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PSEUDOCUMENE IN BRAIN, LIVER, LUNG AND BLOOD OF RATS AFTER SINGLE AND REPEATED INHALATION EXPOSURE

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Abstract. Male Wistar rats were exposed to pseudocumene vapors at nominal concentration of 25, 100 or 250 ppm in the dynamic inhalation chambers for 6 h or 4 weeks (6 h/day; 5 days/week). Following the inhalation exposure, pseudocumene concentrations were estimated in the brain, liver and lung homogenates, as well as in the brain (brainstem, hippocampus, temporal cortex, cerebellum) and blood (arterial, venous) structures. To estimate pseudocumene concentrations in biological material gas chromatography using the headspace technique was applied. The elimination of pseudocumene from venous blood after repeated inhalation exposures followed an open two-compartment model. Venous blood concentration was about twice as high as that in arterial blood. In tissues, the highest values were found in the liver after single exposure to pseudocumene vapor at concentrations of 100 and 250 ppm. There were no statistically significant differences in pseudocumene concentrations between the brain, lungs or arterial blood. In the brain structures of the animals exposed to pseudocumene vapors, significantly higher concentration of psedocumene was found in the brainstem.

Key words:
Pseudocumene, 1,2,4-Trimethylbenzene, Inhalation, Brain, Liver, Lung, Blood, Rat

INTRODUCTION

Pseudocumene (C_9H_{12}, 1,2,4-trimethylbenzene, CAS No. 95-63-6), one of three trimethylbenzene (TMB) isomers, is a component of numerous petroleum-derived organic mixtures. In many commercial solvent mixtures, like Farbasol (Polifarb-Cieszyn S.A., Poland), Jolasol 1000 SOLV (J.L.C. Chemie, Austria), Solvesso 100 (Exxon Chemical, Belgium) and Shellsol A (Shell Netherlands Chemie B.V.), the contents of pseducomuene may reach about 30%. It also occurs, but in small quantities (about 5%), in engine fuels [1–4]. Organic solvents are lipophilic substances and thus surmount easily biological barriers. Bound to lipids and also to membrane proteins, 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, known as a solvent syndrome. These disorders are usually of emotional (emotional liability) or intellectual (concentration difficulties, dysmnesia, impaired learning ability) nature. In inhalation exposure of rats to various aromatic compounds, pseudocumene permeated easily from blood to brain, liver, kidney and fatty tissue, and its concentrations were higher than those of toluene and xylene [5]. The results of acute animal experiments revealed that the pulmonary tract irritation induced in mice by pseudocumene vapor was four and eight times higher than that caused by xylene and toluene, respectively [6]. The results of rotarod performance and hot

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plate behavior tests performed on rats showed that after a 4-h inhalation exposure, the neurotoxic effect of pseudocumene was four times higher than the effect of toluene and twice as high as that of xylene [7]. Subchronic experiments (3 months) on rats showed significantly impaired motor coordination and decreased pain sensitivity after repeated inhalation exposure to pseudocumene at concentrations of 100 and 250 ppm, which indicates a stronger manifestation of the pseudocumene neurotoxic effect than that of toluene or xylene [8]. After a 4-week exposure of rats at concentrations of 100 and 250 ppm, seriously impaired passive avoidance learning, increased emotional reaction to pain and inhibited development of spontaneous cortical spike-wave discharges in EEG recordings were found [9,10].

The aim of the study was to explore pseudocumene concentrations in the brain, liver, lungs and blood of rats after inhalation exposure to pseudocumene vapor, depending on the duration and extent of the exposure. An analysis of pseudocumene concentrations in biological material will facilitate the quantity assessment of the pseudocumene distribution process in rats exposed to its vapors.

MATERIALS AND METHODS

Chemicals

Pseudocumene 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

Male Wistar rats IMP:DAK (four animals in each group) of body weight 152–421 g were exposed to pseudocumene vapors at the nominal concentrations of 25, 100 and 250 ppm in the dynamic inhalation chambers (volume, 250 dm^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 20–23°C and 45–60%, respectively. Pseudocumene 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

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

Biological material	Pseudocumene nominal concentration in inhaled air (ppm)	Pseudocumene actual concentration in inhaled air (ppm)	Body weight (g)
Arterial blood and brain structure collected from animals after a 6-h exposure	25	21 ± 2	219 ± 13
	100	116 ± 5	180 ± 28
	250	215 ± 15	220 ± 24
Arterial blood and brain structure collected from animals after a 4-week exposure	25	24 ± 3	327 ± 21
	100	99 ± 7	295 ± 31
	250	249 ± 19	268 ± 21
Liver, lung and brain homogenates collected from animals after a 6-h exposure	25	28 ± 1	227 ± 15
	100	123 ± 9	246 ± 11
	250	256 ± 7	228 ± 12
Liver, lung and brain homogenates collected from animals after a 4-week exposure	25	25 ± 2	310 ± 10
	100	103 ± 8	328 ± 23
	250	249 ± 13	320 ± 20
Venous blood collected from animals after a 4-week exposure	25	24 ± 3	321 ± 6
	100	99 ± 7	300 ± 22
	250	249 ± 19	373 ± 48

(Hewlett-Packard 5890) with a flame ionization detector (FID) using capillary column (HP-1; 30 m • 0.53 mm • 2.65 μm film thickness) [11].

