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TOXICOKINETICS AND METABOLISM OF PSEUDOCUMENE (1,2,4-TRIMETHYLBENZENE) AFTER INHALATION EXPOSURE IN RATS

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Abstract. The objective of this study was to evaluate the toxicokinetics and metabolism of pseudocumene after 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. Blood samples were collected during (between 1st and 6th h) and after exposure (betwen 6th min and 6th h). Blood concentrations of pseudocumene were estimated by gas chromatography using the headspace technique. During a six-hour exposure, the concentration of pseudocumene in blood increased rapidly within the first 2 h reaching then a plateau. The elimination of pseudocumene from blood followed an open two-compartment model. Urine samples were collected from the exposed animals, and metabolites were analyzed by gas chromatography with a flame ionization detector. Three metabolites were measured in the rat urine after hydrolysis: 3,4-dimethylbenzoic acid (3,4-DMBA), 2,4-dimethylbenzoic acid (2,4-DMBA) and 2,5-dimethylbenzoic acid (2,5-DMBA). A significant linear correlation was found between the level of exposure and the concentration of dimethylbenzoic acids. The enzyme kinetics of pseudocumene biotransformation was calculated by Lineweaver-Burk equation. Metabolic constants, K_m (mg/l) and V_{max} (mg/h/kg), the parameters for pseudocumene biotransformation by rats were estimated (3,4-DMBA – K_m = 28, V_{max} = 96; 2,4-DMBA – K_m = 7, V_{max} = 25; 2,5-DMBA – K_m = 7, V_{max} = 23).

Key words:
Pseudocumene, Urinary metabolite, Toxicokinetic study, Inhalation, Rats

INTRODUCTION

Pseudocumene (C_9H_{12}, 1,2,4-trimethylbenzene, CAS No. 95-63-6), one of three trimethylbenzene isomers occurs in the natural environment mainly as an oil component. Pseudocumene is produced mostly during the catalytic reforming of petroleum. It is a major component of many commonly used commercial solvents, such as Solvesso 100 (Exxon Chemical, Belgium), Shellsol A (Shell Netherland Chemie B. V.), Jolasol (J. L. C. Chemie, Austria) and Farbasol (Polifarb-Cieszyn S. A., Poland).

Pseudocumene undergoes a rapid distribution in the rat body. After a twelve-hour inhalation exposure to pseudocumene at 1000 ppm, high concentrations of the compound were found in the blood, brain and fat tissues of the rat. Following a fourteen-hour repeated exposures to pseudocumene, the brain/blood and fat/blood distribution ratios were 2.0 and 63, respectively [1]. In the human exposure to

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pseudocumene at the concentration of 30 ppm for 8 h, its elimination from blood was rapid within the first 20 min after the termination of exposure and then very slow, which may suggest that pseudocumene accumulates in tissues [2]. Pseudocumene undergoes metabolic transformation in the rat body that leads to the production of dimethylbenzoic acids excreted with urine mostly bound to glycine, glucuronic acid and sulfuric acid [3].

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 180–370 g were exposed to psudocumene vapors at the nominal concentration of 25, 100 or 250 ppm in the dynamic inhalation chambers (volume, 250 dm^3) for 6 h.

The animals were exposed to different pseudocumene concentrations in separate inhalation exposures, depending on the biological material (blood, urine) collected, or the time of blood collection. 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 20–23ºC and 45–60%, respectively. Vapors of pseudocumene 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 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 – argon, constant flow mode, column flow 10 cm^3/min; make-up gas (argon) 20 cm^3/min; air 300 cm^3/min; oven 150ºC; inlet split 200ºC; detector 200ºC. Vapor samples (0.5 dm^3) were adsorbed on solid sorbent tube (charcoal activated for gas chromatography, MERCK, 20–35 mesh, 100 mg/50 mg) and desorbed with carbon disulfide (0.5 cm^3, stand 15 min).

Blood samples

Blood samples drawn from the tail vein were collected during the 1st, 2nd, 3rd, 4th, 5th and 6th h of exposure, as well as 3, 15, 30, 45 min and 1, 2, 3, 4, 5, 6 h after its termination in heparinized glass capillary tubes of 100 µl volume.

Blood concentrations of pseudocumene were estimated by gas chromatography combined with the headspace technique, using p-xylene as an internal standard [4]. Gas chromatograph (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 100ºC. The operating conditions were: carrier – helium, constant flow mode, column flow 10 cm^3/min; make-

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)
Blood collected from animals during a 6-h exposure	25	25 ± 2	200 ± 10
	100	109 ± 10	228 ± 10
	250	262 ± 21	190 ± 12
Blood collected from animals after a 6-h exposure	25	26 ± 3	349 ± 6
	100	101 ± 3	333 ± 18
	250	238 ± 9	336 ± 5
Urine collected from animals after a 6-h exposure	25	27 ± 3	355 ± 10
	100	98 ± 3	338 ± 10
	250	240 ± 7	330 ± 12

up gas (argon) 20 cm^3/min; air 300 cm^3/min; inlet split 180°C; detector 200°C.

