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HEMIMELLITENE (1,2,3-TRIMETHYLBENZENE) IN THE LIVER, LUNG, KIDNEY, AND BLOOD, AND DIMETHYLBENZOIC ACID ISOMERS IN THE LIVER, LUNG, KIDNEY AND URINE OF RATS AFTER SINGLE AND REPEATED INHALATION EXPOSURE TO HEMIMELLITENE

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Abstract
Objectives: The aim of the study has been to explore hemimellitene distribution in blood, liver, lung and kidney as well as toxicokinetics of its elimination from blood of rats after single and repeated inhalation exposure to this compound. Tissue distribution and excretion with urine of 2-dimethylbenzoic acids (2,3-DMBA and 2,6-DMBA) were also evaluated. **Material and Methods:** Male outbred IMP:WIST rats were used in the experiment. The animals were exposed to hemimellitene vapors at the nominal concentration of 25 ppm, 100 ppm, and 250 ppm in the dynamic inhalation chambers for 6 h for single exposure purpose and for 4 weeks (6 h/day for 5 day/week) for repeated exposure purposes. **Results:** Significantly lower concentrations of hemimellitene were detected in the blood and tissues of animals after repeated inhalation exposure of animals to hemimellitene vapors, which points to reduced retention of the chemical in the lungs of the experimental rats. The trend of hemimellitene elimination from the blood depended solely on exposure intensity, irrespective of exposure time, both after single and repeated exposure. As regards the 2 determined hemimellitene metabolites, the major trend of the metabolic transformation involved formation of 2,3-DMBA. **Conclusions:** The significantly higher urinary 2,3-DMBA concentration after repeated exposure shows that hemimellitene induces enzymatic processes in the rat.

Key words:
Rats, Hemimellitene, Pseudocumene, Mesitylene, Inhalation, Toxicokinetic

INTRODUCTION

The volatile organic compounds (VOC) including a very wide use of numerous petroleum products are hazardous to the human health. Trimethylbenzenes (TMBs) belong to a wide category of VOC compounds that are contained in many petroleum products. Hemimellitene (1,2,3-TMB), in addition to pseudocumene (1,2,4-TMB) and mesitylene (1,3,5-TMB), is one of TMB isomers. The continuing growth of petroleum

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products manufacture is directly responsible for an extensive exposure of people involved in their manufacturing and processing in various sectors [1–3]. Many countries have issued regulations governing hygienic standard values for TMB isomers in workplace atmospheres ranging ca. 20–25 ppm [4,5]. However, based on the effects observed in the respiratory and central nervous systems in animals, the threshold limit values of at least 10 ppm should be considered in the occupational exposure to TMB isomers [6].

Hemimellitene, together with some VOCs in the indoor air pollutants, have been reported to induce irritation syndrome, deteriorated performance, and cardiovascular and pulmonary effects [7]. Hemimellitene and organic compounds in urban areas are released into the atmosphere from vaporization of gasoline and diesel fuel and in vehicle exhaust [8–10]. The children living in an ecologically poor area showed sensitization to benzene derivatives, such as TMB, and a high incidence of allergic diseases [11]. Based on a comprehensive review on TMB, the reference concentration (RfC) was calculated and the value of 0.6 ppm was adopted [12].

TMB isomers were similar in their physical properties, such as the boiling point, density, flash point and refraction index [13]. Blood/air, water/air and oil/air partition coefficients for the 3 isomers of TMB were determined *in vitro*. It is worth noting that all those values increase in the order: 1,3,5-TMB < 1,2,4-TMB < 1,2,3-TMB [14].

Some experimental studies on cell cultures and experimental animals revealed significant differences in the toxicity of the TMB isomers. In the Ames test, only the hemimellitene was found to produce mutagenic effects in *Salmonella* Typhimurium cells [15]. Neurotoxic effects of TMB isomers in male rats were investigated in conditions of acute and subchronic inhalation exposure. Neurotoxic effect of hemimellitene was more pronounced than that of pseudocumene and mesitylene [16].

The toxicokinetic data of experimental human exposure to TMB isomers has made it possible to estimate the proposed value of biological exposure limit (BEL). The values of BEL for urinary metabolites of hemimelitene and mesitylene were low in comparison to pseudocumene [17]. The Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area recommends – in terms of the biological tolerance value – determination of dimethylbenzoic acids (sum of all isomers after hydrolysis) in urine of people exposed to TMB isomers [18].

The aim of the study has been to explore the distribution of hemimellitene and dimethylbenzoic acids (DMBA) isomers in tissues as well as the kinetics of hemimellitene excretion with blood and DMBA isomers excretion with urine in rats after single and repeated inhalation exposures to hemimellitene at concentrations of 25 ppm, 100 ppm, and 250 ppm. The study sums up our earlier investigations exploring the toxicokinetics of mesitylene and pseudocumene in rats after single and repeated inhalation exposure to TMB isomer vapors [19–22].

