International Journal of Occupational Medicine and Environmental Health, Vol. 8, No 4, 331 – 337, 1995

TOXIC EFFECTS OF ACUTE INHALATION EXPOSURE TO 1,2,4-TRIMETHYLBENZENE (PSEUDOCUMENE) IN EXPERIMENTAL ANIMALS

ZOFIA KORSAK, RADOSŁAW ŚWIERCZ and KONRAD RYDZYŃSKI

Department of Toxicity Evaluation
The Nofer Institute of Occupational Medicine
Lodz, Poland

Key words: 1,2,4-trimethylbenzene (pseudocumene), Acute exposure, Nervous system, Respiratory system, Rats, Mice

Abstract. Neurotoxic and sensory respiratory irritation effects of 1,2,4-trimethylbenzene (pseudocumene) in male rats and male Balb/C mice were investigated in condition of acute inhalation exposure.

Rotarod performance and pain sensitivity behaviour were tested in rats exposed to pseudocumene at concentrations of 1230 – 9840 mg/m^3 (250 – 2000 ppm) immediately after termination of a four-hour exposure. The respiratory rate was measured in mice by the whole body plethysmographic method in 6 min duration exposure to various concentrations of pseudocumene. Exposure to pseudocumene resulted in concentration-dependent disturbances in rotarod performance, decrease in pain sensitivity in rats and depression of respiratory rate in mice.

The EC_{50} value for rotarod performance behaviour disturbances was 4693 mg/m^3 (954 ppm) and for decrease pain sensitivity EC_{50} was 5682 mg/m^3 (1155 ppm).

The concentration depressing the respiratory rate to 50% (RD_{50}) was 2843 mg/m^3 (578 ppm). As based on RD_{50} value the MAC values for pseudocumene 85 mg/m^3 (17.0 ppm) is suggested.

INTRODUCTION

The trimethylbenzene isomers are produced mostly during catalytic reforming of petroleum and they enter into the composition of many commonly used commercial solvent mixtures like Solvesso 100 (Exon Chemical Belgium) Shellsol A (Shell Netherland Chemie B.V.), Jolasol (J.L.C Chemie, Austria) and Farbasol (Polifarb--Cieszyn S.A., Poland). Among others pseudocumene is one of the substantial constituent of these solvent mixtures.

Adress reprint requests to Zofia Korsak M.D., Department of Toxicity Evaluation, Nofer Institute of Occupational Medicine, P.O. Box 199, 8 Teresy Str., 90-950 Lodz, Poland.

It has been reported in 27 industrial workers, exposed for 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 mentioned) symptoms and signs of impairment of the respiratory, the hematopoietic (probably due to benzene contaminations) and the nervous systems. The levels of hydrocarbon vapours determined in the atmosphere ranged from 49–295 mg/m^3 (10–60 ppm) (6,7).

The assumption of additivity of health effects has often been used in industrial hygiene to cope with the problem of combined exposure to solvents (11).

The ACGIH TLV-TWA concentration of 125 mg/m^3 (25 ppm) for single trimethylbenzene isomers (pseudocumene, mesitylene and hemimellitene) has been established on the grounds of incomplete, uncertain and old data (6,7).

Taking under consideration in pseudocumene molecule, three very active methyl groups and wide use of trimethylbenzenes, the objective of the present study was to evaluate the neurotoxic and irritating effects of the respiratory tract in condition of acute inhalation study.

MATERIALS AND METHODS

Pseudocumene pure p.a. was supplied by Fluka.

Male wistar rats of IMP: DAK stock outbred body weight 250–300 g 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 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 use and were used within 4 weeks upon arrival. Rats were exposed to pseudocumene at tested concentrations of 250–2000 ppm for 4 hours. Animals were exposed to vapours of pseudocumene in a dynamic inhalation chamber (volume of 1.3 m^3, 12 to 15 air changes per hour). Vapours of pseudocumene were generated by heating of liquid solvent in washers. The desired concentrations of vapours were obtained by diluting them in the air. Concentration of solvent in vapours in the exposure chamber were measured every 30 min with a gas chromatograph Hewlett-Packard with flame-ionization detector using 5 m metal column with 10% OV-17 on chromosorb WHD (80/100 mesh) as a stationary phase at column temperature of 150°C.

