

Interactions of Some Organic Solvents: Hydrocarbons and Chloroalkene

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Metabolic and toxicodynamic interactions of some organic solvents in rats repeatedly treated with medium dose levels were examined. It was shown that both n-hexane and ethylbenzene significantly inhibited tetrachloroethylene metabolism during a 2-week period. n-Hexane and tetrachloroethylene enhanced metabolism of ethylbenzene whereas ethylbenzene suppressed n-hexane metabolism only at the end of the experiment. Biochemical changes, especially the drop in the level of non-protein sulfhydryl groups in tissues of rats treated with organic solvent mixtures, were significantly less pronounced than those observed after these chemicals were administered separately. These results demonstrate that metabolic interactions between hydrocarbons and chloroalkene may lead to a modification of the biological response to these compounds.

n-hexane	ethylbenzene	tetrachloroethylene	metabolic interactions
		toxicodynamic interactions	

This study is part of the National Strategic Programme "Occupational Safety and Health Protection in the Working Environment," supported in 1998-2001 by the State Committee for Scientific Research of Poland. The Central Institute for Labour Protection is the Programme's main co-ordinator.

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1. INTRODUCTION

n-Hexane, ethylbenzene, and tetrachloroethylene are widely used in industry. They are often used as solvents for rubber, dyes, resins, lacquers, and stains. They are also present in thinners, varnish removers, cleaning agents, products for treatment of textiles and leathers, and are ingredients of glues for the shoe industry. The favourable solvent properties of these compounds make them very useful for extracting vegetable oils, manufacture of tablets in the pharmaceutical industry, and also in the perfume industry (Jorgensen & Cohr, 1981; Wesołowski & Gromiec, 1997).

n-Hexane is well known to cause peripheral polyneuropathy. It is metabolised to 2,5-hexanedione (HD). This metabolite was shown to be neurotoxic (Jorgensen & Cohr, 1981; Ogata, Tomokuni, & Takatsuka, 1969; Perbellini, De Grandis, Sementzato, Rizzuto, & Simonati, 1978). The neurotoxicity of n-hexane may be modified by co-exposure to other organic solvents. Altenkirch, Stoltenberg, and Wagner (1978) reported that clinical symptoms and polyneuropathological changes after co-exposure to n-hexane and methyl ethyl ketone (MEK) were found much earlier than those after n-hexane alone. Takeuchi et al. (1983) showed that exposure to the mixture of these two solvents in rats lowered nerve conduction velocity in comparison with rats exposed to n-hexane alone. Iwata, Takeuchi, Hisanaga, and Ono (1983) reported that the metabolite levels of n-hexane significantly decreased after co-exposure to n-hexane and MEK, although there was some evidence that MEK increased the neurotoxicity of n-hexane. Iwata, Takeuchi, Hisanaga, and Ono (1984) reported also that during long-term (33 weeks) exposure to both n-hexane and MEK, the amount of HD in urine significantly decreased in the early stage of exposure and later gradually increased but never exceeded that after n-hexane alone. It was demonstrated, in rats, that toluene suppressed metabolism of n-hexane and led to less pronounced neurological disturbances than n-hexane alone (Takeuchi et al., 1993).

Ethylbenzene is a strong irritant to skin and eyes. It is also neurotoxic in high concentrations. Its metabolic pathway includes oxidation to methylphenylcarbinol with subsequent conversion into mandelic acid in a human being or hippuric acid (HA) in rats (Engström, 1984). The combined exposure of four volunteer participants to a mixture of m-xylene and ethylbenzene in equimolar concentrations (655 mg/m^3) decreased the amounts of urinary metabolites of both compounds. This indicates a reciprocal suppression of biotransformation between m-xylene and ethylbenzene (Engström, Riihimäki, & Laine, 1984).

The toxic effects of tetrachloroethylene include disturbances in the central nervous system and changes in the parenchymal organs, especially in the liver and kidneys. It is metabolised by the mixed-function oxidases system (MFO) to perchloroethylene oxide and then, by rearrangement to trichloroacetyl chloride followed by its hydrolysis, to trichloroacetic acid (TCA; Daniel, 1963). The parent compound, similarly as n-hexane and ethylbenzene, is able to accumulate in lipid-rich tissues, whereas TCA may react with cellular nucleophils (Marth, 1987). The toxicity of various combinations of trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane was examined in rats *in vivo* and *in vitro*. For each pair and for all solvents together, toxicodynamic interactions manifested by a release of potassium anion and increase of indicator enzyme activities from hepatocytes were shown (Stacey, 1989). Metabolic and toxicodynamic interactions between trichloroethylene and tetrachloroethylene in male rats subcutaneously treated with these chemicals were also found (Starek, 1998).