MATERIAL AND METHODS

Chemicals

Hemimellitene (No. CAS: 526-73-8) was supplied by Aldrich (Cat. No. T7,320-2), its purity was ≥ 90%. The conversion factors for hemimellitene: 1 ppm ~ 4.92 mg/m^3, 1 mg/m^3 ~ 0.20 ppm.

Animals and inhalation exposure monitoring

Male Wistar rats IMP:WIST (5 animals in each group), body weight of 200–360 g (2–3 months old) were exposed to hemimellitene vapors at the nominal concentration of 0 ppm, 25 ppm, 100 ppm, or 250 ppm in the dynamic inhalation chambers (volume of 0.25 m^3) for 6 h or 4 weeks (6h/day, 5 days/week). In the inhalation chambers, the animals were placed in stainless-steel wire mesh cages [22]. The animals were given standard laboratory diet and water *ad libitum*, except for the exposure to hemimellitene vapors in the dynamic inhalation chambers. Body weight of the rats was measured once a week.

The Local Ethics Committee for Experiments on Animals approved the study protocol (Opinion No. Ł/BD/269).

Hemimellitene vapors were generated by heating liquid solvents in a washer. The desired concentrations of vapors were obtained by diluting them with the air. Concentrations of solvent vapors in the exposure chamber were measured every 30 min by gas chromatography (Hewlett-Packard 5890) with a flame ionization detector (FID) using a capillary column (HP-1; 30 m × 0.53 mm × 2.65 μm film thickness). The operating 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. Vapor samples (0.5 dm^3) were absorbed on a solid sorbent tube (charcoal activated for gas chromatography, MERCK, 20–36 mesh, 1st layer, 100 mg and 2nd layer, 50 mg) and desorbed with carbon disulfide (0.5 cm^3, 15 min).

Biological material collection and analysis for hemimellitene

Samples of the liver, lung and kidney (5 animals in each group) were collected from hemimellitene-exposed rats immediately after termination of exposure and decapitation. Samples were stored in glass vessels at –80°C. The tissues were homogenized before the determination of hemimellitene. In about 100 mg of organ homogenate, hemimellitene was quantitatively assessed. Venous blood samples drawn from the tail vein (5 animals in each group) were collected 3 min, 15 min, 30 min, and 45 min and 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after termination of exposure to hemimellitene vapors into 100 μl heparinized glass capillary tubes. The collected samples were stored at +5°C until the determinations. Blood and tissue hemimellitene concentrations were estimated by gas chromatography combined with the headspace technique, using p-xylene as an internal standard [23]. Gas chromatography (Hewlett-Packard 5890 Series II) was equipped with FID. The working temperature of the 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 hemimellitene was 0.05 μg/g of wet tissue and was the same as for blood analysis.

Biological material collection and analysis for DMBA isomers

Samples of the liver, lung and kidney (5 animals in each group) were collected from hemimellitene-exposed rats immediately after termination of exposure and decapitation. Samples were stored in glass vessels at –80°C. The tissues were homogenized before the determination of hemimellitene metabolite. Urine samples (5 animals in each group) were collected 18 h after termination of exposure in metabolic cages (TECNIPLAST). Urine samples were stored in glass vessels at –20°C.

Two metabolites of hemimellitene were measured in urine and tissue samples: 2,6-dimethylbenzoic acid (2,6-DMBA) and 2,3-dimethylbenzoic acid (2,3-DMBA). The metabolites were measured by means of gas chromatography equipped with FID (Hewlett-Packard 5890 Plus, Chem Station Rev A. 08.03), using 2-naphthol (Fluka) as internal standard, 2,3-DMBA (Fluka) and 2,6-DMBA (Fluka) as standards [17].

Tissues (0.25–2 g) or urine samples (2 ml) were hydrolyzed (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 a HP-PONA methyl siloxane capillary column (50 m × 0.2 mm × 0.5 μm film thickness); the 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 of wet tissue and was the same as for urine analysis.

Statistical analysis

A 2-way analysis of variance with simple effects to evaluate 2×3 factorial experiment having 5 observations per cell and log-linear models was used to describe association patterns among categorical variables (6-h and 4-week) and concentrations (25 ppm, 100 ppm, and 250 ppm) [24,25]. When interaction was significant, Student's t-test was performed [26]. A value of $p < 0.05$ was considered to indicate statistical significance. The kinetic analysis of hemimellitene in blood was calculated on an open 2-compartment model, using SigmaPlot 4.0 (Jandel Corporation) for Windows.

RESULTS

All the rats survived inhalation exposure to hemimellitene. The Table 1 gives nominal and actual hemimellitene concentrations in toxicological chambers and the mean values of body mass of the rats, from which biological material was collected for further analysis. The chamber relative temperature and humidity were maintained at 20–23°C and 30–45%, respectively.

Masses of tissues collected from animals after termination of exposure to hemimellitene are given in the 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 (Figure 1).

