International Journal of Occupational Medicine and Environmental Health, Vol. 13, No. 4, 307 – 315, 2000

STUDIES ON DERMAL, OCULAR AND RESPIRATORY EFFECTS OF 4-ETHYLTOLUENE IN EXPERIMENTAL ANIMALS

RADOSŁAW ŚWIERCZ [1], KONRAD RYDZYŃSKI [1], JOLANTA JAJTE [2], JAN STETKIEWICZ [3] and WANDA MAJCHEREK [1]

[1] Department of Toxicology and Carcinogenesis
[2] Department of Physical Hazards
[3] Department of Pathomorphology
The Nofer Institute of Occupational Medicine
Łódź, Poland

Key words: 4-Ethyltoluene, Inhalation toxicity, Respiratory irritation, Mice, Bronchoalveolar lavage, Rat, Skin and eye irritation, Rabbit

Abstract. The toxicity of 4-ethyltoluene to experimental animals was studied after single and repeated exposures. It was found that 4-ethyltoluene can be classified as a very mild skin and eye irritant. Sensory respiratory irritation of 4-ethyltoluene was studied in Balb/C male mice using the plethysmographic method. The concentration at which the respiratory rate decreased to 50% (RD_{50} value) was determined to be 4216 mg/m^3 (2795 – 5850 mg/m^3 for 95% confidence interval). To study repeated-dose inhalation toxicity, male and female outbred Wistar rats were exposed in a dynamic inhalation chamber to 4-ethyltoluene vapours at concentrations of 477 or 2337 mg/m^3, 6 h/day, 5 days/week for 4 weeks (20 exposure days). No significant changes were observed in food consumption and body weight gain. Statistically significant, concentration-dependent changes in the number of total cells, as well as of macrophages, polymorphonuclear leucocytes and lymphocytes were found in bronchoalveolar lavage. In the fluid of bronchoalveolar lavage, a significant, concentration-related increase was noted in total protein and mucoproteins and the activity of β-glucuronidase, γ-glutamyl transferase, lactate dehydrogenase and acid phosphatase. Histopathology revealed an increased rate of bronchitis and pneumonia and perivascular lymphoid infiltrations in rats exposed to 2337 mg/m^3 of 4-ethyltoluene.

INTRODUCTION

4-Ethyltoluene (C_9H_{12}, 4-ET, CAS No. 622-96-8) is one of three ethyltoluene isomers which in the natural environment occurs mainly as an oil component. As the products of oil cracking have extremely wide industrial use (e.g. solvent mixtures: Solvesso 100, Exxon Chemical, Belgium; Shellsol A, Shell Netherland Chemie B.V.; Jolasol, J.L.C Chemie, Austria; and Farbasol, Petrochemia Płocka S.A., Poland),

Address reprint requests to Dr. R. Świercz, Department of Toxicology and Carcinogenesis, The Nofer Institute of Occupational Medicine, PO Box 199, 90-950 Łódź, Poland.

ethyltoluene isomers are frequently encountered in the work environment. The solvent mixtures of ethyltoluene are predominantly used in the production of paints and lacquers.

The literature data on the toxicity of ethyltoluene isomers in experimental animals are very scarce. For that reason, an experimental animal study was undertaken to investigate the toxicity of 4-ethyltoluene. It might be assumed that toxic effects produced by any other ethyltoluene isomer should be similar to those of 4-ET.

MATERIALS AND METHODS

Chemicals

4-Ethyltoluene was supplied by ALDRICH (Cat. No. 4,980-0); its purity was 96% (2-ethyltoluene $\approx$ 2.5%, 3-ethyltoluene $\approx$1.5).

The conversion factors for 4-ET : 1 ppm $\approx$ 4.92 mg/m^3, 1 mg/m^3 $\approx$ 0.20 ppm.

Animals

The experimental animals were male Balb/C mice, male and female outbred Wistar rats of Imp:DAK stock, chinchilla and New Zealand white rabbits. All animals were supplied from the Animal Husbandry of The Nofer Institute of Occupational Medicine. Relative temperature and humidity in home cages were maintained at 20–23°C and 45–60%, respectively with a 12-h light/dark cycle (light on from 6 a.m. to 6 p.m.).