The results of the aforementioned studies provide evidence that some organic solvents can modify one another's metabolism and toxicity. The present study was undertaken to obtain data on n-hexane, ethylbenzene, and tetrachloroethylene metabolic and toxicodynamic interactions in rats during 28-day exposure.

2. MATERIALS AND METHODS

2.1. Chemicals

n-Hexane, ethylbenzene, tetrachloroethylene, and HD, purity greater than 99%, were purchased from Riedel-de Haën Company (Germany). The other chemicals, that is, HA, TCA, L-(-glutamyl-p-nitroanilide, reduced glutathione (GSH), and tetrachloroethyleneaethoxypropane (TEP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animals and Treatment

Three-month old male Wistar rats were used. The mean weight variation did not exceed $\pm 20\%$. The rats were housed in steel cages in groups of 5 with a 12-hr light/dark cycle (6.00–18.00 light) at $22 \pm 3^\circ\text{C}$ and relative humidity of 45–55%. They were allowed free access to commercial food (Murigran, Poland) and tap water.

The experiments were performed on 35 rats divided into seven groups of 5 animals each. Control rats were treated subcutaneously with olive oil (1.0 ml/kg), whereas the others received in the same manner n-hexane (450 $\mu\text{mol/kg}$), ethylbenzene (450 $\mu\text{mol/kg}$), tetrachloroethylene (450 $\mu\text{mol/kg}$), or two-component mixtures of these chemicals in equimolar doses (450 + 450 $\mu\text{mol/kg}$) in oil solutions for 28 consecutive days. The dose of the examined compounds was selected on the basis of a preliminary experiment.

2.3. Urine Collection and Analysis

During exposure, urine was collected from the rats placed in glass metabolic cages at 1-week intervals. The concentration of n-hexane, ethylbenzene, and tetrachloroethylene metabolites, that is, HD, HA, and TCA in urine was determined by methods of Saito et al. (1991), Ogata et al. (1969), and Ogata, Tomokuni, and Asahara (1974), respectively. The excretion rate of metabolites determined was expressed as $\mu\text{mol/kg/hr}$.

2.4. Blood and Tissue Sampling and Measurements

On the day following the last exposure the rats were anaesthetised with methohexital sodium (40 mg/kg i.p.), bled by cardiac puncture, and killed. The blood was allowed to clot and the serum, obtained after centrifugation, was stored at 2–6 °C for maximum 2 days for the assay of alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), glutamic dehydrogenase (GIDH), γ -glutamyltranspeptidase (GTP), total-cholesterol (T-C), high-density-lipoprotein-cholesterol (HDL-C), triglyceride (TG), and malondialdehyde (MDA).

The liver, kidneys, testes, and spleen were removed, washed in cold saline, blotted dry, and weighed. These organs were kept frozen (–25 °C) and prior to the assay homogenised in 9 vol. of 1.15% KCl. One part of the homogenate was directly used to assay non-protein sulfhydryl groups (NPSH), whereas the other was briefly kept for the determination of the MDA level.

The ALT activities as well as T-C, HDL-C, and TG concentrations in the serum were determined using enzymatic test kits (Alpha Diagnostics, Poland). The low-density-lipoprotein-cholesterol (LDL-C) concentration was calculated from T-C, HDL-C, and TG concentrations. Serum activities of

SDH, GIDH, and GTP were assayed according to Gerlach (1963), Schmidt (1963), and Orłowski and Meister (1965), respectively. The NPSH level was determined by Sedlak and Lindsay's (1968) method. GSH was used as the standard. The MDA level was measured by Mihara, Uchiyama, and Fukuzawa's (1980) method with modification given in a former paper (Starek, 1998). TEP was used as the standard.

2.5. Statistics

The obtained results were statistically evaluated by means of Fischer *F*, Cochran *C* and Cox, and Student's *t* tests.

3. RESULTS

In rats subcutaneously treated with n-hexane and ethylbenzene the urinary excretion rate of HD was reduced only at termination of exposure (Figure 1).

