Biomarkers, November–December 2005; 10(6): 456–463

Taylor & Francis
Taylor & Francis Group

Increased serum bile acids as a possible biomarker of hepatotoxicity in Brazilian workers exposed to solvents in car repainting shops

M. J. NUNES DE PAIVA[1] & M. E. PEREIRA BASTOS DE SIQUEIRA[2]

[1]*Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil,* [2]*College of Pharmacy, Federal University of Alfenas, Alfenas, Minas Gerais, Brazil*

Abstract

The objective was to evaluate total serum bile acids (SBA) as a biological marker of hepatotoxicity in car painters exposed to organic solvents and to compare their performance with classic biochemical parameters of liver function. SBA were analysed in a selected group of workers ($n = 57$) occupationally exposed to a mixture of organic solvents and in a control group ($n = 51$). In addition, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TB), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP) were determined in the two groups. Urinary hippuric acid was measured in all samples. Statistical analysis of the data revealed a significant increase in the concentration of SBA, AST, ALP and TB in exposed workers compared with controls (Mann–Whitney, $p \leq 0.05$). However, SBA was the parameter most frequently altered in exposed workers and showed higher significance between the two groups (chi-square test) compared with the upper limit of the reference range (8 $\mu mol\,l^{-1}$). In conclusion, SBA can be considered to be a sensitive parameter of hepatotoxicity induced by organic solvents than the traditional tests and it can be used as an biological marker of subclinical liver injury.

Keywords: *Serum bile acids, organic solvents, biological monitoring, liver injury*

(Received 31 May 2005; accepted 29 September 2005)

Introduction

Organic solvents are substances extensively used in paints, thinners, varnishes, hair sprays and glues, among others. Occupational health experience demonstrates that humans are simultaneously or sequentially exposed to multiple solvents rather than to one solvent only (Angerer 1979, Chen et al. 1991, Ikeda 1995, Angerer & Krämer 1997). The composition of solvents varies according to the purpose of the product. However, toluene continues to be the most prevalent substance and is found mixed with other components such as ethers, ketones and hexane (Bergeret & Nestler 1991).

The central nervous system is the target tissue of the toxic action of solvents. Behaviour disturbances can result from prolonged exposure to low doses. Repeated exposure to tolerable levels of solvents may result in toxicity to others organs, such as

Correspondence: M. E. Pereira Bastos de Siqueira, Escola de Farmácia, Universidade Federal de Alfenas, Rua Gabriel Monteiro da Silva, 714. 37130-000 Alfenas, Minas Gerais, Brazil. Tel: 55-35-32-99-13-42. Fax: 55-32-99-10-63. E-mail: marelisa@int.efoa.br

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500500399979

the liver (Bergeret & Nestler 1991, Snyder & Andrews 1996), but this issue is the subject of some debate (Franco et al. 1986).

Biological monitoring of exposure to solvents is currently carried out by measuring biological indicators of the internal dose, which permit the evaluation of exposure to each individual substance, as well as the interpretation of the limit values of the biological indicator (American Conference of Governmental Industrial Hygienists 2003). These indicators correspond to some of the metabolites of the solvent excreted in urine or to the unchanged solvent itself found in blood and/or urine (Jang et al. 1993, Caperos & Fernandes 1997, Gobba et al. 1997, Aitio & Kallio 1999, Ikeda 1999). For toluene, the most widely used metabolites are urinary hippuric acid (HA) and o-cresol (Dossing et al. 1983, Hasegawa et al. 1983, Nise 1992, Kongtip et al. 2001, Çok et al. 2003).

In addition to biological monitoring of the internal dose, the determination of changes caused by the solvents in affected organs, reflecting interactive mechanisms of combined exposures, has been recommended (Franco et al. 1986).

