Polish J. of Environ. Stud. Vol. 15, No. 3 (2006), 485-492

Original Research

Mesitylene (1,3,5-Trimethylbenzene) in the Liver, Lung, Kidney, and Blood and 3,5-Dimethylbenzoic Acid in the Liver, Lung, Kidney and Urine of Rats after Single and Repeated Inhalation Exposure to Mesitylene

R. Świercz*, W. Wąsowicz, W. Majcherek

Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine,
8 Św. Teresy Str., 91-348 Łódź, Poland

Received: June 30, 2005
Accepted: December 8, 2005

Abstract

The aim of our study was to explore the tissue distribution of 3,5-dimethylbenzene acid (3,5-DMBA) and its excretion with urine of rats and to evaluate toxicokinetics of mesitylene in blood of rats after single and repeated inhalation exposure to mesitylene vapours.

Experiments were performed on male outbred IMP:WIST rats. The animals were exposed to mesitylene vapours at the target concentration of 25, 100, and 250 ppm in dynamic inhalation chambers for 6 h at single exposure and for 4 weeks (6 h/day for 5day/week) at repeated exposure.

The study revealed in rats, after inhalation exposure to mesitylene, exposure-dependent increases in 3,5-DMBA tissue concentration and urinary excretion as well as enhanced mesitylene concentration in tissues and blood. After termination of exposure, mesitylene was rapidly eliminated from blood of rats. Mesitylene retention reduced in rat lungs after repeated exposure, as compared to a single exposure, was most likely the reason for its lower concentration in lungs and blood. Compared with single exposure, 3,5-DMBA concentration increased in rat lungs after repeated inhalation exposure to mesitylene at 100 and 250 ppm, and in the liver at 250 ppm, which may be associated with the induction of mesitylene-metabolizing enzymes.

Mesitylene metabolism in the lungs of the rats after repeated exposure to its low concentrations probably had a significant impact on the increased urinary excretion of 3,5-DMBA.

Keywords: mesitylene, inhalation, 3,5-dimethylbenzene acid, liver, lung, kidney, urine, blood, rats

Introduction

A very wide use of petroleum products may be harmful after control to the human body. Mesitylene is a component of many petroleum products. In some organic solvents its share may even reach about 10% and in gasoline of different kinds its content is estimated at about 1% [1–3]. Animal studies show that mesitylene vapours exert neurotoxic and irritating effects on animals. It has been evidenced that the irritating effect of mesitylene on the respiratory tract of mice is four and eight times higher than that of xylene and toluene, respectively [4]. The neurotoxic effect of mesitylene on rats, assessed on the basis of the locomotion coordination test on rotary rollers, after a 4-h inhalation exposure, proved to be four times higher than that of toluene and xylene [5]. Changes in alkaline

*Corresponding author; e-mail: radek@imp.lodz.pl

phosphate (AP) activity in blood serum of rats were observed after single exposure, and the increased aspartate aminotransferase (GOT) after repeated exposure to mesitylene vapours. Neither single nor repeated inhalation exposures to mesitylene vapours had any impact on the haemoglobin level or erythrocyte and leukocyte counts [6, 7]. After repeated administration of mesitylene by gastric tube, the activity of microsomal enzymes of the liver, kidney, and lung was increased in rats [8,9]. In rats, like in humans, biotransformation leads to the production of 3,5-dimethylbenzoic acid (3,5-DMBA), which is excreted with urine in the form of 3,5-dimethylhippuric acid [10, 11].

The aim of the study was to explore the distribution of mesitylene and 3,5-DMBA in tissues as well as the kinetics of mesitylene excretion with blood and 3,5-DMBA excretion with urine in rats after termination of single and repeated inhalation exposures to mesitylene at concentrations of 25, 100, and 250 ppm.

Materials and Methods

Chemicals

Mesitylene (1,3,5-trimethylbenzene, 1.3.5-TMB, No. CAS:108-67-8) was supplied by Fluka (Cat. No. 63910), its purity was ≥ 99%. The conversion factors for mesitylene: 1 ppm ~ 4.92 mg/m^3.