Dermal and ocular irritation studies

Dermal irritation studies were conducted on five New Zealand white rabbits of 3.8 $\pm$ 0.2 kg body weight. The dermal irritation test was based on the method described in OECD guidelines No. 404 (16). In brief, gauze patches (2.5 • 2.5 cm) with 4-ET (0.5 cm^3), its 10%, and 50% olive oil solutions (v/v), and olive oil alone (controls) were applied to the skin. The patches were covered with plastic foil and dressed, then left for a 4-h exposure period. The exposed sites were checked for dermal irritation 1, 24, 48 and 72 h after patch removal. Dermal irritation index (DII) was scored according to Draize et al. (5): slightly irritating – <2; moderately irritating – 2–5; and severely irritating >5. The animals had been observed until the skin changes vanished.

Ocular irritation studies were performed on three chinchilla rabbits of 4.0 $\pm$ 0.1 kg body weight, housed individually in stainless-steel cages. The eye irritation test protocol was based on the method reported in the OECD guidelines No. 405 (17). In brief, 0.1 cm^3 4-ET was instilled in the conjunctival sac of a defect-free eye of the rabbit. The other eye served as a control. The cornea, iris and conjunctiva were scored 1, 24, 48 and 72 h and up to 7 days after-dosing. The eye irritation index (EII) was computed by averaging test scores at the 1st, 24th, 48th and 72nd h. The eye irritation ratings were: no reaction – 0; slightly irritating – 1–16; moderately irritating – 17–35; severely irritating – 36–75; and extremely irritating – 75–110 (19).

Inhalation exposure

The concentration of solvent vapours in the exposure chamber was estimated using a Hewlett-Packard gas chromatograph with a flame-ionization detector (FID) and HP-1 capillary column (30 m • 0.54 mm • 2.65 μm).

The respiratory rate was measured in male Balb/C mice weighing 25 – 30 g, using whole-body plethysmography (1,18). Each animal was placed in a body plethysmograph attached to a dynamic inhalation chamber (0.25 m^3 volume, 12 – 13 air changes per hour) and exposed to 4-ET at various concentrations: 1260 ± 79, 3385 ± 339, 3887 ± 369, 5919 ± 285, 8290 ± 399 or 12615 ± 578 mg/m^3. Each group consisted of 10 mice. The respiratory pattern was recorded by Beckman polyphysiograph. The respiratory rate was recorded 5 min before exposure, during 6 min of exposure and 6 min after the exposure termination.

Bronchoalveolar lavage findings and pathological changes in the lung. To study the inhalation toxicity, male and female outbred Wistar rats of Imp:DAK stock were exposed in a dynamic inhalation chamber to 4-ET vapours at concentrations of 0, 477 ± 79 or 2337 ± 325 mg/m^3 for 4 weeks (6 h/day, 5 days/week). During the exposure, food consumption was observed and body weight was monitored individually.

Bronchoalveolar lavage (BAL). Twenty-four hours after termination of subchronic inhalation exposure to 4-ET the rats were anaesthetised by an i.p. injection of pentobarbital sodium (Sigma) in a dose of 60 mg/kg b.w., and their lungs were lavaged with 5 ml of 0.9% NaCl solution at room temperature. BAL fluid samples were centrifuged at 200 g (acceleration of gravity) for 10 min at 4°C to separate the cells. Smears were prepared from BAL cells, stained according to May-Grunwald and Giemsa method, and differential cell counts were obtained using a light microscope. For evaluating cell viability, the trypan blue exclusion was used. The total protein concentration (14), mucoprotein concentration (7), lactate dehydrogenase (LDH) activity (2) and acid phosphatase activity (4) in the BAL supernatant were determined.