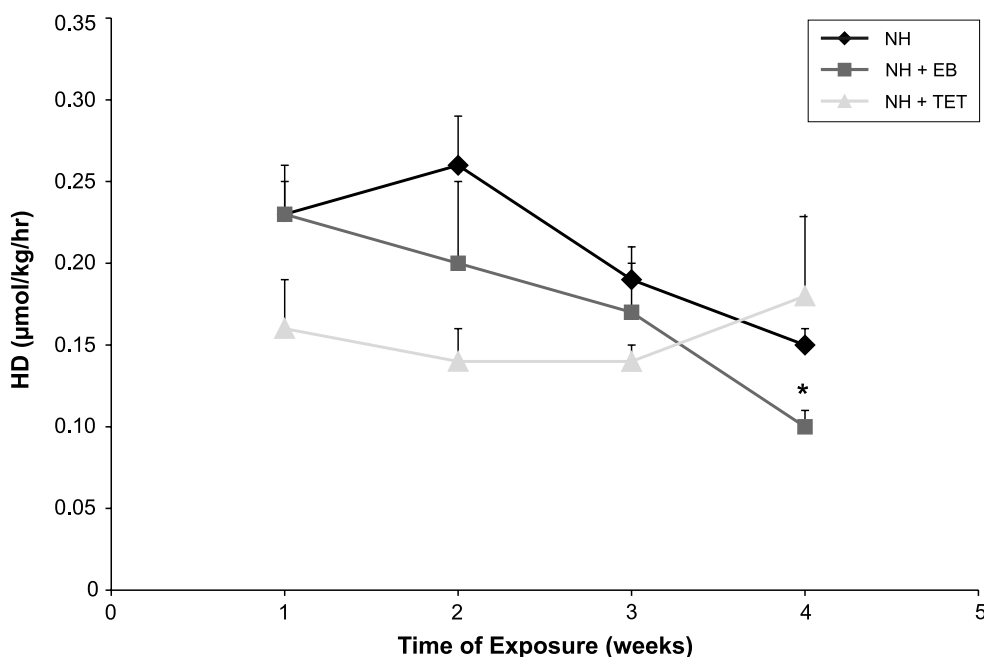


Figure 1. Urinary excretion rate of 2,5-hexanedione in rats subcutaneously treated with n-hexane alone and its mixture with ethylbenzene or tetrachloroethylene in molar ratio 1:1 for 28 days. *Notes.* The results are means $\pm$ SEM. Significance level: * $p < .05$ in comparison with group given n-hexane alone. HD—2,5-hexanedione, NH—n-hexane, EB—ethylbenzene, TET—tetrachloroethylene.

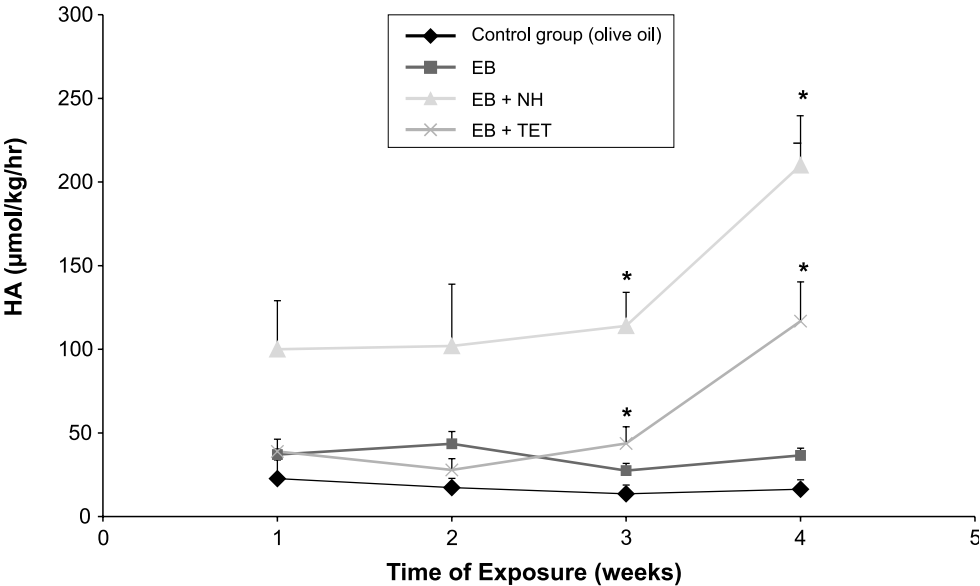


Figure 2. Urinary excretion rate of hippuric acid in rats subcutaneously treated with olive oil or ethylbenzene alone and its mixture with n-hexane or tetrachloroethylene in molar ratio 1:1 for 28 days. Notes. The results are means $\pm$ SEM. Significance level: * $p < .05$, ** $p < .01$ in comparison with group given ethylbenzene alone. HA—hippuric acid, EB—ethylbenzene, NH—n-hexane, TET—tetrachloroethylene.