Animals and Inhalation Exposure Monitoring

Male Wistar rats IMP: WIST (five animals in each group) of body weight 232-350 g (2-3 months old) were exposed to mesitylene vapours at the target concentration of 0, 25, 100, and 250 ppm in the dynamic inhalation chambers (volume 0.25 m^3, 15 changes per hour) for 6 h or 4 weeks (6h/day, 5 days/week). The exposure chambers used on this study were constructed of stainless steel and glass. The chambers were operated in a one-pass, flow-through mode with air flow rates adequate to provide sufficient oxygen for rats and enable adequate distribution of mesitylene in the chambers. The animals were given standard laboratory diet and water *ad libitum* except for the exposure to mesitylene vapours in the dynamic inhalation chambers. Body weight of the rats was measured once per week. Table 1 gives target and actual mesitylene concentrations in toxicological chambers and the mean values of body mass of the rats, from which biological material was collected for further analysis. Chamber relative temperature and humidity were maintained at 21-23°C and 35-45%, respectively. Chamber oxygen concentration was targeted to at lest 19%. Mesitylene vapours were generated by heating liquid solvents in a washer. The desired concentrations of vapours were obtained by diluting them with air. Concentrations of solvent vapours in the exposure chamber were measured every 30 min by gas chromatography (Hewlett-Packard 5890) with a flame ionization detector (FID) using capillary column (HP-1; 30 m, 0.53 mm, 2.65 μm film thickness). The operat-

Table 1. Mean air concentrations (±SD) of mesitylene in inhalation chambers and mean values of body mass (±SD) of rats, from which the biological material was collected.

Biological material	Mesitylene target concentration in inhaled air (ppm)	Mesitylene concentration in inhaled air (ppm)	Body weight (g)
Liver, lung and kidney homogenates collected from animals after a 6-h exposure	Control	0	246 ± 9
	25	25 ± 2	254 ± 11
	100	97 ± 14	242 ± 14
	250	254 ± 20	249 ± 7
Liver, lung and kidney homogenates collected from animals after a 4-week exposure	Control	0	331 ± 17
	25	23 ± 2	311 ± 26
	100	101 ± 8	320 ± 38
	250	233 ± 16	328 ± 21
Blood collected from animals after a 6-h exposure	Control	0	251 ± 7
	25	24 ± 2	250 ± 5
	100	101 ± 7	239 ± 7
	250	240 ± 22	249 ± 10
Blood collected from animals after a 4-week exposure	Control	0	310 ± 9
	25	23 ± 2	307 ± 15
	100	101 ± 8	310 ± 33
	250	233 ± 16	309 ± 19
Urine collected from animals after a 6-h exposure	Control	0	280 ± 9
	25	25 ± 2	278 ± 10
	100	102 ± 10	335 ± 15
	250	238 ± 27	273 ± 18
Urine collected from animals after a 4-week exposure	Control	0	310 ± 10
	25	25 ± 2	295 ± 15
	100	102 ± 10	331 ± 19
	250	238 ± 27	320 ± 28

ing conditions were: carrier gas – helium, constant flow mode, column flow 10 cm^3/min; make-up gas (helium) 20 cm^3/min; air 300 cm^3/min; oven 150°C; inlet split 200°C, detector 200°C. Vapour samples (0.5 dm^3) were absorbed on solid sorbent tube (charcoal activated for gas chromatography, MERCK, 20 – 36 mesh, first layer, 100 mg and second layer, 50 mg) and desorbed with carbon disulphide (0.5 cm^3, stand 15 min).

Biological Material Collection and Analysis for Mesitylene

Samples of the liver, lung and kidney were derived from mesitylene-exposed rats after decapitation. Samples were stored in glass vessels at –80°C. The tissues were homogenized before the determination of mesitylene (ULTRA-TURRAX T8, IKA-WERKE). In about 100 mg of organ homogenate, mesitylene was quantitatively assessed. Venous blood samples drawn from the tail vein were collected 3, 15, 30, and 45 min and 1, 2, 3, 4, 5, and 6 h after termination of exposure to mesitylene vapours in heparinized glass capillary tubes with volume of 100 µl. The collected samples were stored at +5°C until the determinations. Blood and tissue mesitylene concentrations were estimated by gas chromatography combined with the headspace technique, using p-xylene as an internal standard [12]. Gas chromatography (Hewlett-Packard 5890 Series II) was equipped with FID. The working temperature of capillary column (HP-1; 30 m, 0.53 mm, 2.65 µm film thickness) was 100°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 180°C, detector 200°C. The limit of detection of mesitylene was 0.05 µg/g wet tissue and was the same for blood analysis.