Pathological changes in the lung. Twenty-four hours after termination of subchronic inhalation exposure to 4-ET the rats were killed under anaesthetic and tissue samples of the left lung were prepared. The samples were fixed in Carnoy's solution and embedded in paraffin wax. Histological sections were stained with haematoxylin and eosin, and evaluated microscopically.

Statistical analysis

The concentrations that caused a 50% depression in the respiratory rate (RD_{50}) of mice were calculated using the least squares regression (9). Kruskall-Wallis test (8) was applied to evaluate the total protein and mucoprotein levels and enzymatic activity in the cells and BAL fluid. The trend analysis was used to evaluate the relation between the extent of changes in lung cells, enzymatic activity in the BAL fluid and the exposure level (10).

RESULTS

The values of the dermal and eye irritation indices after a single exposure of rabbits indicated that 4-ET can be classified as a substance with a slight irritant effect

(Tables 1 and 2). The eye irritation index was 1.7. Eye changes were minimal, and they were observed no longer than 6 days after application. The dermal irritation index values were concentration-dependent and amounted to: 0.4, 0.8 and 1.3 for 10%, 50% and 100% 4-ET solutions, respectively. Skin changes disappeared before the 7th (10%), 10th (50%) or 14th (100%) day after application.

Table 1. The eye irritation index (EII) of rabbits exposed to 4-ethyltoluene

Time observed (h)	EII
1	2.1
24	1.8
48	2.0
72	0.8
EII (average)	1.7

Table 2. The dermal irritation index (DII) of rabbits exposed to 4-ethyltoluene

Time observed (h)	Concentration (%)		
	10	50	100
1	0	0	0
24	0.4	1.4	1.9
48	0.8	1.3	2.3
72	0.5	0.6	1.1
DII (average)	0.4	0.8	1.3

All mice exposed to 4-ET vapours survived the experiment for 6 min. The effects of a single exposure on the mouse respiratory rate are presented in Fig. 1. The irritant effect of 4-ET on the respiratory system after a single exposure of mice was

