Polish Journal of Occupational Medicine and Environmental Health, Vol 5, No 1, 35-42, 1992

THE TOXIC EFFECTS OF COMBINED EXPOSURE TO TOLUENE AND M-XYLENE IN ANIMALS. IV. LIVER ULTRASTRUCTURE AFTER SUBCHRONIC INHALATORY EXPOSURE

KONRAD RYDZYŃSKI[1,2], ZOFIA KORSAK[1], URSZULA JEDLIŃSKA[2] and JERZY A. SOKAL[1]

[1] Department of Toxicity Evaluation, [2] Department of Pathomorphology, The Nofer Institute of Occupational Medicine, Lodz, Poland

Key words: Toluene, m-Xylene, Combined exposure, Liver, Ultrastructure

Abstract: The effects of combined inhalatory exposure to m-xylene and toluene on the rat hepatocytes were investigated. Limited proliferation of smooth endoplasmic reticulum and increase of the number of lysosomes were observed in hepatocytes after 3-months of single exposure to 1000 ppm of m-xylene and toluene. Moreover, mitochondria increased in most of hepatocytes. After combined exposure to mixture of m-xylene and toluene at concentrations of 500 + 500 ppm, respectively, the types of ultrastructural changes in hepatocytes followed the pattern observed after exposure of each of the single organic solvent. However, proliferation of the smooth endoplasmic reticulum was more eminent.

The hepatocytes ultrastructure of rats exposed to 100 ppm of m-xylene and toluene for 6 months were similar to that observed in rats exposed to 1000 ppm of m-xylene for 3 months. In rats exposed to a mixture of m-xylene and toluene for 6 months, the ultrastructural changes in hepatocytes were a combination of those observed after a single exposure to each of the organic solvent.

It may be concluded that changes in the hepatocytes ultrastructure were an adaptive rather than a toxic effect. Because in the case of combined exposures the doses were half of those used in single exposures, the additive effect on the proliferation of smooth endoplasmic reticulum was observed in the rat liver after combined exposure to toluene and m-xylene using electron microscopy.

INTRODUCTION

Inhalatory exposure to m-xylene and toluene may cause adverse effects on the rat liver morphology. The increased number of mitochondria and their relative cytoplasmic volume as well as increase of nuclear density and of nuclear/cytoplasm ratio were reported after subchronic exposure to toluene (17). Slight hepatocellular effects such as an increase of the rough and smooth endoplasmic reticulum and of peroxisomes were revealed in subchronic inhalatory exposure to xylene (14).

Address reprint requests to K. Rydzyński, Department of Toxicity Evaluation, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Łódź, Poland.

According to our knowledge there are no data illustrating the combined effects on hepatocytes of these widely used industrial organic solvents.

It is assumed that the toxic effects of combined exposure to two or more solvents are additive in nature (9). Nevertheless, this assumption is not sufficiently validated and has been shown not to hold up in the case of selected organic solvents (5, 6, 13). Our earlier observations indicated a more than additive effect on the rat central nervous system and respiratory tract after subchronic inhalatory exposure (7). However, no hepatotoxic effects were observed when routine toxicological and pathological methods were applied (7).

The aim of this paper was to answer the questions of whether combined inhalatory exposure to m-xylene and toluene has the same effect on the hepatocytes as the single exposure of each, and whether the observed effects might be evaluated on a subcellular level. Three and six month exposure periods were chosen to test the hypothesis.

MATERIALS AND METHODS

A detailed description of the procedure of inhalation exposure is presented elsewhere (7). Briefly, male Wistar rats, weighing 200–250 g were exposed to vapours of m-xylene, toluene or their mixture 50/50 vol%, in a dynamic inhalation chamber for 6 hours daily, five times a week, for 3 months (3M) and for 6 months (6M). M-xylene and toluene vapours were at concentrations of 1000 ppm and 100 ppm during single exposures for 3M and 6M exposures, respectively. Concentrations of m-xylene and toulene in 50/50 vol% mixtures were 500 + 500 ppm and 50 + 50 ppm during 3M and 6M exposure, respectively.