Hemimellitene was not found in tissues or blood of the control rats. Hemimellitene concentrations in the liver, lung, kidney, and venous blood collected immediately after termination of exposure are shown in the Table 3 and 4.

Table 1. Air concentrations of hemimellitene in inhalation chambers and body mass of rats

Biological material	Hemimellitene target concentration in inhaled air [ppm]	Hemimellitene concentration in inhaled air [ppm] (M±SD)	Animals treated [n]	Body weight [g] (M±SD)
Liver, lung and kidney homogenates				
after 6-h exposure	control	0	5	226±4
	25	25±5	5	207±5
	100	105±10	5	215±20
	250	242±10	5	205±5
after 4-week exposure	control	0	5	309±26
	25	25±2	5	280±17
	100	97±7	5	323±28
	250	246±16	5	310±13
Blood				
after 6-h exposure	control	0	5	210±7
	25	28±2	5	223±10
	100	110±9	5	214±11
	250	234±26	5	208±5

Table 1. Air concentrations of hemimellitene in inhalation chambers and body mass of rats – cont.

Biological material	Hemimellitene target concentration in inhaled air [ppm]	Hemimellitene concentration in inhaled air [ppm] (M±SD)	Animals treated [n]	Body weight [g] (M±SD)
Blood – cont.				
after 4-week exposure	control	0	5	311±10
	25	24±3	5	333±23
	100	104±6	5	321±22
	250	243±13	5	292±20
Urine				
after 6-h exposure	control	0	5	250±9
	25	21±1	5	243±10
	100	99±3	5	251±15
	250	225±13	5	238±14
after 4-week exposure	control	0	5	310±10
	25	25±2	5	305±15
	100	97±7	5	317±22
	250	246±16	5	284±23

M – mean; SD – standard deviation.

Table 2. Absolute and relative weight of liver, lung and kidney of rats after exposure to hemimellitene

Hemimellitene target concentration in inhaled air [ppm]	Absolute organ weight [g] (M±SD)			Relative organ weight [g/100 g b.w.] (M±SD)		
	liver	lung	kidney	liver	lung	kidney
6-h exposure						
control	9.48±0.63	1.31±0.13	1.83±0.19	4.50±0.41	0.62±0.08	0.87±0.10
25	9.25±0.46	1.17±0.30	1.93±0.15	4.47±0.26	0.57±0.14	0.93±0.07
100	9.09±1.06	1.34±0.29	1.82±0.11	4.27±0.72	0.63±0.17	0.85±0.04
250	9.37±0.61	1.21±0.20	1.87±0.16	4.57±0.35	0.59±0.09	0.91±0.08
4-week exposure						
control	12.63±1.02	1.47±0.24	2.28±0.19	4.09±0.27	0.47±0.06	0.74±0.08
25	11.61±1.62	1.63±0.32	2.07±0.08	4.14±0.50	0.58±0.10	0.74±0.01
100	13.37±2.37	1.54±0.33	2.51±0.32	4.11±0.42	0.48±0.09	0.77±0.04
250	13.15±1.12	1.43±0.33	2.49±0.17	4.24±0.31	0.46±0.09	0.80±0.05

Abbreviations as in Table 1.

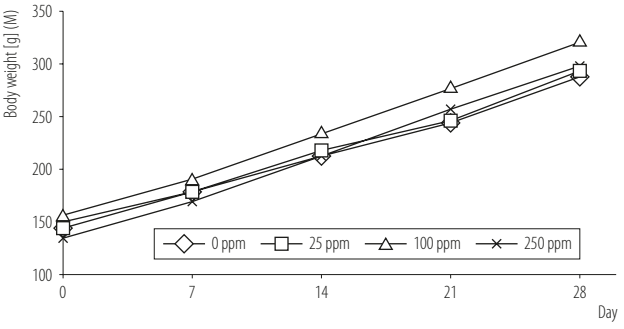

Fig. 1. Mean body weights of rats exposed to hemimellitene at 0 ppm (N = 5), 25 ppm (N = 5), 100 ppm (N = 5) and 250 ppm (N = 5) for 4 weeks

Hemimellitene concentrations in the biological material were dependent on the magnitude of exposure to hemimellitene vapors. After single and repeated exposures to similar concentrations of hemimellitene vapors, their highest levels were found in kidneys of the exposed rats.

