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THE TOXICOKINETICS OF 2-METHYLNAPHTALENE IN RATS

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Abstract
Background: The aim of the study was to evaluate the toxicokinetics of 2-methylnaphtalene (2-MN) during and after inhalation exposure. **Material and Methods:** Male Wistar rats were exposed to 2-MN vapours at nominal concentrations of 200 or 400 mg/m^3 in the dynamic inhalation chamber for 6 hours or 5 days (6 h/day). Blood samples were collected during and after exposure. Blood concentrations of 2-MN were estimated by gas chromatography using the headspace technique. **Results:** During a 6-hour exposure to 200 or 400 mg/m^3, blood 2-MN concentration increased rapidly within the first or second hour of exposure, respectively, after reaching a plateau. The elimination of 2-MN from blood followed an open two-compartment model. **Conclusion: 2**-MN was rapidly eliminated from blood of the animals exposed by inhalation to 2-MN. During exposure, lung retention of the chemical was found to decrease. Under conditions of repeated 2-MN exposure, no significant systemic 2-MN accumulation could be observed.

Key words:
2-Methylnaphtalene, Rats, Inhalation exposure, Blood, Toxicokinetics

INTRODUCTION

2-Methylnaphtalene (2-MN) is a petrol component and it can also be found in numerous commercial solvent mixtures [1,2]. 2-MN is one of the many constituents of tobacco smoke [3]. Human systemic 2-MN penetration is attributable primarily to inhalation exposure. Only a few reports assessing 2-MN toxicity in humans or animals under conditions of inhalation exposure are available in literature [4].

Studies on mice have shown that the major toxic effect of 2-MN is on the lungs, and there has been a strong correlation between 2-MN dose and lung damage [5–7]. Animal inhalation study revealed that a single dose of 2-MN produced a strong irritant effect in mice and a neurotoxic effect in rats [8].

The present paper discusses the toxicokinetics of 2-MN in rat blood under conditions of single or repeated exposure to 2-MN.

MATERIALS AND METHODS

Chemicals

2-Methylnaphtalene (2-MN, CAS No.: 91-57-6) was supplied by Fluka. Its chemical purity was 97%.

Animals and inhalation exposure

Male Wistar IMP: WIST rats weighing 290–380 g (3–4 months old) were exposed to 2-MN vapours at the target concentrations of 200 and 400 mg/m^3 in the dynamic inhalation chamber (volume 0.25 m^3, 15 air changes per hour) for 6 hours or 5 consecutive days (6 hours/day). The animals were given standard laboratory food and water *ad libitum,* except for the time when they were exposed to 2-MN vapours. The relative temperature in the chamber was maintained at 20–22°C and humidity at 40–50%. The required 2-MN vapours were generated by heating 2-MN to 85°C in a glass washer. The desired vapour concentrations were obtained through air dilution. Vapour sample (0.5 dm^3) was absorbed on 2 cm^3 liquid sorbent

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(ethyl alcohol from Polmos, Poland; purity 95%). The concentration of 2-MN vapours in the exposure chamber was measured every 30 min by gas chromatography (Hewlett-Packard 5890) with a flame ionisation detector (FID) using capillary column (HP-1; 5 m, 0.53 mm, 2.65 μm film thickness). The operating conditions were: carrier gas — helium, column flow 10 ml/min; make-up gas (helium) 20 ml/min; air 300 ml/min; oven 150°C; inlet split 220°C, detector 230°C.

