International Journal of Occupational Medicine and Environmental Health, Vol. 11, No 4, 335–342, 1998

TOXIC EFFECTS OF ACUTE INHALATION EXPOSURE TO 1-METHYLNAPHTHALENE AND 2-METHYLNAPHTHALENE IN EXPERIMENTAL ANIMALS

ZOFIA KORSAK, WANDA MAJCHEREK and KONRAD RYDZYŃSKI

Department of Toxicity and Carcinogenesis
The Nofer Institute of Occupational Medicine
Łódź, Poland

Key words: 1-Methylnaphthalene, 2-Methylnaphthalene acute exposure, Nervous system, Respiratory system, Rats, Mice

Abstract. Neurotoxic and sensory respiratory irritation effects of 1-methylnaphthalene and 2-methylnaphthalene in male rats and male Balb/C mice were investigated under conditions of acute inhalation exposure. Rotarod performance and pain sensitivity behaviour were tested in rats exposed to 1-methylnaphthalene at concentrations of 152–407 mg/m^3, and 2-methylnaphthalene at concentrations of 229–522 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 6 min duration exposure to various concentrations of 1-methylnaphthalene and 2-methylnaphthalene. Exposure to both methylated naphthalene derivatives resulted in concentration-dependent decrease in pain sensitivity in rats and depression of respiratory rate and mice.

At the concentrations applied no statistically significant disturbances in rotarod performance behaviour were observed. The concentrations depressing the respiratory rate to 50% (RD_{50}) were 129 mg/m^3 and 67 mg/m^3, for 1-methylnaphthalene and 2-methylnaphthalene, respectively. As based on RD_{50} values, the MAC values of 4 mg/m^3 for 1-methylnaphthalene, and of 2 mg/m^3 for 2-methylnaphthalene are suggested.

INTRODUCTION

The naphthalene derivatives enter into the composition of many commonly used commercial solvent mixtures like Solvesso 150, Solvesso 200 (Exxon Chemical Belgium). The main constituents of these solvent mixtures are methyl- and ethylnaphthalenes (7).

Naphthalene and its methylated derivatives are natural constituents of mineral oil and could be isolated during distilation processes.

Address reprint requests to Dr. Zofia Korsak, Department of Toxicity and Carcinogenesis, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Łódź, Poland.

Among other constituents methylated naphthalene derivatives may reach 60% of Solvesso 200 content (18). Since the MAC values for 1-methylnaphthalene and 2-methylnaphthalene have not been established, the objective of the present study was to evaluate the neurotoxic and irritating effects on the respiratory tract under conditions of acute inhalation study.

MATERIALS AND METHODS

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-h light-dark cycle (lights on 6:00 AM). Animals were acclimatised for 1 week prior to the use and were used within four weeks upon arrival. Rats were exposed to vapours of 1-methylnaphthalene and 2-methylnaphthalene at concentrations of 150–500 mg/m^3 for 4h. Animals were exposed to vapours of solvents in a dynamic inhalation chamber (volume of 0.25 m^3, 12 to 15 air changes per hour). Vapours were generated by heating of solvents in washers to 85°C. 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 with a gas Hewlett-Packard chromatograph with a flame-ionization detector using a 30 m HP-1 column at temperature of 200°C.

1-Methylnaphtalene was supplied by Riedel-Haen, and 2-methylnaphthalene by Fluka.

Rotarod performance was tested according to the principle described by Kaplan and Murphy (9). 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 1-methylnaphthalene, 2-methylnaphthalene 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 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 (16). 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 12 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 1-methylnaphthalene and 2-methylnaphthalene are expressed in mg/m^3

conversion factors: 1 mg/m^3 = 0.172 ppm
1 ppm = 5.813 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 (8). The Kruskal-Wallis test (17) was applied for evaluating the decrease in sensitivity to pain.

RESULTS

All rats exposed for 4 h to both 1-methylnaphthalene and 2-methylnaphthalene at all concentrations applied survived the exposure. Maximal 1-methyl- and 2-methylnaphthalene concentrations, attainable in the exposure chamber, were 407 mg/m^3 and 527 mg/m^3, respectively.

1-Methylnaphthalene, at maximal attainable exposure concentration, did not influence the rotarod performance behaviour in rats (Table 1). 2-Methylnaphthalene at maximal concentration of 527 mg/m^3 disturbed the rotarod performance behaviour but the changes were not statistically significant (Table 2).

The pain sensitivity measured as latency of the paw-lick response changed in rats exposed to 1-methylnaphthalene and 2-methylnaphthalene. The observed decrease in sensitivity to pain was concentration-dependent and statistically significant at concentrations of 253 and 407 mg/m^3 for 1-methylnaphthalene and of 352 and 525 mg/m^3 for 2-methylnaphthalene (Tables 3 and 4).

