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Letter to the Editor

Dimethylbenzoic Acid Isomers in Lung, Kidney, Liver and Urine of Rats after Single and Repeated Inhalation Exposure to Pseudocumene

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Abstract

The objective of our study was to estimate metabolites of pseudocumene in the lung, liver, kidney and urine of rats after single and repeated inhalation exposure.

Male Wistar rats were exposed to pseudocumene vapors at nominal concentrations of 25, 100 or 250 ppm in the dynamic inhalation chambers for 6 h or 4 weeks (6h/day; 5 days/week). Following exposure, three metabolites were measured in biologic material after hydrolysis: 3,4-dimethylbenzoic acid (3,4-DMBA), 2,4-dimethylbenzoic acid (2,4-DMBA) and 2,5-dimethylbenzoic acid (2,5-DMBA). The metabolites were analyzed by gas chromatography with a flame ionization detector.

The study showed no significant increase in concentrations of the analyzed DMBA metabolites in the liver and lungs of rats exposed to repeated inhalation exposures to pseudocumene as compared to a single exposure. It was found that metabolic transformation of pseudocumene, leading to the production of 3,4-DMBA in the rats kidneys, are more pronounced after repeated inhalation exposure.

Keywords: pseudocumene, dimethylbenzoic acid, rats, urine, liver, kidney, lung

Introduction

The wide use of petroleum-derived organic compounds is a major source of environmental exposure to xenobiotics in humans. These are usually substances that easily permeate the human organism via the pulmonary system. Due to their lipophilicity these compunds are able to surmount biological barriers. Bound to lipids and protein elements of cellular membranes, they disrupt the ionic balance and intercellular information flow. A large proportion of lipids in nervous tissues makes this system particularly susceptible to harmful effects of solvents. Solvent exposure may lead to long-term and even irreversible functional disorders of the nervous system (emotional liability, concentration difficulties, dysmnesia or impaired learning ability), known as a solvent syndrome. A better knowledge of xenobiotic transformation in human and animal organisms allows for an in-depth assessment of risks associated with exposure to organic compounds.

Trimethylbenzene (TMB) isomers: 1,2,4-TMB (pseudocumene), 1,3,5-TMB (mesitylene), and 1,2,3-TMB (hemimellitene) belong to a group of organic compounds that occur in the natural environment mainly as oil components. In some oil-derived products, used now in Poland, their proportion may reach even 44%, including 29.3% of pseudocumene; 3.6% of hemimellitene; and 11.1% of mesitylene. TMBs are essential components of different liquid fuels [1-3]. In Poland, TMB isomers have been classified as hazardous factors, that might occur in the work environ-

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ment. Therefore, a maximum allowable concentration (MAC) has been set at 100 mg/m^3 for the sum of TMB isomers. At the same time, a possible additional TMB penetration of an organism via skin was noted [4]. The maximum allowable biological concentration (MABC) of TMB metabolites in the urine of TMB-exposed persons has also been set. The MABC value for pseudocumene was based on the determination of the sum of three metabolites of dimethylbenzoic acid in the fraction of urine collected during the last four hours of exposure. The adopted MABC value for urine pseudocumene metabolites is 170 mg/h [5]. In spite of the fact that a large number of workers is exposed to this group of compounds, the data on their adverse effects on human health are far from being complete. The epidemiological studies, carried out almost 50 years ago, revealed functional disorders of respiratory, hematopoietic and nervous systems in workers exposed for years to solvent vapors composed in 80% from trimethylbenzenes at concentrations of 10-60 ppm [6, 7]. A single exposure of volunteers to pseudocumene vapors showed that pseudocumene is characterized by high retention (68%) and rapid permeability into human blood circulation during inhalation exposure [8, 9]. Pseudocumene is metabolized in the human body, which leads to the production of dimethylbenzoic acid isomers, mainly in the form of dimethyl derivatives of hippuric acid excreted with urine [10, 11]. Studies performed on rats revealed that, like in humans, rapid blood absorption of pseudocumene occurred during a six-hour inhalation exposure at concentrations of 25, 100 and 250 ppm. After termination of exposure, it is rapidly eliminated from animal blood [12]. After repeated exposures to pseudocumene, the increase in its concentration in the rats tissues that could depend on the prolonged duration of exposure was not significant [13-15]. In rats, like in humans, biotransformation leads to the production of dimethylbenzoic acid (DMBA) isomers [16, 17].