Some studies have investigated the relationship between liver injury and combined exposure to multiple solvents present in paints and glues. Most of these studies involved the evaluation of classic and poorly sensitive biochemical parameters, mainly transaminases, alkaline phosphatase and gamma-glutamyltransferase activities (Chen et al. 1991, Driscoll et al. 1995, Liu et al. 1996, Neghab & Stancey 2000). Studies involving non-halogenated solvents have provided poor evidence of hepatotoxicity, although the negative results might be due to the inability of traditional tests to detect liver injury in an early stage (Franco et al. 1989, Bai et al. 1992, Azer et al. 1993, Neghab & Stancey 2000).

The need for an early detection of hepatic dysfunction has become more important in the last few years when restrictions in the use of more hazardous solvents and increasing application of industrial hygiene measures has been implemented. Thus, there is greater concern regarding the chronic effects resulting from exposure to low environmental solvent concentrations that may cause subclinical diseases or chronic liver injury.

More sensitive tests of hepatotoxicity, such as the determination of bile acids, have been implemented. Since regulation of bile acid metabolism is one of the main functions of the liver, detectable changes in bile acid levels reflect dysfunction of this organ (Franco et al. 1986, Bai et al. 1992, Liu et al. 1996, Neghab et al. 1997, Neghab & Stancey 2000). Cholestasis is the principal cause of SBA elevation in serum and is caused by hepatocyte retention (Tolman & Rej 1999).

An increase in blood bile salts seems to result from changes in their hepatocellular uptake when induced by chemical substances. Investigations of the mechanism by which SBA increases in solvent exposure pointed out to selective dose-related and reversible inhibition of bile acid uptake at the sinusoidal domain of the hepatocyte plasma membrane (Neghab & Stancey 2000). Carbon tetrachloride and other halogenated solvents have been shown to cause significant changes in bile salt concentrations (Kukongviriyapan et al. 1990, Bai et al. 1992, Neghab et al. 1997). However, no concrete data are available regarding the usefulness of this biological marker in the detection of liver injury during exposure to multiple non-halogenated solvents such as *n*-hexane, toluene, ketones and xylenes. Neghab and Stancey (1997) demonstrated in rats that toluene exposure raises SBA serum levels.

Therefore, the objective of the present study was to determine the usefulness of serum bile acids in the detection of liver injury in workers exposed to organic solvents at motor vehicle repainting shops compared with traditional liver function tests.

Materials and Methods

The exposed group consisted of 57 workers from 21 motor vehicle repainting shops, with a mean $\pm$SD age of 33.8 $\pm$ 9.9 years and a mean $\pm$SD work time of 187.7 $\pm$ 125 months, who were exposed to solvents during different tasks. The control group consisted of 51 randomly selected volunteers with a mean $\pm$SD age of 34.4 $\pm$ 12.6 years who were not occupationally exposed to any solvent. All individuals were males. Thirty-six workers of the exposed group used active carbon masks during paint application.

In 11 shops, paint was applied in a painting cabin measuring 105–320 m^3. The walls and roof of the cabin were equipped with exhausts and sealed glass windows to prevent dispersal of the vapours to other places in the workshop. In the other shops, paint application was carried out in a common area used for all activities (body work and painting). In all shops without a painting cabin, the site with the best ventilation was chosen for painting. The duration of paint application varied depending on the size of the piece to be painted and the number of vehicles to be repaired.

All volunteers were asked to reply to a toxicological protocol at the time the samples were taken, giving information about their personal habits (smoking, alcohol and other beverages intake, hobbies activities), residence, age, type of work developed in the shop, kinds of food consumed in the last 2 days, especially canned and/or conserved foods, tomato sauce, pharmacies ingestion, clinical complaints, etc. All individuals who related the use of pharmacies or food that clearly could elevate the HA levels were excluded from the group (ten volunteers), as well as those presenting hepatic, renal, cardiac or pulmonary diseases (two volunteers).

Spot urine samples were collected at the end of the last day of the working week, i.e. in the fifth or sixth day of the week, between 16.00 and 18.00 hours. Urine was collected into polyethylene flasks and transported in a polystyrene box on dry ice to the laboratory. Specific gravity and creatinine were measured before the samples were frozen at $-20^{\circ}C$. Creatinine in urine was measured using Bioclin® kit test, based on the Jaffé reaction with picric acid.