Table 1. Rotarod performance in rats exposed to 1-methylnaphthalene vapours for 4 h

Control	1-methylnaphthalene	
No. of failures/ No. of tested animals	Concentration (mg/m^3)	No. of failures/ No. of tested animals
0/10	152	0/10
0/10	253	0/10
0/10	074	0/10

Table 2. Rotarod performance in rats exposed to 2-methylnaphthalene vapours for 4 h

Control	2-methylnaphthalene	
No. of failures/ No. of tested animals	Concentration (mg/m^3)	No. of failures/ No. of tested animals
0/10	229	0/10
0/10	352	0/10
0/10	522	1/10

Table 3. Latency of the paw-lick response (hot-plate behaviour) in rats exposed to 1-methylnaphthalene vapours for 4 h

Group		Latency of the paw-lick response sec	Decrease in sensitivity to pain %[a]
control	(n = 50)	10.2 ± 2.3	
152 mg/m^3	(n = 10)	12.8 ± 2.9	4.8
253 mg/m^3	(n = 20)	24.8 ± 15.9***	29.0
407 mg/m^3	(n = 20)	36.1 ± 18.6***	51.8

Statistically significant difference as compared to the control *** $p \leq 0.001$.

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

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

Group		Latency of the paw lick response sec	Decrease in sensitivity to pain %[a]
control	(n = 20)	10.5 ± 2.6	
229 mg/m^3	(n = 10)	13.9 ± 3.3	6.8
352 mg/m^3	(n = 10)	25.7 ± 6.3***	30.7
525 mg/m^3	(n = 20)	33.3 ± 19.9***	46.0

Statistically significant difference as compared to the control *** $p \leq 0.001$.

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

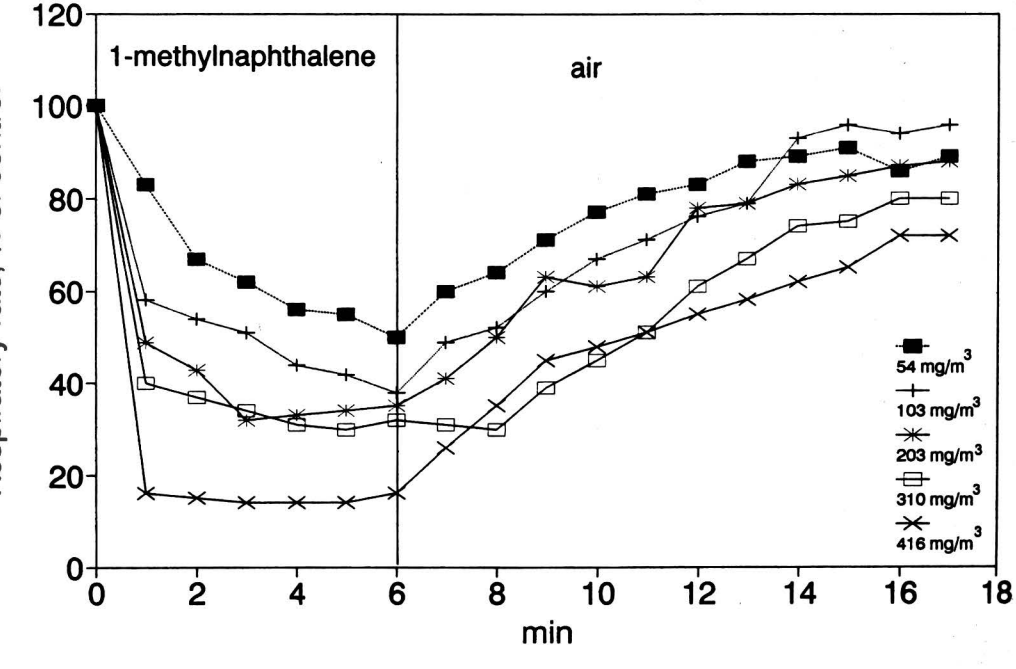

Fig. 1. Time-response relationship for the effect of 1-methylnaphthalene on the respiratory rate in mice. Each point represents the mean value in 8–10 mice.

