THE JOURNAL OF PREVENTIVE MEDICINE
2003; 11 (1): 53-60

METABOLIC ASPECTS IN RATS EXPOSED INDIVIDUALLY AND SIMULTANEOUSLY TO ORGANIC SOLVENTS

Brigitte Scutaru[1], Mirela Ghițescu[1], Doina Hăvârneanu[1], A. Maftei[1], Mihaela Romanic[2]

1. Public Health Institute Iasi 2. Technical University "Gh. Asachi" Iasi

Abstract. The present study describes the influence of combined exposure to organic solvents on their urinary excretion. Wistar male rats were repeatedly exposed to toluene (TO), xylene (XY) or acetone (AC), individually and simultaneously, or to a mixture of TO and benzene (BE), by intraperitoneal injection (1 injection/week for 12 weeks). Each solvent and mixture was administered in two doses: the high (H) dose was equally to the current occupational exposure limit, the low (L) one was half of the high dose. The rats were divided into 12 groups as follows: (1) 850 (TO_H) and (2) 425 (TO_L) mg/kg toluene; (3) 1500 (XY_H) and (4) 750 (XY_L) mg/kg xylene; (5) 1500 (AC_H) and (6) 750 (AC_L) mg/kg acetone; (7) 1200 (MI_H) and (8) 600 (MI_L) mg/kg mixture (M) I (30 % TO, 30% XY, 40 % AC); (9) 500 (MII_H) and (10) 250 (MII_L) mg/kg mixture II (85 % TO, 15 % BE); (11) control oil (CO) (0.5 ml oil/100 g body weight) and (12) unexposed control (C). Urine samples were collected for 24 h after the last administration. Major metabolites of TO and XY (hippuric acid and methylhippuric acid, respectively) and urinary AC were measured by spectrophotometric methods. The relationship exposure–excretion was examined by simple regression analysis. The correlation of the solvent exposure doses with the concentrations of the corresponding metabolites was close for TO ($r = 0.97$; $p < 0.001$) and XY ($r = 0.95$; $p < 0.05$) both in single and in combined administration. Compared with single treatment, simultaneous exposure resulted in higher amounts of excreted hippuric acid (195 %) and methylhippuric acid (200 %) in urine in the immediate post–exposure period. The excretion of hippuric acid in urine from rats co-injected with mixture II was not modified at both doses. These results suggest metabolic interactions between TO, XY and AC that alter initially the excretion of both metabolites and ultimately the biological monitoring of an exposure to a combination of solvents. No apparent interactive effects between TO and BE have been noted.
Key words: organic solvents mixtures, metabolic aspects, experimental study

Rezumat. Studiul de faţă a urmărit descrierea influenţei expunerii simultane la solvenţi organici asupra excreţiei urinare a acestora. Şobolani masculi din rasa Wistar au fost expuşi repetat la toluen (TO), xilen (XY) sau acetonă (AC), individual şi simultan, sau la un amestec de TO şi benzen (BE), prin injectare intraperitoneală (1 injecţie/săptămână timp de 12 săptămâni). Fiecare solvent şi amestec a fost administrat în două doze: doza mare (H) a fost echivalentă cu concentraţia maximă admisă din mediul ocupaţional, iar cea mică (L) reprezintă 1/2 din doza mare. Şobolanii au fost grupaţi în 12 loturi, după cum urmează: (1) 850 (TO_H) şi (2) 425 (TO_L) mg/kg toluen; (3) 1500 (XY_H) şi (4) 750 (XY_L) mg/kg xilen; (5) 1500 (AC_H) şi (6) 750 (AC_L) mg/kg acetonă; (7) 1200 (MI_H) şi (8) 600 (MI_L) mg/kg amestec (M) I (30 % TO, 30 % XY, 40 % AC); (9) 500 (MII_H) şi (10) 250 (MII_L) mg/kg amestec II (85 % TO, 15 % BE); (11) martor ulei (CO) (0,5 ml ulei/100 g greutate corporală) şi (12) martor neexpus (C). Probele de urină au fost colectate timp de 24 h după ultima administrare.