Urine samples

Urine samples were collected 18 h after the termination of exposure in metabolic cages (TECNIPLAST). Three metabolites of pseudocumene (3,4-dimethylbenzoic acid (3,4-DMBA), 2,4-dimethylbenzoic acid (2,4-DMBA) and 2,5-dimethylbenzoic acid) were measured by gas chromatography equipped with FID (Hewlett Packard 6890 Plus, Chem Station Rev. A. 08. 03) using 2-naphthol as an internal standard [2]. Urine samples (2 cm^3) were hydrolyzed (2 cm^3 11 mol NaOH, 1 h at 100°C). After cooling, 5 cm^3 of 6 N H_2SO_4 with 0.5 g NaCl was added and then extracted (10 cm^3 ethyl ether, 10 min). The ether layer of 5 cm^3 was collected, after evaporation of ethyl ether the residue was silylated for 30 min (70°C) with 0.5 cm^3 N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Samples were separated using a HP-PONA methyl siloxane capillary column (50 m • 0.2 mm • 0.5 μm film thickness); programmed temperature: initial oven temperature – 40°C/0.5 min; rate A – 5°C/min to 85°C, held for 0 min; rate B – 3°C/min to 220°C, held for 14 min; rate C – 20°C/min to 240°C, held for 25 min; programmed pressure: initial pressure – 2.047 bar/39 min; rate A – 5 bar/min to 0.8 bar, held for 40 min; rate B – 5 bar/min to 2.047 bar, held for 1 min. Split injection with a split ratio of 5:1 and helium at nominal initial flow of 0.9 ml/min was used as carrier gas.

Calculations

The kinetic analysis of pseudocumene in blood was calculated on an open two-compartment model using SigmaPlot 4.0 (Jandel Corporation) for Windows. The Michaelis-Menten parameters (K_m and V_{max}) for pseudocumene metabolism were estimated by analyzing Lineweaver-Burk plots using the Microsoft Excel 5.0.

RESULTS

Blood pseudocumene concentrations in the rats during a six-hour inhalation exposure to its vapors at nominal concentrations of 25, 100 or 250 ppm are summarized in Table 2. The analysis showed that after a single exposure, the increase in the pseudocumene concentration was dependent on the level of exposure. During a six-hour exposure to 25, 100 or 250 ppm, the blood pseudocumene concentration increased rapidly within the first 2, 4 or 5 h, respectively, reaching then a plateau. The kinetics of changes in pseudocumene blood concentration during exposure, expressed in equations, is presented in Table 3. The half-life ($t_{1/2}$) of pseudocumene in blood increased with increasing level of inhalation exposure.

Table 2. Blood pseudocumene concentrations during a 6-h inhalation exposure to pseudocumene

Time	Pseudocumene (mg/l)		
	25 ppm	100 ppm	250 ppm
15 (min)	0.22 ± 0.07	1.12 ± 0.80	4.02 ± 0.85
30	0.33 ± 0.08	1.99 ± 1.09	4.87 ± 1.61
45	0.49 ± 0.16	3.56 ± 0.49	6.97 ± 1.22
1 (h)	0.53 ± 0.14	4.29 ± 0.60	8.67 ± 0.54
2	0.73 ± 0.16	5.10 ± 0.34	14.5 ± 2.6
3	0.80 ± 0.17	6.22 ± 0.70	17.8 ± 1.6
4	0.72 ± 0.15	7.40 ± 1.05	20.0 ± 0.5
5	0.79 ± 0.22	7.72 ± 1.48	23.3 ± 2.6
6	0.94 ± 0.16	8.32 ± 1.34	23.6 ± 1.8

Results are presented as mean ±SD.

Table 3. Toxicokinetics of pseudocumene absorption in blood during a 6-h inhalation exposure to pseudocumene

Exposure (ppm)	Absorption equation	Half-life
25	$A = 0.83(1 - e^{-1.08t})$	38 min
100	$A = 8.10(1 - e^{-061t})$	1 h 8 min
250	$A = 25.8(1 - e^{-0.41t})$	1 h 41 min

Pseudocumene concentrations in blood after a six-hour inhalation exposure to its vapors at 25, 100 or 250 ppm are presented in Table 4. The elimination of pseudocumene from blood followed an open two-compartment model. The half-life of pseudocumene in blood of laboratory animals increased with increasing level of exposure in both phases: I (rapid) and II (slow) (Table 5).