Rotarod performance was tested according to the principle described by Kaplan and Murphy (13). 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 from 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 pseudocumene 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 (8). The rat was placed on

the hot-plate within the plastic enclosure and after occurrence of the expected response – licking the foot, or arfter 60 sec, 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. For calculation of ED_{50} value the observed latency elongation over control was used.

The respiratory rate was measured in Balb/C male mice weighing 25–30 g by the use of plethysmographic method (19). 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 the use of 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 second minute of exposure, was used.

Exposure concentrations of pseudocumene are expressed in ppm;
conversion factors: 1 ppm $\approx$ 4.92 mg/m^3
1 mg/m^3 $\approx$ 0.20 ppm

STATISTICS

Probit analysis was applied to determine the medial effective concentration EC50, value in a rotarod performance test (10). The concentration depressing the respiratory rate in mice to 50% (RD50) and concentration increasing the latency of the paw-lick response (decrease of sensitivity to the pain) to 50% over control (EC50) were calculated from least squares regression lines of concentration – effect relationship (12).

RESULTS

All rats exposed for 4 hours to the concentrations of pseudocumene used, survived the exposure. Pseudocumene caused concentration-dependent disturbance in the rotarod performance of rats (Fig. 1). All control animals performed correctly in the test throughout the experiment. Rotarod performance behaviour disturbance EC value determined with its 95% confidence intervals amounted to 4693 mg/m^3 (3891–5493 mg/m^3) 954 ppm (791–1113 ppm).

The pain sensitivity measured as latency of the paw-lick response was changed in rats exposed to pseudocumene. Pseudocumene decreases sensitivity to the pain and changes were concentration-dependent (Fig. 2). ED value with its 95% confidence intervals amounted to 5682 mg/m^3 (2715–7596 mg/m^3) 1155 ppm (552–1544 ppm).

Pseudocumene caused concentration-dependent decrease in respiratory rate in mice (Figs 3,4). The maximum decrease of respiratory rate was always observed in the 1–2 min of exposure (Fig. 3). The concentration depressing the respiratory rate in mice to 50% (RD50) with its 95% confidence intervals was 578 ppm (311–793 ppm).

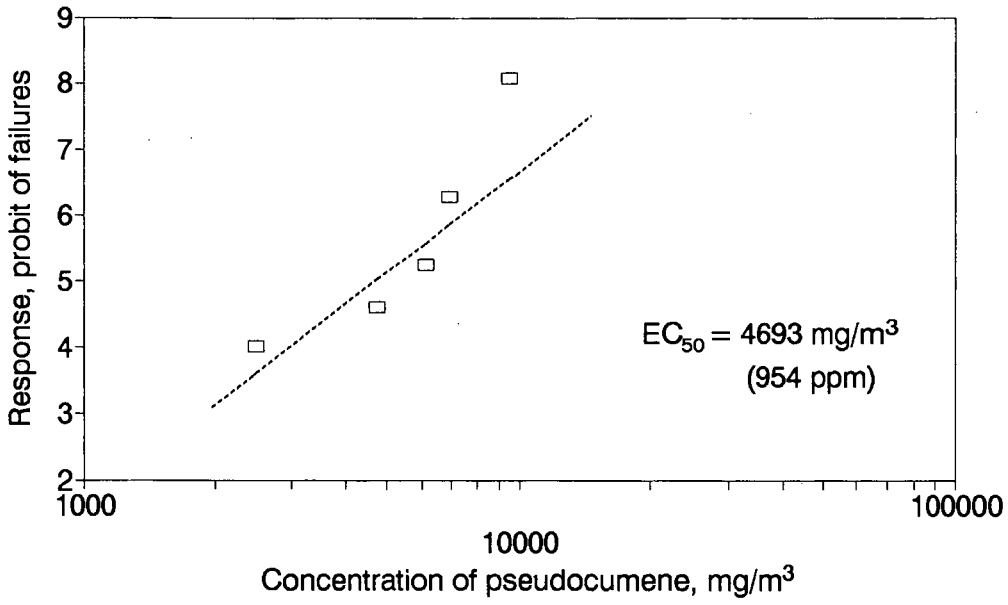

Fig. 1. Rotarod performance of rats exposed to pseudocumene. Rats were exposed to vapours of solvent for 4 hours. Rotarod performance was tested immediately after termination of exposure. Each point represents probit of failures on rotarod in a group of 10 rats.