Metaboliții majori ai TO şi XY, şi anume acidul hipuric, respectiv, acidul metilhipuric, precum şi AC urinară au fost determinați prin metode spectrofotometrice. Asocierea dintre expunere şi excreție a fost examinată prin analiza de regresie simplă. Corelarea dintre dozele de expunere la solvenți şi concentrațiile corespunzătoare ale metaboliților a fost foarte strânsă pentru TO ($r = 0,97$; $p< 0,001$) şi XY ($r = 0,95$; $p< 0,05$), atât în expunerea singulară cât şi în cea combinată. Comparativ cu administrarea individuală, tratamentul simultan a condus la cantități crescute de acid hipuric (195 %) şi metilhipuric (200 %) în urina recoltată imediat după ultima injectare. Excreția urinară de acid hipuric la animalele coinjectate cu amestec II a fost nemodificată la ambele doze. Rezultatele sugerează interacțiuni metabolice între TO, XY şi AC care afectează, în primul rând, excreția ambilor metaboliți şi, prin umare, monitorizarea datelor de expunere la o combinație de solvenți. Aparent nu s-au observat efecte interactive între TO şi BE.

Cuvinte cheie: amestecuri solvenți organici, aspecte metabolice, studiu experimental

INTRODUCTION

It is well recognized in industrial health that man may be exposed simultaneously to more than one chemical. Interactions may take place in the metabolism of chemicals absorbed in combination or in sequence, especially when the chemicals share similar structures. It is further conceivable that the extent of possible metabolic interaction will depend on the intensity of exposure. Moreover, the metabolism of chemicals may be modified by social habits, especially alcohol consumption and smoking (1). No systemic and comprehensive studies however have been reported in literature, possibly because the combination of the chemicals is various and the exposure intensities vary greatly (2). Mainly, the available data arrive from experimental studies on laboratory animals and volunteers (3,4,5).

The repeated single and combined exposure of adult male rats to 150 ppm TO and 150 ppm XY resulted in significantly higher concentrations of TO (210 %) and XY (240 %) in blood of animals in comparison with repeated exposure to each solvent administered singly. These results suggest toxicokinetic interactions between TO and XY that affect the urine excretion of their major metabolites, hippuric acid and methylhippuric acid, respectively (6).

Tardif et al. (7) reported significant increasing of blood concentrations of the aromatic solvents at rats treated with binary mixtures containing TO and XY, after the addition of a third solvent.

In order to estimate the role of cytochrome P_{450} in the activation of XY and TO metabolites in the conditions of combined exposures, Nedelcheva (8) examined the influence of AC on the capacity of TO and XY to induce several forms of cytochrome P_{450} in rats liver. The addition of AC potentiated the induction effect of TO and XY, conducting to higher amounts of excreted hippuric and methylhippuric acid, respectively.

Binary mixtures of TO and BE were studied on different experimental models. There were observed metabolic inhibitions for the majorities of cases (2,9,10), but also noncompetitive

interactions between the substrates for the enzymes implicated in the metabolic activations of the solvents (11,12).

One of the key risk assessment issues for mixtures is that of the extrapolation data from animal to human. The physiologically based toxicokinetic model framework is useful for conducting extrapolations of the kinetics of chemicals in mixtures of varying complexities and composition (6,9,13-16). These models may be used to predict the magnitude of interactive effects at different exposure concentrations. Then the most relevant predictions may be tested using selected experiments.

The objectives of the present study were to examine the influence of repeated simultaneous exposure to organic solvents on some aspects of their respective urinary excretion in rats, and the possibility to apply these results to assess health risk associated with occupational exposure to airborne mixtures of organic solvents.

MATERIAL AND METHODS

Animals

Our experiment was carried out on white male Wistar rats (10–12 weeks of age) from the Biobreeding of the Institute of Virology of Bucharest. All animals were housed under constant, identical conditions of temperature (18–20 °C) and humidity (40–60 %) receiving food (a natural diet: milk, bread, oats and carrots) and water ad libitum (17).

Chemicals

The solvents (GC grade) were dissolved in sunflower oil. Toluene and acetone were obtained from Chimopar Bucharest, benzene from Sigma (Germany) and xylene (mixture of the 3 isomers) from UC (Belgium). The standards and Dowex ion exchanger for the metabolites determination were purchased from Aldrich and Fluka (Germany), respectively. The detection of acetone in urine was performed with salicylaldehyd and sulphuric acid from Merck (Germany) and with natrium bisulphite from Serva (Germany).