The objective of the study was to carry out a quantitative assessment of DMBA isomers in the lung, liver, kidney and urine of rats after single and repeated inhalation exposures to pseudocumene at concentrations of 25, 100 and 250 ppm.

Materials and Methods

Chemicals

Pseudocumene (1,2,4-TMB, No. CAS: 95-63-6) was supplied by Fluka (Cat. No. 82542), its purity was ≥ 97%. The conversion factors for pseudocumene: 1 ppm ~ 4.92 mg/m^3, 1 mg/m^3 ~ 0.20 ppm.

Animals and Inhalation Exposure Monitoring

Male Wistar rats IMP: DAK (five animals in each group) of body weight 189-375 g (2-3 months) were exposed to pseudocumene vapors at the nominal concentration of 25, 100 or 250 ppm in the dynamic inhalation chambers (volume, 0.25 m^3) for 6 h or 4 weeks (6 h/day; 5 days/week). Table 1 gives nominal and actual pseudocumene concentrations in toxicological chambers and the mean values of the body mass of rats, from which biological material was collected for further analyses. Chamber relative temperature and humidity were maintained at 21-23°C and 33-45%, respectively. Pseudocumene vapors were generated by heating liquid solvents in a washer. The desired concentrations of vapors were obtained by diluting them

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

Biological material	Pseudocumene target concentration in inhaled air (ppm)	Pseudocumene concentration in inhaled air (ppm)	Body weight (g)
Liver, lungs and kidney homogenates collected from animals after 6-h exposure	Control	0	226 ± 4
	25	25 ± 7	224 ± 10
	100	101 ± 5	226 ± 12
	250	239 ± 6	223 ± 7
Liver, lungs and kidney homogenates collected from animals after 4-week exposure	Control	0	295 ± 15
	25	25 ± 2	293 ± 23
	100	102 ± 10	298 ± 24
	250	238 ± 27	289 ± 22
Urine collected from animals after 6-h exposure	Control	0	210 ± 18
	25	21 ± 2	219 ± 13
	100	116 ± 5	205 ± 13
	250	215 ± 15	214 ± 25
Urine collected from animals after 4-week exposure	Control	0	299 ± 25
	25	24 ± 3	328 ± 21
	100	99 ± 7	295 ± 31
	250	249 ± 19	268 ± 21

with the air. Concentrations of solvent vapors in the exposure chamber were measured every 30 min by means of 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 operating conditions were: carrier gas — argon, constant flow mode, column flow 10 cm^3/min; make-up gas (argon) 20 cm^3 /min; air 300 cm^3 /min; oven 150°C; inlet split 200°C; detector 2000C. Vapor samples (0.5 dm^3) were adsorbed 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 disulfide (0.5 cm^3, stand 15 min).

Biological Material Collection and Analysis for DMBA Isomers

Samples of the liver, lung and kidney were derived from pseudocumene-exposed rats after decapitation. Samples were stored in glass vessels at -80°C. The tissues were homogenized before the determination of pseudocumene metabolites. Urine samples were collected 18 h after the termination of exposure in metabolic cages (TECNIPLAST). Urine samples were stored in glass vessels at -20°C. Three metabolites of pseudocumene were measured in urine and tissue samples: 3,4-dimethylbenzoic acid (3,4-DMBA), 2,4-dimethylbenzoic acid (2,4-DMBA) and 2,5-dimethylbenzoic acid (2,5-DMBA). The metabolites were measured by gas chromatography equipped with FID (Hewlett Packard 6890 Plus, Chem Station Rev. A. 08.03) using 2-naphthol (Fluka) as an internal standard and three isomers of DMBA (Fluka) as standards [8]. 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 H2SO4 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 silytated 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 temperature was 40°C/0.5 min; rate A was 5°C/min to 100 °C, held 1 min; rate B was 3°C/min to 150 °C, held 10 min; rate C was 3°C/min to 160 °C, held 30 min; and rate D was 20°C/min to 240°C, held 30 min. Split injection with a split ratio of 5: 1 and helium at the constant flow of 0.6 ml/min was used as carrier gas. The limit of detection for all determined metabolites were 0.25 µg/g wet tissues and was the same for urine analysis.

