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CASE REPORT

Radhouane Chakroun,[1] *M.Sc.; Fayçal Faidi,*[1] *M.Sc; Abderrazek Hedhili,*[2] *Ph.D.; Kaouther Charbaji,*[2] *M.D.; Habib Nouaigui,*[1] *M.D.; and Mohamed Ben Laiba,*[1] *M.D.*

Inhalant Abuse Detection and Evaluation in Young Tunisians

ABSTRACT: Occupational exposure biological monitoring techniques were applied for the diagnosis of inhalation abuse and for the evaluation of the levels of exposure to benzene, toluene, ethylbenzene, xylenes, and *n*-hexane, in 44 Tunisian adolescents and children suspected for volatile substance addiction. Urinary *trans,trans*-muconic acid, hippuric acid (HA), mandelic acid, and methylhippuric acids determinations were performed by high performance liquid chromatography with a photodiode array detector, and urinary *o*-cresol (*o*-Cr) and 2,5-hexanedione (HD) were extracted simultaneously and measured using gas chromatography with a flame ionization detector. Given the high linearity ranges, HD and *o*-Cr occupational exposure monitoring techniques could be applied without modification. However, urinary sample dilution was necessary before HA analysis. Concentrations were compared with the maxima of normal values (MNVs) in the general population and to the biological exposure indices (BEIs) used in occupational toxicology. Values as high as 6610-fold the MNV and 68 times the BEI were registered. The subjects showed high exposure to toluene and hexane. Measured metabolites HA and/or *o*-Cr and HD enabled the easy detection and evaluation of exposure levels. The problem of inhalant abuse should be given more attention and treated through an effective prevention strategy.

KEYWORDS: forensic science, inhalant abuse, solvents, biological monitoring, *trans,trans*-muconic acid, mandelic acid, hippuric acid, *o*-cresol, methylhippuric acids, 2,5-hexanedione

The intentional inhalation of products containing substantial amounts of volatile organic solvents so as to achieve euphoria is a significant international public health problem reported in most parts of the world. Numerous household products may be abused via inhalation. Some such products include gasoline, thinners, correction fluid, and especially glue. The availability and low cost of these products have caused them to be widely abused among children and adolescents. Abused inhalants may contain toxic organic solvents including aliphatic (*n*-hexane), aromatic (toluene, xylene, benzene), or halogenated hydrocarbons (chloroform, dichloromethane, trichloroethylene). However, toluene is the main constituent of many abused substances. Several adhesives may also contain significant quantities of *n*-hexane.

Immediate effects of inhalants are euphoria frequently with hallucinations, followed by drowsiness and sleep (1). High doses may lead to depressed respiration, convulsions, and coma. Even death may ensue in severe cases (2). Chronic poisoning results mainly in central nervous system disorders including dementia and cerebellar dysfunction that reflect brain damage (3–5). *n*-Hexane peripheral neuropathy from glue- and naphtha-sniffing remains an important health effect for inhalant abusers (6–8). Other cardiovascular, nephrotoxic, and hepatotoxic effects have also been reported (9,10). Moreover, benzene that is still found in gasoline and in low-purity industrial solvents has been proved to be carcinogenic for humans (11).

Although it is an important health problem particularly among children and adolescents, volatile substance abuse is still an unrecognized problem in Tunisia and receives less public attention than illegal drug use. Because detection procedures and national epidemiological data on inhalant abuse are not available, the aim of this study was, therefore, to adapt occupational exposure monitoring techniques for the diagnosis of inhalation abuse and for the evaluation of the levels of exposure to toluene, benzene, ethylbenzene, xylenes, and *n*-hexane.

[1]Laboratoires de Biologie et de Toxicologie Professionnelle, Institut de Santé et de Sécurité au Travail, UR-06-ISST-01, 5 Boulevard M. Khaznadar 1007 Tunis, Tunisia.

[2]Unité de Recherche Toxicologie et Environnement, CAMU, Laboratoire de Toxicologie, 10 Rue Aboulkacem Chabbi 1008 Tunis, Tunisia.

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Materials and Methods

Subjects

Forty male and four female Tunisian adolescents, young adults and children, aged 11–23 years, and suspected as drug addicts were studied. Patients came to the medical surgery for legal medicine expertise or from the childhood protection society.

For all the subjects, information about social conditions, district of residence, and scholar level was collected using a questionnaire.