For the determination of bile acids and other biochemical parameters, fasting blood samples were collected from both groups on the same day of urine collection, in the morning and in fasting condition. Serum was separated by centrifugation at 3040 g and stored at $-20^{\circ}C$ for SBA determination.

The biochemical liver function parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TB), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP), were determined using an autoanalyser Bioplus 2000® (Bioplus, Baruer, Brazil) materials from Bioclin (Quimica Base).

HA was determined according to the gas-chromatographic method of Alvarez-Leite et al. (1994), with small modifications, i.e. using a capillary HP-1 column (300 $\times$ 0.32 mm, 0.25 μm film) (initial 170°C for 2 min, raising 30°C min^{-1} until 200°C, holding for 6 min and raising 40°C min^{-1} until 240°C) and a nitrogen flow of 3 ml min^{-1}, and split 1/10, instead of the packed column SE 30 3% in Chromossorb WHP reported by the authors. The volume of methylating agent (thrimethylphenylammonium hydroxide) was increased (120–200 μl). The HA extraction from urine

was performed according to the method of Kira (1977). All chemicals were from Merck, except thrimethylphenylammonium and HA, from Sigma-Aldrich (St. Louis, MO, USA). Total serum bile acids (SBA) were measured by an enzymatic method using the Sigma Diagnostic® kit, which is based on the method of Mashige et al. (1981). SBA calibrators in concentrations of 5, 25, 50, 100 and 200 μmol l^{-1} from Sigma Diagnostics (Sigma-Aldrich, Dorset, UK), No.s 061k6043, 061k6044, 061k6045, 061k6046 and 061k6047, respectively; reagent A from Sigma Diagnostic, No. 119H6059; reagent B Sigma Diagnostics, No. 039H6131; stop reagent.

The data showed no normal distribution (Shapiro–Wilk test). The non-parametric Mann–Whitney test was used to evaluate the influence of personal and work habits on the results of the biological parameters, as well as to determine differences between the two groups. The χ^2-test was applied to determine the significance of each parameter studied in relation to the number of individuals with values higher than the upper limit of its reference range. Statistical analysis was performed with the SAS® program, version 6.11.

Results

Table I shows SBA levels in exposed and control individuals, which differed significantly between the two groups (Mann–Whitney test, $p \leq 0.05$). Mean urinary hippuric acid levels, enzyme activities and bilirubin concentrations of the exposed and control groups are shown in Table II. A significant difference was observed for hippuric acid, AST, ALP and TB (Mann–Whitney test, $p \leq 0.05$).

No normal distribution of bile acids was observed for the exposed or control group. There was also no normal distribution of the other variables (Shapiro–Wilk, $p < 0.0421$), except for total bilirubin in the exposed group.

Table III shows the prevalence of the number of individuals whose biochemical parameters were above and below the reference range. The number of individuals with increased SBA was significantly higher in the exposed group than in the control group. No significant difference between exposed and control individuals was observed for the other parameters studied, except for ALP activity.

Individual habits, consumption of alcoholic beverages (>20 g per week), smoking (more than five cigarettes per day), time of work or use of masks had no significant effect on the majority of the parameters studied. A significant difference was observed in terms of ALP according to the use of masks ($p = 0.0229$) and for HA according to the intake of alcoholic beverages ($p = 0.0229$).

Table I. Serum bile acids (μmol l −1) in the controls and workers exposed to solvents.

Parameters	Exposed group	Control group
Mean ± SD	10.34 ± 6.73	5.62 ± 3.70
Median	7.93*	4.57*
Geometric mean	8.59	4.61
Maximum	30.94	17.44
Minimum	1.80	1.04
Confidence range 95%	8.59–12.09	4.60–6.63
Percentile 10%	4.27	2.23
Percentile 90%	19.9	11.38

*Significant difference (Mann–Whitney, $p = 3.09 \times 10^{-6}$).