Experiment

The animals were repeatedly exposed to TO, XY, AC, individually and simultaneously, or to a mixture of TO and BE, by intraperitoneal injection (1 injection/week for 12 weeks). Each solvent and mixture was administered in two doses: the high (H) dose was equally to the current occupational exposure limit, the low (L) one represents 1/2 of the high dose. The equivalent doses were calculated according to Rumiantev (18) applying the transformation relations between the threshold limit values (TLV), lethal doses and concentrations (LD_{50}, LC_{50}), relations validated by INRS (19). The rats were divided into 12 groups, as follows: (1) 850 (TO_H) and (2) 425 (TO_L) mg/kg toluene; (3) 1500 (XY_H) and (4) 750 (XY_L) mg/kg xylene; (5) 1500 (AC_H) and (6) 750 (AC_L) mg/kg acetone; (7) 1200 (MI_H) and (8) 600 (MI_L) mg/kg mixture (M) I (30 % TO, 30% XY, 40 % AC); (9) 500 (MII_H) and (10) 250 (MII_L) mg/kg mixture II (85 % TO, 15 % BE); (11) control oil (CO) (0.5 ml oil/100 g

body weight) and (12) unexposed control (C). Urine samples were collected for 24 h after the last administration. Major metabolites of TO and XY (hippuric acid and methylhippuric acid, respectively) and urinary AC were measured by spectrophotometric methods according to Cotrau (20).

Statistical analysis

Data were expressed as the mean ± standard deviation (S.D.) of eight animals from each group and were compared statistically by Student's *t* test. The exposure–excretion relationship was examined by simple regression analysis.

RESULTS AND DISCUSSION

The results in Table 1 show that the mean concentrations of the metabolites and of the acetone in the urine of the treated rats are significantly different from those of the unexposed control (Table 1).

Tablel 1: Mean urinary concentrations of metabolites and acetone in exposed *vs* unexposed rats

Group	Hippuric acid (g/l)	Methylhippuric acid (g/l)	Acetone (mg/l)
TO_H	6.32±1.10*	-	-
TO_L	3.90±0.43*	-	-
XY_H	-	4.56±1.17*	-
XY_L	-	3.22±0.83*	-
AC_H	-	-	102.32±40.48**
AC_L	-	-	118.32±37.62*
MI_H	3.44±1.66**	3.07±1.46**	65.40±8.80*
MI_L	2.57±0.60*	2.14±0.75*	69.20±11.30*
MII_H	4.20±1.03*	-	-
MII_L	3.45±0.40*	-	-
C	0.19±0.11	0.20±0.12	17.82±15.10

*p < 0.001; ** p < 0,01

Although the two biomarkers of the aromatic solvents are also endogenous urinary compounds and the administered doses were relatively low (equally or below current occupational threshold limit values), the amounts of hippuric and methylhippuric acid are in line with the exposure levels. The correlation of the solvent exposure doses with the concentrations of corresponding metabolites was close for TO (fig. 1) and XY (fig. 2), also in single as well as in combined administration.

Compared with single treatment, the simultaneous exposure resulted in higher amounts of excreted hippuric acid (195%) and methylhippuric acid (200%) in urine in the immediate post–exposure period.

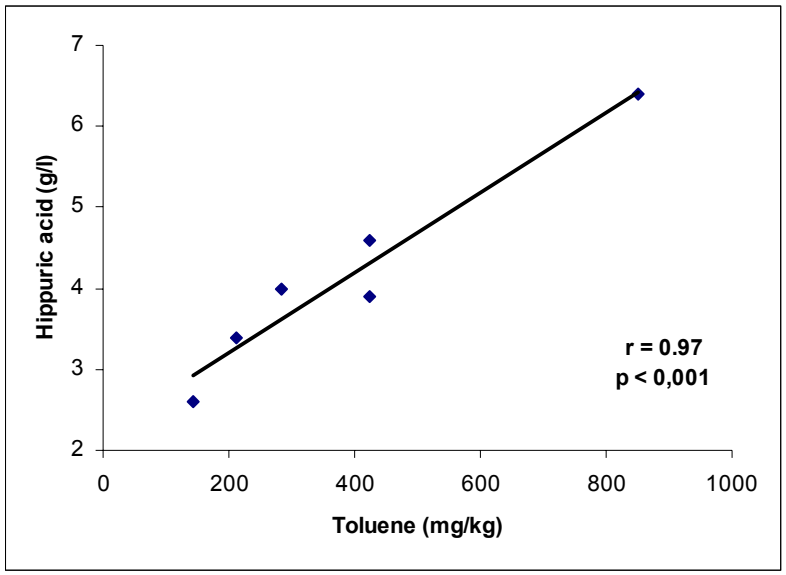

Figure 1: Correlation of the toluene exposure levels (regardless the mode of administration) with the concentrations of the hippuric acid in the urine