Biological Material Collection and Analysis for 3,5-DMBA

Samples of the liver, lung and kidney were derived from mesitylene-exposed rats after decapitation. Samples were stored in glass vessels at –80°C. The tissues were homogenized before the determination of mesitylene metabolite (ULTRA-TURRAX T8, IKA-WERKE). Urine samples were collected 18 h starting immediately after termination of exposure in metabolic cages (TECNIPLAST). Urine samples were stored in glass vessels at –20°C. 3,5-DMBA, mesitylene metabolite, was measured in urine and tissue samples. The metabolite was measured by gas chromatography equipped with FID (Hewlett-Packard 5890 Plus, Chem Station Rev A. 08.03), using 2-naphthol (Fluka) as internal standard and 3,5-DMBA (Fluka) as standard [13]. Tissues (0.25 – 2 g) or urine samples (2 ml) were hydrolysed (2 ml 11 mol NaOH, 2 h at 95°C). After cooling, 5 ml of 6 N H_2SO_4 with 0.5 g NaCl was added and then extracted (10 ml diethyl ether, 10 min). The ether layer of 5 ml was collected after evaporation of diethyl ether, the residue was silylated for 30 min (70°C) with 0.5 ml N,O-bis(trimethylsilyl)trifluoroacetamine (BSTFA; Fluka). Samples were separated, using an HP-PONA methyl siloxane capillary column (50 m, 0.2 mm, 0.5 µm film thickness); programmed temperature: initial oven, 40°C/0.5 min; rate A, 5°C/min to 100°C, held 1 min; rate B, 3°C/min to 150°C, held 10 min; rate C, 3°C/min to 160°C, held 30 min; rate D, 20°C/min to 240°C, held 30 min. Split injection with a split ratio of 10:1 and helium at the constant flow of 0.6 ml/min was used as carrier gas. The limit of detection for all metabolites was 0.25 µg/g wet tissue and was the same for urine analysis.

Statistical Analysis

A two-way analysis of variance with simple effects to evaluate 2 x 3 factorial experiment having five observations per cell and log-linear models to describe association patterns among categorical variables (6-h and 4-week) and concentrations (25, 100, and 250 ppm) [14, 15]. When interaction was significant, Student's t-test was performed [16]. A value of $p < 0.05$ was considered to indicate statistical significance. The kinetic analysis of mesitylene in blood was calculated on an open two-compartment model, using SigmaPlot 4.0 (Jandel Corporation) for Windows.

Results

All the rats survived inhalation exposure to mesitylene. Masses of tissues collected from animals after termination of exposure to mesitylene are given in Table 2. Compared with controls, no statistically significant changes were found either in tissue masses, or in body mass of exposed animals during a 4-week exposure (Fig. 1).

Mesitylene was not found in tissues or blood of the control rats. Mesitylene concentrations in the liver, lung, kidney, and venous blood collected immediately after termination of exposure are shown in Table 3. Mesitylene concentrations in the biological material were dependent on the magnitude of exposure to mesitylene vapours. After single and repeated exposures to similar concentrations of mesitylene vapours, their highest levels were found in kidneys of the exposed rats. In the majority of cases, mesitylene concentrations were similar. The mean mesitylene partition coefficients of kidney/liver, kidney/lung, and kidney/blood after a 6-h exposure were similar within its magnitude and they decreased with increasing exposure, accounting for 25 ppm, 15.0, 14.5, and 14.5; for 100 ppm, 4.3, 4.6, and 4.3; and for 250 ppm, 1.9, 1.8, and 2.4, respectively; after four weeks of exposure they were evidently lower, 7.8, 4.1, and 5.6 for 25 ppm, but higher for 100 and 250 ppm, 5.2, 7.8, and 6.8 and 2.8, 3.2 and 4.8, respectively. After repeated exposure at 250 ppm in blood and at 25 ppm in kidneys, significatly lower con-

Table 2 Mean values (±SD) of liver, lung and kidney of rats exposed to mesitylene.