Table II. Mean levels ± SD of liver enzyme activities aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), total bilirubin (TB), and hippuric acid (HA) in the controls and workers exposed to solvents.

	HA ($g\,g^{-1}$ creatinine)	AST ($U\,l^{-1}$)	ALT ($U\,l^{-1}$)	GGT ($U\,l^{-1}$)	ALP ($U\,l^{-1}$)	TB ($mg\,dl^{-1}$)
Exposed	0.95 ± 0.89	15.5 ± 6.02	16.88 ± 7.03	37.41 ± 20.62	41.33 ± 12.36	0.74 ± 0.22
Control	0.47 ± 0.67	12.7 ± 3.86	17.58 ± 8.24	31.27 ± 12.04	33.83 ± 8.50	0.58 ± 0.17
p	8.81×10^{-6}*	0.01439*	0.93133	0.32630	0.00078*	0.00082*

*Statistical significance (Mann–Whitney test).

Significant correlation ($p \leq 0.05$) was observed, in the exposed group, between GGT and TB ($r = 0.6192$), GGT and AST ($r = 0.5307$), and GGT and ALT ($r = 0.6961$). In controls, SBA and AST ($r = 0.6099$), GGT and AST ($r = 0.6740$), GGT and ALP ($r = 0.5887$), and GGT and ALT ($r = 0.7728$) showed significant correlations.

Discussion

Biological monitoring of workers occupationally exposed to solvents is carried out by measuring biological indicators of internal dose and effect. Biological markers of effect include traditional liver function tests; however, studies regarding the usefulness of these parameters in the detection of liver injury caused by organic solvents are conflicting (Franco et al. 1986, Chen et al. 1991, Driscoll et al. 1995, Liu et al. 1996, Neghab et al. 1997).

In motor vehicle repainting shops, workers are exposed to multiple solvents. Lepera (1997), investigating the contamination of the work environments of motor vehicle repaint, found the following solvents to be present in different types of paints used in Brazil: toluene and xylene; ethanol; iso- and *n*-butanol; butyl-, ethyl- and amylacetate; methylethylketone, and butyl- and methylethylene glycol ether and acetate. Toluene was detected in 100% of the samples. The author concluded that urinary levels of hippuric acid, the main toluene metabolite, in samples collected at the end of a work week showed a significant correlation with exposure to toluene and could therefore be considered a 'tracer' of solvent exposure during repainting operations.

Hippuric acid is still an indicator in biomonitoring of workers exposed to toluene, because of the good correlation it shows with the exposure level, in spite of its lack of specificity to toluene or occupational exposure (De Rosa et al. 1987). The most important sources of background hippuric acid are environmental toluene

Table III. Prevalence of subjects with serum bile acids (SBA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) and total bilirubin (TB) above the upper limit of the reference range of each parameter.

	SBA ($\mu mol\,l^{-1}$)		AST ($U\,l^{-1}$)		ALT ($U\,l^{-1}$)		GGT ($U\,l^{-1}$)		ALP ($U\,l^{-1}$)		TB ($mg\,dl^{-1}$)	
	≤8.0	>8.0	≤34	>34	≤36	>36	≤43	>43	≤43	>43	≤1.2	>1.2
Exposed	31	26	56	01	55	02	42	15	38	19	56	01
Control	44	07	51	0	49	02	43	08	45	06	51	0
P	0.0024		n.s.		n.s.		n.s.		0.0153		n.s.	

Statistic significance appraised for the $\chi 2$ test; n.s., not significant.

contamination and diet, as found in some fruits (plum and peach), coffee green beans presenting quinic acids and other benzoic acid liberators, such as phenylalanine, aspirin or ethyl benzene (Nise 1992, Truchon et al. 1999). Then, all volunteers who declared the ingestion of medicines or foods that raise HA levels, until 4 days before sample collection, were excluded from the study.