The mean hemimellitene partition coefficients of kidney/liver after a 6-h exposure were similar, 1.7, 1.9 and 1.5 for 25 ppm, 100 ppm and 250 ppm, respectively. The mean hemimellitene partition coefficients of kidney/lung, and kidney/blood after a 6-h

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

Hemimellitene target concentration in inhaled air	Hemimellitene concentration (M±SD)			
	liver [μg/g tissue]	lung [μg/g tissue]	kidney [μg/g tissue]	blood [μg/ml]
6-h exposure				
25 ppm	1.66±0.48	0.62±0.08	2.81±0.40	0.76±0.09
100 ppm	4.20±0.85	2.57±0.40	7.78±3.17	3.82±0.94
250 ppm	20.75±3.30	18.73±2.81	31.16±3.84	10.73±1.30
4-week exposure				
25 ppm	1.18±0.28	0.83±0.11**	4.55±0.32***	0.58±0.08**
100 ppm	2.68±0.76*	2.17±0.24	10.07±0.67	3.14±0.61
250 ppm	11.30±3.42**	17.28±6.02	29.99±8.00	6.87±1.05***

Abbreviations as in Table 1.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ – significantly different from the single exposure (Student's t-test).

Table 4. Statistics of hemimellitene concentration in liver, lung, kidney homogenates and venous blood of rats after exposure to hemimellitene

Statistics	p			
	liver	lung	kidney	blood
Main effects				
exposure	< 0.001	n.s.	n.s.	< 0.001
concentration	< 0.001	< 0.001	< 0.001	< 0.001
Interaction effect				
exposure by concentration	< 0.001	n.s.	n.s.	< 0.001
Simple effects				
concentration within 6-h exposure	< 0.001	< 0.001	< 0.001	< 0.001
concentration within 4-week exposure	n.s.	< 0.001	< 0.010	< 0.050

Table 4. Statistics of hemimellitene concentration in liver, lung, kidney homogenates and venous blood of rats after exposure to hemimellitene – cont.

Statistics	p			
	liver	lung	kidney	blood
Exposure within concentration				
25 ppm	n.s.	n.s.	n.s.	n.s.
100 ppm	n.s.	n.s.	n.s.	n.s.
250 ppm	< 0.050	n.s.	n.s.	n.s.

n.s. – not statistically significant ($p > 0.05$).

exposure were decreased with increasing exposure, and were: 4.5 and 3.7 for 25 ppm; 3.0 and 2.0 for 100 ppm; and, 1.7 and 2.9 for 250 ppm. After 4 weeks of exposure, partition coefficients of kidney/liver, kidney/lung, and kidney/blood were evidently lower with increasing exposure, 3.9, 5.5, 7.8 for 25 ppm; 3.8, 4.6, 3.2 for 100 ppm; and 2.7, 1.7, 4.4 for 250 ppm, respectively.

After repeated exposure at 25 ppm and 250 ppm in blood and at 100 ppm and 250 ppm in liver, significantly lower concentrations of hemimellitene were found as compared to those observed after single inhalation exposure. After repeated exposure at 25 ppm in lung and kidneys, significantly higher levels of hemimellitene were found as compared to those after single inhalation exposure.

Hemimellitene concentrations in blood collected from the tail vein after termination of single and repeated inhalation exposure to hemimellitene vapors are given in the Tables 5 and 6.

During the 1st hour after exposure termination, hemimellitene was rapidly eliminated from blood of the rats exposed to its different concentrations. The elimination was calculated using a 2-compartment model. The kinetic equations are presented in the Tables 7 and 8.

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

Time [h (min)]	Hemimellitene concentration [μg/ml] (M±SD)		
	25 ppm exposure	100 ppm exposure	250 ppm exposure
0 (3)	0.76±0.09	3.82±0.94	10.73±1.30
0 (15)	0.75±0.08	3.21±0.91	9.56±1.40
0 (30)	0.67±0.14	2.83±0.35	7.09±1.70
0 (45)	0.52±0.14	2.76±0.47	6.73±1.16
1 (0)	0.50±0.03	2.29±0.34	7.71±0.58
2 (0)	0.45±0.15	1.63±0.16	5.10±0.62
3 (0)	0.26±0.06	1.32±0.23	3.50±0.71
4 (0)	0.18±0.08	0.87±0.03	3.13±0.45
5 (0)	0.12±0.10	0.55±0.10	1.51±0.39
6 (0)	0.07±0.05	0.48±0.14	1.25±0.30

Abbreviations as in Table 1.

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

Time [h (min)]	Hemimellitene concentration [μg/ml] (M±SD)		
	25 ppm exposure	100 ppm exposure	250 ppm exposure
0 (3)	0.58±0.09	3.14±0.70	6.87±1.05
0 (15)	0.40±0.07	2.77±0.50	6.04±0.80
0 (30)	0.42±0.10	2.03±0.15	4.56±0.73
0 (45)	0.43±0.10	1.78±0.18	4.02±0.91
1 (0)	0.43±0.13	1.80±0.24	3.45±0.74
2 (0)	0.30±0.06	1.38±0.30	3.04±0.32
3 (0)	0.30±0.03	1.03±0.15	2.43±0.37
4 (0)	0.25±0.03	0.85±0.10	2.04±0.67
5 (0)	0.19±0.06	0.82±0.16	1.66±0.36
6 (0)	0.18±0.07	0.75±0.21	1.56±0.37

Abbreviations as in Table 1.