Mesitylene target concentration in inhaled air (ppm)		Liver (g)	Lung (g)	Kidney (g)
Absolute organ weight				
Animals at 6-h exposure	0 (control)	10.59 ± 1.52	1.53 ± 0.56	1.98 ± 0.18
	25	12.49 ± 1.12	1.69 ± 0.37	1.96 ± 0.08
	100	11.42 ± 0.98	1.66 ± 0.35	1.93 ± 0.17
	250	11.53 ± 1.44	1.39 ± 0.12	1.99 ± 0.15
Animals at 4-week exposure	0 (control)	12.28 ± 1.05	1.95 ± 0.39	2.17 ± 0.25
	25	10.23 ± 1.13	1.82 ± 0.48	2.28 ± 0.27
	100	11.84 ± 1.51	1.85 ± 0.37	2.28 ± 0.15
	250	11.87 ± 1.18	1.72 ± 0.15	2.44 ± 0.21
Relative organ weight				
Animals at 6-h exposure	0 (control)	4.30 ± 0,52	0.63 ± 0.18	0.80 ± 0.04
	25	4.89 ± 0.26	0.66 ± 0.13	0.77 ± 0.01
	100	4.92 ± 0.48	0.69 ± 0.13	0.80 ± 0.10
	250	4.77 ± 0.59	0.56 ± 0.06	0.80 ± 0.05
Animals at 4-week exposure	0 (control)	3.71 ± 0.20	0.58 ± 0.14	0.66 ± 0.08
	25	3.29 ± 0.15	0.58 ± 0.10	0.73 ± 0.03
	100	3.70 ± 0.17	0.53 ± 0.02	0.72 ± 0.04
	250	3.62 ± 0.29	0.59 ± 0.13	0.74 ± 0.02

Table 3. Concentrations of mesitylene in liver, lung, kidney homogenates and venous blood of rats after exposure to mesitylene.

Mesitylene target concentration in inhaled air (ppm)		Liver (µg/g tissue)	Lung (µg/g tissue)	Kidney (µg/g tissue)	Blood (µg/ml)
Animals at 6-h exposure	25	0.30 ± 0.07 [A]	0.31 ± 0.12	4.49 ± 1.93	0.31 ± 0.12
	100	3.09 ± 0.50	2.87 ± 0.57	13.32 ± 2.58	3.06 ± 0.65
	250	17.00 ± 6.08	17.36 ± 5.56	31.80 ± 9.44	13.36 ± 1.54
Animals at 4-week exposure	25	0.22 ± 0.01	0.42 ± 0.12	1.73 ± 0.30*	0.31 ± 0.08
	100	3.01 ± 0.58	1.99 ± 0.75	15.61 ± 2.14	2.30 ± 0.52
	250	12.98 ± 4.16	11.20 ± 3.61	35.97 ± 8.53	7.55 ± 1.43**
Statistics					
Main effects:					
Exposure		NS	$p < 0.05$	NS	$p < 0.001$
Concentration		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Interaction effects: Exposure by concentration		NS	$p < 0.05$	NS	$p < 0.001$
Simple effects:					
Concentration within 6-h exposure		$p < 0.001$	$p < 0.01$	$p < 0.01$	$p < 0.001$
Concentration within 4-week exposure		$p < 0.01$	$p < 0.05$	$p < 0.001$	$p < 0.05$
Exposure within concentration:					
25 ppm		NS	NS	NS	NS
100 ppm		NS	NS	NS	NS
250 ppm		NS	NS	NS	NS

[A] – mean ± SD; NS – not significant ($p > 0.05$), * $p < 0.05$; ** $p < 0.01$ – significantly different from the single exposure (Student's t-test).

centrations of mesitylene were found compared to those observed after single inhalation exposure.

3,5-DMBA was not observed in tissues or urine of control animals. Concentrations of 3,5-DMBA in the liver, lung, and kidney as well as its excretion with urine after termination of exposure to mesitylene are summarized in Table 4. Tissue concentrations of 3,5-DMBA and its excretion with urine increased with increasing magnitude of the rats' exposure. After a 6-h exposure to mesitylene at 25 and 100 ppm, 3,5-DMBA concentrations in the liver and kidney were similar and in the lung several times lower; at 250 ppm the highest values were observed in the kidney, and the calculated average partition coefficients of 3,5-DMBA in the kidney/liver and kidney/lung were 1.6 and 4.5, respectively. In the rats exposed to the same level of mesitylene vapours for 4 weeks, the high-

est values of 3,5-DMBA concentrations were found in the kidney compared to the level of its metabolite in the liver and lungs. Partition coefficients of 3,5-DMBA in kidney/lung explicitly increased (3.0, 3.5, and 4.1) with increasing exposure to mesitylene, whereas partition coefficients kidney/liver were similar (1.7, 1.4, and 1.5). Compared to single exposures at 25 and 100 ppm, 3,5-DMBA concentrations were significantly lower in the liver of rats after repeated exposure to mesitylene. After a 4-week exposure, 3,5-DMBA concentrations were significantly increased in rats compared to those observed in lungs after 6-h exposures at 100 and 250 ppm and in the liver after exposure at 250 ppm. In kidneys, 3,5-DMBA concentrations were increased after repeated exposure compared to single exposure, but the increase was not statistically significant. The increase in 3,5-DMBA excretion with urine was observed in rats after repeated exposure to mesitylene within the whole range of its concentrations under the study and it was significant in rats exposed to mesitylene at 25 ppm.