Statistics

The two-way analysis of variance with simple effects to evaluate 2×3 factorial experiment having five observations per cell and log-linear models to describe association patterns among categorical variables: exposure (6-h and 4-week), concentration (25, 100 and 250 ppm) and effect (0, 1) [18, 19]. When interaction was significant, Student's t-test was performed [20]. P values of < 0.05 were considered significant.

Results

Masses of tissues collected from animals after the termination of exposure to pseudocumene are given in Table 2. No statistically significant changes in masses of the tissues in animals under examination and as compared to control groups were observed. They did not vary within the groups with single and repeated exposure to different concentrations of psedocumene.

No metabolites of pseudokumene concentration in tissues of control animals were observed. Concentrations of the three pseudocumene metabolites in the liver of rats after the termination of exposure are summarized in Table 3. It was found that in the liver of rats exposed to pseudocumene, concentrations of DMBA isomers increased with increasing extent of exposure. Of the three pseudocumene metabolites, determined in the liver of rats under similar extent of exposure, 2,4-DMBA showed the highest concentration. The prolonged exposure did not induce any significant change in 2,4-DMBA concentration in rat liver. 3,4-DMBA occupied the second place as to its concentration in the liver. The statistical analysis revealed that after the termination of a four-week cycle

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

Pseudocumene target concentration in inhaled air (ppm)		Liver (g)	Lung (g)	Kidney (g)
Animals a 6-h exposure	0 (control)	9.12 ± 0.47	1.28 ± 0.12	1.78 ± 0.07
	25	9.74 ± 0.74	1.18 ± 0.34	1.99 ± 0.13
	100	9.30 ± 0.90	1.26 ± 0.16	1.83 ± 0.09
	250	8.92 ± 0.64	1.10 ± 0.10	1.76 ± 0.12
Animals a 4-week exposure	0 (control)	11.05 ± 1.53	1.59 ± 0.39	2.21 ± 0.23
	25	11.43 ± 1.72	1.65 ± 0.44	2.17 ± 0.12
	100	12.14 ± 1.68	1.56 ± 0.48	2.29 ± 0.12
	250	9.69 ± 1.84	1.44 ± 0.24	2.23 ± 0.20

Table 3. Concentrations of DMBA isomers in the liver of rats after exposure to pseudocumene.

Pseudocumene target concentration in inhaled air (ppm)		Liver concentration of metabolites (μg/g tissue)		
		2,5-DMBA	2,4-DMBA	3,4-DMBA
Animals at 6-h exposure	25	n.d.	5.14 ± 2.31 (5)	2.05 ± 0.41 (5)
	100	0.80 ± 0.40 (3)[A]	26.50 ± 6.75 (5)	5.77 ± 1.23 (5)
	250	1.46 ± 0.45 (5)	36.10 ± 5.01 (5)	14.02 ± 3.99 (5)
Animals at 4-week exposure	25	n.d.	4.36 ± 1.44 (5)	1.09 ± 0.28 (5)**
	100	0.53 ± 0.12 (5)	23.83 ± 3.37 (5)	3.30 ± 0.45 (5)**
	250	1.21 ± 0.27 (5)	31.34 ± 6.66 (5)	8.22 ± 2.66(5)*
Statistics				
Main effects:				
Exposure		NS	NS	$p < 0.0005$
Concentration		$p < 0.0005$	$p < 0.0005$	$p < 0.0005$
Interaction effects:				
Exposure by concentration		NS	NS	$p < 0.05$
Simple effects:			$p < 0.0005$	$p < 0.0005$
Concentration within 6-h exposure		$p < 0.01$		
Concentration within			$p < 0.001$	$p < 0.05$
4-week exposure		$p < 0.05$		
Exposure within concentration:				
25 ppm			NS	NS
100 ppm		NS	NS	NS
250 ppm		NS	NS	$p < 0.05$

[A] — mean ±SD (number of animals with detected metabolites), NS — not significant, ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$ — significantly different from the single exposure (Student's t-test), n.d. — not detected.

