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IS IT SAFE TO APPLY THE ADDITIVITY RULE TO EVALUATING HEALTH EFFECTS OF EXPOSURE TO FARBASOL?

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Abstract. Neurotoxic and sensory respiratory irritation effects of Farbasol in male rats and male Balb/C mice were investigated in condition of acute inhalation exposure. Rotarod performance and pain sensitivity behaviour were tested in rats exposed to Farbasol at concentrations of 4665–9622 mg/m^3 immediately after termination of a four-hour exposure. The respiratory rate was measured in mice by the whole body plethysmographic method in 6 min duration exposure to various concentrations of Farbasol. Exposure to Farbasol resulted in concentration-dependent disturbances in rotarod performance and decrease in pain sensitivity in rats, and depression of respiratory rate in mice. The EC_{50} value for rotarod performance behaviour disturbances was 5497 mg/m^3 and for pain sensitivity EC_{50} was 6589 mg/m^3. The concentration depressing the respiratory rate to 50% (RD_{50}) was 3139 mg/m^3. The comparison of Farbasol EC50 and RD50 values and its individual constituents such as trimethylbenzene isomers and 4-ethyl toluene established in similar experimental conditions indicate their similar toxic effects potency. Application of additivity rule for establishing the MAC value for Farbasol seems to be safe.

INTRODUCTION

Farbasol is a very popular, commercial organic solvent mixture, used in Poland. Similar organic solvent mixtures used are: Solvesso 100 (Exon Chemical, Belgium), Shellsol A (Shell Netherland Chemie B.V) and Jolasol (JLC Chemie, Austria). Trimethylbenzene isomers and ethylotoluene are the substantial constituents of these solvent mixtures.

Combined exposure to various mixtures of organic solvents frequently occurs under industrial conditions whereas exposure limits are set separately for single solvents. To overcome this inconsistency a well known additivity formula is often used for the evaluation of the total exposure:

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$$\frac{C_1}{MAC_1} + \frac{C_2}{MAC_2} + \ldots \frac{C_n}{MAC_n} < 1$$

It is belived that toxic effects of combined exposure to two or more solvents are additive in nature due to similarities in their toxic effect.

The additivity formula is adopted in the regulations of the State Sanitary Inspectorate in Poland (4) and widely used in hygiene practice. However, there are scientific and regulatory bodies which do not readily accept this formula. The German MAC-Commission, for instance, takes the view that additivity rule is not based on any sufficient scientific grounds (9). We share this opinion, however, with the reservation that the application of MAC values for single solvents only, could lead to underestimation of actual exposure and consequently to adverse health effects.

It is generally recognised that toxicant interactions may occur during any of the toxicological processes that take place with a single compound: absorption, distribution, metabolism, excretion and activity at the receptor site (5).

If nothing is known about an interaction between components of solvent mixture (such is the case of Farbasol) the application of additivity rule shall go together with an anxiety for over- or underestimation of MAC value for the mixture. Underestimation of solvent mixtures MAC value may result in adverse health effects.

In our earlier studies (7,8,10,11) the neurotoxic and irritating effects of trimethylbenzene isomers and 4-ethyltoluene, which are the main components of Farbasol, were evaluated in conditions of acute and sub-chronic inhalation exposure.

Based on obtained data the MAC values for ethyltoluene was proposed and reevaluated for trimethylbenzene isomers.

To compare the risk of over- or underestimation of Farbasol exposure health effects, with their single constituents, neurotoxic and irritating effects on the respiratory tract of this mixture in conditions of acute inhalation exposure in rats and mice were studied (7,8,10,11).