Samples were taken from the liver right lobe and immediately fixed in 3.6% glutaraldehyde solution in 0.1 M cacodylate buffer, pH 7.2–7.4, overnight. After few subsequent washes in the buffer they were postfixed in 1% osmium teroxide, dehydrated and embedded in Epon; one μm sections were cut and stained with toluidine blue for inspection in light microscopy. Ultrathin sections were cut, poststained with uranyl acetate and lead citrate and examined with JOEL 100C electron microscope at 80 kV.

The distribution and severity of the observed effects compared to the controls were assessed by semiquantitative validation. Slight and focal changes were denoted as +, whereas moderate and multifocal as + +.

RESULTS

Three-month exposure

The ultrastructure of hepatocytes after 3M exposure to 1000 ppm of m-xylene differed only slightly from controls. Limited proliferation of smooth endoplasmic reticulum and an increase of number of lysosomes were observed. In most of the cells there were no changes in ultrastructure of mitochondria and other cytoplasmic organella and components (Fig. 1). A few cells revealed autophagous bodies.

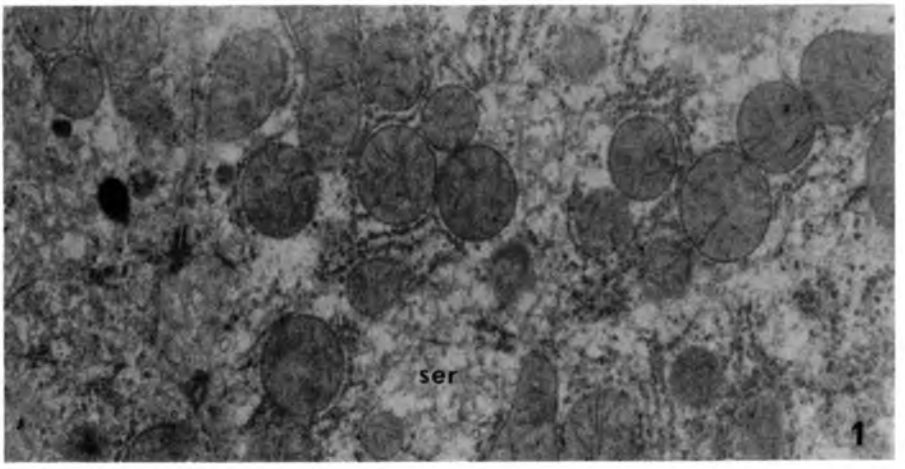

Fig. 1. Electron micrograph of centrilobular hepatocyte of rat exposed for 3 months to 1000 ppm of m-xylene. Note minor proliferation of smooth endoplasmic reticulum (ser.) Mitochondria of number and configurational states typical to this region of hepatocyte. Magnification, × 20000.

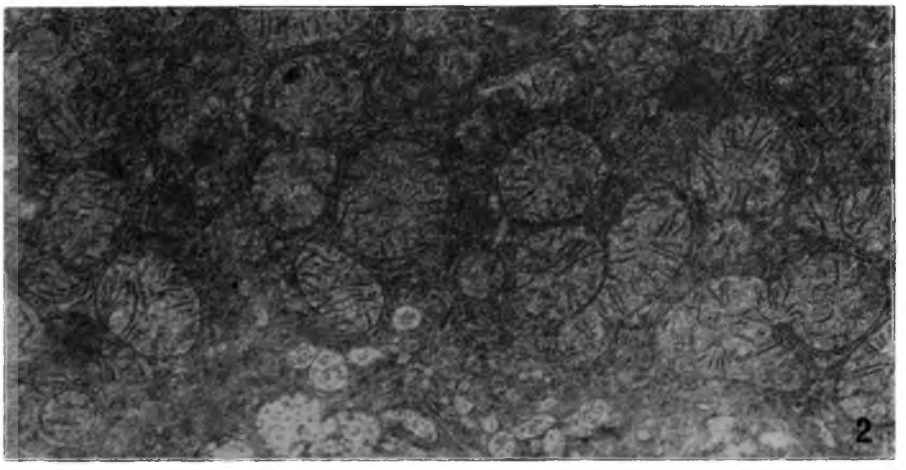

Fig. 2. Increased number of swollen mitochondria in hepatocyte after 3 -month exposure to 1000 ppm of toluene. Magnification, × 20000.