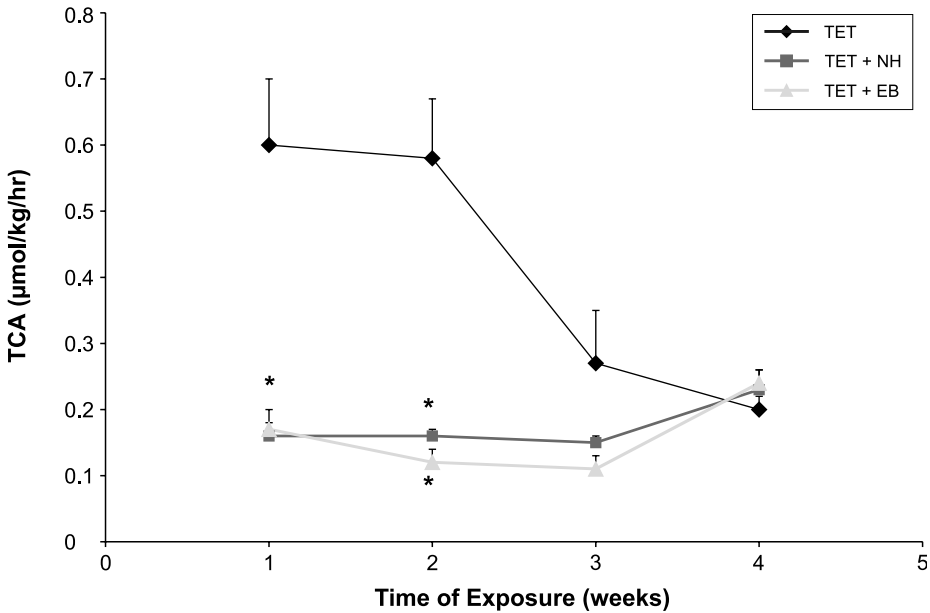


Figure 3. Urinary excretion rate of trichloroacetic acid in rats subcutaneously treated with tetrachloroethylene alone and its mixture with n-hexane or ethylbenzene in molar ratio 1:1 for 28 days. Notes. The results are means $\pm$ SEM. Significance level: * $p < .05$, ** $p < .01$ in comparison with group given tetrachloroethylene alone. TCA—trichloroacetic acid, TET—tetrachloroethylene, NH—n-hexane, EB—ethylbenzene.

The excretion rates of HA in rats exposed to a mixture of n-hexane and ethylbenzene as well as ethylbenzene and tetrachloroethylene were elevated from the third week of exposure (Figure 2). The amounts of TCA excreted in rats treated with both tetrachloroethylene and n-hexane or tetrachloroethylene and ethylbenzene were partly diminished, especially during the first 2 weeks of the treatment (Figure 3).

Neither any solvent alone, nor their mixtures, did affect the indicator enzyme activity in the serum of rats treated with these chemicals (Figure 4). In rats given tetrachloroethylene alone, HDL-C concentration was elevated, whereas the LDL-C level diminished. The LDL-C level in rats exposed to n-hexane alone was lower, whereas after co-exposure to ethylbenzene and tetrachloroethylene it was higher than in the control group (Figure 4).