MATERIALS AND METHODS

Farbasol was supplied by Petrochemia, Płock and consisted of:

1) o-xylene		5.1%
2) cumene		1.4%
3) n-propylbenzene		7.6%
4) ethyltoluene		40.9%
2-ethyltoluene	7.0%	
3-ethyltoluene	23.4%	
4-ethyltoluene	10.5%	
5) trimethylbenzene isomers		44.0%
hemimellitene	3.6%	
pseudocumene	29.3%	
mesitylene	11.1%	
6) diethylbenzene isomers		0.9%

Concentrations of the Farbasol components were measured by a gas chromatograph Helwett-Packard with a flame-ionization detector using a capilary column (HP-5 40 m · 0.2 mm). Operating conditions were: carrier gas (argon)

flow – 0.4 cm^3/min, make-up gas (argon) – 30 cm^3/min, hydrogen – 30 cm^3/min, air – 300 cm^3/min; injector – 250°C; detector – 220°C. The oven temperature was programmed as follows: initial oven temperature was set at 32°C for 1 min and than increased at the rate of 2°C/min up to t_1 = 115°C and held for 1 min; then increased 15°C/min up to t_1 = 180°C and held for 3 min.

Male Wistar rats of Imp:DAK stock outbred body, weighing 250–300 g and Balb/C male mice, weighing 25–30 g, were used. Animals were housed in wire mesh stainless steel cages. LSK lab chow and water were provided *ad libitum*. Animal rooms were maintained at 22-25°C with a 12-hr light-dark cycle (lights on 6:00 AM). Animals were acclimated for 1 week prior to the experiment and were used within 4 weeks upon arrival. Rats were exposed to Farbasol at tested concentrations of 4665–9622 mg/m^3 for 4 hours. Animals were exposed to vapours of Farbasol in a dynamic inhalation chamber (volume of 1.3 m^3, 12 to 15 air changes per hour). Vapours of Farbasol were generated by heated atomizer. The desired concentrations of Farbasol vapours were obtained by diluting them in the air. Concentration of solvent vapours in the exposure chamber were measured every 30 min with a gas chromatograph Hewlett-Packard with a flame-ionization detector using 30 m HP-1 column at temperature of 135°C.

Rotarod performance was tested according to the principle described by Kaplan and Murphy (6). The rotarod apparatus used consisted of an 8-cm diameter wooden rod rotating at 12 rpm and suspended horizontally 20 cm above the floor which was constructed from metal bars connected to a power source of 80 V and 2 mA. The ability of rats to remain on the rotating rod for 2 min was taken as an index of normal neuromuscular function. Before the experiment, the animals were trained and only those rats which could perform normally on the rotarod for at least 10 consecutive days were used in the experiment. Rotarod performance was tested before exposure and immediately after termination of exposure to several concentrations of Farbasol and in sham exposed control animals for four hours. Each group consisted of 10 rats.

Hot plate behaviour was tested immediately after termination of exposure. The hot-plate test was used to measure the level of analgesia (2). The rat was placed on the hot-plate within the plastic enclosure and after occurrence of the expected response – licking the foot, or after 60 sec, the animal was removed. The latency of the paw-lick response was measured at plate temperature of 54.5°C. Each group consisted of 10 rats.

The respiratory rate was measured in Balb/C male mice by the use of plethysmographic method (11). Each animal was placed in a body plethysmograph attached to a small dynamic inhalation chamber (volume of 2.3 dm^3). A Stattham pressure transducer was attached to each plethysmograph. The respiratory pattern was recorded by the use of a Beckman polyphysiograph. The respiratory rate was recorded continuously before the exposure to solvent, during 6 min of exposure and 6 min after termination of exposure.

Each exposure group consisted of 8–10 mice. For calculation of RD_{50} value, the maximum decrease in the respiratory rate, observed in the second minute of exposure, was used.

Statistical analysis

Probit analysis was applied to determine the medial effective concentration EC_{50} value in rotarod performance test (3). The concentration depressing the respiratory

rate in mice to 50% (RD_{50}) and concentration increasing the latency of the paw-lick response (decrease in sensitivity to the pain) to 50% (EC_{50}) were calculated from least squares regression lines of concentration-effect relationship (5).

RESULTS

All rats exposed to the concentrations of Farbasol ranging from 4665 to 9622 mg/m^3 for 4 hours, survived the exposure. Farbasol caused concentration-dependent disturbance in the rotarod performance of rats (Table 1, Fig. 1). All control animals performed correctly in the test throughout the experiment. Rotarod performance behaviour disturbance EC_{50} value determined with its 95% confidence intervals amounted 5497 mg/m^3 (3778 – 6243 mg/m^3). Rotarod performance behaviour disturbance EC_{50} value was higher than that of 4-ethyltoluene and trimethylbenzene isomers (Fig. 1).