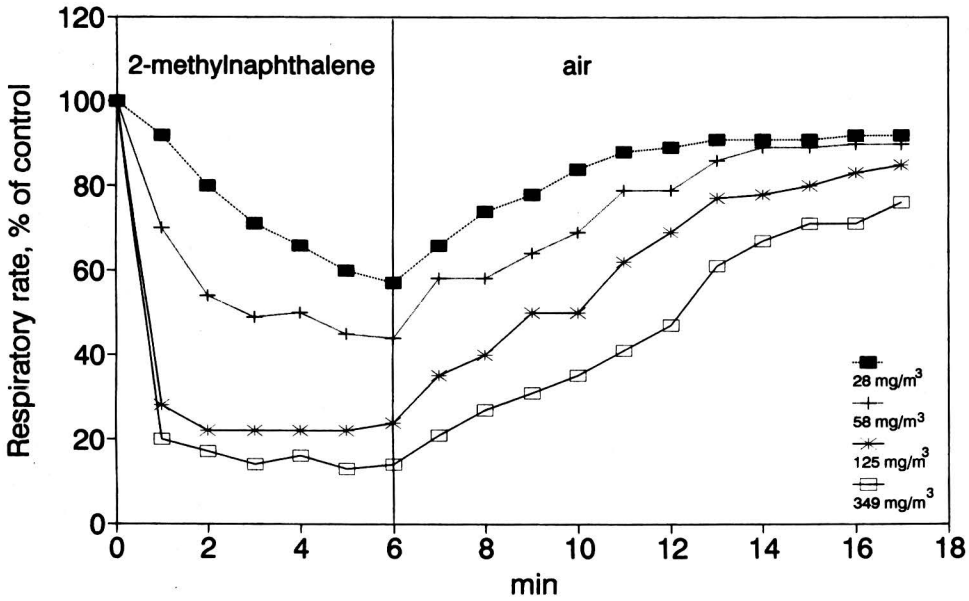

Fig. 2. Time-response relationship for the effect of 2-methylnaphthalene on the respiratory rate in mice. Each point represents the mean value in 8—10 mice.

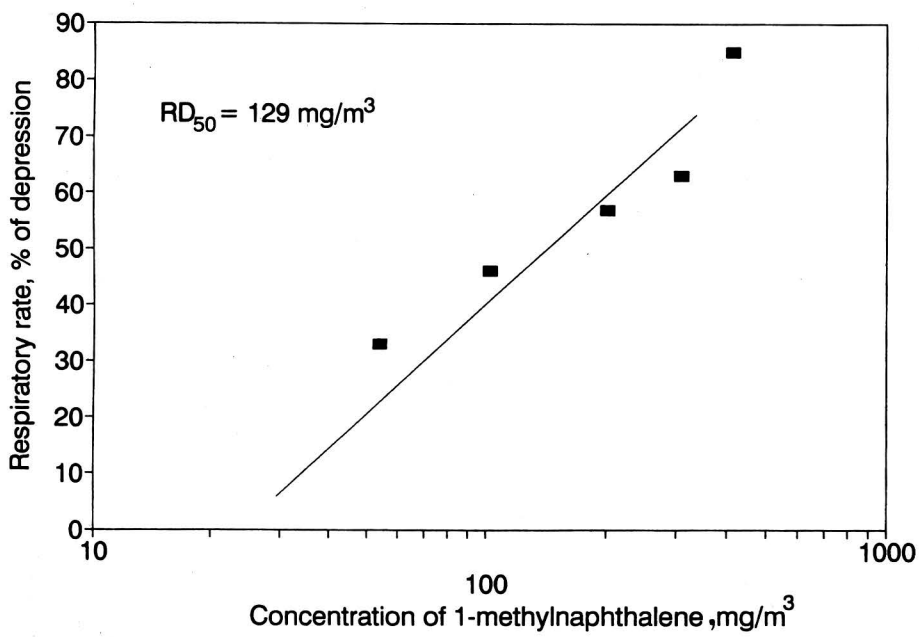

Fig. 3. The respiratory rate of mice exposed to 1-methylnaphthalene. Each point represents the mean value of separate measurements in 8—10 mice. The decrease in the respiratory rate observed in the 2nd min of exposure was taken into consideration. The regression line was determined by the least squares procedure.