The concentrations of dimethylbenzoic acid (DMBA) in urine after termination of exposure to pseudocumene at concentrations of 25, 100 or 250 ppm are given in Table 6. Among the three metabolites measured in urine after exposure termination, 3,4-DMBA was the key one. Table

Table 4. Blood pseudocumene level after a 6-h inhalation exposure to pseudocumene

Time	Pseudocumene (mg/l)		
	25 ppm	100 ppm	250 ppm
3 (min)	0.68 ± 0.09	4.44 ± 1.54	20.9 ± 4.03
15	0.47 ± 0.04	3.72 ± 0.96	20.7 ± 5.13
30	0.40 ± 0.05	2.98 ± 0.88	17.1 ± 4.71
45	0.36 ± 0.04	2.89 ± 0.86	15.9 ± 5.74
1 (h)	0.34 ± 0.03	1.79 ± 0.49	14.9 ± 3.77
2	0.23 ± 0.04	1.25 ± 0.33	10.2 ± 3.04
3	0.17 ± 0.04	0.88 ± 0.29	8.05 ± 2.25
4	0.12 ± 0.02	0.61 ± 0.20	6.13 ± 1.64
5	0.10 ± 0.02	0.41 ± 0.14	3.98 ± 0.43
6	0.08 ± 0.02	0.33 ± 0.06	3.20 ± 0.52

Results are presented as mean ±SD.

Table 5. Toxicokinetics of pseudocumene elimination from blood after a 6-h inhalation exposure to pseudocumene

Exposure (ppm)	Elimination equation	Half-life	
		Phase I (min)	Phase II
25	$E = 0.60e^{-4.09t} + 0.30e^{-0.18t}$	10	3 h 37 min
100	$E = 4.50e^{-1.47t} + 0.95e^{-0.13t}$	28	20 min
250	$E = 20.0e^{-0.73t} + 5.00e^{-0.04t}$	57	17 h 20 min

7 shows the statistically significant linear correlation between pseudocumene exposure, urinary concentration of 2,5-DMBA, 2,4-DMBA, 3,4-DMBA and the sum of isomeric dimethylbenzoic acids. The correlation coefficients between pseudocumene in air and urinary simple DMBA and the sum of DMBA were similar.

Lineweaver-Burk plots for urinary pseudocumene metabolites are shown in Fig. 1. The results of metabolic constants (V_{max} and K_m) are given in Table 8.

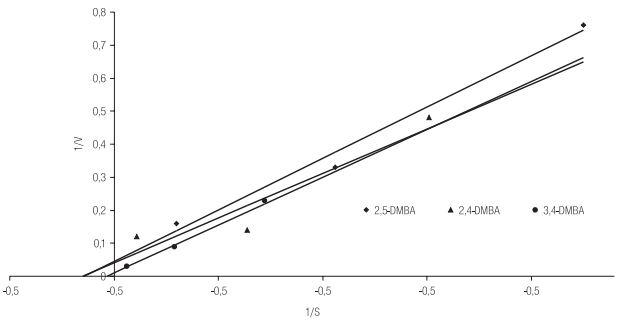

Fig. 1. Lineweaver-Burk plots for the formation of dimethylbenzoic acid (DMBA) by the rats. V = mg/h/kg b.w.; S = substrate concentration, mg/l.

Table 6. Urine concentrations of dimethylbenzoic acid (DMBA) after the termination of pseudocumene exposure

Pseudocumene (ppm)	2,5-DMBA (mg/l)	2,4-DMBA (mg/l)	3,4-DMBA (mg/l)
25	23.6 ± 8.6	37.6 ± 12.9	79.9 ± 33.3
100	54.0 ± 5.4	130.9 ± 22.1	200.8 ± 25.8
250	109.4 ± 71.1	308.8 ± 220.1	571.8 ± 381.6

The values of metabolic constants were much higher for 3,4-DMDA than those for 2,5-DMBA and 2,4-DMBA.

DISCUSSION

The study revealed a rapid increase in blood pseudocumene concentrations in a single inhalation exposure of rats, especially during the first hours of exposure. The half-life increased with the increasing level of exposure. Pseudocumene concentrations in different tissues, including blood, after inhalation exposure have been analyzed by Zahlsen et al. [5]. They found that after exposure at 100 ppm for 12 h, the blood pseudocumene concentration accounted for 1.7 ± 0.08 mg/kg. In our study, the values of psedocumene concentrations in rats after exposure at a similar level were four times higher. It is likely that the differences result from different methods of blood collection (in the study carried out by Zahlsen et al. [5], blood was collected after decapitation), or from the differences in time of blood collection after exposure termination. A similar dynamics of increase in psedocumene concen-

Table 7. The relationship between pseudocumene exposure and urinary concentration of dimethylbenzoic acid

Metabolites	Regression equations y (mg/l); x (ppm)	Correlation coefficient r	Significance level p <
2,5-DMBA	y = 0.41x + 13.4	0.80	0.002
2,4-DMBA	y = 1.37x + 4.41	0.81	0.002
3,4-DMBA	y = 2.31x + 0.29	0.82	0.002
2,5-, 2,4-; 3,4-DMBA	y = 3.99x + 18.1	0.82	0.002

Table 8. Michaelis-Menten parameters estimated for pseudocumene biotransformation by rats after inhalation exposure to pseudocumene.