After 3M exposure to 1000 ppm of toluene the smooth endoplasmic reticulum and lysosomes were affected similarily as after exposure to m-xylene. Moreover, mitochondria increased in most of hepatocytes; in some cells they were polymorphic, predominantly of swollen conifiguration (Fig. 2).

After combined 3M exposure to a mixture of m-xylene and toluene at concentrations of 500 + 500 ppm, respectively, the types of ultrastructural changes in hepatocytes followed the pattern observed after single exposure to each of the organic solvents. However, the proliferation of the smooth endoplasmic reticulum was more eminent (Fig. 3). Those observations presented as semiquantitative results, are shown in Table 1.

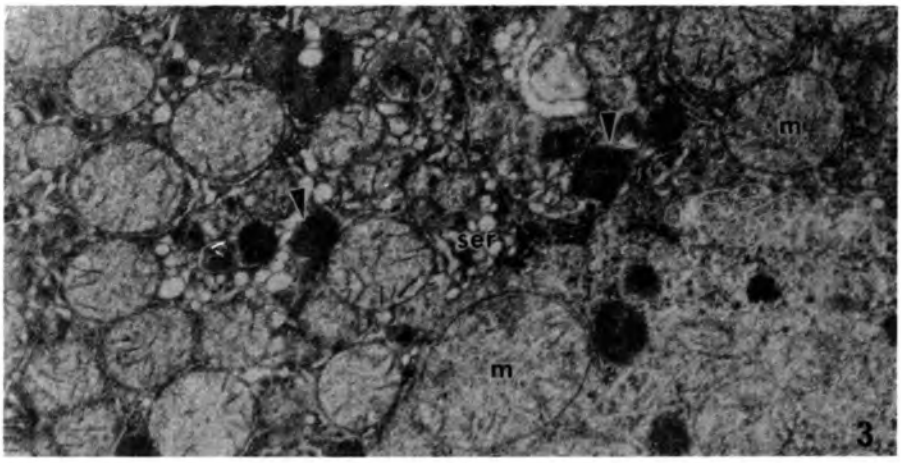

Fig. 3. Hepatocyte of rat exposed for 3 months to 1000 ppm of the 50/50 vol% m-xylene/toluene mixture. Increased proliferation and dilation of SER (ser), swollen mitochondria (m) and abundant lysosomes (arrows). Magnification, × 20000.

Table 1. The effects of m-xylene, toluene and their 50/50% vol mixtures inhalations on the rat hepatocytes. Changes in hepatocytes smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), lysosomes (Lys), mitochondria (Mit), peroxisomes (Per) and nucleus (Nu) were estimated.

	Exposition 1000 ppm/3 months						Exposition 100 ppm/6 months					
	SER	REF	Lys	Mit	Per	Nu	SER	RER	Lys	Mit	Per	Nu
m-xylene	+	NC	+	NC	NC	NC	+	NC	+	NC	NC	NC
toluene	+	NC	+	+	NC	+	+	NC	+	+	NC	+
m-xylene/ toluene	++	NC	+	+	NC	+	++	NC	+	+	NC	+

NC – not changed as compared to controls
\+ – slight and focal changes
++ – moderate and multifocal

Six-month exposure

The hepatocytes ultrastructure of rats exposed to 100 ppm of m-xylene for 6M were similar to that observed in rats exposed for 3M to 1000 ppm of m-xylene. A minor proliferation of smooth endoplasmic reticulum and lysosomes were observed; mitochondria were not affected (Fig. 4).

Ultrastructural changes in hepatocytes after toluene exposure included in--creased number of mitochondria, swelling of some of them and a slight proliferation of smooth endoplasmic reticulum. In some hepatocytes indentations of the nucleus were seen (Fig. 5).