Biological material collection and analysis

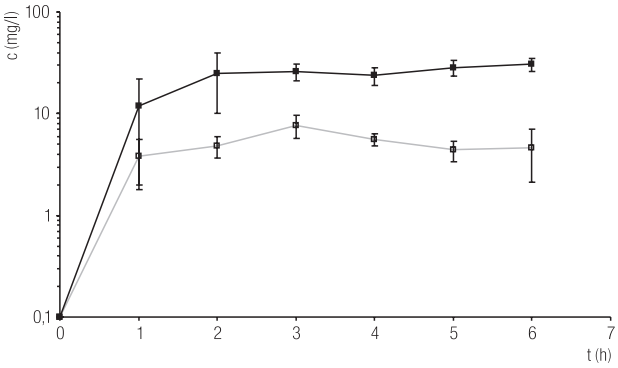
Venous blood samples drawn from the tail vein were collected before (0 h), during (1, 2, 3, 4, 5, 6 h) and after (0.05, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6 h) exposure to 2-MN vapours into 100 μl heparinized glass capillary. The collected samples were stored at +5°C until the determination. Blood 2-MN concentrations were estimated by gas chromatography combined with the headspace technique, using naphthalene as an internal standard [9]. Gas chromatograph (Hewlett-Packard 5890 Series II) was equipped with FID. The operating temperature of the capillary column (HP-1; 30 m, 0.53 mm, 2.65 μm film thickness) was 150°C. The operating conditions were: carrier gas — helium, constant flow mode, column flow 10 ml/min; make-up gas (helium) 20 ml/min; air 300 ml/min; inlet split 220°C, detector 240°C. The limit of detection for 2-MN was 0.01 mg/l for blood analysis.

Statistical analysis

An open two-compartment model plotted with SigmaPlot 4.0 for Windows (Jandel Corporation) was used for the kinetic analysis of 2-MN in blood. The differences in 2-MN blood concentrations between the days of exposure were estimated using Student t-test [10]. $P < 0.05$ was considered significant.

RESULTS

2-MN concentrations in rat blood during a six-hour inhalation exposure to 2-MN vapours at the nominal concentrations of 200 or 400 mg/m^3 and the elimination kinetics data are presented in Figure 1 and Table 1. During a six-hour exposure to 168 or 404 mg/m^3 of 2-MN, blood concentration of the chemical increased rapidly within the 1st or 2nd hour of exposure, respectively, and then reached a plateau. The increase in 2-MN concentration in rat blood was dependent on the magnitude of exposure. The kinetics analysis showed that the half-life and the area under the curve (AUC) of 2-MN in blood increased with a rising level of inhalation exposure.



Results are presented as mean ±SD; four animals per group.

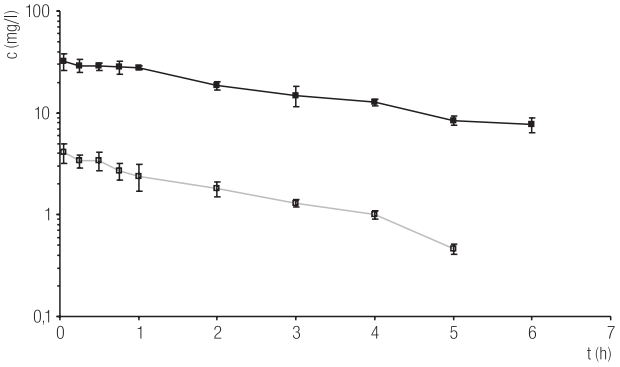
Fig. 1. 2-MN concentration in rat blood during 6-hour inhalation exposure to 2-MN vapours at the target concentrations of 200 (blank rectangle) and 400 (filled rectangle) mg/m^3.

Blood 2-MN concentrations after a six-hour inhalation exposure to 2-MN vapours at nominal concentrations of 200 or 400 mg/m^3 and the elimination kinetics data are displayed in Figure 2 and Table 2. A rapid decrease in blood 2-MN levels was noted within the first hour after a single exposure to low 2-MN levels. In the animals exposed to high 2-MN concentrations, this process was noted during the first two hours of observation. During

Table 1. Toxicokinetics of 2-MN absorption in rat blood during 6-hour inhalation exposure to 2-MN vapours at the target concentrations of 200 and 400 mg/m^3

Parameter	2-MN concentration in inhaled air (mg/m^3)	
	168±55	404±26
	absorption equation: $y = a \times (1-e^{-k \times t})$	
a	5.60±1.40	29.20±0.90
k	1.20±0.30	0.51±0.15
Half-life (h)	0.62±0.20	1.46±0.52
AUC (0 → 6 h)	28.90±8.50	119.00±18.80

AUC — area under curve.