Table 1. Rotarod performance in rats exposed to the vapours of Farbasol for 4 hours

Control	Farbasol	
No. of failures/ No. of tested animals	Concentration mg/m^3	No. of failures/ No. of tested animals
0/10	4665	4/10
0/10	5497	5/10
0/10	6635	8/10
0/10	9622	10/10

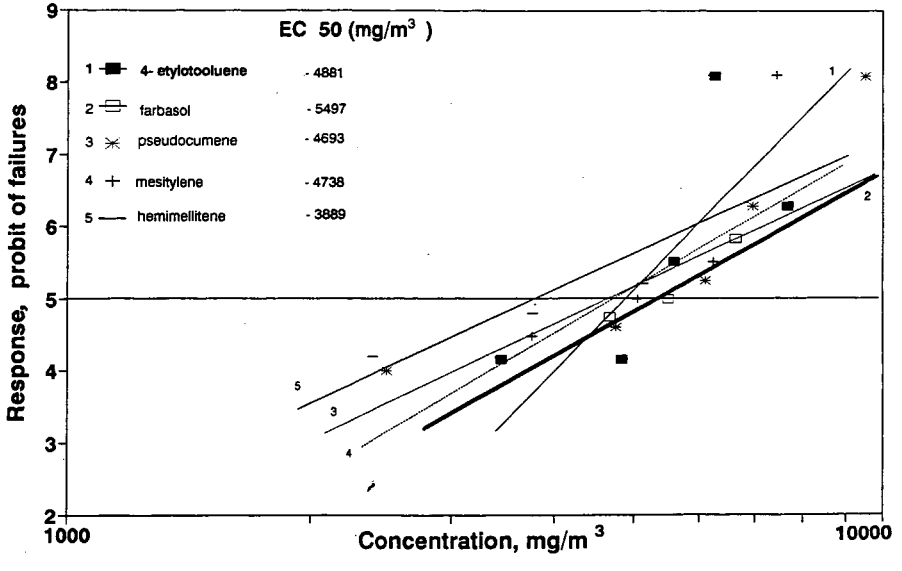

Fig. 1. The EC_{50} values calculated for disturbances in rotarod performance of rats exposed to Farbasol trimethylbenzene isomers (7) and 4-ethylotoluene (10). Rats were exposed to vapours of each solvent for 4 hours. Rotarod performance was tested immediately after termination of exposure. Each point represents probit of failures on rotarod in a group of 10 rats.

The pain sensitivity measured as latency of the paw-lick response was changed in rats exposed to Farbasol. Farbasol like its individual constituents decreased sensitivity to the pain and changes were concentration-dependent (Table 2, Fig. 2). EC_{50} value with 95% confidence intervals amounted to 6589 mg/m^3 (5707 – 10528 mg/m^3).

The pain sensitivity decrease EC_{50} value was higher than that of 4-ethyltoluene and trimethylbenzene isomers (Fig. 2).

Table 2. Latency of the paw-lick response (hot plate behaviour) in rats exposed to the vapours of Farbasol for 4 hours

Group	Latency of the paw-lick response, sec	Decrease in the pain sensitivity, %
Control (n = 40)	10.0 ± 2.3	
4665 mg/m^3 (n = 10)	17.2 ± 4.5	14.4
5497 mg/m^3 (n = 10)	20.4 ± 6.4	20.8
6635 mg/m^3 (n = 10)	36.9 ± 13.0	53.8
9622 mg/m^3 (n = 10)	60	100