In rats exposed to a mixture of m-xylene and toluene for 6 months the

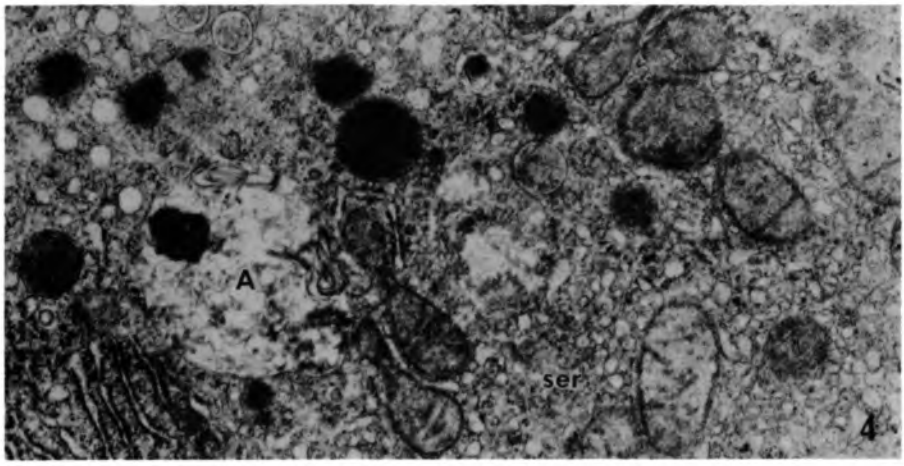

Fig. 4. Electron micrograph of hepatocyte of rat exposed to 100 ppm of m-xylene for 6 months. Dilation and proliferation of smooth endoplasmic reticulum (ser) and increased number of lysosomes. Autophagou s body is also seen (A). Magnification, × 20000.

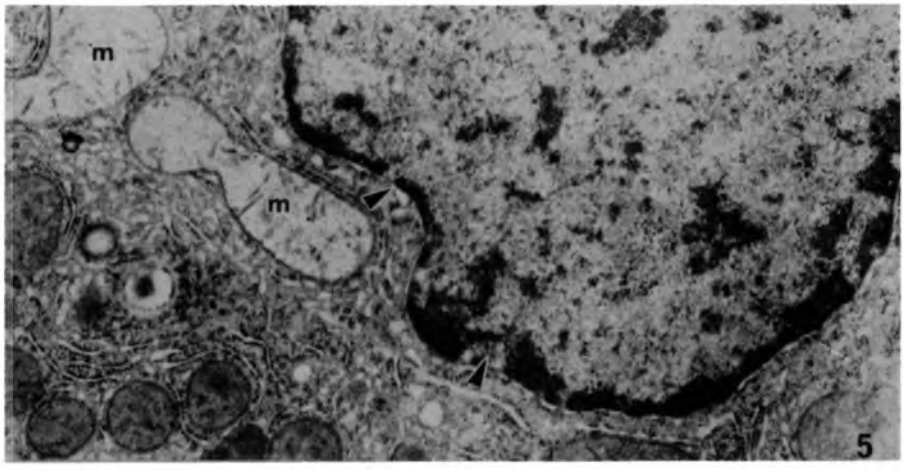

Fig. 5. Hepatocyte of rat exposed for 3 months to 100 ppm of toluene. Indentation of nucleus pointed by arrows. Note swollen mitochondria (m) in the perinuclear region, and dilated cisterns of SER (ser). Magnification, × 20000.

ultrastructural changes in hepatocytes were a combination of those observed after single exposure to each of the organic solvents. There were dilation and proliferation of the smooth endoplasmic reticulum, and increased number of lysosomes and mitochondria. In some hepatocytes giant, swollen mitochondria up to μm were seen (Fig. 6).

Those observations presented as semiquantitative results are shown in Table 1.