Results are presented as mean ±SD; four animals per group.

Fig. 2. 2-MN concentration in rat blood after 6-hour inhalation exposure to 2-MN vapours at the target concentration of 200 (blank rectangle) and 400 (filled rectangle) mg/m^3.

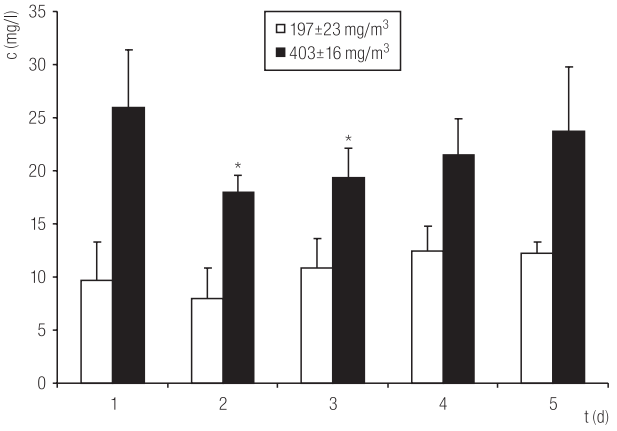
the next few hours of observation, blood 2-MN levels of the exposed rats gradually decreased. The rate of elimination was calculated using an open two-compartment model. The half-lives (phase I and II) of the decline in blood of 2-MN concentration and AUC were dependent on the exposure level.

Figure 3 presents 2-MN concentrations in the blood collected from the tail vein during repeated inhalation exposure to 2-MN vapours. No significant differences in blood 2-MN concentrations could be detected between the consecutive days after daily 6-hour exposure to low-level 2-MN. Animals exposed to high 2-MN concentrations

Table 2. Toxicokinetics of 2-MN elimination from rat blood after 6-hour inhalation exposure to 2-MN vapours at target concentrations of 200 and 400 mg/m^3

Parameter	2-MN concentration in inhaled air (mg/m^3)	
	168±55	413±43
	elimination equation: $y = a \times e^{-k \times t} + b \times e^{-l \times t}$	
a	3.15±0.44	28.80±2.50
k	1.37±0.20	0.65±0.19
b	1.73±0.37	10.60±2.40
l	0.15±0.04	0.05±0.003
Half-life, phase I (h)	0.52±0.08	1.13±0.31
Half-life, phase II (h)	4.96±1.05	13.97±0.97
AUC (0 → 6 h)	9.84±2.09	99.70±11.30

AUC — area under curve.

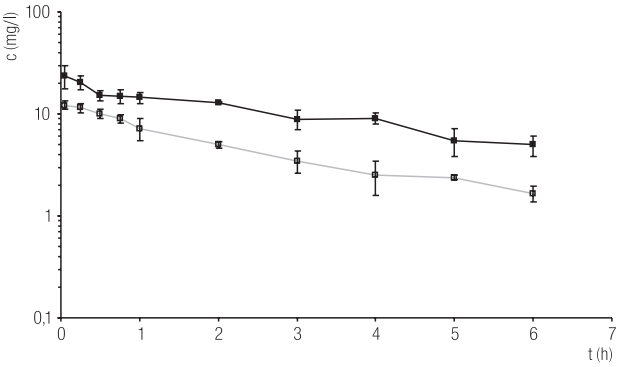


Results are presented as mean ±SD, six animals per group.
* Significantly different from day 1 of exposure at $p < 0.05$.

Fig. 3. 2-MN concentration in rat blood during repeated inhalation exposure (5 days, 6 h/day) to 2-MN vapours at target concentrations of 200 (blank rectangle and column) and 400 (filled rectangle and column) mg/m^3.

had higher blood 2-MN levels after the first day of exposure compared to the following days. Statistical analysis showed significantly lower blood 2-MN concentrations on exposure days 2 and 3 compared to day 1. At both exposure levels, 2-MN was not detected in the blood during the consecutive days following exposure to 2-MN.