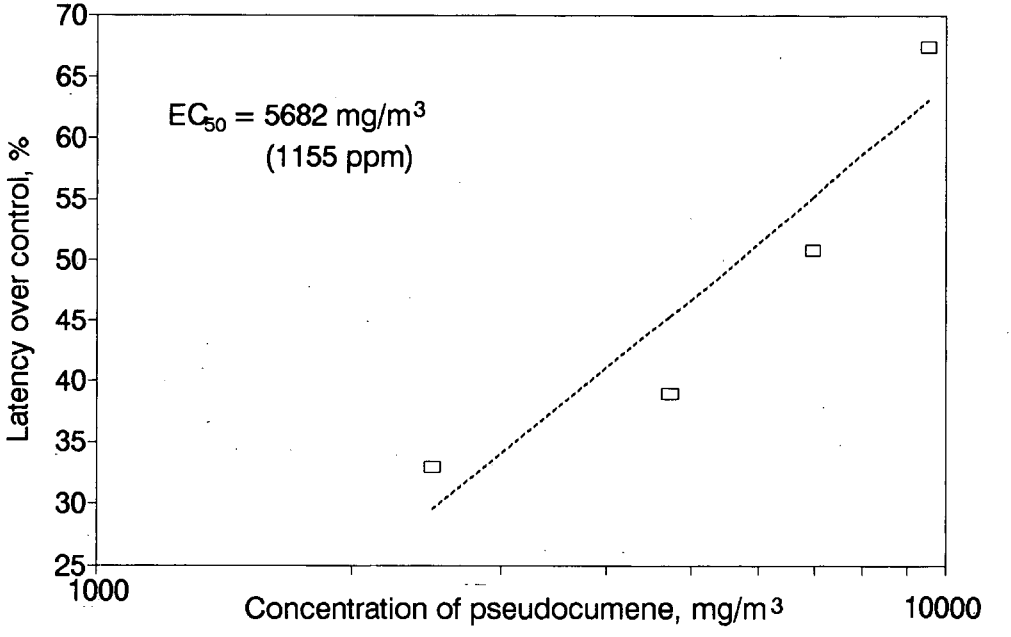

Fig. 2. Hot-plate behaviour in rats exposed to pseudocumene. Rats were exposed to vapours of solvent for 4 hours. Hot-plate behaviour was tested immediately after termination of exposure. Each point represents the mean value of separate measurements of latency over the control in 10 rats.

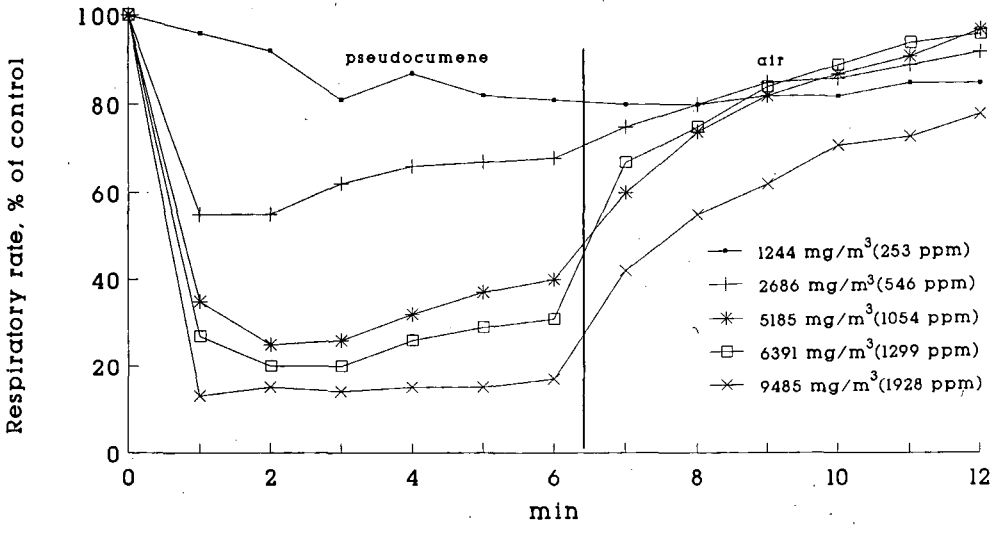

Fig. 3. Time response relationship for the effect of pseudocumene on respiratory rate in mice. Each point represents the mean value in 8 – 10 mice. After termination of 6 min exposure recovery of respiratory rate was observed.