The concentration of hippuric acid found in exposed workers was quite low, with none of the individuals showing levels above the maximum established in Brazil, i.e. 2.5 g g^{-1} creatinine (Brazil 1994). These data indicate low absorption of toluene, the same probably applying to other solvents present in material used during repainting activities. The low solvent absorption may be attributed to the fact that painting is carried out in open environments in half the shops, to the exhaustion in cabins, as well as to the use of masks by part of the workers. On the other hand, paints have not much toluene on their composition.

Statistically significant differences between controls and exposed groups were found for SBA (Table I). SBA was the most frequently altered biochemical parameter in exposed workers with 45.6% of them showing results above the upper reference limit (8.0 μmol l^{-1}), followed by ALP (Table III).

Among the classical biochemical parameters of liver function, ALP, TB and AST showed significant differences between the two groups (Table II). Liu et al. (1996) reported differences between controls and exposed groups (appendices spray painters) only for GGT activity. Kurppa and Husman (1982) did not detect a significant difference in classic biochemical liver function parameters between workers exposed to solvents at body vehicle shops and in a control group, concluding that these tests should not be used for the early detection of the hepatotoxicity due to solvents. Similar results were reported by Franco et al. (1986) for workers exposed to multiple organic solvents. Paints have a varied composition, but in general they are constituted by solvents, additives and pigments. It is important to take into account that colour pigments of the paints (more than 10% in volume) may contain different heavy metals that could alter liver function (Lepera 1997).

SBA levels were only correlated significantly with AST activity in the controls. Gammaglutamyltransferase was the parameter more well correlated with others classical biochemical parameters as TB, AST, ALT (in exposed workers) and with AST and ALP in the controls.

Where classified as smokers, people smoked more than five cigarettes per day ($n = 21$, a mean of 11 cigarettes per day) and as drinkers ($n = 29$), people consumed some kind of alcohol beverage at least four times a week (minimum 20 g alcohol a week, mean of 85 g $week^{-1}$).

A significant difference in HA excretion between subgroups alcohol drinkers/no drinkers was observed (Mann–Whitney, $p = 0.0229$). There was no difference between smokers/no smokers. There are conflicting reports about the influence of alcohol and tobacco on the metabolism of toluene affecting the elimination of its metabolites, mainly in the situation of experimental and occupational exposure (Nise 1992, Inoue et al. 1993, Bazzano et al. 1994, Kawamoto et al. 1996). Only one paper reported that the individual habits of smoking and drinking, either separately or in combination, did not alter significantly the basal levels of HA (Alvarez-Leite et al. 1999).

Alcohol consumption, smoking or use of a protective mask did not show a significant influence on SBA levels. Chen et al. (1991) observed changes in SBA after ethanol consumption. These differences are probably due to the different volumes and

types of beverage ingested by the volunteers, as well as to the different frequency and duration of drinking.

The length of work in the offices also showed no significant correlation with any of the biochemical parameters studied in the total group or when subdivided in five subgroups, except ALP in workers working more than 12 years (subgroups 3–5; p between 0.008 and 0.051). Perhaps it can be due to modifications of work conditions over the years, the recent introduction of the carbon masks (in the last 6 months) and change in the activities of the worker in the office. It was not possible to obtain information about the real duration of exposure specifically to solvents.

Finally, among the biochemical parameters studied, SBA showed the highest sensitivity in the detection of liver injury, with a higher statistical significance between the exposed and control groups (χ^2, $p = 0.0024$), followed by ALP ($p = 0.0153$) (Table III). These results indicate that SBA may represent a valuable marker of hepatotoxicity that should be better explored for the application to health surveillance activities for workers exposed to organic solvents.

References

Aitio A, Kallio A. 1999. Exposure and effect monitoring: a critical appraisal of their practical application. Toxicology Letters 108:137–147.

