All rats exposed for 4 hours to durene at all concentrations applied survived the exposure. Maximum durene concentration, attainable in exposure chamber was 1280 mg/m^3.

Durene at attainable exposure concentrations of 1100 mg/m^3 and 1280 mg/m^3 disturbed the rotarod performance behaviour in rats (Table 1), but the changes were not statistically significant.

Table 1. Rotarod performance in rats exposed to durene vapours for 4 hours

Control	Durene	
No. of failures/ No. of animals tested	Concentration	No. of failures/ No. of animals tested
0/10	880	0/10
0/10	1100	1/10
0/10	1280	1/10

The pain sensitivity measured as latency of the paw-lick response changed in rats exposed to durene. The observed decrease in sensitivity to pain was concentration-dependent and statistically significant at durene concentrations of 1100 and 1280 mg/m^3 (Table 2).

Table 2. Latency of the paw-lick response (hot-plate behaviour) in rats exposed to durene vapours for 4 hours

Group		Latency of the paw-lick response, sec	Decrease in the pain sensitivity %[a]
Control	(n = 30)	10.7±2.1	
880 mg/m^3	(n = 10)	13.2±4.3	5.0
1100 mg/m^3	(n = 10)	16.4±5.3*	11.5
1280 mg/m^3	(n = 9)	19.8±4.5**	18.4

Statistically significant difference as compared to control *$p \leq 0.01$, **$p \leq < 0.001$

[a] Latency elongation to 60 sec over the control was taken as 100% decrease in sensitivity to pain.

Durene caused concentration-dependent decrease in respiratory rate in mice (Figs. 1 and 2). The maximum decrease in the respiratory rate was always observed in 2–3 min of exposure (Fig.1).

The concentration depressing the respiratory rate in mice to 50% (RD_{50}) with its 95% confidence intervals was 838 mg/m^3 (647–1378 mg/m^3).

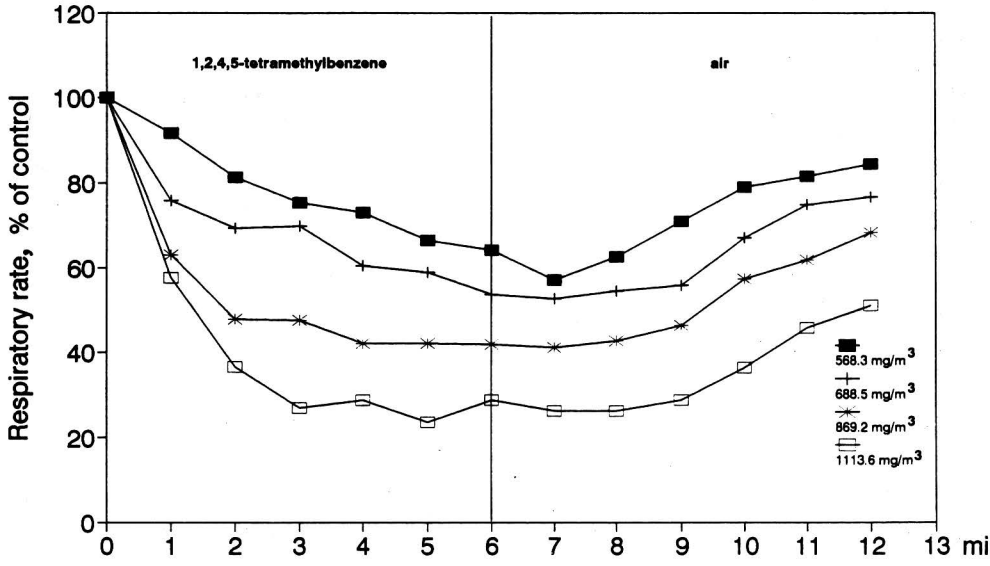

Fig. 1. Time response relationship for the effect of 1,2,4,5-tetramethylbenzene (durene) on the respirat rate in mice. Each point represents the mean value in 8—10 mice.