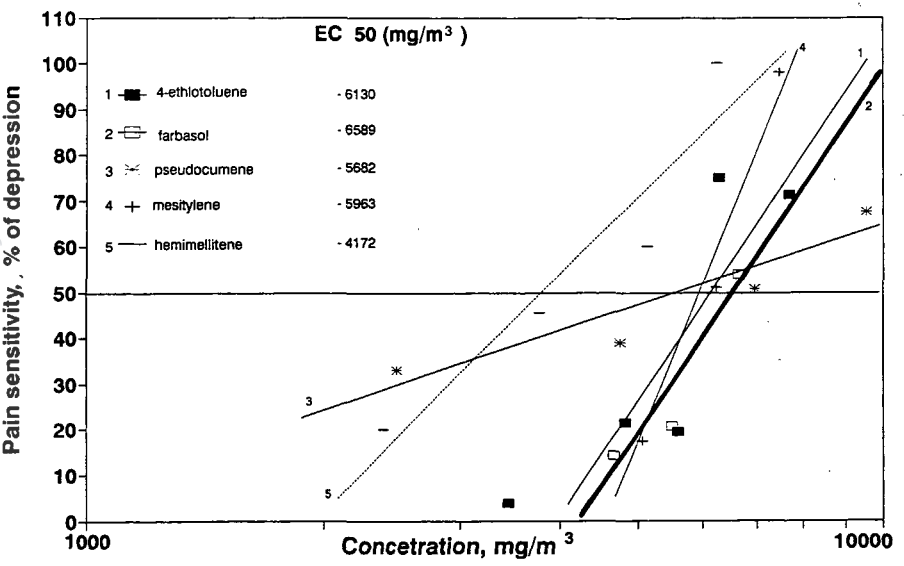

Fig. 2. The EC_{50} values established for the decrease in the pain sensitivity of rats exposed to Farbasol, trimethylbenzene isomers (7) and 4-ethyltoluene (10). Rats were exposed to vapours of each solvent for 4 hours. Hot-plate behaviour was tested immediately after termination of exposure. Each point represents the mean value of separate measurements of latency over the control in 10 rats.

Farbasol caused concentration-dependent decrease in the respiratory rate in mice (Figs. 3 and 4). The maximum decrease in the respiratory rate was observed in the second minute of exposure (Fig. 3). The concentration depressing the respiratory rate in mice to 50% (RD_{50}) with its 95 confidence intervals was 3137 mg/m^3 (1902 – 6672 mg/m^3) (Fig. 4).

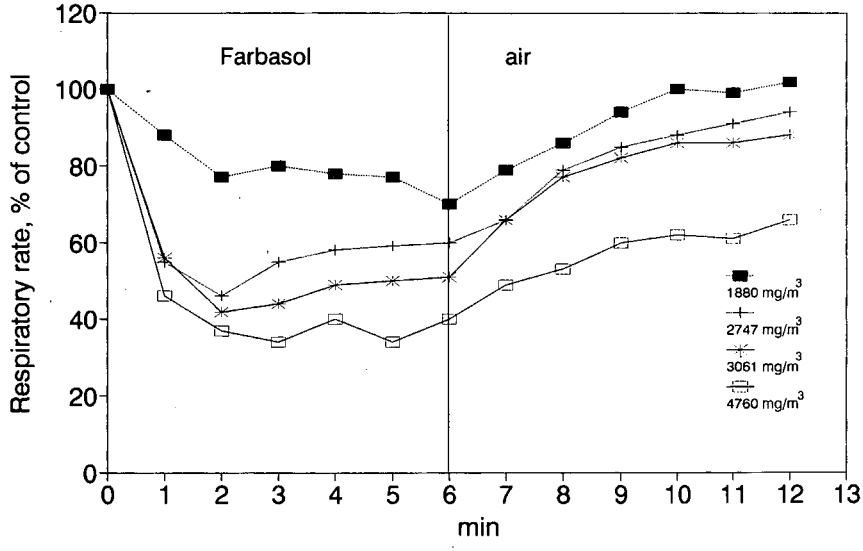

Fig. 3. Time response relationship for the effect of Farbasol on the respiratory rate in mice. Each point represents the mean value in 8–10 mice.