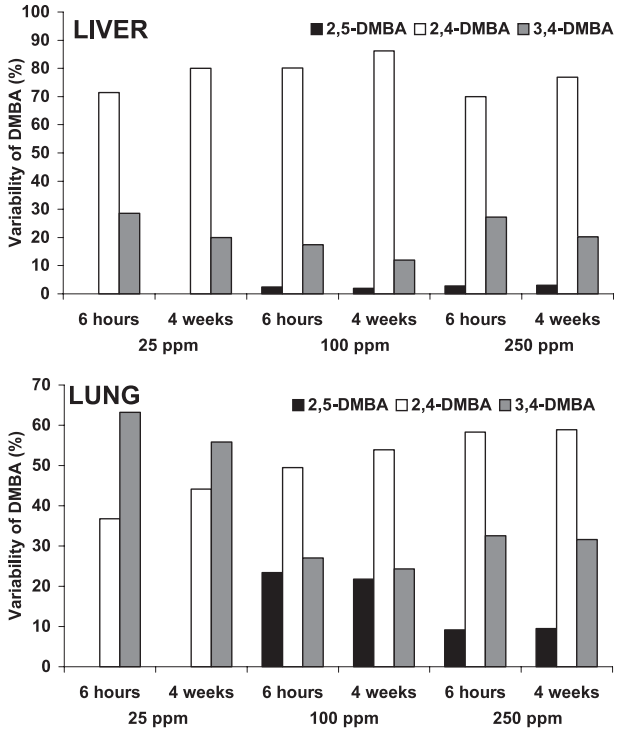

Fig. 1. Variability of DMBA rate in liver and lung of rats after single and repeated inhalation exposure to pseudocumene.

of exposures, 3,4-DMBA concentration significantly decreased compared to that observed after a single exposure. 2,5-DMBA reached the lowest concentration in the liver of rats exposed to pseudocumene, below the level of detection of the applied analytical method in both single and repeated exposures at 25 ppm. The proportion of DMBA isomers fell within the range of 0-3% for 2,5-DMB; 70-86% for 2,4-DMBA and 12-29% for 3,4-DMBA (Fig. 1).

Table 4 summarizes the results of the study of DMBA isomers in the lungs of rats exposed to pseudocumene. At the concentration of 25 ppm, the highest value was obtained for 3,4-DMBA and after exposure at 100 and 250 ppm the highest values were found for 2,4-DMBA. Concentrations of 2,4-DMBA and 3,4-DMBA increased with increasing extent of exposure. After repeated inhalation exposure, 2,4-DMBA concentrations varied significantly. 3,4-DMBA showed lower values after four weeks of exposure than those observed after a single exposure. The values of 2,5 DMBA concentration were low and not dependent on the extent and duration of exposure. At 25 ppm, its concentration was below the level of detection of the applied analytical method. The proportion of the produced DMBA isomers in rat lungs was dependent on the extent of exposure. After exposure to psedocumene at 25 ppm, 3,4-DMBA showed values higher (56-63%) than those of 2,4-DMBA (37-44%) (Fig. 1). At 100 and 250 ppm, the proportion of 3,4-DMBA was lower than that of 2,4-DMBA and ranged from 24 to 33% and from 50 to 59%, respectively. After exposure to pseudocumene at concentrations of 25, 100 and 250 ppm, 2,5-DMBA demonstrated low values, 0, 22 and 9%, respectively.

Table 4. Concentrations of DMBA isomers in the lung of rats after exposure to pseudocumene.