Biological material

Samples of the arterial blood, brain, liver and lung were derived from pseudocumene-exposed rats after decapitation. Arterial blood was collected to heparinized glass capillary tubes. 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 pseudocumene vapors in heparinized glass capillary tubes with volume of 100 μl. The collected samples were stored at $+5^0$C until the determinations. The brain was homogenized or divided into anatomical structures: brainstem, hippocampus, temporal cortex and cerebellum. The liver and lung were also homogenized before the pseudocumene determinations. In about 100 mg of each brain structure or organ homogenate, pseudocumene was quantitatively assessed. Samples were stored in glass tubes at -20^0C. In the blood and tissues, pseudocueme 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 100^0C [11].

Statistical analysis

The kinetic analysis of pseudocumene in blood was calculated on an open two-compartment model using SigmaPlot 4.0 (Jandel Corporation) for Windows. The differences in pseudocumene concentrations between brain structures and tissues were estimated by employing one-way analysis of variance (ANOVA) [13].

RESULTS

Venous blood pseudocumene concentrations after repeated inhalation exposure (4 weeks) at 25, 100 and 250 ppm are summarized in Table 2. The concentration and speed of pseudocumene elimination from blood depended on the magnitude of pseudocumene exposure. The elimination of pseudocumene from blood after repeated exposure was calculated on an open two-compartment model. The kinetic equations are presented in Table 3. The half-lives ($t_{1/2}$) of pseudocumene in blood after termination of repeated exposure to pseudocumene vapors at 25, 100 and 250 ppm were 9, 32 and 68 min for phase I (rapid) and 2 h 53 min, 5 h 47 min and 9 h 54 min for phase II.

Psedocumene concentrations in the liver, lung and venous blood collected immediately after termination of exposure are shown in Table 4. Psedocumene concentrations in the biological material were dependent on the magnitude of

Table 2. Venous blood pseudocumene concentrations after a 4-week inhalation exposure to pseudocumene

Time	Pseudocumene (mg/l)		
	25 ppm	100 ppm	250 ppm
3 (min)	0.56 ± 0.18	4.06 ± 0.46	13.77 ± 3.34
15	0.43 ± 0.10	3.73 ± 1.21	11.82 ± 3.05
30	0.33 ± 0.03	3.02 ± 1.43	8.28 ± 2.07
45	0.28 ± 0.05	2.86 ± 0.89	7.21 ± 1.84
1 (h)	0.22 ± 0.02	2.62 ± 0.82	6.27 ± 1.72
2	0.17 ± 0.06	1.83 ± 0.17	4.50 ± 1.04
3	0.11 ± 0.04	0.88 ± 0.24	3.17 ± 0.76
4	0.07 ± 0.04	0.64 ± 0.21	1.73 ± 0.37
5	0.07 ± 0.01	0.39 ± 0.11	1.30 ± 0.22
6	0.06 ± 0.02	0.37 ± 0.14	1.25 ± 0.22

Results are presented as mean ± standard deviation.

Table 3. Toxicokinetics of pseudocumene elimination from venous blood after a 4-week exposure to pseudocumene

Exposure (ppm)	Elimination equation	Half-life	
		Phase I (min)	Phase II
25	$E = 0.55e^{-4.42t} + 0.25e^{-0.24t}$	9	2 h 53 min
100	$E = 4.50e^{-1.31t} + 1.10e^{-0.12t}$	32	5 h 47 min
250	$E = 10.0e^{-0.61t} + 1.50e^{-0.07t}$	68	9 h 54 min

exposure to its vapors. In the lung and brain, concentrations were similar after single and repeated exposures of similar magnitude. The arterial blood pseudocumene concentration was lower than that observed in the brain, liver and lungs. In the liver, pseudocumene levels were lower after repeated exposure to pseudocumene vapors at 100 ppm ($F(1,6) = 34.667$; $p < 0.05$), and 250 ppm than after single exposure.

Substantially lower concentration of pseudocumene was found in blood collected after decapitation than in blood derived from the tail vein of rats with repeated exposure to similar amount of pseudocumene vapors. The venous blood/arterial blood distribution ratio for pseudocumene after repeated exposure at 25, 100 and 250 ppm was 1.7, 2.6 and 1.8, respectively.