Both the huffing and sniffing modes of abuse are used by the inhalant abusers. Some of them declared adding solvents or polish to their meals.

Measurement of Urinary Biomarkers

Spot urine samples were collected in a 60-mL polyethylene bottle for the determination of urinary metabolites of solvents: *trans, trans*-muconic acid (t,t-MA) from benzene, mandelic acid (MA) from ethylbenzene and styrene, hippuric acid (HA) and *o*-cresol (*o*-Cr) from toluene, methylhippuric acids (MHAs) from xylenes, and 2,5-hexanedione (HD) from *n*-hexane.

Measurements of *o*-Cr and HD were performed mainly from the methods of Truchon et al. (12) and Kawai et al. (13), respectively. In a test tube, 10 mL of urine was acidified to pH 5 with hydrochloric acid. For specific hydrolysis of glucuronide and sulfate

conjugates of HD and *o*-Cr, 20 µL of 1/10 diluted β-glucuronidase/sulfatase enzymatic solution (≥100,000 U/mL β-glucuronidase/≤7500 U/mL sulfatase; G0876 from Sigma, Gillingham, Dorset, UK) was added. The enzymatic catalysis was carried out for 20 h at 37°C. Six calibration solutions of *o*-Cr (0.125–4 mg/L) and HD (0.97–9.7 mg/L) were prepared by aqueous dilution of a master standard solution, in a final volume of 10 mL. To all the tubes (blank, standard, and sample), 100 µL internal standard solutions was added (i.e., 410 mg/L 1-hexanol solution or 810 mg/L 2-methyl-3-pentanone solution for HD, and 100 mg/L 2,3,5-trimethylphenol solution for *o*-Cr). Hydrolysis was completed by adding 2 mL of hydrochloric acid and by heating for 30 min at 100°C. The combination of the two hydrolysis procedures provided better recoveries than simple acidic attack.

After cooling to room temperature, analytes were extracted using 2 mL dichloromethane and centrifuged for 10 min at 345 × *g*. Two microliter of the organic layer was injected with an Agilent 6890 automatic liquid sampler, in the splitless mode into a 6890 Agilent gas chromatograph (GC) equipped with a Chrompack-CPSil 8 CB 50 m × 320 µm × 0.40 µm column.

For HD analysis, the injector and detector temperatures were set at 270°C and 280°C respectively. The furnace temperature was kept at 40°C for 5 min after injection, increased at a rate of 5°C/min to 120°C, immediately increased again at a rate of 50°C/min to 250°C, and then kept for 36 min at this temperature to elute all heavy and high-boiling point compounds and clean the column for the next injection. In addition, a pressure program was applied. Initial head pressure was set at 10 psi for 21 min followed by a pressure gradient of 4 psi/min to 21 psi.

Typical chromatograms from a control subject and from an inhalant abuser are shown in Figs. 1 and 2. The calibration curve was linear in the standards concentrations range (r = 0.9989). However, the method was linear in the concentration interval of 0.1–50 mg/L (r = 0.9998). The detection limit derived from a minimum signal to noise ratio of 3 (n = 15) was 60 µg/L.

Coefficients of variation (CVs) were evaluated by analyzing six aliquots of a spiked urine sample at low, medium, and high HD concentrations (1.9, 5.8, and 9.7 mg/L). CVs were respectively 0.5%, 4.9%, and 0.1%.

Different GC parameters were set for *o*-Cr analysis. The injector port temperature was 220°C, and the detector set at a temperature of 240°C. The column head pressure was 7 psi. The oven temperature was held at 50°C for 3 min, ramped at 3°C/min to 100°C, and kept at 100°C for 5 min. Then the temperature was increased at a rate of 15°C/min to 200°C, and held at 200°C for 10 min. Finally, the temperature was ramped again, at a rate of 25°C/min to 220°C. The final temperature was kept for 3 min. Figures 3 and 4 respectively illustrate the chromatograms of inhalant abuser and control's urine samples.

The calibration curve was linear in the standards concentrations range (r = 0.9999). However, the linearity interval ranged from 0.2 to 34 mg/L (r = 0.9991). The detection limit was as low as 3 µg/L. CVs evaluated by analyzing 10 aliquots of a spiked urine sample at the concentrations levels of 0.2, 1.0, and 4.0 mg/L were respectively 5.8%, 3.0%, and 0.6%.