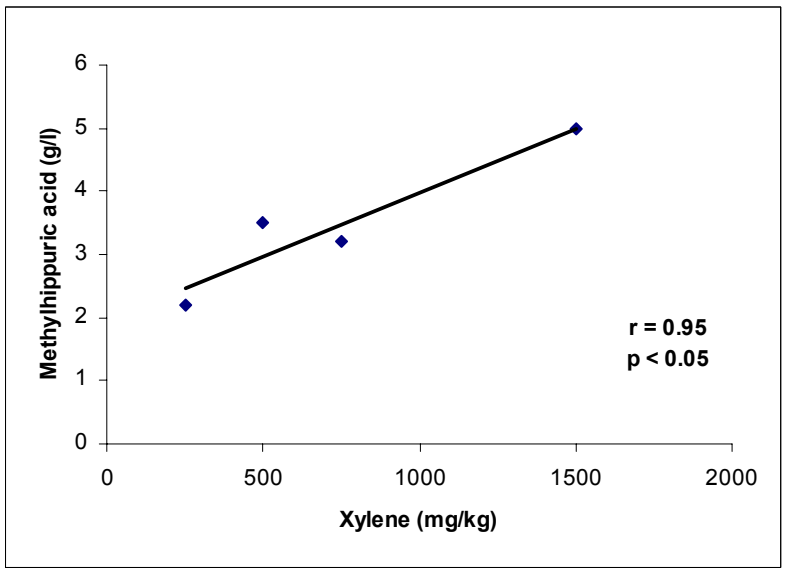

Figure 2: Correlation of the xylene exposure levels (regardless the mode of administration) with the concentrations of the methylhippuric acid in the urine

Generally, experimental data on kinetic of binary mixtures containing TO and XY showed the occurrence of metabolic inhibition or of non-competitive interactions, depending on the proportion between components and the intensity of exposure (3,4). The addition of AC in the experiment protocol produced modifications in the metabolic disposition of both aromatic solvents. We estimate that the presence of AC increased the solubility of TO and XY in blood and, at the same time, intensified their induction effect on cythocrome P_{450}. This aspect was demonstrated by Nedelcheva, too (8). The biological oxidations, involved in the bio-transformation of a large number of xenobiotics, imply essentially a hydroxylation mechanism, where cytocrom P_{450}, a flavoproteine and NADPH-dinucleotide are implicated. The enzymatic induction of the cytocrome P_{450} leading to the rapid consumption of the exogene substrates (e. g. toluene and xylene) and to the stimulation of microsomal enzyme activities, results in a faster formation of metabolites. We consider that this mechanism was responsible for the increased quantities of hippuric and methylhippuric acid found in groups treated with mixture I.

Not any metabolic interactions between the solvents have been detected in TO and BE simultaneous administration. The amounts of excreted hippuric acid in the urine of mixture II co-injected animals were unmodified at both doses. As literature data mentioned (2), even for the binary mixtures of benzene–toluene, the extent of the possible metabolic interactions will depend on the intensity of exposure, on the balance of activation processes, such as enzymatic oxidation, and deactivation processes, like conjugation and excretion.

CONCLUSIONS

1. No apparent interactive effects between toluene and benzene were observed.
2. Our results suggest metabolic interactions between toluene, xylene and acetone that affect initially the excretion of both metabolites and ultimately the biological monitoring of data of exposure to a combination of solvents. The presence of acetone in mixture I was, probably, responsible for the induction of the enzymatic oxidation of the aromatic solvents, resulting in higher metabolites amounts in co-injected rats than in single exposed groups.
3. This study shows that the greater risk of toxicity often thought to be associated with exposures to complex mixtures should not only be related to the magnitude of interactive effects among components resulting from combined exposures, but also should take into account the internal dose of toxic chemicals in target organs/ tissues.
4. The results of this research represents an useful tool for screening the chemicals for which metabolic interactions are likely to be important in the context of combined exposures and mixture risk assessment. A research strategy

involving hypothesis generation through quantitative modeling and testing through laboratory-based experiments may be the most effective one for the complex issue of human health risks from exposure to chemical mixtures.