Pseudocumene concentrations in the brain structures of rats after single and repeated inhalation exposure to pseudocumene vapors of different magnitudes are given in Table 5. After single exposure at 25 ppm ($F(3,12) = 7.914$; $p < 0.05$) and 250 ppm ($F(3,12) = 17.714$; $p < 0.05$), lower concentrations of pseudocumene were found in temporal cortex, hippocampus and cerebellum than in brainstem, whereas at 100 ppm, significantly lower values of pseudocumene were observed in hippocampus and cerebellum

Table 4. Liver, lung, brain homogenates and arterial blood pseudocumene concentrations after inhalation exposure to pseudocumene

Exposure		25 ppm	100 ppm	250 ppm
6 h	Blood (mg/l)	0.31 ± 0.12	1.24 ± 0.41	7.76 ± 1.64
4 weeks		0.33 ± 0.11	1.54 ± 0.32	7.52 ± 2.11
6 h	Brain (mg/kg)	0.49 ± 0.06	2.92 ± 0.73	18.34 ± 1.92
4 weeks		0.45 ± 0.05	2.82 ± 0.40	18.63 ± 4.27
6 h	Liver (mg/kg)	0.44 ± 0.01	7.13 ± 1.31	28.18 ± 5.34
4 weeks		0.45 ± 0.15	3.00 ± 0.49*	22.47 ± 4.10
6 h	Lung (mg/kg)	0.43 ± 0.11	4.14 ± 0.54	18.90 ± 3.72
4 weeks		0.47 ± 0.20	3.74 ± 0.82	22.47 ± 4.10

Results are presented as mean ± standard deviation.
* $p < 0.05$ in comparison to a 6-h exposure.

Table 5. Pseudocumene concentrations in brain structures after inhalation exposure to pseudocumene

Exposure	Brain structures	Pseudocumene (mg/kg)		
		25 ppm	100 ppm	250 ppm
6 h				
	Brainstem	0.54 ± 0.11	3.38 ± 0.84	26.91 ± 5.33
	Temporal cortex	0.31 ± 0.06*	2.30 ± 0.71	13.54 ± 2.33*
	Hippocampus	0.28 ± 0.09*	1.89 ± 0.29*	12.99 ± 2.18*
	Cerebellum	0.32 ± 0.09*	1.99 ± 0.40*	12.91 ± 2.05*
4 weeks				
	Brain stem	0.38 ± 0.23	2.33 ± 1.24	21.95 ± 3.81
	Temporal cortex	0.25 ± 0.07	2.03 ± 0.66	15.71 ± 3.54
	Hippocampus	0.41 ± 0.27	3.03 ± 0.48	12.44 ± 2.63*
	Cerebellum	0.33 ± 0.05	3.20 ± 0.40	10.85 ± 2.47*

Results are presented as mean ± standard deviation.
* $p < 0.05$ in comparison to the brainstem.

($F(3,12) = 5.116$; $p < 0.05$) than in brainstem. In the brain structures collected from rats exposed repeatedly to pseudocumene vapors at only 250 ppm and decapitated immediately after exposure termination, lower psedocumene concentrations in hippocampus and cerebellum than in brainstem ($F(3,12) = 9.607$; $p < 0.05$) were found.

DISCUSSION

The results of our study revealed a rapid elimination of pseudocumene from venous blood of rats after termination of repeated exposure to pseudocumene vapors at 25, 100 and 250 ppm. When comparing the results of our previous study of pseudocumene elimination from venous blood after termination of single exposure with this compound elimination after repeated exposure to pseudocumene vapors at 25 and 100 ppm, described here, one may conclude that the dynamics of the elimination process was similar in both cases, and the values of half-lives for phases I and II were close to each other [11]. Phase II of pseudocumene elimination from venous blood after repeated exposure to pseudocumene vapors at 250 ppm was evidently shorter than after single exposure. In animals with exposure to pseudocumene vapors of similar magnitude, pseudocumene concentrations in arterial blood were lower than those found in venous blood. This difference may result from possible mixing arterial blood with other body fluids during decapitation of the rat. Zahlsen et al. [5] obtained similar results when estimating pseudocumene concentration in the blood of rats exposed to pseudocumene vapors at 100 ppm and decapitated to draw blood after 12 h of inhalation exposure. Blood pseudocumene concentrations were 1.7 ± 0.08 mg/kg on average.

The studies showed that after single and repeated exposures to pseudocumene vapors at 25, 100 and 250 ppm, pseudocumene concentrations in the brain and liver homogenates were close to the maximum values determined in blood drawn from the tail vein. In our study like in reports of other authors, pseudocumene concentration in the brain homogenate was about twice as high as in blood drawn after decapitation [5,14].

In the biological material, the highest concentrations of pseudocumene were found in the liver of the rats after single exposure to pseudocumene vapors at 100 and 250 ppm. After repeated exposure, pseudocumene concentrations in the liver, lungs and brain were similar, depending on the exposure magnitude. Pseudocumene concentration in the liver was significantly lower after repeated than after single exposure. This may point to the induction of enzymes, which metabolize pseudocumene in the liver of animals exposed repeatedly to pseudocumene vapors at 100 and 250 ppm.

After termination of inhalation exposure to pseudocumene vapors, significantly lower concentrations of the compound were found in the brainstem. This was observed particularly after a single exposure. The higher concentrations of pseudocumene in brainstem can be associated with higher fat affinity of this structure as compared to the other structures under study. Brainstem is a structure which cooperates with the regions of brain responsible for emotional processes. The earlier study showed that the behavioral effects of pseudocumene could be described as a reduction of ability to inhibit or to reduce motor reaction in a stressful situation [9].

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