The t,t-MA analysis was performed according to the method of Ducos et al. (14), slightly modified. Briefly, 1 mL of urine sample was submitted to a clean-up procedure with a Bond-Elut solid phase extraction column filled with 500 mg strong anion exchange (SAX) sorbent previously conditioned by 3 mL methanol and 3 mL distilled water. The column was later washed with 1% acetic acid solution. Finally t,t-MA was eluted with 10% acetic acid solution. The extract was adjusted to 5 mL with distilled water. Twenty

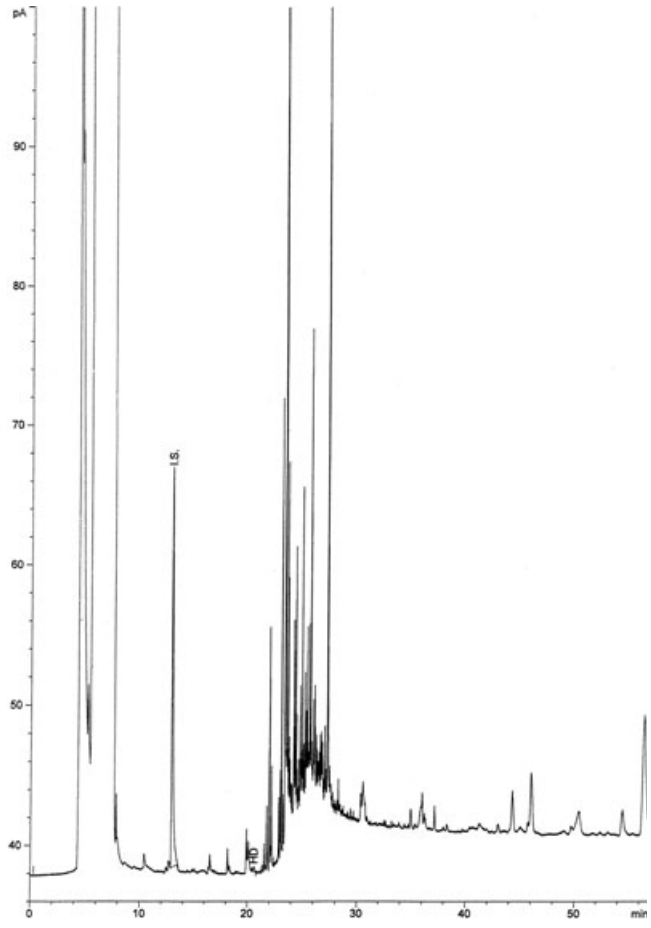

FIG. 1—*Chromatogram of urine sample from a nonexposed subject. HD, 2,5-hexanedione; IS, internal standard (2-methyl-3-pentanone).*

microliter of this solution was injected into the high performance liquid chromatography (HPLC) system, which consisted of a system controller (Waters 600), a gradient HPLC pump (Waters 600), a column oven (Waters 600), and a photodiode array detector (Waters 996). Chromatographic separation was performed on a 250 × 4.6 mm column packed with 5 µm Waters Spherisorb ODS2 (Waters, Wexford, Ireland). The mobile phase was 1% acetic acid/methanol solution (90:10 V/V) with a flow rate of 1 mL/min. The analytical wavelength for peak detection was set at 264 nm. The detection limit of the method was 0.02 mg/L. Calibration curve was linear in the concentration range of 0.05–5 mg/L (r = 0.9995). An example of chromatograms from a control and from an inhalant abuser is represented in Fig. 5.

CVs for standards (n = 10) at the concentrations levels of 0.1, 0.8, and 2.0 mg/L were respectively 1.0%, 1.0%, and 0.3%.

Simultaneous determination of urinary MA, HA, and MHAs was performed as described in a previous paper (15). Briefly, 1 mL of the urinary sample was mixed with an equal volume of methanol, and the mixture was spun at 1600 × *g* for 10 min to eliminate salts. An aliquot (5 µL/injection) of the supernatant solution was introduced to the HPLC system for analysis. The analysis was achieved on a Waters Symmetry 3.9 × 150-mm, 5µm, C_{18} column. The mobile phase employed was a gradient mixture (4:96 to 10:90) of acetonitrile: 150 µl/L perchloric acid solution, and was allowed to flow at a constant rate of 1.2 mL/min. The effluent was monitored at a wavelength of 200 nm. The detection limit was 0.2 mg/L for MHAs, and 0.1 mg/L for MA and HA. Correlation