Mesitylene concentrations in blood collected from the tail vein after termination of single and repeated inhalation exposure to mesitylene vapours are given in Tables 5 and 6. During the first hour after exposure termination, mesitylene was rapidly eliminated from blood of the rats exposed to its different concentrations. The elimination was calculated on an open two-compartment model. The kinetic equations are presented in Tables 7 and 8. The half-lives of decline in blood mesitylene concentration were similar in phase I and were not dependent on the magnitude and duration of exposure, whereas in phase II, they evidently increased with increasing magnitude of exposure to mesitylene after both 6-h and 4-week exposures.

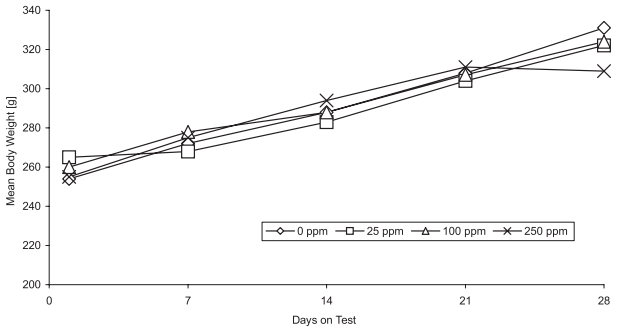

Fig. 1. Mean body weights of rats exposed to mesitylene concentrations of 0, 25, 100 or 250 ppm for 4 weeks.

Discussion and Conclusions

A number of authors have observed the increased cytochrome P-450 concentration and induced specific enzymes associated with xenobiotic metabolism not only in the liver, but also in the kidney and lung after repeated administration of mesitylene to rats by gastric tube [8, 9]. Thus it could be assumed that repeated inhalation exposure to mesitylene vapours would generate higher 3,5-DMBA concentrations in the rat liver and increased excretion of this metabolite with urine compared to a single exposure due to induction of mesitylene-metabolizing enzymes. In our study we found 3,5-DMBA concentrations in the liver of rats significantly lower after repeated exposures to me-

Table 4. Concentrations of 3,5-DMBA in liver, lung, kidney and excretion of 3,5-DMBA in urine of rats after exposure to mesitylene.

Mesitylene target concentration in inhaled air (ppm)		Liver (μg/g tissue)	Lung (μg/g tissue)	Kidney (μg/g tissue)	Urine (mg/18 h)
Animals at 6-h exposure	25	12.62 ± 1.62 [A]	2.87 ± 0.55	8.77 ± 0.99	0.52 ± 0.03
	100	26.05 ± 2.77	5.50 ± 0.55	27.01 ± 9.86	3.66 ± 0.57
	250	36.92 ± 1.61	13.39 ± 1.90	60.91 ± 19.78	10.99 ± 3.90
Animals at 4-week exposure	25	6.52 ± 0.67**	3.69 ± 1.21	11.06 ± 4.33	0.83 ± 0.15*
	100	21.67 ± 3.14**	8.90 ± 0.98**	31.03 ± 18.56	4.36 ± 0.86
	250	53.07 ± 5.41**	19.79 ± 2.70**	82.10 ± 14.48	11.92 ± 3.05
Statistics					
Main effects:					
Exposure		NS	p < 0.001	NS	NS
Concentration		p < 0.001	p < 0.001	p < 0.001	p < 0.001
Interaction effects: Exposure by concentration		p < 0.001	p < 0.005	NS	NS
Simple effects:					
Concentration within 6-h exposure		p < 0.05	p < 0.05	p < 0.05	p < 0.001
Concentration within 4-week exposure		p < 0.001	p < 0.001	p < 0.001	p < 0.001
Exposure within concentration:					
25 ppm		NS	NS	NS	NS
100 ppm		NS	NS	NS	NS
250 ppm		NS	NS	NS	NS

[A] – mean ± SD; NS – not significant (p > 0.05), * p < 0.05; ** p < 0.01 – significantly different from the single exposure (Student's t-test).

Table 5. Venous blood mesitylene concentrations after 6-h inhalation exposure to mesitylene.