Alvarez-Leite EM, Duarte A, Barroca MM, Silveira NS. 1999. Possible effects of drinking and smoking habits on hippuric acid levels in urine of adults with no occupational toluene exposure. Journal of Occupational Health 41:112–114.

Alvarez-Leite EM, França LS, Monteiro RB, Cioletti AG, Barroca MM. 1994. Determination of urinary hippuric acid by gas chromatography after derivatization with trimethylphenylammonium hydroxide. Toxirama 6:5–8.

American Conference of Governmental Industrial Hygienists. 2003. Threshold limit values and biological exposure indices for 2003. Cincinnati, OH: ACGIH.

Angerer J. 1979. Occupational chronic exposure to organic solvents. VII. Metabolism of toluene in man. International Archives of Occupational and Environmental Health 43:63–67.

Angerer J, Krämer A. 1997. Occupational chronic exposure to organic solvents XVI. Ambient and biological monitoring of workers exposed to toluene. International Archives of Occupational and Environmental Health 69:91–96.

Azer SA, Murray M, Farrell G, Stacey NH. 1993. Selectivity and sensitivity of changes in serum bile acids during induction of cirrhosis in rats. Hepatology 18:1224–1231.

Bai C, Canfield PJ, Stancey NH. 1992. Individual serum bile acids as early indicators of carbon tetrachloride and chloroform-induced liver injury. Toxicology 75:221–234.

Bazzano P, Perico A, Li-Donn V, Colzi A. 1994. Exposizione professionali e fattori individuali che condizionano l'eliminazione urinaria di acido ippurico. Giornal Italiani del Medicina del Lavoro 16:57–61.

Bergeret K, Nestler K. 1991. Solvent uptake in relation to physical activity. Science of the Total Environment 101:111–115.

Brazil. 1994. Ministério do trabalho. Secretaria de relações do trabalho Segurança e Medicina do Trabalho: Portaria n. 24, de 29 de dez. 1994. Diário Oficial, Brasília, 30 de dez. 1994. Seção 1. p. 21278–21279.

Caperos JR, Fernandez JG. 1997. Simultaneous determination of toluene and xylene metabolites in urine by gas-chromatography. British Journal of Industrial Medicine 34:229–233.

Chen JD, Wang JD, Jang JP, Chen YY. 1991. Exposure to mixture of solvents among paint workers and biochemical alterations of liver function. British Journal of Industrial Medicine 48:696–701.

Çok I, Dagdelen A, Gokçe E. 2003. Determination of urinary hippuric acid and o-cresol levels as biological indicator of toluene exposure in shoe workers and glue sniffers. Biomarkers 8:119–127.

De Rosa E, Bartolucci GB, Sigon M, Callegaro R, Perbellini L, Brugnone F. 1987. Hippuric acid and o-cresol as biological indicators of occupational exposure to toluene. American Journal of Industrial Medicine 11:529–537.

Dossing M, Baelum J, Hansen SH, Lundqvist GR, Andersen NT. 1983. Urinary hippuric acid and ortho cresol excretion in man during experimental exposure to toluene. British Journal of Industrial Medicine 40:470–473.

Driscoll TR, Hamdan HH, Wang G, Wright PF, Stancey NH. 1995. Concentration of individual serum or plasma bile acids in workers exposed to chlorinated aliphatic hydrocarbons. British Journal of Industrial Medicine 25:15–27.

Franco G, Fonte R, Tempini G, Candura F. 1986. Serum bile acid concentrations as a liver function test in workers occupationally exposed to organic solvents. International Archives of Occupational and Environmental Health 58:157–164.

Franco G, Santagostino G, Lorena M, Imbriani M. 1989. Conjugated serum bile acid concentrations in workers exposed to low doses of toluene and xylene. British Journal of Industrial Medicine 46:141–142.

Gobba F, Ghittori S, Imbriani M, Maestri L, Capodaglio E, Cavalleri A. 1997. The urinary excretion of solvents and gases for the exposure biological monitoring of occupational exposure: a review. Science of the Total Environment 199:3–12.