In rats treated with n-hexane or ethylbenzene alone, the liver NPSH level was significantly lower in relation to the control group. The kidney

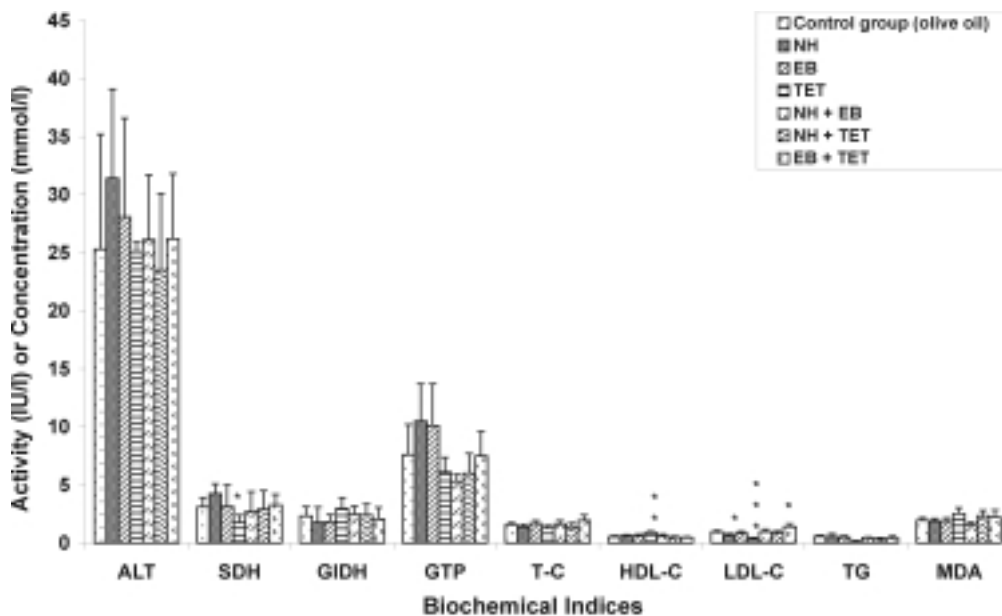


Figure 4. Biochemical indices in the serum of rats subcutaneously treated with n-hexane, ethylbenzene, or tetrachloroethylene as well as their two-component mixtures in molar ratio 1:1 for 28 days. *Notes.* Malondialdehyde level was expressed in $\mu\text{mol/l}$. The results are means $\pm$ SD. Significance level: * $p < .05$, ** $p < .01$, *** $p < .001$ in comparison with control group given olive oil alone. NH—n-hexane; EB—ethylbenzene; TET—tetrachloroethylene; ALT—alanine aminotransferase; SDH—sorbitol dehydrogenase; GIDH—glutamic dehydrogenase; GTP— γ -glutamyl-transpeptidase; T-C—total-cholesterol; HDL-C—high-density-lipoprotein-cholesterol; LDL-C—low-density-lipoprotein-cholesterol; TG—triglyceride; MDA—malondialdehyde.

NPSH concentration in rats exposed to n-hexane or tetrachloroethylene alone as well as treated with a mixture of n-hexane and ethylbenzene was reduced. A similar effect was also observed in the spleen after treatment with tetrachloroethylene alone. The decreases in NPSH levels in rats exposed to mixtures of the examined chemicals were less pronounced than when each compound was given alone (Figure 5). The liver MDA level in rats given tetrachloroethylene alone or a mixture of n-hexane and tetrachloroethylene was increased. The kidney MDA level in rats treated with n-hexane or tetrachloroethylene alone as well as the testicle MDA concentration in animals simultaneously treated with n-hexane and tetrachloroethylene was higher than in the control group. The increases in MDA levels in rats exposed to mixtures of the examined compounds were less pronounced than in rats treated with each chemical separately (Figure 6).