Time	Mesitylene (μg/ml)		
	25 ppm	100 ppm	250 ppm
3 (min)	0.31 ± 0.12	3.06 ± 0.65	13.36 ± 1.54
15	0.26 ± 0.13	2.51 ± 0.17	13.05 ± 1.61
30	0.15 ± 0.04	2.35 ± 0.57	12.06 ± 1.23
45	0.10 ± 0.03	1.41 ± 0.27	10.53 ± 1.71
1 (h)	0.06 ± 0.02	1.35 ± 0.30	8.85 ± 0.90
2	0.04 ± 0.02	1.34 ± 0.39	6.14 ± 0.53
3	n.d.	0.79 ± 0.30	4.54 ± 0.67
4	n.d	0.57 ± 0.14	3.49 ± 1.16
5	n.d	0.38 ± 0.14	2.31 ± 0.67
6	n.d	0.20 ± 0.04	0.76 ± 0.06

Results are presented as mean ± SD; n.d. – not detected.

Table 6. Venous blood mesitylene concentrations after 4-week inhalation exposure to mesitylene.

Time	Mesitylene (μg/ml)		
	25 ppm	100 ppm	250 ppm
3 (min)	0.31 ± 0.08	2.30 ± 0.52	7.55 ± 1.43
15	0.26 ± 0.03	1.83 ± 0.47	6.51 ± 1.50
30	0.19 ± 0.02	1.57 ± 0.39	4.56 ± 0.98
45	0.17 ± 0.03	1.41 ± 0.13	3.65 ± 0.62
1 (h)	0.12 ± 0.03	1.33 ± 0.15	3.69 ± 1.25
2	0.05 ± 0.01	0.95 ± 0.22	3.14 ± 0.64
3	n.d.	0.72 ± 0.17	2.28 ± 0.19
4	n.d.	0.41 ± 0.11	1.74 ± 0.17
5	n.d.	0.39 ± 0.05	1.23 ± 0.34
6	n.d.	0.29 ± 0.13	1.14 ± 0.20

Results are presented as mean ± SD; n.d. – not detected.

Table 7. Toxicokinetics of mesitylene elimination from blood after 6-h inhalation exposure to mesitylene.

Exposure (ppm)	Elimination equation	AUC	Half-life	
			Phase I (min)	Phase II
25	$E = 0.37e^{-3.34t} + 0.08e^{-0.31t}$	0.33	12	2 h 40 min
100	$E = 2.80e^{-3.91t} + 1.50e^{-0.22t}$	5.72	11	3 h 9 min
250	$E = 15.50e^{-2.54t} + 7.00e^{-0.17t}$	32.46	16	4 h 5 min

AUC – area under curve.

Table 8. Toxicokinetics of mesitylene elimination from venous blood after 4-week exposure to mesitylene.

Exposure (ppm)	Elimination equation	AUC	Half-life	
			Phase I (min)	Phase II
25	$E = 0.30e^{-1.78t} + 0.08e^{-0.29t}$	0.40	23	2 h 23 min
100	$E = 2.50e^{-5.23t} + 1.10e^{-0.15t}$	4.84	8	4 h 37 min
250	$E = 7.50e^{-4.17t} + 3.50e^{-0.15t}$	15.67	10	4 h 37 min

AUC – area under curve.

sitylene at 25 and 100 ppm, than after single exposure. Significantly increased 3.5-DMBA concentrations in the liver were observed after the four-week exposure to mesitylene vapours at 250 ppm. Significantly increased excretion of 3.5-DMBA with urine was also observed after the four-week exposure to mesitylene vapours at 25 ppm. It is likely that significantly lower concentrations of 3,5-DMBA in the liver of the rats exposed to mesitylene vapours at 25 and 100 ppm resulted from the saturation of metabolic routes leading to 3,5-DMBA generation as well as from the lack of induction of mesytelene-metabolising enzymes, which occurred most probably only after repeated inhalation exposure to high concentrations of mesitylene vapours.

Unlike in the liver, mesitylene metabolism in the lungs of the rats was higher after the four-week inhalation exposure than that after a 6-hour inhalation almost within the whole range of mesitylene concentrations in the air. The amount of 3,5-DMBA excreted with urine of animals exposed to mesitylene vapours could probably be influenced by lungs. Other authors also observed that the role of lung tissues played in metabolism of organic solvents and drugs has a significant impact on metabolism of xenobiotics [17, 18].