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

Exposure [ppm]	Elimination (E) equation	$AUC_{0\rightarrow 6\ h}$ [mg×h/l]	Half-life	
			phase I [min]	phase II [h (min)]
25	$E = 0.60e^{-3.04t}+0.52e^{-0.23t}$	1.89	14	3 (4)
100	$E = 3.05e^{-2.23t}+2.00e^{-0.19t}$	8.53	19	3 (42)
250	$E = 9.00e^{-1.28t}+4.00e^{-0.13t}$	23.70	32	5 (20)

AUC – area under curve.

Table 8. Toxicokinetics of hemimellitene elimination from venous blood after a 4-week inhalation exposure to hemimellitene

Exposure [ppm]	Elimination (E) equation	$AUC_{0\rightarrow 6\ h}$ [mg×h/l]	Half-life	
			phase I [min]	phase II [h (min)]
25	$E = 0.58e^{-23.35t}+0.40e^{-0.12t}$	1.75	2	5 (52)
100	$E = 2.70e^{-5.09t}+1.80e^{-0.15t}$	7.66	8	4 (34)
250	$E = 7.00e^{-3.24t}+3.00e^{-0.09t}$	16.09	13	7 (58)

AUC – area under curve.

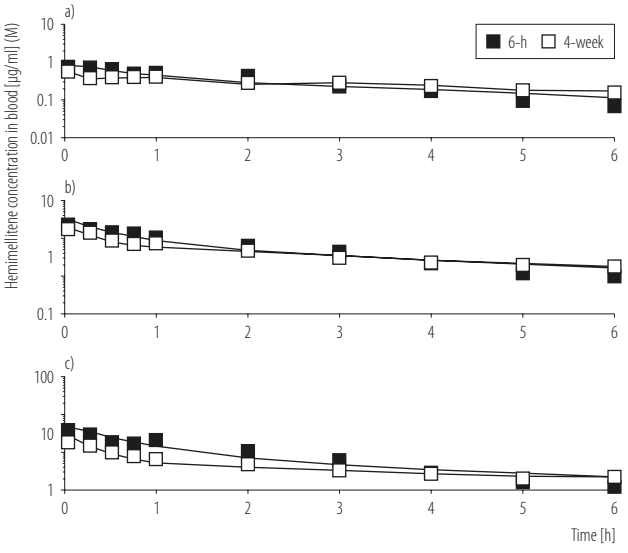

Fig. 2. Kinetics of hemimellitene elimination from venous blood of rats after termination of 6-h and 4-week exposures to hemimellitene vapors at nominal concentration of: a) 25 ppm (N = 5), b) 100 ppm (N = 5) and c) 250 ppm (N = 5)

Phase I and II half-lives of hemimellitene in blood were dependent on the magnitude and duration of exposure to hemimellitene. The half-lives evidently increased with increasing magnitude of exposure to hemimellitene after both 6-h and 4-week exposures. The trend of its elimination rate at the same magnitude of exposure and its different duration was similar (Figure 2).

The DMBA isomers were not observed in tissues or urine of control animals. The 2,6-DMBA was not observed in the liver, lung or kidney of rats after termination of exposure to hemimellitene. Concentrations of 2,3-DMBA in the liver, lung, and kidney after termination of exposure to hemimellitene are summarized in the Table 9 and 10.

After single and repeated exposure to hemimellitene at 25 ppm and 100 ppm, the 2,3-DMBA was not observed in the lung of rats. The 2,3-DMBA concentrations in the liver and kidney were similar, and its concentrations increased with increasing magnitude of the exposure of the rats. As compared to single exposures at 100 ppm and 250 ppm, 2,3-DMBA concentrations were significantly lower in the liver and kidney of rats after repeated exposure to hemimellitene. However, after 6-h and 4-week exposures to hemimellitene at 25 ppm, 100 ppm and 250 ppm, average partition coefficients of 2,3-DMBA in kidney/liver were similar (after a 6-h exposure 0.72, 1.11 and 1.04; after 4-week exposure 0.80, 0.81 and 1.03, respectively).

Concentrations of 2,3-DMBA and 2,6-DMBA in the urine after termination of exposure to hemimellitene are given in the Table 11 and 12.

Urine concentration of DMBA isomers increased with increasing magnitude of the exposure of the rats. As

Table 9. Concentration of 2,3-dimethylbenzoic acid (2,3-DMBA) in liver, lung and kidney of rats after exposure to hemimellitene

Hemimellitene target concentration in inhaled air	2,3-DMBA concentration [µg/g tissue] (M±SD)		
	liver	lung	kidney
6-h exposure			
25 ppm	7.68±1.64	n.d.	5.52±0.77
100 ppm	21.19±0.59	n.d.	23.59±3.33
250 ppm	27.66±3.62	3.23±0.56	28.69±6.55
4-week exposure			
25 ppm	8.54±1.17	n.d.	6.84±0.76
100 ppm	13.78±2.84**	n.d.	11.19±1.58***
250 ppm	17.93±4.33**	2.82±0.44	18.53±2.31*

n.d. – not detected. Other abbreviations as in Table 1 and 3.