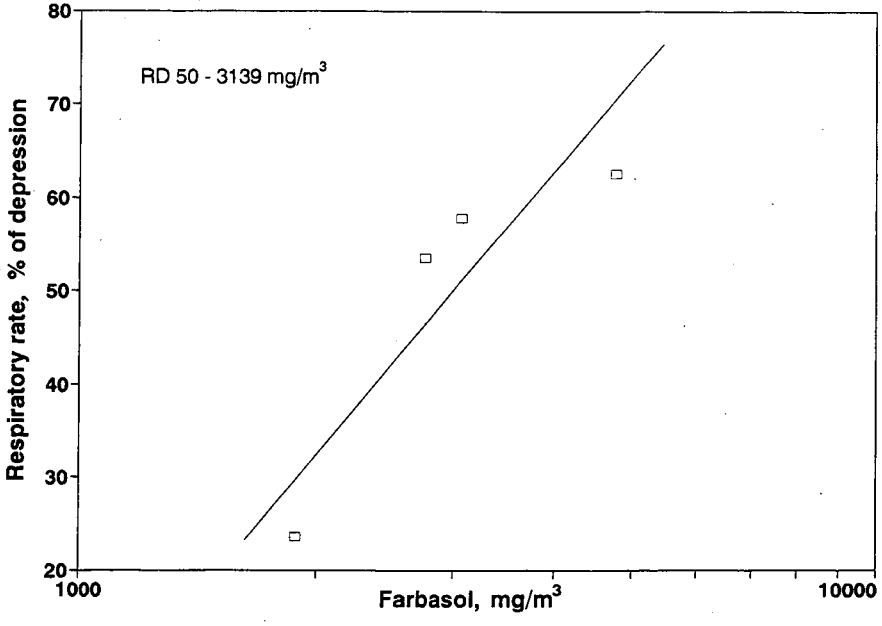

Fig. 4. The respiratory rate of mice exposed to Farbasol. Each point represents the mean value of separate measurements in 8–10 mice. The maximal decrease in the respiratory rate observed after 2 min of exposure was taken into consideration. The regression line was determined by the least squares procedure.

The established Farbasol RD_{50} value was higher than that of trimethylbenzene isomers and lower than that of 4-ethyltoluene (Fig. 5).

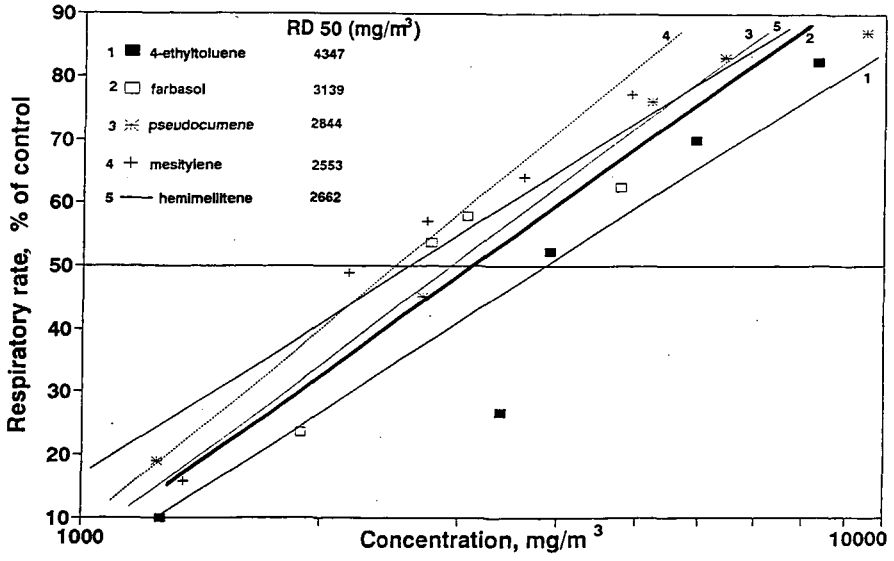

Fig. 5. The RD_{50} values calculated for the respiratory rate in mice exposed to Farbasol, trimethylbenzene isomers (8) and 4-ethyltoluene (10). The regression lines were determined by the least squares procedure.