Our earlier studies indicated that metabolic transformations of pseudocumene (1,2,4-TMB) in rats, leading to the production of three DMBA isomers, are specific and their intensity differs depending on the liver, lungs, and kidneys [19]. It might be thought that metabolism in lungs was responsible for significantly increased excretion of 3,5-DMBA with urine after repeated exposure of rats to mesitylene vapours. The more so that in the liver after the four-week exposure of rats to mesitylene at 25 ppm, 3,5-DMBA concentrations were significantly lower than those determined after single exposure. Metabolism in lungs of rats after repeated exposure to high concentrations of mesitylene was significantly more intensified than after single exposure despite the fact that lower concentrations of mesitylene were determined in the animal lungs. The lower mesitylene concentrations in lungs of rats after repeated exposure could be associated with significantly reduced retention of mesitylene in lungs, which most likely induced a twofold decrease in mesitylene concentration in blood of rats after repeated exposure to high concentrations of this compound. After termination of single and repeated exposures of rats to mesitylene vapours, a rapid

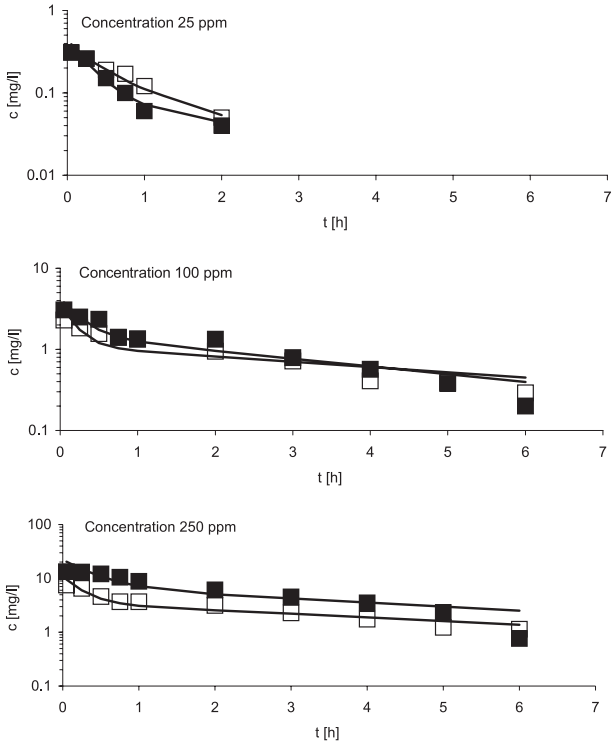

Fig. 2. Kinetic of mesitylene elimination from venous blood of rats after termination of 6-h (full rectangle) and 4-week (empty rectangle) exposures to mesitylene vapours at nominal concentrations of 25, 100 or 250 ppm.

elimination of mesitylene from blood was observed. The dynamics of its elimination rate at the same magnitude of exposure and its different duration was similar (Fig. 2). A rapid elimination of mesitylene from blood of the rats may evidence equally rapid elimination of this compound from fat tissue and probably very low accumulation in tissues. The more so that the observed elimination of mesitylene from blood was explicitly more rapid than that found of pseudocumene despite its similar concentration in blood of the animals after similar magnitude of exposure [20]. Pseudocumene is characterized by higher than mesitylene partition coefficients of blood/air, water/air, and oil/air [21]. The prolonged elimination of pseudocumene from the animal blood compared to mesitylene, may be due to a higher affinity of pseudocumene for fat present in blood

and fat tissue. The major problem related with exposure to vapours of organic solvents involves the risk for long-term disturbances in the higher functions of the central nervous system, which is characterized by a substantial amount of fat in its structure [22, 23]. Pseudocumene with its strong affinity for fat better permeates cellular walls and thus its elimination from cells is much less rapid. Consequently, a direct toxic effect of pseudocumene on humans would probably be stronger than that of its isomer – mesitylene.

In conclusion, the studies have revealed that in rats after inhalation exposure to mesitylene, 3,5-DMBA concentrations in tissues and urine as well as mesitylene concentrations in tissue and blood increased with increasing magnitude of exposure. After termination of exposure, mesitylene was eliminated from the blood of rats more rapidly. Lower concentrations of mesitylene in the lungs and blood of rats after repeated exposure to this compound at 100 and 250 ppm are most likely linked with its diminished retention in lungs. In the lungs of rats after repeated inhalation exposure to mesitylene at 100 and 250 ppm as well as in the liver at 250 ppm, 3,5-DMBA concentrations were significantly higher than after single exposure, which may be associated with the induction of mesitylene-metabolizing enzymes. Mesitylene metabolism in the lungs of rats after repeated exposure to low concentrations probably had a significant impact on the enhanced urinary excretion of 3,5-DBMA.