Pseudocumene target concentration in inhaled air (ppm)		Lung concentration of metabolites (μg/g tissue)		
		2,5-DMBA	2,4-DMBA	3,4-DMBA
Animals a 6-h exposure	25	n.d.	0.43 ± 0.01 (2)	0.74 ± 0.36 (5)
	100	0.97 ± 0.30 (2)[A]	2.05 ± 0.76 (4)	1.12 ± 0.24 (5)
	250	0.64 ± 0.21 (5)	4.08 ± 0.71 (5)	2.28 ± 0.40 (5)
Animals a 4-week exposure	25	n.d.	0.34 ± 0.07 (2)	0.43 ± 0.03 (5)
	100	0.61 ± 0.06 (2)	1.51 ± 0.41 (5)	0.68 ± 0.15 (5)**
	250	0.51 ± 0.07 (5)	3.17 ± 0.61 (5)	1.70 ± 0.81 (5)
Statistics				
Main effects:				
Exposure		NS	NS	p < 0.005
Concentration		NS	p < 0.0005	p < 0.0005
Interaction effects:				
Exposure by concentration		NS	NS	NS
Simple effects:				
Concentration within 6-h exposure		NS	p < 0.0005	p < 0.005
Concentration within 4-week exposure		NS	p < 0.01	p < 0.01
Exposure within concentration:				
25 ppm			NS	NS
100 ppm		NS	NS	NS
250 ppm		NS	NS	NS

[A] — mean ±SD (number of animals with detected metabolites), NS — not significant, ($p > 0.05$), ** $p < 0.01$ — significantly different from the single exposure (Student's t-test), n.d. — not detected

Table 5. Concentrations of DMBA isomers in the kidney of rats after exposure to pseudocumene.

Pseudocumene target concentration in inhaled air (ppm)		Kidney concentration of metabolites (μg/g tissue)		
		2,5-DMBA	2,4-DMBA	3,4-DMBA
Animals a 6-h exposure	25	1.78 ± 0.43 (4)[A]	4.67 ± 0.83 (5)	5.48 ± 1.59 (5)
	100	2.26 ± 1.02 (5)	10.31 ± 3.89 (5)	9.31 ± 4.19 (5)
	250	2.89 ± 1.54 (5)	16.76 ± 7.27 (5)	19.84 ± 8.89 (5)
Animals a 4-week exposure	25	0.91 ± 0.28 (3)*	1.82 ± 0.62 (5)***	3.06 ± 1.20 (5)*
	100	3.02 ± 1.83 (5)	12.26 ± 7.95 (5)	23.36 ± 16.07 (5)
	250	4.33 ± 1.74 (5)	23.26 ± 9.18 (5)	49.12 ± 30.34 (5)
Statistics				
Main effects:				
Exposure		NS	NS	p < 0.05
Concentration		p < 0.001	p < 0.0005	p < 0.001
Interaction effects:				
Exposure by concentration		NS	NS	NS
Simple effects:				
Concentration within 6-h exposure		NS	NS	NS
Concentration within 4-week exposure		p < 0.001	p < 0.0005	p < 0.001
Exposure within concentration:				
25 ppm		NS	NS	NS
100 ppm		NS	NS	NS
250 ppm		NS	NS	p < 0.05

[A] — mean ±SD (number of animals with detected metabolites), NS — not significant, ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$ — significantly different from the single exposure (Student's t-test).

Table 5 gives the results of the analysis of DMBA isomers in the kidneys of the pseudocumene-exposed rats. After a single exposure, the values of 2,4-DMBA and 3,4-DMBA were close to each other and concentrations of isomers increased with increasing extent of the exposure. 2,5-DMBA showed lower values then observed in 2,4-DMBA

Table 6. The urinary excretion of dimethylbenzoic acid after exposure to pseudocumene.