Metabolite	Metabolic constant	
	K_m (mg/l)	V_m (mg/h/kg)
2,5-DMBA	7	23
2,4-DMBA	7	25
3,4-DMBA	28	96

trations in capillary blood, collected in a group of volunteers, was observed by Kostrzewski et al. [2] and Järnberg et al. [6] during an eight-hour exposure at 30 ppm and a two-hour exposure at 25 ppm, respectively.

Like in laboratory animals, in humans the blood pseudocumene concentrations increased rapidly within the first hours of exposure. Both teams of researchers, Kostrzewski et al. [2] and Järnberg et al. [6] observed in volunteers a rapid elimination of pseudocumene from blood. In the study by Kostrzewski et al. [2], the kinetics of pseudocumene elimination from blood was presented on a triphasic model with half-lives of 10 min, 20 min and 44 h. Järnberg et al. [6], analysing this process (25 ppm, 2-h exposure), used a four-phase model resulting in half-lives of 1.3 min, 21 min, 3.6 h and 87 h. In our study, the elimination of pseudocumene from blood on an open two-compartment model was proposed.

The differences in pseudocumene elimination from blood between animals and humans is probably associated with a more rapid metabolism in rats and different permeability of pseducumene to and from tissues. Zahlsen et al. [5] observed high pseudocumene concentrations in different animal tissues after the termination of exposure (100 ppm, 12-h exposure); 12 h after the exposure termination, they were low or close to zero.

Pseudocumene metabolism in rats leads to the production of isomeric dimethylbenzoic acids, trimethylphenols and dimethylbenzyl alcohols. They are excreted with urine mostly as glucuronide, sulfate, mercapturic acid and dimethylhippuric acid [7]. Both in laboratory animals and in humans, pseudocumene metabolism is mainly directed towards isomeric dimethylbenzoic acids, which may be an indicator of the level of pseudocumene exposure.

Fukaya et al. [8] suggest that the urinary concentration of 3,4-dimethylhippuric acid (3,4-DMHA) may be a useful indicator for biological monitoring of pseudocumene exposure. Kostrzewski et al. [2] propose the value of biological exposure limit for pseudocumene as the sum of 2,4-DMBA, 2,5-DMBA and 3,4-DMBA metabolites. The analysis of the concentrations of pseudocumene metabolites revealed the relationship between the concentration of pseudocumene in the air and the concentration of DMBA acids in urine of animals exposed to pseudocumene. It is likely that the determination of at least one of the three isomeric dimetylbenzoic acids could be a useful indicator for biological monitoring of pseudocumene exposure.

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REFERENCES

1. Zahlsen K, Nilsen AM, Eide I, Nilsen OG. *Accumulation and distribution of aliphatic (n-nonane), aromatic (1,2,4-trimethylbenzene) and naphthenic (1,2,4-trimethylcyclohexane) hydrocarbons in the rat after repeated inhalation.* Pharmacol Toxicol 1990; 67: 436–40.
2. Kostrzewski P, Wiaderna-Brycht A, Czerski B. *Biological monitoring of experimental human exposure to trimethylbenzene.* Sci Total Environ 1997; 199: 73–81.

3. Mikulski PI, Wilgusz R. *The comparative metabolism of mesitylene, pseudocumene and hemimellitene in rats.* Toxicol Appl Pharmacol 1975; 31: 21–31.
4. Radzikowska-Kintzi H, Jakubowski M. *Internal standarization in the Head Space analysis of organic solvents in blood.* Int Arch Occup Environ Health 1981; 49: 115–21.
5. Zahlsen K, Eide I, Nilsen AM, Nilsen OG. *Inhalation kinetics of C6 to C10 aliphatic, aromatic and naphthenic hydrocarbons in rats after repeated exposures.* Pharmacol Toxicol 1992; 71: 144–9.
6. Järnberg J, Johanson G, Löf. *Toxicokinetics of inhaled trimethylbenzene in man.* Toxicol Appl Pharmacol 1996; 140: 281–8.
7. Huo JZ, Aldous S, Campbell K, Davies N. *Distribution and metabolism of 1,2,4-trimethylbenzene (pseudocumene) in the rat.* Xenobiotica 1989; 19; 161–70.
8. Fukaya Y, Saito I, Matsumoto T, Takeuchi Y, Tokudome S. *Determination of 3,4-dimethylhippuric acid as a biological monitoring index for trimethylbenzene exposure in transfer printing workers.* Int Arch Occup Environ Health 1994; 65: 295–7.

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