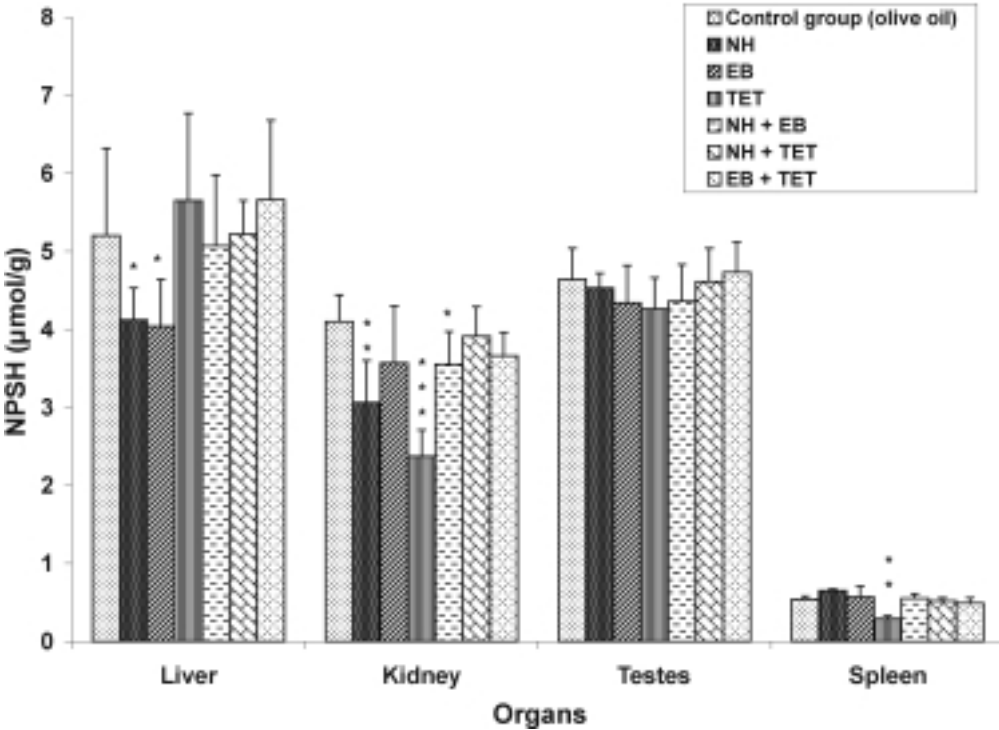


Figure 5. Organ non-protein sulfhydryl group levels in rats subcutaneously treated with n-hexane, ethylbenzene, or tetrachloroethylene as well as their two-component mixtures in molar ratio 1:1 for 28 days. *Notes.* The results are means $\pm$ SD. Significance level: * $p < .05$, ** $p < .01$, *** $p < .001$ in comparison with control group given olive oil alone. NPSH—non-protein sulfhydryl groups, NH—n-hexane; EB—ethylbenzene; TET—tetrachloroethylene.

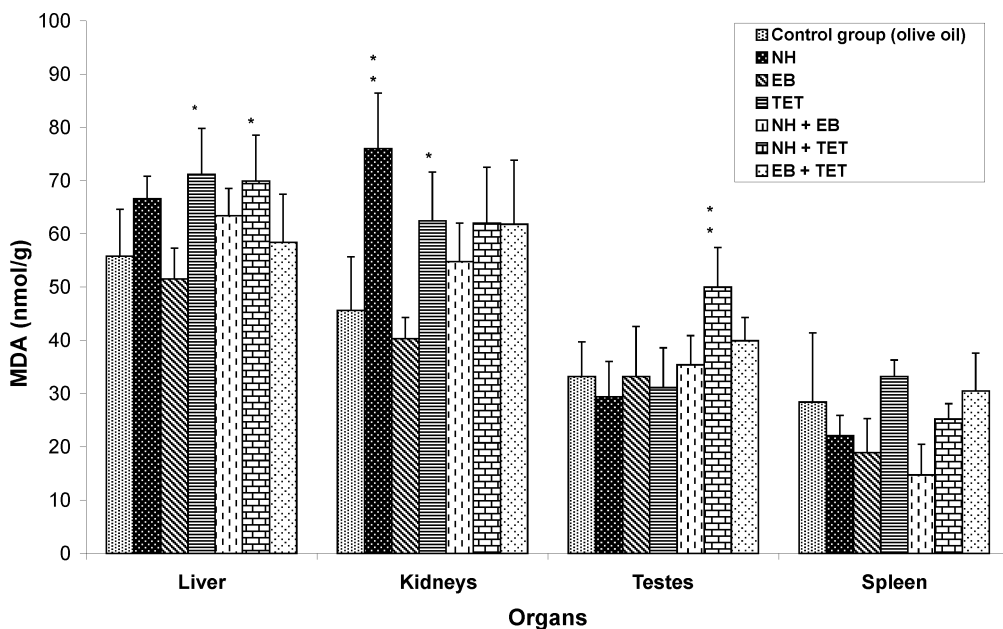


Figure 6. Tissue malondialdehyde levels in rats subcutaneously treated with n-hexane, ethylbenzene, or tetrachloroethylene as well as their two-component mixtures in molar ratio 1:1 for 28 days. Notes. The results are means $\pm$ SD. Significance level: * $p < .05$, ** $p < .01$ in comparison with control group given olive oil alone. MDA—malondialdehyde; NH—n-hexane; EB—ethylbenzene; TET—tetrachloroethylene.