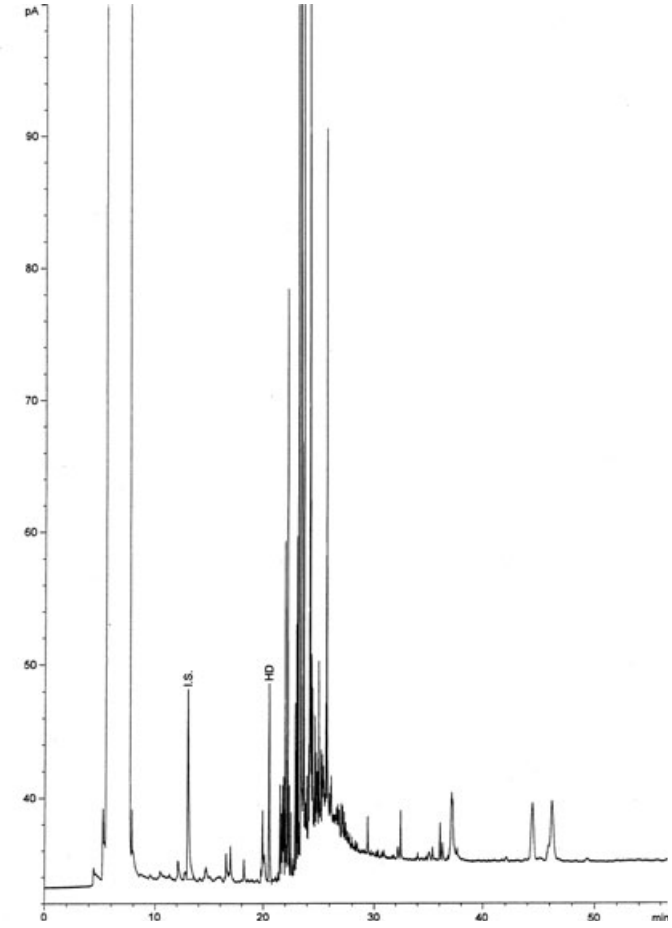

FIG. 2—*Chromatogram of urine sample from an inhalant abuser. HD, 2,5-hexanedione; IS, internal standard (2-methyl-3-pentanone).*

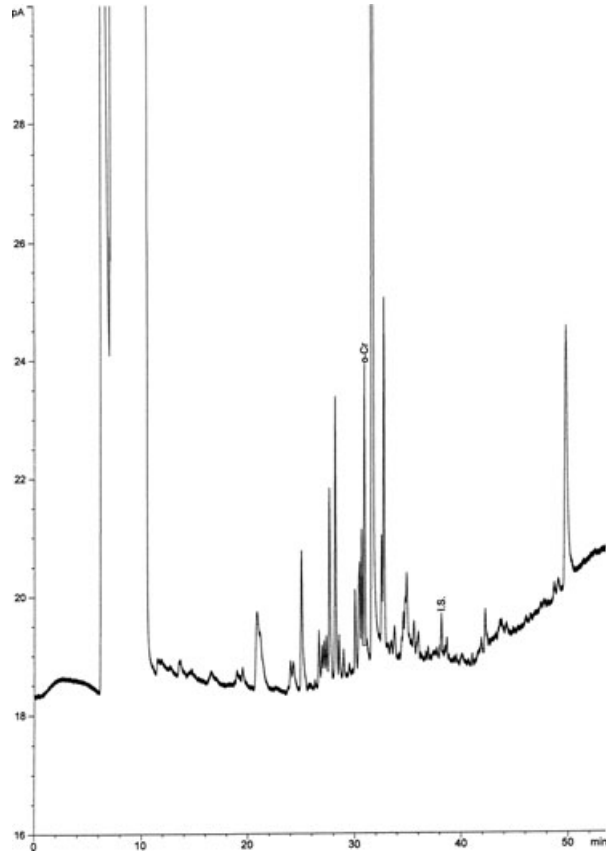

FIG. 3—*Chromatogram of urine sample from an inhalant abuser. o-Cr, o-cresol; IS, internal standard (2,3,5-trimethylphenol).*

coefficients in the concentrations intervals from 10 to 800 mg/L were greater than 0.999 for all the metabolites. CVs calculated for control urine samples, spiked at the concentrations levels of 50, 200, and 500 mg/L ranged respectively from 2.5% to 3.4% for MA, 2.7% to 4.3% for HA, and 2.6% to 4.8% for MHAs (15). Typical chromatograms from a control and from an inhalant abuser are shown in Fig. 6.