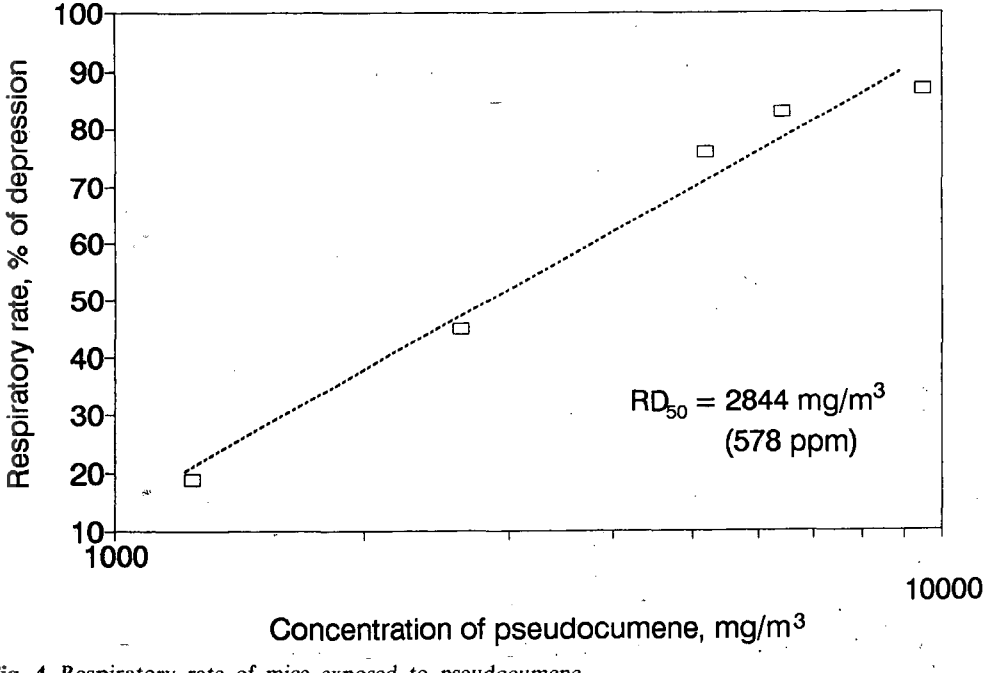

Fig. 4. Respiratory rate of mice exposed to pseudocumene.
Each point represents mean value of separate measurements in 8 – 10 mice. The decrease of respiratory rate observed in the 1st minute of exposure was taken for consideration. The regression line was determined by the least squares procedure.

DISCUSSION AND CONCLUSION

Results of rotarod performance and hot plate behaviour tests in rats exposed for 4 hours to vapours of pseudocumene at studied concentrations provide an evidence of pseudocumene neurotoxic effects. The observed disturbances in rotarod performance and decrease in pain sensitivity were concentration dependent. The established pseudocumene 954 ppm ED50 value for rotarod performance behaviour indicates more pronounced toxic effect of pseudocumene than that of toluene (ED50 – 4050 ppm) and xylene (ED50 – 1982 ppm) in similar experimental conditions (14,15).

As in the case of motor coordination, ED50 value for the pain sensitivity of pseudocumene was at the same level of exposure 5682 mg/m^3 (1155 ppm). Irritation effect of pseudocumene was quantifield by measurements of respiratory rate in mice. It provided good evidence that depression of respiratory rate in mice correlates well with the extent of eye and respiratory irritation in man (2,18).

The determined concentration depressing the respiratory rate in mice to 50% (RD50) amounted to 578 ppm and was about 8 and 4 times lower than that of toluene (RD50 – 4750 ppm) and xylene (2440 ppm) established in similar experimental conditions (14).

In the case of these organic solvents (toluene, xylene, trimethylbenzene) correlation between the potency of the irritating effect and number of methyl groups seems to be very likely and agree with the belief that methylated substances are characterized by stronger biological activity. Our results show that pseudocumene is a potent respiratory irritant. It is suggested that sensory irritation can occur due to activation of so called "the sensory irritant receptor" (1,16,17). 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 for potent irritants. A model for the receptor protein has been proposed with two main sites for benzene moieties and thiol group (2,3,12).