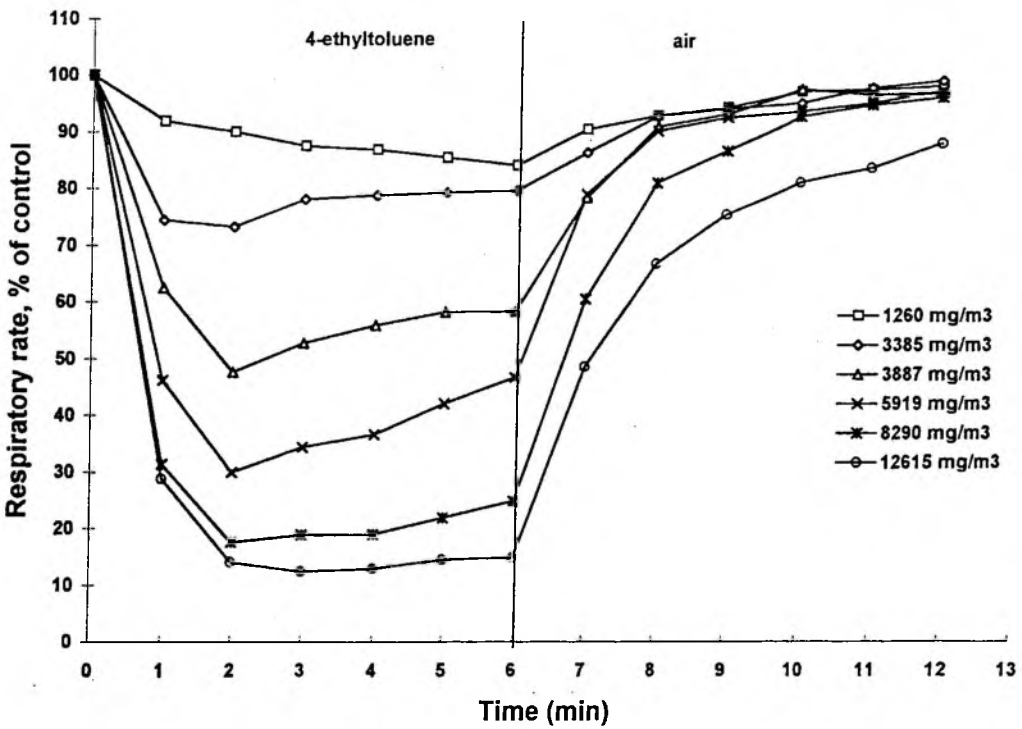

Fig. 1. Time-response relationship for the effect of 4-ethyltoluene on the respiratory rate in mice. Each point represents the mean value for 10 mice. After termination of a 6-min exposure a recovery of the respiratory rate was observed.

manifested by a concentration-dependent decrease in the breath rate. The maximum decline in the respiratory rate (about 85) was noted when 4-ET concentration reached its highest level (12625 mg/m^3). In most cases the lowest respiratory rate in mice could be found in the 2nd min of exposure and then it reached the plateau. The RD50 concentration (95 CI) was 4216 mg/m^3 (2795 – 5850 mg/m^3) (Fig. 2).

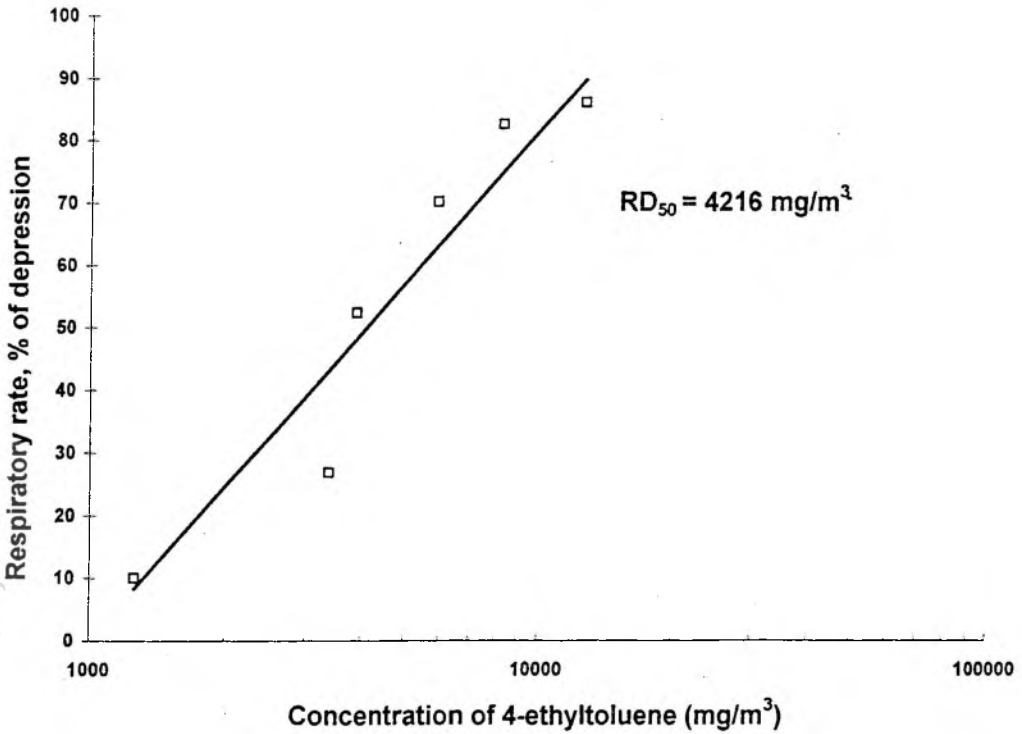

Fig. 2. Respiratory rate of mice exposed to 4-ethyltoluene. Each point represents the mean value for 10 mice. The decrease in the respiratory rate observed in the 2nd min of exposure.