DISCUSSION AND CONCLUSIONS

Results of rotarod performance and hot plate behaviour tests in rats exposed to vapours of Farbasol at studied concentrations provide an evidence of its neurotoxic effects. The observed disturbances in rotarod performance behaviour and decrease in pain sensitivity were concentration-dependent and indicated a smaller neurotoxic effect of Farbasol than of trimethylbenzene isomers or 4-ethylotoluene (Figs. 1 and 2). The Farbasol EC_{50} values for rotarod performance behaviour and pain sensitivity were 5497 mg/m^3 and 6589 mg/m^3, respectively and thus were higher than that of trimethylbenzene and 4-ethylotoluene established in similar experimental conditions (7,10). Farbasol showed irritating effect on the respiratory tract and caused concentration-dependent decrease in the mice respiratory rate (Figs. 3 and 4). The concentration depressing the respiratory rate in mice to 50% (RD_{50}) was 3139 mg/m^3 and was higher than that of trimethylbenzene isomers and lower than that of 4-ethyltoluene (8,11). If calculations of Farbasol occupational exposure limit were based on prevention of sensory irritation and set at proposed value of 0.03 RD_{50} (1) the Farbasol MAC value should be around 100 mg/m^3. When compared with trimethylbenzene isomers and ethyltoluene, the irritating effect of Farbasol was similar or less potent.

Occupational exposure limits for most of the organic solvents are predominantly based on the protection against their neurotoxic effects.

The established Farbasol EC_{50} values for rotarod performance and pain sensitivity behaviour indicate similar or even less potent neurotoxic effect of Farbasol than that of its individual constituents such as trimethylbenzene isomers and 4-ethyltoluene. The comparison of neurotoxic and sensory irritation effects of Farbasol multicomponent organic solvent mixture and of its main individual constituents in rats and mice, observed in conditions of acute inhalation exposure,

give no indication of Farbasol synergistic or potentiating toxic effects, but indicate their similar toxic effect potency.

Acute exposure to toxic effects of Farbasol mixture and its main constituents indicate that application of the additivity rule for Farbasol in industrial hygiene seems to be safe.

REFERENCES

1. Alarie Y. Establishing threshold limit values for airborne sensory irritants from an animal model and the mechanism of action of sensory irritants. Adv Environ Toxicol 8: 153–167, 1984.
2. Bodnar RJ. Types of stress which induce analgesia. In: Stress Induced Analgesia. MD Tricklebank, G Curzon, eds. Willey, London, 1984.
3. Finey DJ. Probit Analysis. Cambridge University Press, Cambridge, 1971.
4. Guidelines of the Chief Sanitary Inspector for measurements and assessment of harmful substance concentrations and for physical agents at workplace environment. The Nofer Institute of Occupational Medicine, Łódź, 1993 (in Polish).
5. Jędrychowski R. Statistical methods for evaluation of toxicity. In: Industrial Toxicology, J. Indulski, J.K. Piotrowski, eds. The Nofer Institute of Occupational Medicine, Łódź, 1983 (in Polish).
6. Kaplan ML, Murphy SD. Effect of acrylamide on rotarod performance and sciatic nerve beta-glucuronide activity of rats. Toxicol Appl Pharmacol 22: 259–266, 1972.
7. Korsak Z, Rydzyński K. Neurotoxic effects of acute and subchronic inhalation exposure to trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) in rats. Int J Occup Med Environ Health 9: 341–349, 1996.
8. 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.
9. Maximum Concentrations at the Workplace and Biological Tolerance Values for Working Materials 1989 – Report No XXV, Commission for Investigation of Health Hazards of Chemical Compounds in the Work Area, Verlag Chemie, 1989.
10. Rydzyński K et al. Acute and 28-day inhalation toxicity of 4-ethyltoluene in experimental animals. Toxicol Letters Supp 1/88: 44, 1996.
11. Świercz R, Jajte J, Rydzyński K, Stetkiewicz J. Inhalation toxicity study of 4-ethylotoluene in experimental animals. Toxicol Letters Suppl 1/78: 1995.
12. Tomas T, Oliskiewicz W, Czerczak S, Sokal JA. Decrease in the mice's respiration rate as an index of chemical irritating effects on the upper respiratory tract. Med Pr 36: 295–302, 1985 (in Polish).

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