During the first hour after repeated exposure at different 2-MN concentrations, the chemical was rapidly eliminated from blood (Figure 4). The elimination rate was calculated using an open two-compartment model. The kinetics equations are presented in Table 3. The half-lives



Results are presented as mean ±SD, six animals per group.

Fig. 4. 2-MN concentration in rat blood after repeated inhalation exposure (5 days, 6 h/day) to 2-MN vapours at target concentrations of 200 (blank rectangle) and 400 (filled rectangle) mg/m^3.

Table 3. Toxicokinetics of 2-MN elimination from rat blood after repeated inhalation exposure (5 days, 6 h/day) to 2-MN vapours at target concentrations of 200 and 400 mg/m^3

Parameter	2-MN concentration in inhaled air (mg/m^3)	
	197±23	403±16
	elimination equation: $y = a \times e^{-k \times t} + b \times e^{-l \times t}$	
a	9.43±0.85	17.80±2.60
k	0.73±0.18	1.38±0.21
b	3.47±0.15	9.00±1.41
l	0.14±0.02	0.071±0.010
Half-life, phase I (h)	1.00±0.26	0.51±0.09
Half-life, phase II (h)	5.18±0.79	9.97±1.45
AUC (0 → 6 h)	28.30±3.40	57.20±8.80

AUC — area under curve.

in phase I of 2-MN elimination from blood were similar and did not depend on the magnitude of exposure. After repeated exposure to 2-MN, the half-lives in phase II and the AUC value evidently increased with an increasing 2-MN concentration.

DISCUSSION AND CONCLUSIONS

A rapid increase in blood 2-MN concentration during the first hours of exposure was noted in the animals exposed by inhalation to different levels of 2-MN (Figure 1). In guinea pigs, a quick penetration of 2-MN to the blood was noted after oral administration of titrated 2-MN. The distribution of the radioactivity in blood and lungs was at similar levels at all the time points analyzed [11]. Thus, it is very likely that 2-MN concentrations in the blood of animals exposed by inhalation are similar to 2-MN concentrations in the pulmonary parenchyma, which makes one conclude that the retention of 2-MN vapours in rat airways seems to be high.

In the animals subjected to a single or repeated exposure to low-dose 2-MN, the trends of its elimination from blood were fairly similar. The half-lives for phase I and II in those experiments were alike despite the fact that blood 2-MN concentrations at the analyzed time points were different. The differences in blood 2-MN concentrations affected the calculated AUC values. After repeated exposure to 2-MN, the AUC was almost three times as high as that after a single exposure. Two major factors may have accounted for the increase in the AUC value and blood 2-MN concentration in the repeatedly exposed rats. The first one was the difference in the magnitude of exposure to 2-MN during a single and repeated exposure. The mean concentration of 2-MN under conditions of repeated exposure was ca. 15% higher than that during a single exposure. The other factor was the high affinity of 2-MN to the kidney, liver and adipose tissue [6]. The particularly high 2-MN affinity to the liver and kidney, belonging to the rapidly equilibrating compartments, could affect 2-MN distribution and elimination in the rats repeatedly exposed to 2-MN, which has resulted in higher blood 2-MN levels and greater AUC value.

The animals repeatedly exposed to high 2-MN concentrations showed decreased blood 2-MN levels on the consecutive days after the daily 6-hour period of exposure (Figure 3). This has resulted in shorter half-lives of elimination (phase I and II) and a lower AUC value compared with a single-dose exposure (Tables 2 and 3). The underlying factors include lower lung retention of 2-MN in the rats exposed to higher 2-MN concentrations, as well as faster 2-MN metabolism and quicker 2-MN removal attributable to the high 2-MN affinity to the kidney [6].

To sum up, 2-MN was rapidly eliminated from the blood of animals subjected to inhalation exposure. Under conditions of inhalation exposure to 2-MN, lung retention of the chemical decreased. In repeated exposure, no significant systemic 2-MN accumulation in the rats could be observed.

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