4. DISCUSSION

The metabolism-effect relationship plays an important role in the toxicity of numerous organic solvents. n-Hexane and tetrachloroethylene are examples of these chemicals due to their metabolic activation to the metabolite or the intermediate, which directly exert toxic effects on the organism (Abou-Donia, Makkaway, & Graham, 1982; Buben & O'Flaherty, 1985). In contrast, the metabolism of ethylbenzene displays a classic inactivation process (Elovaara, Engström, Nickels, Aito, & Vainio, 1985).

The results of some studies demonstrate that formation of the reactive intermediate or final metabolite, whatever their chemical structure, must be proportional to the overall amount of metabolism, as when metabolism reaches a constant maximum value toxicity does not increase further, either (Buben & O'Flaherty, 1985; Mitchell, Snodgrass, & Gillette, 1976).

With respect to n-hexane and tetrachloroethylene, both chemicals are believed to be metabolised by MFO (Monster, 1979). The metabolism of

n-hexane and tetrachloroethylene represents typical saturable kinetics. This pattern suggests that hepatic extraction and metabolism of these chemicals is not efficient. The extent of n-hexane and tetrachloroethylene metabolism is dose dependent, even at low concentrations. The liver has a low capacity for metabolising these compounds. This limited capacity, especially for tetrachloroethylene metabolism, has been observed in numerous species (Daniel, 1963; Monster, 1979).

The present study confirms a previous paper (Starek, 1998) that repeated subcutaneous exposure to low doses of tetrachloroethylene causes moderate functional disturbances in the liver demonstrated by changes in serum lipoprotein-cholesterol concentrations and also in the depletion of the organ NPSH levels, and poorly marked increase in tissue MDA levels. A similar, but less pronounced, effect was seen in rats treated with n-hexane alone.

The results of this study demonstrate that ethylbenzene poorly inhibits oxidation of n-hexane to HD, whereas n-hexane or ethylbenzene partly reduces biotransformation of tetrachloroethylene to TCA. On the other hand, n-hexane or tetrachloroethylene exerts a stimulatory effect on the conversion of ethylbenzene to HA. The mechanism of the metabolic interactions of the examined chemicals is not clear. It seems that this may be due to n-hexane, ethylbenzene, or tetrachloroethylene binding to the active sites of MFO taking part in oxidation of these compounds (Soni, Nomiyama, & Nomiyama, 1990).

It is interesting that the depletion of the organ NPSH levels and lipid peroxidation, expressed by an increase of tissue MDA levels, in rats exposed to mixtures of the examined chemicals, was less pronounced than when each compound was given separately. It seems that metabolic interactions between the examined chemicals may lead to a limitation of the direct reactions of their active intermediates with GSH and may protect cells from oxidative stress.

Our results confirm the view that extrapolation of risk would be based on metabolism rather than on dose for those chemicals whose toxicity or carcinogenicity has been demonstrated to depend on metabolic activation (Buben & O'Flaherty, 1985). For risk assessment purposes, metabolic studies should be undertaken to relate the administration dose to the significant internal end-point of metabolism, especially in the case of combined exposure. This principle may also apply to genotoxic carcinogens. Many of these chemicals are first metabolised to toxic intermediates, which react with cellular DNA and RNA. The extent of their metabolism would likely be more directly related to their carcinogenic potency than would dose.

The practical interest of the present study is difficult assess. This results from the difficulty of interpreting animal studies, especially on rodents, for human. Nevertheless it seems that the observed metabolic and toxicodynamic interactions between examined organic solvents are favourable. This means that the examined solvents should be used in a mixture and not alone. It would be advisable to examine people occupationally exposed to these substances.

5. CONCLUSIONS

1. Both n-hexane and ethylbenzene diminish tetrachloroethylene metabolism in rats.
2. Ethylbenzene may suppress n-hexane metabolism in vivo.
3. n-Hexane and tetrachloroethylene enhance metabolism of ethylbenzene.
4. Biochemical disturbances, especially changes in serum concentrations of lipoprotein-cholesterol fractions and drop in organ non-protein sulfhydryl groups levels in rats treated with organic solvent mixtures are significantly less pronounced than that after these compounds are administered separately.
5. It appears that metabolic interaction between hydrocarbons and chloroalkene may lead to a modification of a biological response of an organism to these chemicals.

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