Table 10. Statistics of 2,3-dimethylbenzoic acid (2,3-DMBA) concentration in liver, lung and kidney of rats after exposure to hemimellitene

Statistics	p		
	liver	lung	kidney
Main effects			
exposure	< 0.001		< 0.001
concentration	< 0.001		< 0.001
Interaction effect			
exposure by concentration	< 0.001		< 0.001
Simple effects			
concentration within 6-h exposure	< 0.001		< 0.001
concentration within 4-week exposure	n.s.		n.s.
Exposure within concentration			
25 ppm	n.s.		n.s.
100 ppm	n.s.		< 0.050
250 ppm	< 0.050		n.s.

n.s. – not statistically significant ($p > 0.05$).

Table 11. Urinary excretion of dimethylbenzoic acid (DMBA) isomers after exposure to hemimellitene

Hemimellitene target concentration in inhaled air	Urine [mg/18 h] (M±SD)	
	2,6-DMBA	2,3-DMBA
6-h exposure		
25 ppm	n.d.	0.07±0.01
100 ppm	0.17±0.03	0.58±0.06
250 ppm	0.59±0.26	2.19±0.66
4-week exposure		
25 ppm	n.d.	0.11±0.005***
100 ppm	0.39±0.13*	1.60±0.40**
250 ppm	0.58±0.14	2.79±0.76

Other abbreviations as in Table 1 and 3.

compared to single exposures at 100 ppm for 2,6-DMBA and 50 ppm and 100 ppm for 2,3-DMBA, the concentrations were significantly high in the urine of rats after repeated exposure to hemimellitene.

DISCUSSION

The use of biomarkers in toxicology is becoming increasingly important [27]. Quantitative analysis of volatile neurotoxic chemicals in blood is a good marker of their

Table 12. Statistics of urinary excretion of dimethylbenzoic acid (DMBA) isomers after exposure to hemimellitene

Statistics	p	
	2,6-DMBA	2,3-DMBA
Main effects		
exposure	n.s.	< 0.005
concentration	< 0.001	< 0.001
Interaction effect		
exposure by concentration	n.s.	n.s.
Simple effects		
concentration within 6-h exposure	< 0.050	< 0.050
concentration within 4-week exposure	n.s.	< 0.001
Exposure within concentration		
25 ppm		n.s.
100 ppm	n.s.	n.s.
250 ppm	n.s.	n.s.

n.s. – not statistically significant ($p > 0.05$).

effective doses. Blood concentrations of the solvents were found to be dose-related in the experimental animals after single administration of the TMB isomers [28]. The current study and our earlier investigations on the assessment of pseudocumene and mesitylene concentrations in rat blood and tissues that were performed in similar conditions point to distinct differences of TMB isomer levels in the case of single and repeated inhalation exposures [20,22].

The Table 13 presents trends of pseudocumene, mesitylene and hemimellitene concentration variations in selected tissues, including blood of animals exposed to single, as relative to repeated exposure to TMB isomers.

When analyzing single vs. repeated exposure to one of the studied 3 TMB isomers, the concentration of hemimellitene in blood and liver after repeated exposure was significantly lower as compared to single exposure practically for all 3 exposure levels. A similar, although less intensive pattern of changes was observed after exposure to mesitylene. The patterns of changes of pseudocumene, concentrations in the solid tissues and blood were ambivalent or varied at similar levels that depended on the exposure magnitude instead of inhalation exposure time.

If it is assumed that the neurotoxic effect after 4-week exposure depends on the level of TMB isomers in blood, then the most potent isomer is pseudocumene, the blood level of which was dependent on exposure magnitude and practically independent of exposure time.

The Table 14 sums up toxicokinetic characteristics of TMB isomers.

After comparing the toxicokinetic parameters of TMB isomers, it is evident that there are no differences in the area under the curve (AUC) and half-life values calculated from the kinetic equations after 6-h and 4-week exposures to TMB isomers at 25 ppm and 100 ppm. The AUC evidently decreased after 4-week exposure as compared to 6-h exposure to TMB isomers at 250 ppm. The trend of elimination of TMB isomers from blood of rats exposed to TMB isomers at 250 ppm is specific for each solvent.