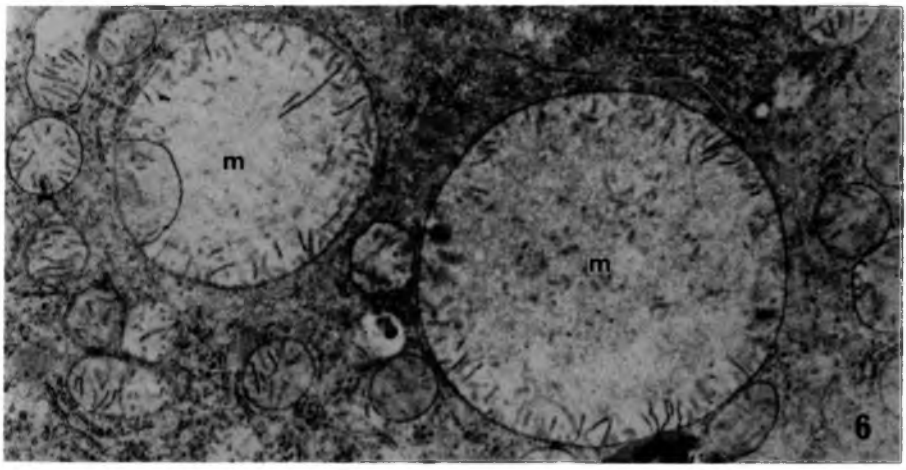

Fig. 6. Swollen, giant mitochondria (m) in hepatocyte of rat exposed for 6 months to 100 ppm of the 50/50 vol% m-xylene/toluene mixture. Magnification, × 20000.

DISCUSSION

The bulk of toxicological science data base is generated from studies of individual chemicals. However, in the real world, most chemical exposures are to mixtures rather than to single agents. Exposure to combinations of toxicants result in the possibility of various interactions due to different cellular mechanisms.

Electron microscopy is one of the method used to examine morphological alterations on a cellular level.

Studies on rats indicate that mixtures of xylenes alike individual xylene isomers generally induce a wide variety of hepatic enzymes and increase hepatic cytochrome P−450 content (2, 3, 11, 15, 16, 19). Upon histopathological examination no treatment-related effects were noted in rats following 13 weeks' exposure to concentrations as high as 810 ppm of mixed xylene (1) or 12 weeks' exposure to 1000 ppm of m-xylene (13).

Electron microscopic examination of liver tissue revealed a proliferation of the smooth endoplasmic reticulum and increased numbers of peroxisomes (14). We have found similar effects except peroxisome proliferation.

Observed hepatic effects in animals following exposure to xylene can be ascribed to the increased metabolism of the solvent and are not necessarily adverse effects (5, 14). It is an adaptive rather than a toxic effect.

Any specific histopathological changes were not found in rats in long-term studies on toluene toxicity (4,8).

However, proliferation of the smooth endoplasmic reticulum and the increased number and relative cytoplasmic volume of mitochondria as well as an increase of nuclear density and of nuclear/cytoplasm ratio were reported after subchronic exposure to toluene (17, 18, 19). These changes were observed in both sexes and were dose-related and reversible. Our studies confirm these observation; however in our experiments, the nuclear changes were less evident.

It should be noted that in both experiments, i.e. 3M and 6M, the similar effects of combined exposure were observed.

The mechanism often proposed for observed differences in chemically induced toxic responses comes from metabolic studies, whereby a chemical may modify its own toxicity or that of other chemicals by altering metabolism through the induction of metabolizing enzymes.

From the study of Ogata and Fuji (10) it appears that metabolisms of these solvents do not significantly interfere with each other. It was confirmed by Pathiratne et al. (12) who investigated the effects of benzene, toluene and xylenes on rat liver metabolism. Benzene was better at inducing conjugating systems, whereas xylenes were more potent at inducing cytochrome P-450-dependent enzymes. Toluene was equally good at inducing both enzyme types. In our study it seems that observed changes in the hepatocytes ultrastructure were adaptive rather than a toxic effect.

Because, in the case of combined exposures, the doses were half of those used in single exposures, the additive effect on the proliferation of the smooth endoplasmic reticulum was observed in rat liver after combined exposure to toluene and m-xylene as revealed with electron microscopy.