All rats exposed to 4-ET vapours for 28 days survived the experiment. Clinical observations did not reveal any significant toxicological findings. No important changes were found in food consumption and body weight gain. The results of the total differential number and cell viability in BAL are shown in Table 3. Statistically significant, concentration-dependent increase in the number of total cells, as well as of macrophages, polymorphonuclear leucocytes and lymphocytes were noted in BAL from male rats. No substantial changes were observed in BAL of female rats exposed to 4-ethyltoluene for 28 days. Table 4 presents changes in total protein, mucoprotein and urea and the activity of enzymes in the BAL fluid. Statistically significant, concentration-dependent changes in total protein and activity of β-glucuronidase and γ-glutamyl transferase were observed in BAL fluid of male and female rats. Mucoprotein concentration and activity of lactate dehydrogenase and acid phosphatase were increased in BAL fluid of male rats. Rats exposed to 4-ethyltoluene at concentration of 2337 mg/m^3 showed statistically significant increase in mucopro-

Table 3. Total number and viability of lung cells obtained from bronchoalveolar lavage fluid of rats exposed to 4-ethyltoluene for 4 weeks

	Control (n = 5)	477 mg/m³ (n = 7)	2337 mg/m³ (n = 7)	Jonckheere's trend test
Body weight (g)				
Males	279 ± 18	284 ± 15	262 ± 31	
Females	225 ± 17	194 ± 12	210 ± 18	
Total cells (10^6/ml)				
Males	3.08 ± 1.02	6.66 ± 2.83	25.8 ± 23.0**	$p_J = 0.0006$
Females	2.18 ± 0.33	3.27 ± 1.44	3.24 ± 1.27	$p_J = 0.0999$
Macrophages (10^6/ml)				
Males	2.62 ± 0.81	5.99 ± 3.13	13.61 ± 9.22**	$p_J = 0.0009$
Females	2.07 ± 1.04	3.11 ± 1.41	2.98 ± 1.17	$p_J = 0.2312$
Polymorphonuclear leucocytes (10^6/ml)				
Males	0.09 ± 0.04	0.34 ± 0.54	9.24 ± 12.8*	$p_J = 0.0050$
Females	0.03 ± 0.03	0.04 ± 0.05	0.07 ± 0.09	$p_J = 0.3415$
Lymphocytes (10^6/ml)				
Males	0.07 ± 0.06	0.36 ± 0.41	2.99 ± 4.92*	$p_J = 0.0065$
Females	0.10 ± 0.04	0.13 ± 0.10	0.19 ± 0.15	$p_J = 0.1619$
Cell viability (%)				
Males	97.6 ± 2.8	95.6 ± 3.7	94.8 ± 4.7	$p_J = 0.0772$
Females	98.8 ± 0.8	98.4 ± 2.1	97.3 ± 1.4	$p_J = 0.0637$

*, ** Significantly different from control at $p < 0.05$ and $p < 0.01$,
Results expressed as the mean ± SD.

Table 4. Total protein, mucoprotein and urea levels and enzymatic activity in the BAL fluid of rats exposed to 4-ethyltoluene for 4 weeks

	Control (n = 5)	477 mg/m³ (n = 7)	2337 mg/m³ (n = 7)	Jonckheere's trend test
Total protein (mg/ml)				
males	0.15 ± 0.02	0.27 ± 0.15	0.71 ± 0.29	$p_J = 0.0001$
females	0.06 ± 0.03	0.06 ± 0.04	0.21 ± 0.12	$p_J = 0.0043$
Mucoproteins (mg/ml)				
males	0.14 ± 0.03	0.17 ± 0.03	0.52 ± 0.29**	$p_J = 0.0002$
females	0.11 ± 0.001	0.12 ± 0.002	0.12 ± 0.004	$p_J = 0.3821$
Urea (μg/ml)				
males	0.63 ± 0.32	0.78 ± 0.37	1.04 ± 0.52	$p_J = 0.0602$
females	0.48 ± 0.18	0.53 ± 0.22	0.60 ± 0.22	$p_J = 0.1454$
Lactate dehydrogenase (mU/ml)				
males	35.4 ± 9.0	36.9 ± 4.6	94.7 ± 65.8**	$p_J = 0.0006$
females	29.3 ± 6.3	29.9 ± 6.3	29.1 ± 6.4	$p_J = 0.4042$
γ-Glutamyl transferase (mU/ml)				
males	3.2 ± 1.1	5.5 ± 1.9	7.6 ± 5.2	$p_J = 0.0234$
females	3.8 ± 1.3	4.6 ± 1.8	5.3 ± 1.0	$p_J = 0.0535$
β-Glucuronidase (mU/ml)				
males	0.33 ± 0.05	0.46 ± 0.16	1.31 ± 0.67**	$p_J = 0.0002$
females	0.27 ± 0.03	0.24 ± 0.04	0.31 ± 0.02*	$p_J = 0.0101$
Acid phosphatase (mU/ml)				
males	1.14 ± 0.20	1.15 ± 0.19	1.40 ± 0.19	$p_J = 0.0179$
females	0.81 ± 0.16	0.72 ± 0.10	0.84 ± 0.08	$p_J = 0.2334$