REFERENCES

1. Bolt HM, Riemer K, Schaller KH: *Wechselwirkungen von Arbeitsstoffen untereinander und Beeinflussung durch Lifestyle-Faktoren,* Arbeitsmed. Sozialmed. Umweltmed., 1994, 29: 320-323.
2. Ikeda M: *Exposure to complex mixtures: implications for biological monitoring,* Toxicol. Lett., 1995, 77: 85-91.
3. Schaller KH, Triebig G: *Arbeitsmedizinische Bewertung von organischen Losungsmittelgemischen*, Arbeitsmed. Sozialmed. Umweltmed., 1996, 31: 504-512.
4. Takeuchi A, Kawai T, Zhang ZW, Ikeda M: *Toluene, xylenes and xylenes isomers in urine as biological indicators of low-level exposure to each solvent,* Int. Arch. Environ. Health, 2002, 75: 387-393.
5. Zhao W, Misumi J, Yasui T, Aoki K, Kimura T: *Effects of methyl ethyl ketone, acetone, or toluene coadministration on 2,5-hexanedione concentration in the sciatic nerve, serum and urine of rats*, Int. Arch. Occup. Environ. Health, 1998, 71: 236-244.
6. Tardif R, Plaa GL, Brodeur J: *Influence of various mixtures of inhaled toluene and xylene on the biological monitoring of exposure to these solvents in rats,* Can. J. Physiol. Pharmacol., 1992, 70: 385-393.
7. Tardif R, Charest–Tardif G, Brodeur J:: *Comparison of the influence of binary mixtures versus a ternary mixture of inhaled aromatic hydrocarbons on their blood kinetics in the rat,* Arch. Toxicol., 1996, 70: 405-413.
8. Nedelcheva V: *Effects of acetone on the capacity of o-xylene and toluene to induce several forms of cytochrome P_{450} in rat liver,* Cent. Eur. J. Public Health, 1996, 4: 119-122.
9. Haddad S, Charest–Tardif G, Tardif R, Krishnan K: *Validation of a physiological modeling framework for simulating the toxicokinetics of chemicals in mixtures,* Toxicol. Appl. Pharmacol., 2000, 167: 199-209.
10. Purcell KJ, Cason GH, Gargas ML, Andersen ME, Travis CC: *In vivo metabolic interactions of benzene and toluene*, Toxicol. Lett., 1990, 52: 141-152.
11. Medinsky MA, Schlosser PM, Bond JA: *Critical issues in benzene toxicity and metabolism: the effect of interactions with other organic chemicals on risk assessment,* Environ. Health Perspect., 1994, 102: 119-124.
12. Pathiratne A, Puyear RL, Brammer J D: *A comparative study of the effects of benzene, toluene and xylenes on their in vitro metabolism and drug-metabolizing enzymes in rat liver*, Toxicol. Appl. Pharmacol., 1986, 82: 272-80.
13. Bond JA, Medinsky MA: *Health risk assessment of chemical mixtures from a research perspective,* Toxicol. Lett., 1995, 83: 521-525.
14. Haddad S, Beliveau M, Tardif R, Krishnan K: *A PBPK modeling–based approach to account for interactions in the health risk assessment of chemical mixtures*, Toxicol. Sci., 2001, 63: 125-131.
15. Haddad S, Tardif R, Viau C, Krishnan K: *A modeling approach to account for toxicokinetic interactions in the calculation of biological hazard index*

for chemical mixtures, Toxicol. Lett., 1999, 108: 303-308.
16. Krishnan K, Hadadd S, Beliveau M, Tardif R: *Physiological modeling and extrapolation of pharmacokinetic interactions from binary to more complex chemical mixtures,* Environ. Health Perspect. 2002, 110: 989+994.
17. Sos J, Szelenyi I: *Diets for animal experiments,* ed. Akad. Kiado, Budapest, 1974: 11-21.
18. Rumianțev GI: *The toxicity of chemical compounds administered on different pathways*, Gig. i. Sanit., 1975, 2: 25-30.
19. INRS: *Essai de détermination d'une valeur limite calculée d'exposition*, Cahiers de notes documentaries, 1978, 93: 550-552.
20. Cotrau M, Proca M: *Analytical Toxicology,* Medical Publishing House, Bucureşti, 1988: 149-183 (in Romanian).