Samples out of the linearity range were reanalyzed after appropriate dilution.

Results and Discussion

Although in occupational monitoring of exposure to solvents sample dilution is generally not needed for the measure of the urinary metabolites concentrations, in inhalant abusers, 17 analyzed samples had to be diluted up to 20-fold to meet the HA linearity range. To prevent contamination and related problems, urine samples were systematically diluted 1:10 in pure water. If necessary, an appropriate second dilution is performed.

Only one sample (41.4 mg/L) was diluted for HD analysis. However, sample dilution may be avoided as the HD analysis method is linear up to 50 mg/L.

The concentrations of t,t-MA, MA, HA, and MHAs measured in inhalant users were compared with the maxima of normal values (MNV) found in the previous works for Tunisians unexposed to solvents (15,16). The concentrations of *o*-Cr and HD not yet measured in the general Tunisian population were compared with normal values reported elsewhere (17,18). Inhalant abuser metabolites concentrations were also compared with the American Conference of Governmental Industrial Hygienists biological exposure indices (BEIs), used for the monitoring of occupational exposure to the studied solvents (19). The BEIs are reference values intended as guidelines for the evaluation of potential health hazards in the practice of occupational hygiene. These values represent the levels of determinants which are most likely to be observed in specimens collected from healthy workers exposed to threshold limit value level concentrations of chemical pollutants. The results are summarized in Table 1. Among the 44 subjects studied, three males were monitored twice at different periods. Normal metabolite concentrations were found in 18 samples (from 14 males and four females). This is probably because of the sample timing. Some of the urine samples were collected several days after inhalant intake, whereas the half-life of the studied metabolites vary between 3 (*o*-Cr) and 30 h (MHAs), with a total elimination within 48 h except MA which takes 4 days to be totally eliminated (20). In 29 samples (from 28 males and one female), at least one of the metabolite concentrations was higher than the MNV.

Very high concentrations were found in several samples. MA reached values as high as 6610-fold the MNV and *o*-Cr, 68-fold the BEI (Table 2). Three subjects had t,t-MA values greater than BEI which means that they had been exposed to gasoline vapors or glue made with low-quality solvents having high benzene impurity.

HA and *o*-Cr concentrations higher than BEIs were registered in 13 and 12 subjects, respectively. In the monitoring of occupational exposure to toluene, *o*-Cr is preferred to HA because it is more specific and not influenced as HA by dietary intake (21–24). Other

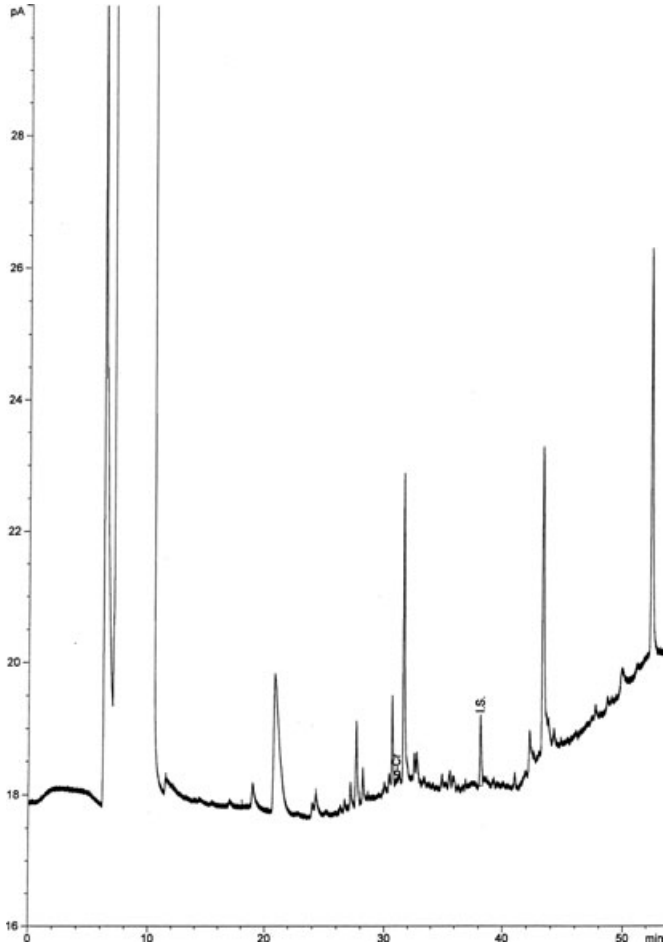

FIG. 4—*Chromatogram of urine sample from a nonexposed subject. o-Cr, o-cresol; IS, internal standard (2,3,5-trimethylphenol).*