Hasegawa K, Shiojima S, Koizumi A, Ikeda M. 1983. Hippuric acid and o-cresol in the urine of workers exposed to toluene. International Archives of Occupational and Environmental Health 52:197–208.

Ikeda M. 1995. Exposure to complex mixtures: implications for biological monitoring. Toxicology Letters 77:85–91.

Ikeda M. 1999. Solvents in urine as exposure markers. Toxicology Letters 108:99–106.

Inoue O, Seiji K, Watanabe T, Nakatsuka H, Jin C, Liu SJ, Ikeda M. 1993. Effects of smoking and drinking on excretion of hippuric acid among toluene exposed workers. International Archives of Occupational and Environmental Health 64:425–430.

Jang J, Kang S, Watanabe T. 1993. Biological exposure indices of organic solvents for Korean workers. International Archives of Occupational and Environmental Health 65:S219–S222.

Kawamoto T, Koga AM, Oyama T, Kodama Y. 1996. Habitual and genetic factors that affect urinary background levels of biomarkers for organic solvent exposure. International Archives of Occupational and Environmental Health 30:114–120.

Kira S. 1977. Measurement by gas chromatography of urinary hippuric acid and methylhippuric acid as indices of toluene and xylene exposure. British Journal of Industrial Medicine 34:305–309.

Kongtip P, Vararussami J, Pruktharathikul V. 2001. Modified method for determination of hippuric acid and methylhippuric acid in urine by gas chromatography. Journal of Chromatography B 751:199–203.

Kukongviriyapan V, Kukongviriyapan U, Stancey NH. 1990. Interference with hepatocellular substrate uptake by 1,1,1-trichloroethane and trichloroethylene. Toxicology and Applied Phamacology 102:80–90.

Kurppa K, Husman K. 1982. Car painters' exposures to a mixture of organic solvents. Scandinavian Journal of Work and Environmental Health 8:137–140.

Lepera JS. 1997. Avaliação da exposição ocupacional a solventes e a material particulado em procedimento de repintura automotiva. Thesis, University of São Paulo.

Liu JJY, Bai C, Williamson AM, Qu SX, Hamdan H, Stancey NH. 1996. Individual serum bile acids in apprentice spray painters in association with solvent exposure. International Archives of Occupational and Environmental Health 69:21–26.

Mashige F, Tanaka N, Maki A, Kamei S, Yamanaka M. 1981. Direct spectrophotometry of total bile acids in serum. Clinical Chemistry 27:1352–1356.

Neghab M, Qu S, Bai CL, Caples J, Stancey NH. 1997. Raised concentration of serum bile acids following occupational exposure to halogenated solvents, 1,1,2-trichloro-1,2,2-trichloroethane and trichloroethylene. International Archives of Occupational and Environmental Health 70:187–197.

Neghab M, Stancey NH. 1997. Toluene-induced elevation of serum bile acids: relationship to bile acid transport. Journal of Toxicology and Environmental Health 52:249–268.

Neghab M, Stancey NH. 2000. Serum bile acids as a sensitive biological marker for evaluating hepatic effects of organic solvents. Biomarkers 5:81–107.

Nise G. 1992. Urinary excretion of o-cresol and hippuric acid after toluene exposure in rotogravure printing. International Archives of Occupational and Environmental Health 63:377–381.

Snyder R, Andrews LS. 1996. Toxic effects of solvents and vapours. In: Klaassen CD, editor. Cassarett & Doull's toxicology: The basic science of poisons. 5th edition. New York, NY: McGraw-Hill. p. 737–771.

Tolman KG, Rej R. 1999. Liver function. In: Burtis CA, Ashwood ER, editors. Textbook of clinical chemistry. 3rd edn. Philadelphia, PA: Saunders. p. 1125–1177.

Truchon G, Tardiff R, Brodeur J. 1999. O-cresol: a good indicator of exposure to low levels of toluene. Applied Occupational and Environmental Hygiene 14:677–681.