References

1. WESOŁOWSKI W., CZERSKI B. Exposure to organic solvent vapours in the production of lacquers and paints. Med. Pr. **XLIII** (2), 129, **1992** [in Polish].
2. WESOŁOWSKI W., GROMIEC J.P. Prediction of exposure to engine fuel vapours. Med. Pr. **XLVII** (1), 19, **1996** [in Polish].
3. WESOŁOWSKI W., GROMIEC J.P. Occupational exposure in Polish paint and lacquer industry. Int. J. Occup. Med. Environ. Health **10** (1), 79, **1997.**
4. KORSAK Z., RYDZYŃSKI K., JAJTE J. Respiratory irritative effects of trimethylbenzenes: an experimental animal study.Int. J. Occup. Med. Environ. Health **10** (3), 303, **1997**.
5. KORSAK Z., RYDZYŃSKI K. Neurotoxic effects of acute and subchronic inhalation exposure to trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) in rats. Int. J. Occup. Med. Environ. Health **9** (4), 341, **1996**.
6. WIGLSZ R., DELĄG G., MIKULSKI P. Serum enzymes activity of mesitylene vapour treated rats. Bull. Inst. Marit. Top. Med. Gdynia **26** (3-4), 303, **1975**.
7. WIGLSZ R., KIENITZ M., DELĄG G., GAŁUSZKO E., MIKULSKI P. Peripheral blood of mesitylene vapour treated rats. Bull. Inst. Marit. Top. Med. Gdynia **26** (3-4), 315, **1975**.
8. PYYKKÖ K. Effects of methylbenzenes on microsomal enzymes in rat liver, kidney and lung. Biochim. Biophys. Acta **633** 1, **1980**.
9. ZAJWORONIUK H., RZECZYCKI W. Effect of mesitylene on ethanol metabolism in rats liver microsomes. Acta Biochimica Polonica **39** (4), 335, **1992**.
10. MIKULSKI P.I., WIGLUSZ R. The comparative metabolism of mesitylene, pseudocumene and hemimelitene in rats. Toxicol. Appl. Pharmacol. **31**, 21, **1975**.
11. JÄRNBERG J., JOHANSON G., LÖF A., STÅHLBOM B. Urinary excretion of dimethylhippuric acids in humans after exposure to trimethylbenzenes. Int. Arch. Occup. Environ. Health. **69**, 491, **1997**.
12. RADZIKOWSKA-KINTZI H., JAKUBOWSKI M. Internal standardization in the Head Space analysis of organic solvent in blood. Int. Arch. Occup. Environ. Health **49** 115, **1981**.
13. KOSTRZEWSKI P., WIADERNA-BRYCHT A., CZERSKI B. Biological monitoring of experimental human exposure to trimethylbenzene. Sci. Total Environ. **199** 73, **1997**.
14. WINER B.J., BROWN D., MICHELS K. Statistical Principles in Experimental Design. McGraw-Hill Book Company, Inc., New York, **1991**.
15. AGRESTI A., Categorical Data Analysis. Wiley, New York, **1990**.
16. SENDECOR G.W., COCHRAN W.G. Statistical methods, 6th edn., Iowa State University Press, Ames, IA, **1967**.
17. POWLEY M. W., CARLSON G. P. Species comparison of hepatic and pulmonary metabolism of benzene. Toxicology **139** 207-217. **1999**.
18. DE KANTER R., DE JAGER M. H., DRAAISMA A. L., JURVA J. U., OlINGA P., MEIJER D. K. F., GROOTHUIS G. M. M. Drug-metabolizing activity of human and rat liver, lung, kidney and intestine silces. Xenobiotica **32** (5), 349, **2002**.
19. ŚWIERCZ R., WĄSOWICZ W., MAJCHEREK M.: Dimethylbenzoic acid isomers in lung, kidney, liver and urine of rats after single and repeated inhalation exposure to pseudocumene. Pol. J. Environ. Studies **14** (4), **2005.** [in press]
20. ŚWIERCZ R., WIADERNA D., WĄSOWICZ W., RYDZYŃSKI K. Pseudocumene in brain, liver, lung and blood of rats after single and repeated inhalation exposure. Int. J. Occup. Med. Environ. Health **16** (1), 61, **2003**.
21. JÄRNBERG J., JOHANSON G. Liquid/air partition coefficients of the trimethylbenzenes. Toxicology and Industrial Health **11** (1), 81, **1995**.
22. FOO S. C., JEYARATNAM J., KOH D. A. D. Chronic neurobehavioral effects of toluene. Br. J. Ind. Med. **47**, 480, **1990**.
23. IKEDA M. Public health problems of organic solvents. Toxicology Letters, **64/65** 191, **1992**.