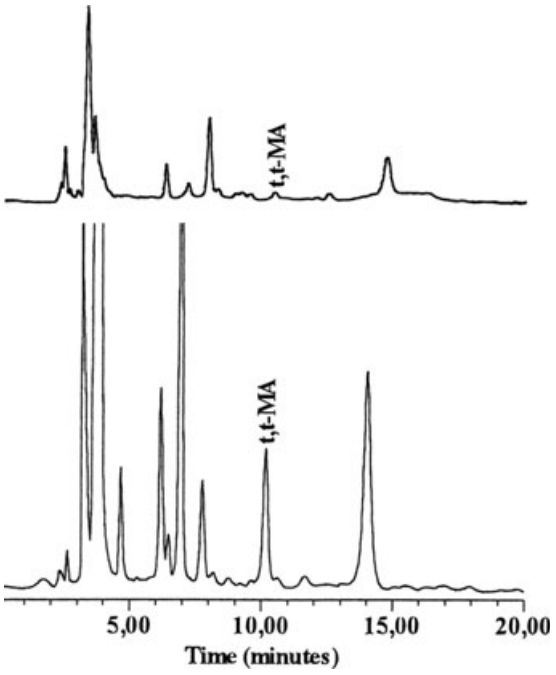

FIG. 5—*High-performance liquid chromatograms of a nondiluted sample from a nonexposed subject (top) and a 20-fold diluted sample from an inhalant abuser (down). t,t-MA, trans,trans-muconic acid.*

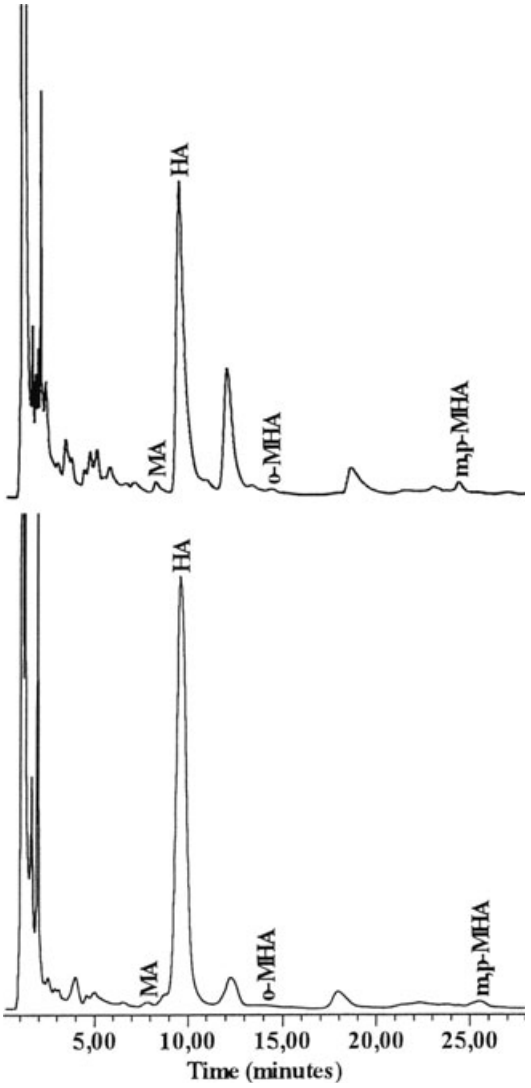

FIG. 6—*High-performance liquid chromatograms of a nondiluted sample from a nonexposed subject (top) and a 20-fold diluted sample from an inhalant abuser (down). MA, mandelic acid; HA, hippuric acid; o-MHA, o-methylhippuric acid; m,p-MHA, m- and p-methylhippuric acids.*