*, ** Significantly different from control at $p < 0.05$ and $p < 0.01$,
Results expressed as the mean ± SD.

teins and lactate dehydrogenase activity (male) and β-glucuronidase activity (male and female) as compared with controls. The high incidence ratio of bronchitis and bronchopneumonia, as well as perivascular infiltration by lymphocytes was noted in the lungs of rats exposed for 4 weeks to 4-ET at concentration of 2337 mg/m^3. Only the lungs of single rats in this group remained intact. There were no marked pathological changes in the lungs of rats exposed to 4-ET at concentration of 477 mg/m^3 (Table 5).

Table 5. The number of rats with pathological changes in lungs after 28 days of 4-ethyltoluene exposure

	Control (n = 7)	477 mg/m^3 (n = 7)	2337 mg/m^3 (n = 8)
Lungs and bronchi within limits			
males	5	6	1
females	5	5	1
Proliferation of bronchi associated lymphatic tissue			
males	0	1	1
females	0	0	0
Alveolar macrophages			
males	1	0	1
females	0	1	2
Bronchitis and/or pneumonia			
males	0	0	4
females	2	0	1
Perivascular infiltration by lymphocytes			
males	1	0	1
females	0	1	4

DISCUSSION

The dermal and eye irritation indices after single exposures of rabbits indicate that 4-ET can be classified as a substance with a very mild irritant effect. A similar effect was found in rabbits exposed to toluene, xylene and ethylbenzene after single dermal and eye application of these substances (6,20).

The irritant effect of 4-ET on the respiratory rate in mice was demonstrated by a decrease in the breath ratio, depending on the vapour concentration. The 4-ET concentration of 4216 mg/m^3 depressed the respiratory rate in mice to 50 (RD_{50}). The values of RD_{50} were lower for trimethylbenzene isomers (pseudocumene – 2844 mg/m^3, mesitylene – 2553 mg/m^3, hemimellitene – 2662 mg/m^3) and much higher for toluene and xylene (17893 mg/m^3 and 10590 mg/m^3) (11,12,13). Similar RD_{50} values of toluene and xylene were reported by other authors (3,15). Nielsen and Alarie (15) suggest that the irritation effect of a given compound on the respiratory tract increases with elongation of the hydrocarbon chain connected with benzene ring. Moreover, it is suggested that this effect increases, depending on the number of methyl groups contained in the molecule (3,11,13,15). The results of our study on 4-ET suggest that the number of methyl groups in the molecule rather than in the elongate of the hydrocarbon chain influences the respiratory irritant potency of benzene derivatives.

The enzymatic activity, total protein, mucoprotein concentration and cytologic data on the lavage fluid of the rats exposed to 4-ET for 4 weeks indicated a linear dose-response relationship. The response in lavage fluid preceded histopathological changes observed in the lung tissue. It can be concluded from the study that changes, indicating pulmonary injury in BAL, emerged quite early and that the method in question can be used to detect an inflammatory response in the lung.