Table 13. Changes of trimethylbenzene (TMB) isomers in tissues and blood of rats after 6-h vs. 4-week exposure to isomers of TMB

TMB isomer	Changes of TMB isomers concentration [%]								
	25 ppm exposure			100 ppm exposure			250 ppm exposure		
	lung	blood	liver	lung	blood	liver	lung	blood	liver
Pseudocumene[a]	9 ↑	6 ↑	2 ↑	10 ↓	24 ↑	58 ↓↓	19 ↑	3 ↓	20 ↓
Mesitylene[b]	35 ↑	0	27 ↓	31 ↓	25 ↓	3 ↓	35 ↓	43 ↓↓	24 ↓
Hemimellitene	34 ↑↑	24 ↓↓	29 ↓	29 ↑	18 ↓	36 ↓↓	4 ↓	36 ↓↓	46 ↓↓

↑ – insignificant increase; ↑↑ – significant increase; ↓ – insignificant decrease; ↓↓ – significant decrease.
[a] Świercz et al., 2003 [20]; [b] Świercz et al., 2006 [22].

Table 14. Toxicokinetics of trimethylbenzene (TMB) isomers elimination from venous blood after 6-h or 4-week exposure to isomers of TMB

TMB isomer	Toxicokinetics of TMB isomers					
	25 ppm exposure		100 ppm exposure		250 ppm exposure	
	6-h	4-week	6-h	4-week	6-h	4-week
Pseudocumene[a]						
$AUC_{0\rightarrow 6\,h}$ [mg×h/l]	1.25	0.92	7.02	8.14	53.74	23.33
half-life [h (min)]						
phase I	0 (10)	0 (9)	0 (28)	0 (32)	0 (57)	1 (8)
phase II	3 (51)	2 (53)	5 (20)	5 (47)	17 (20)	9 (54)
Mesitylene[b]						
$AUC_{0\rightarrow 6\,h}$ [mg×h/l]	0.33	0.40	5.72	4.84	32.46	15.67
half-life [h (min)]						
phase I	0 (12)	0 (23)	0 (11)	0 (8)	0 (16)	0 (10)
phase II	2 (40)	2 (23)	3 (9)	4 (37)	4 (5)	4 (37)
Hemimellitene						
$AUC_{0\rightarrow 6\,h}$ [mg×h/l]	1.89	1.75	8.53	7.66	23.70	16.09
half-life [h (min)]						
phase I	0 (14)	0 (2)	0 (19)	0 (8)	0 (32)	0 (13)
phase II	3 (4)	5 (52)	3 (42)	4 (34)	5 (20)	7 (58)

AUC – area under curve.
[a] Świercz et al., 2002, 2003 [19,20]; [b] Świercz et al., 2006 [22].

After 4-week exposure, as compared to 6-h exposure, the value of half-life calculated for mesitylene phase II was similar, while for pseutocumene and hemimellitene, it was lower and higher, respectively.

The reduction of mesitylene and hemimellitene concentrations in the blood in the case of repeated exposure as compared to single-exposed animals is likely to result in a weaker neurotoxic activity of those compounds in

the case of repeated exposure as compared to single exposures. The more so that in many studies involving repeated inhalation exposure to TMB isomers, the behavioral changes of experimental animals pointed to the non-linear character of the concentration/effect relationships for those compounds [29–30]. For acute inhalation exposure to TMB isomers, the neurotoxic effects in rats were concentration-dependent for pesudocumene, hemimellitene and mesitylene [16].

Low concentrations of TMB isomers were observed to induce the behavioral changes. The authors suggest that prolonged exposures for low TMB isomer concentrations are likely to produce significant changes in the function of the rats' central nervous system [31].

In our earlier studies, we had detected significant changes in the results of neurobehavioral tests, during which rats exposed by inhalation to pseudocumene or hemimellitene were given amphetamine, which is a strong psychostymulant. For each of the 2 solvents, the concentration-effect relationship was nonlinear. Out of the 3 concentrations used: 25 ppm, 100 ppm and 250 ppm, the concentration of 100 ppm appeared to be the most effective. The alterations induced by exposure to 100 ppm hemimellitene or pseudocumene go in opposite directions: exposure to hemimellitene results in increased, and exposure to pseudocumene in decreased behavioral sensitivity to amphetamine and susceptibility to sensitization by a repeated amphetamine treatment [32]. A reduction of hemimellitene and pseudocumene concentration observed after repeated exposure in the blood of rats exposed to high concentrations of the solvents could be responsible for no dose/response relationship in the behavioral tests of the animals.

Differences in the absorption and specific metabolic activity of TMB isomers in rats affected formation of the metabolites in the biological material and the removal of the metabolites with urine. The Table 15 shows trends in TMB metabolite changes in the biological material after 4-week exposure as compared to 6-h inhalation exposure to the tested TMB isomer. Lower concentrations of pseudocumene metabolites in urine found in our study 4 weeks after exposure termination as compared with

Table 15. Changes of dimethylbenzoic acid (DMBA) isomers in tissues and urine of rats after 6-h vs. 4-week exposure to isomers of trimethylbenzene (TMB)