Pseudocumene target concentration in inhaled air (ppm)		Urine (mg/18 h)		
		2,5-DMBA	2,4-DMBA	3,4-DMBA
Animals a 6-h exposure	25	0.08 ± 0.03 (5)[A]	0.18 ± 0.07 (5)	0.32 ± 0.09 (5)
	100	0.56 ± 0.09 (5)	1.41 ± 0.29 (5)	2.04 ± 0.48 (5)
	250	0.90 ± 0.49 (5)	3.27 ± 1.69 (5)	5.17 ± 1.92 (5)
Animals a 4-week exposure	25	0.03 ± 0.01 (5)*	0.17 ± 0.05 (5)	0.21 ± 0.06 (5)
	100	0.30 ± 0.05 (5)***	0.94 ± 0.15 (5)**	1.37 ± 0.21 (5) *
	250	0.99 ± 0.14 (5)	2.85 ± 0.51 (5)	6.21 ± 0.63 (5)
Statistics				
Main effects: Exposure Concentration		NS p < 0.0005	NS p < 0.0005	NS p < 0.0005
Interaction effects: Exposure by concentration		NS	NS	NS
Simple effects: Concentration within 6-h exposure Concentration within 4-week exposure Exposure within concentration: 25 ppm 100 ppm 250 ppm		 p < 0.005 p < 0.0005 NS NS NS	 p < 0.001 p < 0.01 NS NS NS	 p < 0.01 p < 0.0005 NS NS NS

[A] — mean ±SD (number of animals with detected metabolites), NS — not significant, (p > 0.05), * p < 0.05, ** p < 0.01, *** P < 0.001 — significantly different from the single exposure (Student's t-test).

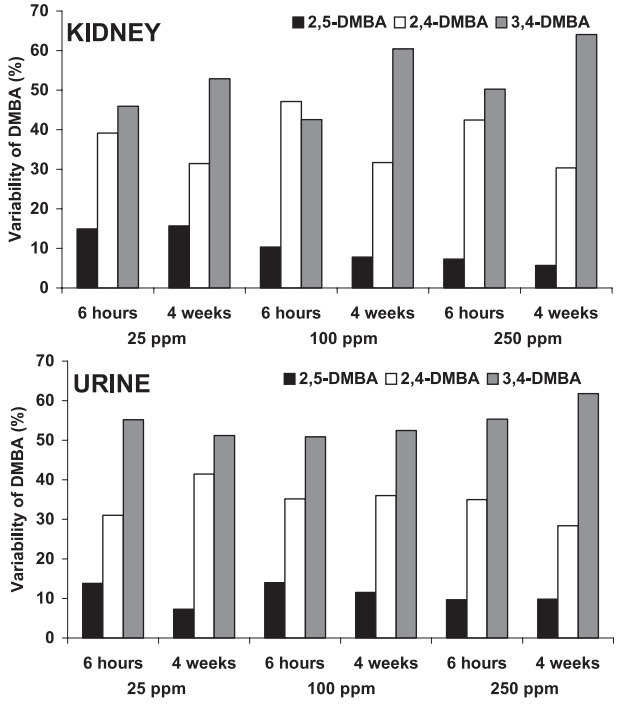

Fig. 2. Variability of DMBA rate in kidney and urine of rats after single and repeated inhalation exposure to pseudocumene.

and 3,4-DMBA. After repeated exposure to pseudocumene, 3,4-DMBA concentration in rat kidneys was higher than that of 2,4-DMBA. After termination of a four-week exposure to psedocumene at 25 ppm, concentrations of DMBA isomers were lower than those noted in animals after a single exposure. The proportions of metabolites present in kidneys after a single exposure to pseudocumene were similar and accounted for 7-15% for 2,5-DMBA; 39-47% for 2,4-DMBA; and 43-50% for 3,4-DMBA (Fig. 2). After four weeks of exposure, the proportions of compounds changed in favor of 3,4-DMBA and ranged between 8-16% for 2,5-DMBA; 30-32% for 2,4-DMBA; and 53-64% for 3,4-DMBA.