REFERENCES

1. Carpenter CP, Kinkead ER, Geary DJ et al. Petroleum hydrocarbon toxicity studies. V. Animal and human response to vapours of mixed xylenes. Toxicol Appl Pharmacol 33: 543–558, 1975.
2. Elovaara E. Dose-related effects of m-xylene inhalation on the xenobiotic metabolism of the rat. Xenobiotica 12: 345–352, 1982.
3. Elovaara E, Collan Y, Pfaffli P et al. The combined toxicity of technical grade xylene and ethanol in the rat. Xenobiotica 10, 435–445, 1980.
4. Gibson JE, Hardisty JF. Chronic toxicity and oncogenicity bioassay of inhaled toluene in Fischer–344 rats. Fundam Appl Toxicol 3: 315–319, 1983.
5. Holmberg B, Lundberg P. Exposure limits for mixtures. Ann Am Ind Hyg 12: 111–120, 1985.
6. Ikeda M. Interactions and health aspects of exposure to mixtures of organic solvents. In: Health effects of combined exposure to chemicals in work and environments. WHO, Regional Office, Europe, Copenhagen, Interim Document 11: 77–96, 1983.
7. Korsak Z, Sokal JA, Górny R. Toxic effects of combined exposure to toluene and m-xylene in animals. III. Subchronic inhalation study. Pol J Occup Med Environ Health, 5: 29–36, 1992.
8. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of toluene in F344/N rats and B6C3F$_1$ mice. NTP Technical Raport no. 371. U.S. Department of Health and Human Services. Public Health Service, National Institutes of Health, Research Triangle Park, NC. 1990.
9. Nordberg G, Astill I, Barlows S et al. Use of toxicity data on single chemicals to predicit the effects of mixtures. In: Methods for assesing the effects of mixtures of chemicals, eds VB Vouk, GC Butler, AC Upton, DV Parke, SC Asher, SCOPE, 99–114, 1987.
10. Ogata M, Fuji T. Urinary excretion of hippuric acid and m-metylhippuric acid after administration of toluene and m-xylene mixtures to rats. Int Arch Occupat Environ Health 43: 45–51, 1979.
11. Patel JM, Harper C, Drew RT. The biotransformation of p-xylene to a toxic aldehyde. Drug Metab Dispos 6: 368–374, 1978.
12. Pathiratne A, Puyear RL, Brammer JD. A comparative study of the effects of benzene, toluene, and xylenes and their *in vitro* metabolism and drug-metabolizing enzymes in rat liver. Toxicol Appl Pharmacol. 82: 272–280, 1986.
13. Sokal JA, Korsak Z. The additivity rule in the evaluation of health effects of combine exposure to solvents – does it hold? Pol J Occup Med 3: 333–336, 1990.
14. Tatrai E, Ungvary G. Changes induced by o-xylene inhalations in the rat liver. Acta Med Acad Sci Hung 37: 211–216, 1980.

15. Toftgard R, Nilsen OG, Gustafsson JA. Changes in rat liver microsomal cytochrome P-450 and enzymatic activities after the inhalation of m-hexane, xylene, methyl ethyl ketone and methylchloroform for four weeks. Scand J Work Environ Health 7: 31–37, 1981.
16. Toftgard R, Nilsen OG. Effects of xylene and xylene isomers on cytochrome P-450 and *in vitro* enzymatic activities in rat liver, kidney and lung. Toxicology 23: 197–212, 1982.
17. Ungvary G, Hudak A, Bors Z, Folly G. The effect of toluene on the liver assayed by quantitative morphological methods. Exp Mol Pathol 25: 49–59, 1976.
18. Ungvary G, Tatrai E, Szeberenyi Sz, Rodics K, Lorincz M, Barcza GY. Effect of toluene exposure on the liver under different experimental conditions. Exp Mol Pathol 36: 347–360, 1982.
19. Ungvary G, Manya S, Tatrai E, Szeberenyi Sz, Csehi RJ, Molnari J, Folly G. Effect of toluene inhalation on the liver of rats – dependence of sex, dose and exposure time. J Hyg Epidem Microbiol Immunol (Praha), 24: 242–252, 1980.

Received for publication: February 26, 1992
Accepted for publication: March 31, 1992