A twenty-eight-day inhalation exposure to 4-ET revealed its irritative effect on the lungs. An elevated rate of bronchitis and pneumonia, as well as perivascular infiltration by lymphocytes was found histopathologically in rats exposed to 4-ET at concentration of 2337 mg/m^3. Whereas 4-ET concentration of 477 mg/m^3 induced no significant changes in the respiratory tract of male and female rats.

ACKNOWLEDGEMENTS

This study was supported by grant No. 0925/S4/95/07 from the State Committee for Scientific Research, and project 1.5/95 of The Nofer Institute of Occupational Medicine. The authors are grateful to Elżbieta Rajkowska MSc, Janina Kłosek and Krzysztof Mader for their technical assistance.

REFERENCES

1. Alarie Y. Irriting properties of airborne materials to upper respiratory tract. Arch Environ Health 13: 433–449, 1966.
2. Bergmeyer HU. Methods of enzymatic analysis. Verlag Chemie GmbH, 574–580,1974.
3. Bos PMJ, Zwart A, Reuzel PGJ, Bragt PC. Evaluation of the sensory irritation test for the assessment of occupational health risk. Toxicology 21: 423–449, 1992.
4. Dingle IT. Lysosomes. A laboratory handbook 113. Elsevier North-Holland Biomedical Press, 1977.
5. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharm Exp Ther 82: 377–390, 1944.
6. Ethel Browning's Toxicity and Metabolism of Industrial Solvents I: Hydrocarbons, Ed. R Snyder R. Elsevier, Amsterdam-New York-Oxford, 1987.
7. Homolka J. Clinical Biochemistry. PZWL, Warszawa, 386–387, 1971 (in Polish).
8. Hollander M, Wolfe DA. Nonparametric Statistic Methods. Wiley, New York, 1973.
9. Jędrychowski R. Statistic methods in evaluation of toxicity. In: Industrial Toxicology. Eds. J Indulski, JK Piotrowski. The Nofer Institute of Occupational Medicine, Łódź, 78–81, 1993 (in Polish).
10. Jonckheere AR. A distribution – free k-sample test against ordered alternatives. Biometrica 41: 135–45, 1954.
11. Korsak Z, Sokal J, Dedyk A, Tomas T, Jędrychowski R. Toxic effects of combined exposure to toluene and xylene in animals. I. Acute inhalation study. Pol J Occup Med 1: 45–50, 1988.
12. Korsak Z, Świercz R, Rydzyński K. Toxic effects of acute inhalation exposure to 1,2,4-trimethylbenzene (pseudocumene) in experimental animals. Int J Occup Med Environ Health 8: 331–337, 1995.
13. Korsak Z, Rydzyński K, Jajte J. Respiratory irritative effects of trimethylbenzenes: an experimental animal study. Int J Occup Med Environ Health 10: 303–311, 1997.
14. Lowry AH, Rosebrough NJ, Farr AL, Randa LL. Protein measurement with the Folin phenyl reagent. J Biol Chem 193: 265–275, 1951.
15. Nielsen GD, Alarie Y: Sensory irritation, pulmonary irritation and respiratory stimulation by airborne benzene and alkylbenzenes: prediction of safe industrial exposure levels and correlation with their thermodynamic properties. Toxicol Appl Pharm 65: 459–477, 1982.
16. OECD guidelines for the testing of chemicals, No 404 OECD, Paris, 1993.
17. OECD guidelines for the testing of chemicals, No 405 OECD, Paris, 1993.

18. Rydzyński K, Jędrychowski R: Sensory irritating properties of cyanuric chloride as revealed with plethysmographic method. Int J Occup Med Environ Health 7: 149–154, 1994.
19. Sokal J, Knapek R, Majka J, Barański B, Jędrychowski R. Scope and Methods in Toxicity Testing of Industrial Chemicals. 6. The Nofer Institute of Occupational Medicine, Łódź, 1990 (in Polish).
20. Toluene. Environmental Health Criteria. No. 52. WHO, Geneva, 1985.

Received for publication: September 26, 2000
Approved for publication: November 30, 2000