In the rats urine collected for 18 h after termination of exposure to pseudocumene, the increase in all DMBA isomers was related to the extent of exposure, and the values of 3,4-DMBA were evidently higher than those of 2,4-DMBA and 2,5-DMBA (Table 6). Having compared the urinary excretion of DMBA isomers after single and repeated exposure, it was found that the concentrations of all the compounds were significantly lower after four weeks of exposure to pseudocumene at 100 ppm and those of 2,5-DMBA at 25 ppm. After repeated and single exposure, the proportion of DMBA isomers in urine ranged from 7 to 14% for 2,5-DMBA; from 28 to 42% for 2,4-DMBA and from 51 to 62% for 3,4-DMBA (Fig. 2).

Discussion

Metabolism of pseudocumene in laboratory animals and in humans leads to the production of three major metabolites of DMBA isomers, which are excreted with urine at different amounts. Studies carried out on animals

orally administered with pseudocumene revealed its metabolic transformation, resulting in the production of isomers: dimethylbenzoic acid, trimethylphenol and dimethylbenzoic alcohol as well as 4-methylphthalic and 4-methylisophthalic acids [16, 17, 21-22].

Having analyzed the results of the quantitative assay of metabolite concentrations in tissues and urine of animals exposed to pseudocumene at different concentrations and for shorter or longer duration, significant differences were found in transformations leading to the production of the three pseudocumene metabolites. Interestingly and contrary to expectations, 2,4-DMBA, not 3,4-DMBA (whose highest concentrations were determined in urine), appeared to be the metabolite with the highest concentration in the liver. This provides evidence that kidneys play a vital role in the process of pseudocumene transformation in the rat body, and may also be in humans. The studies performed in a group of volunteers exposed to pseudocumene at the concentration of 150 mg/m^3 showed that urinary excretion of individual isomers changed in successive hours following the termination of inhalation exposure; during the first 8 h, excretion of 3,4-DMBA isomer was faster compared to the sum of 2,4-DMBA and 2,5-DMBA, whereas in consecutive hours, the excretion of the latter was faster than that of the former [8]. Determination of DMBA isomer concentrations in rat lungs show a significant role of the lungs in the process of metabolic transformation of pseudocumene in animals. Lower concentrations of pseudocumene metabolites in lungs found in our study four weeks after exposure termination compared with those after a single exposure may evidence the saturation of pseudocumene metabolic transformation in lung tissues (Table 4). In the liver, the major organ responsible for metabolism of xenobiotics, the 2,4-DMBA production dependent on exposure duration predominated (Table 3). The production of 3,4-DMBA in the liver of animals exposed to pseudocumene was significantly lower after repeated exposures than after a single one. The inhibition of this trend of pseudocumene transformation may result from the reduced enzymatic ability of the liver to produce 3,4-DMBA. No increase in the concentration of any of the three pseudocumene metabolites was observed in the liver of animals after repeated exposures, which may evidence the stimulation of enzymatic processes leading to higher concentrations of the compounds in question. Some findings on the increased activity of microsomal monooxygenases in rat tissues (lung, liver and kidney) after acute and chronic exposure to pseudocumene at the concentration of 10 mg/m^3 have been reported [23]. After repeated exposure to pseudocumene at 100 and 250 ppm, concentrations of all the three DMBA isomers in the rat kidneys were higher than after a single exposure, which indicates a possible stimulation of pseudocumene metabolism (Table 5). The increased concentration of 3,4-DMBA after repeated inhalation exposure, compared to other DMBA isomers, indicates that this trend of pseudocumene transformation predominates in the rat kidneys. Numerous authors have suggested that determination of 3,4-DMBA (in free form or combined with hippuric acid) or the sum of DMBA isomers in urine of persons exposed to this solvent may be regarded as a biological marker of pseudocumene exposure [8, 11]. It is possible that during repeated inhalation exposure to pseudocumene, metabolism of this compound increases in kidneys in both animals and humans.

In conclusion, the results of the study indicate that metabolic transformations of pseudocumene in rats, leading to the production of three DMBA isomers, are specific and their intensity differs depending on the organ (liver, lung or kidney), and the production of 3,4-DMBA in rat kidneys, are more pronounced after repeated inhalation exposure.

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