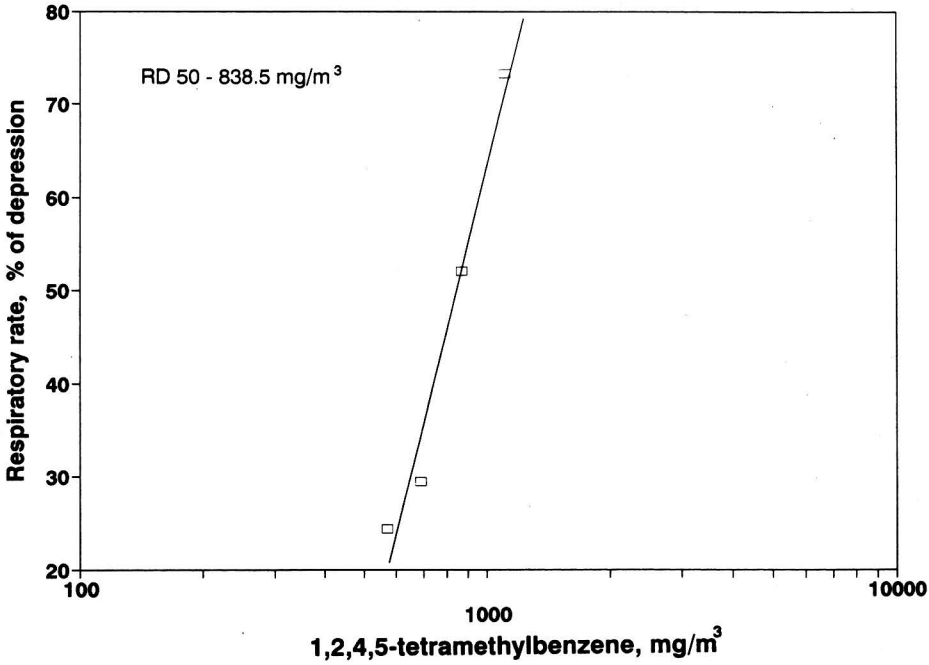

Fig. 2. The respiratory rate of mice exposed to 1,2,4,5-tetramethylbenzene (durene). Each point repres the mean value of separate measurements in 8—10 mice. The decrease in the respiratory rate observed the 3rd minute of exposure was taken into consideration. The regression line was determined by the le squares procedure.

DISCUSSION AND CONCLUSION

Results of rotarod performance and hot-plate behaviour test in rats at the exposure attainable concentrations indicated neurotoxic effects of durene.

Durene like most organic solvents (11,12,13) disturbed the rotarod performance behaviour and decreased the pain sensitivity.

At durene maximum attainable concentrations the disturbances in rotarod performance behaviour were slight and not statistically significant. The decrease observed in sensitivity to pain was concentration-dependent and statistically significant but the extent of the decrease was not sufficient to calculate the EC_{50} value.

For better evaluation of durene neurotoxic effects a subchronic exposure experiment is necessary.

The irritation effect of durene was quantified by measurements of the respiratory rate in mice. It has been well evidenced that the respiratory rate depression in mice correlates well with the extent of the eye and respiratory irritation in man (2,17).

The determined concentration, depressing the respiratory rate in mice to 50% (RD_{50}), amounted to 838 mg/m^3 (150 ppm) and indicated strong irritative respiratory effect of durene. Durene RD_{50} value was about 4, 16 and 32 times lower than that of trimethylbenzene isomers (RD_{50} – 578, 519, 541 ppm), xylene (RD_{50} – 2440 ppm) and toluene (RD_{50} – 4750 ppm), established in similar experimental conditions (11,14).

In the case of organic solvents in question (toluene, xylene, trimethylbenzene, durene), the correlation between the potency of the irritating effect and the number of methyl groups seems to be very likely and in accordance with the belief that methylated substances are characterized by a stronger biological activity. Our results show that durene is a potent respiratory irritant.

It is suggested that sensory irritation can occur due to activation of so called 'sensory irritant receptor' (1,15,16). The receptor can be activated only due to physical adsorption of the agonist or physical adsorption and chemical reaction to 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 tiol group (2,3).

It has been suggested that an occupational exposure limit (OEL) be based on the prevention of sensory irritation between 0.01 RD_{50} and 0.1 RD_{50} (5). Later on, Alarie (4) argued that better way of predicting OEL from the test system was to take a single value instead of a whole range of values. The factor of 0.03 RD_{50} was proposed as the highest level acceptable for OEL, unless other toxic effects occur in the respiratory system at exposure concentrations lower than those at which sensory irritation occurs. A good correlation was reported (correlation coefficient: 0.92) between the logarithm of RD_{50} and the logarithm of the ACGIH TLV of 40 chemicals. This is not surprising since, according to our knowledge, 60–70% of TLV values and of the OSHA Toxic Substances list are based on the irritation effect, mostly sensory irritation.

Taking into consideration this assumption, MAC of 25 mg/m^3 (4.5 ppm) for 1,2,4,5-tetramethylbenzene (durene), as based on RD_{50} value, should be considered.

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