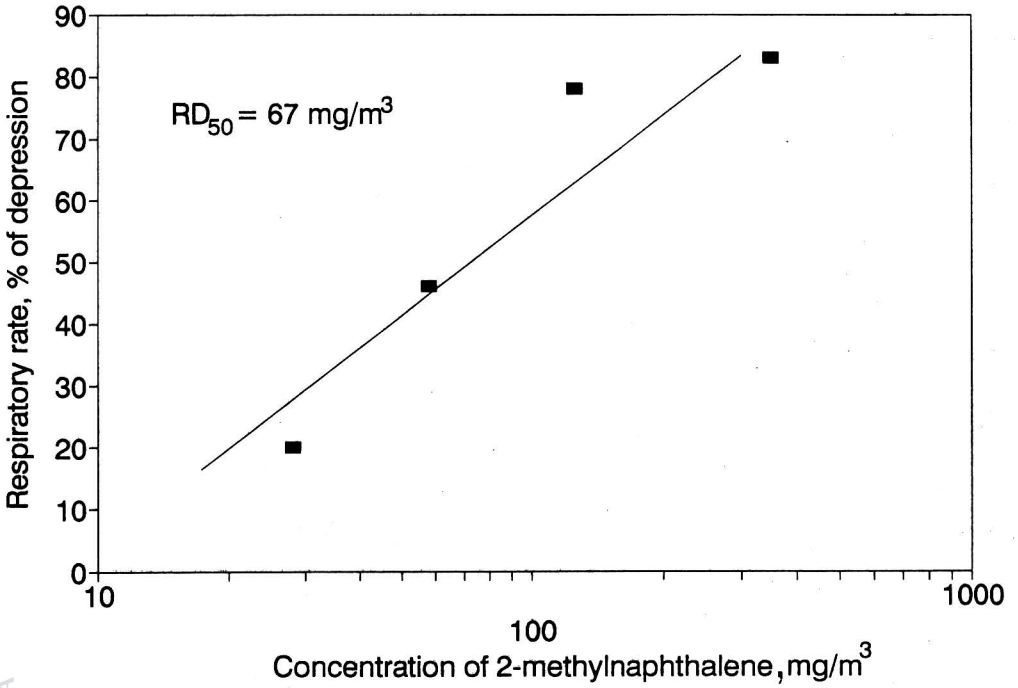

Fig. 4. The respiratory rate of mice exposed to 2-methylnaphthalene. Each point represents the mean value of separate measurements in 8–10 mice. The decrease in the respiratory rate observed in the 2nd min of exposure was taken into consideration. The regression line was determined by the least squares procedure.

1-Methylnaphthalene and 2-methylnaphthalene caused concentration-dependent decrease in the respiratory rate in mice (Figs. 1–4). The maximum decrease in the respiratory rate was observed in the 1–2 min of exposure (Figs. 1 and 2).

The concentration depressing the respiratory rate in mice to 50% (RD_{50}) with its 95% confidence intervals was 129 mg/m^3 (61–228 mg/m^3) for 1-methylnaphthalene and 67 mg/m^3 (80–81 mg/m^3) for 2-methylnaphthalene.

DISCUSSION AND CONCLUSION

The results of rotarod performance and hot-plate behaviour test in rats exposed for 4 h to vapours of 1-methylnaphthalene and 2-methylnaphthalene at concentrations studied indicated their neurotoxic effects.

Methylnaphthalenes in question like most of organic solvents (10–12) decreased the pain sensitivity and disturbed the rotarod performance behaviour.

At a maximal attainable concentration 2-methylnaphthalene influenced the rotarod performance behaviour but changes were slight and not statistically significant, while 1-methylnaphthalene showed no effect. Both 1-methylnaphthalene and 2-methylnaphthalene decreased the pain sensitivity in rats. The observed decrease in sensitivity to pain was concentration-dependent and statistically significant, however, the extent of the decrease was not sufficient for the calculation

of EC_{50} value. Because of low acute attainable concentrations of methylnaphthalenes further experiments under conditions of subchronic inhalation exposure are necessary for better evaluatin of their neurotoxic effects.

The irritation effect of 1-methylnaphthalene and 2-methylnaphthalene were quantified by measurements of the respiratory rate in mice. They provided good evidence that depression of the respiratory rate in mice correlates well with the extent of eye and respiratory irritation in man (2,15).

The concentrations depressing the respiratory rate in mice to 50% (RD_{50}) amounted to 129 mg/m^3 and 67 mg/m^3 for 1-methylnaphthalene and 2-methylnaphthalene, respectively. The irritation effect of 2-methylnaphthalene was twice as strong as that of 1-methylnaphthalene. Our results show that methylated naphthalene derivatives are a potent irritant.

It is suggested that sensory irritation may occur due to activation of so called 'sensory irritant receptor' (1,13,14). 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 tiol group (2,3).

It has been proposed 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, 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 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 (correlation coefficient: 0.92) was reported between the logarithm of RD_{50} and the logarithm of the ACGIH TLV of 40 chemicals. This is not surprising since it has already been proved that 60–70% of TLV values and those of the OSHA Toxic Substances list are based on irritation, mostly sensory irritation.

Taking into consideration this assumption, and based on RD_{50} value, the MAC values of 4 mg/m^3 and 2 mg/m^3 for 1-methylnapthalene and 2-methylnapthalene, respectively, should be considered.

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Received for publication: August 20, 1998
Approved for publication: October 15, 1998