It has been proposed that an Occupational Exposure Limit (OEL) be based on prevention of sensory irritation between 0.01 RD50 and 0.1 RD50 (5). Later, Alarie (4) argued that better way of predicting on OEL from the test system was to take a single volume instead of a range. The factor of 0.03 RD50 was proposed as the highest level acceptable for on OEL, unless other toxic effects occur in the respiratory system at exposure concentrations lower than those at which sensory irritation occurs. It was reported a good correlation (correlation coefficient: 0.92) between the logarithm of RD50 and the logarithm of the ACGIH TLV of 40 chemicals. 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. Taking into consideration this assumption, the MAC value for 1,2,4-trimethylbenzene as based on RD50 value should be 85 mg/m^3 (17.0 ppm) – considerably lower than the ACGIH TLV value (125 mg/m^3 – 25 ppm) and than the Polish MAC value (100 mg/m^3 – 20 ppm) (9).

However, due to pseudocumene neurotoxic and suspected hematopoietic impairment further animal study in condition of subchronic exposure are necessary.

REFERENCES

1. Alarie Y. Irritating properties of airborne materials to the upper respiratory tract. Arch Environ Health 13: 433–449, 1966.
2. Alarie Y. Sensory irritation of the upper airways by airborne chemicals. Toxical Appl Pharmcol 24: 279–297, 1973.
3. Alarie Y. Sensory irritation by airborne chemicals. Crit Rev Toxicol 2: 299–363, 1973.
4. Alarie Y. Establishing threshold limit values for airborne sensory irritants from an animal model and the mechanism of action of sensory irritants. Adv Environ Toxicol 8, 153, 1984.
5. Barrow CS, Alarie Y, Warrick JC, Stock MF. Comparison of the sensory irritation response in mice to chlorine and hydrogen chloride. Arch Environ Health 32: 68–76, 1977.
6. Battig K, Grandjean E, Turrian V. Gesundheitschaden nach langdauernder Trimethylbenzol Exposition in einer Malerwerkstatt. Z Praventiv Med 1: 389–403, 1956.
7. Batting K, Grandjean E, Rossil L, Rickenbacher J. Toxikologische Untersuchungen uber Trimethylbenzol. Arch. Gewerbepathol. Gewerbehyg 16: 555–566, 1958.
8. Bodnar RJ. Types of stress which induce analgesia. In: Stress Induced Analgesia. MD Tricklebank, G Curzon, Willey, eds. London 1984.
9. Law Gazette no 69, Warsav, 29 July, 1995 (in Polish).
10. Finey DJ. Probit Analysis. Cambridge University Press, Combridge, 1971.
11. Ikeda M. Interactions and health aspects of exposure to mixtures of organic solvents. In: Health effects of combined exposures to chemicals in work and community environments. Interim Document 11. WHO Regional Office, Europe, Copenhagen, 1983.
12. Jędrychowski R. Statistic methods in evaluation of toxicity. In: Industrial Toxicology, J. Indulski, JK. Piotrowski, ed. The Nofer Institute of Occupational Medicine, Lodz 1983 (in Polish).
13. Kaplan ML, Murphy SD. Effect of acrylamide on rotarod performance and sciatic nerve beta-glucuronide activity of rats. Toxicol Appl Pharmacol 22: 259–266, 1972.
14. Korsak Z, Sokal J, Dedyk A, Tomas T, Jędrychowski R. Toxic effects of combined exposure to toluene and xylene in animals. I. Acute inhalation study. Pol J Occup Med 1: 45–50, 1988.
15. Korsak Z, Świercz R, Jędrychowski R. Effects of acute combined exposure to n-butyl alcohol and m-xylene. Pol J Occup Med Environ Health 6: 35–41, 1993.
16. Nielsen GD. Mechanisms of activation of the sensory irritant receptor by airborne chemicals. Crit Rev Toxicol 21, 183–208, 1991.
17. Nielsen GD, Alarie Y. Sensory irritation, pulmonary irritation, and respiratory stimulation by airborne benzene and alkylbenzenes: prediction of safe industrial exposure levels and correlation with their thermodynamic properties. Toxicol Appl Pharmacol 65, 459–477, 1982.
18. Nielsen GD, Bakbo JCh. Exposure limits for irritants. Ann Am Conf Ind Hyg 12, 119–126, 1985.
19. Tomas T, Oliszkiewicz W, Czerczak S, Sokal JA. Decrease in the mice's respiration rate as an index of chemical substances irritating effects upon the upper respiratory tract. Med Pr 36: 295–302, 1985 (in Polish).

Received for publication: July 31, 1995
Approved for publication: September 27, 1995