DMBA-isomer	TMB concentration in inhaled air											
	25 ppm exposure				100 ppm exposure				250 ppm exposure			
	lung	liver	kidney	urine	lung	liver	kidney	urine	lung	liver	kidney	urine
Pseudocumene[a] [%]												
2,5-DMBA	n.d.	n.d.	49 ↓↓	62 ↓↓	37 ↓	34 ↓	34 ↑	46 ↓↓	20 ↓	17 ↓	50 ↑	10 ↑
2,4-DMBA	21 ↓	15 ↓	61 ↓↓	6 ↓	26 ↓	10 ↓	19 ↑	33 ↓↓	22 ↓	13 ↓	39 ↑	13 ↓
3,4-DMBA	42 ↓	47 ↓↓	44 ↓↓	34 ↓	39 ↓↓	43 ↓↓	151 ↑	33 ↓↓	25 ↓	43 ↓↓	148 ↑	20 ↑
Mesitylene[b] [%]												
3,5-DMBA	29 ↑	48 ↓↓	26 ↑	60 ↑↑	62 ↑↑	17 ↓↓	15 ↑	19 ↑	48 ↑↑	44 ↑↑	35 ↑	8 ↑
Hemimellitene [%]												
2,6-DMBA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	129 ↑↑	n.d.	n.d.	n.d.	2 ↓
2,3-DMBA	n.d.	11 ↑	24 ↑	57 ↑↑	n.d.	35 ↓↓	53 ↓↓	176 ↑↑	13 ↓	35 ↓↓	35 ↓↓	27 ↑

Abbreviations as in Table 9 and 10.
[a] Świercz et al., 2005 [21]; [b] Świercz et al., 2006 [22].

those after a single exposure may evidence the saturation of pseudocumene metabolic transformation in tissues.

Some findings on the increased activity of microsomal monooxygenases in rat tissues after chronic (4-month) exposure to pseudocumene at the concentration of 10 mg/m^3 (c.a. 2 ppm) have been reported [33]. After repeated exposure to pseudocumene at 100 ppm and 250 ppm, concentrations of all the 3 DMBA isomers in the rat kidneys were higher than after a single exposure, which indicates a possible stimulation of pseudocumene metabolism.

Repeated inhalation exposure to mesitylene and hemimellitene vapors usually in the majority of experimental animals generated higher metabolite concentrations in the rat tissue and increased excretion of those metabolites with urine as compared to a single exposure due to induction of mesitylene and hemimellitene metabolizing enzymes. 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 [34,35]. Nevertheless, at elevated hemimellitene concentration following repeated exposure as compared to single exposure, lower concentrations of its metabolite were detected in the tissues, indicating possible saturation of the metabolic processes occurring in the studied rat tissues.

By analogy to the differences observed in rats, humans are also likely to show significant differences in the absorption, metabolism and removal of TMB isomers under circumstances of repeated inhalation exposure to those compounds. Elevated metabolic hemimellitene and mesitylene efficiency may lead to the erroneous assessment of biomonitoring results.

The American Conference of Governmental Industrial Hygienists (ACGIH) and Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) publish values of admissible concentrations of various chemical compounds in the biological material [4,18]. Out of those 2 organizations, the DFG has decided to express biological tolerance values of TMB isomers exposures in terms of determined dimethylbenzoic acid concentrations (sum of all isomers after hydrolysis) in urea [18].

Human studies show that the urinary excretion of dimethylhippuric acid isomers and dimethylbenzoic acid isomers in humans after short-term-exposure is a good indicator of TMB exposure [17,36]. The simulation model of accretion and excretion of dimethylbenzoic acids in human urine during a working week points to increased removal of 2,6-DMBA, one of 2 hemimellitene metabolites [17]. Our animal studies have confirmed increased removal of 2,6- and 2,3-DMBA with urine of rats repeatedly exposed to hemimellitene. To determine the biological tolerance values for TMB, an individual approach, i.e., considering each TMB isomer separately, seems preferable.

CONCLUSIONS

In conclusion, the studies have revealed that in rats after inhalation exposure to hemimellitene, the detected significantly lower hemimellitene concentrations in the blood and solid tissues of the repeatedly exposed animals may point to a reduced hemimellitene retention in the lungs of the rats. Hemimellitene elimination from the blood of rats after single and repeated inhalation exposure depended on the exposure magnitude and not on exposure time. For the 2 hemimellitene metabolites determined in rat urine, the main trend of their metabolism in the animal tissues involved formation of 2,3-DMBA. The significantly higher urinary 2,3-DMBA concentration after repeated inhalation exposure to hemimellitene as compared to the single exposure points to the induction of enzymatic processes in the rats exposed by inhalation to hemimellitene.

The results of the study indicate that metabolic transformations of TMB isomers in rats, leading to the production of DMBA isomers, are specific and their intensity differs depending on the organ (liver, lung, or kidney). The analysis of the concentration of TMB isomer metabolites in

urine of animals indicates that the use of the value of biological exposure limit specified in terms of the total for all isomers may result in over- or underestimation of the risk of exposure to TMB isomers under circumstances of repeated inhalation exposure to those chemical compounds.

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