TABLE 1—*Frequencies of high metabolites concentrations in inhalant abusers.*

Metabolite [unit] (Number of Samples)	MNV	BEI	Range	% >MNV	% >BEI
HD [mg/g.c.] (33)	0.795*	5	[<LD–30.89]	33.33	15.15
t,t-MA [mg/g.c.] (27)	0.39	0.5	[<LD–4.43]	22.22	11.11
HA [g/g.c.] (36)	1.29	1.60	[0.05–11.52]	38.89	36.11
o-Cr [mg/g.c.] (33)	0.3	0.5	[<LD–34.12]	36.36	36.36
MA [mg/g.c.] (36)	0.1*	800	[<LD–14.91]	11.11	0.00
MHAs [mg/g.c.] (36)	22.4	1500	[<LD–341.86]	30.56	0.00

g.c., gram creatinine; MNV, maximum normal value, BEI, biological exposure index; LD, limit of detection.

*Value in mg/L.

investigators found that *o*-Cr is a better indicator of toluene exposure than is HA in glue sniffers (25). In the present study, HA allowed easy detection of toluene inhalation abuse. Moreover, an excellent correlation was found between HA and *o*-Cr ($r = 0.88$) (Fig. 7). However, *o*-Cr is still a more sensitive biomarker. Despite

TABLE 2—*Concentration ratios of MM with regard to MNVs and BEIs in subjects having high values.*

Metabolite	[MM/MNV] (*n*)	[MM/BEI] (*n*)
HD	[1.2–52.1] (11)	[1.0–6.2] (5)
t,t-MA	[1.0–11.4] (4)	[1.3–8.9] (3)
HA	[1.0–9.2] (14)	[1.0–7.4] (13)
o-Cr	[1.4–113.7] (12)	[1.1–68.2] (12)
MA	[199–6610] (4)	(0)
MHAs	[1.0–15.3] (11)	(0)

MM, measured metabolites; MNV, maximum normal value, BEI, biological exposure index; *n*, number of samples.

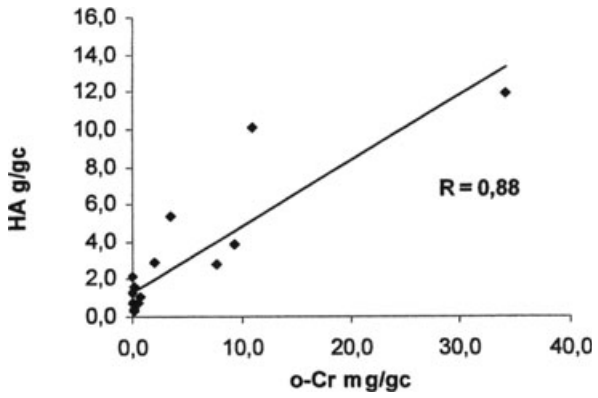

FIG. 7—*Regression line representing relation between urinary concentrations of o-cresol (mg/g creatinine) and hippuric acid (g/g creatinine) in inhalant abusers.*

the lower half-life of *o*-Cr, higher ratios of measured concentrations with regard to MNVs and BEIs were observed (Table 2).

In all cases, a simultaneous rise of concentration of both metabolites is supplementary evidence of toluene abuse. This solvent is a constituent of paints, thinners, and polyurethane and neoprene-type adhesives frequently used by abusers. The cheap abused neoprene glue may also contain *n*-hexane. HD values higher than the MNV were observed in 11 subjects. Some of the measured concentrations reached 3 to 6 times the BEI.

Finally, four subjects had excessively high MA values with regard to MNV. MHA values higher than MNV were also observed in 11 individuals. However, all the MA and MHA registered concentrations were lower than the BEIs. These results show that most of the studied subjects inhaled glues, and none of them has sniffed paints which contain xylenes and ethylbenzene.

Socioeconomic conditions such as educational failure and poverty are factors that contribute to inhalant abuse. In the present study, all abusers live in urban settings, and among the group of individuals with abnormal values, 37.5% are orphans or their parents divorced. Educational level varied from illiterate to 9th grade. The mean educational level is 5.8 ± 2.3 years of education. Therefore, the recommendation of the Tunisian health authorities to prohibit the sale of glues containing solvents to children and adolescents is not sufficient. Much more effort has to be put into selecting appropriate prevention strategies for specific social environments. Education, particularly if initiated before the usual age of experimentation, seems to be the most effective strategy (26). Recently, the Tunisian ministry of health had successfully initiated a prevention program against tobacco in elementary school. Pupils were educated about the harmfulness of tobacco and were given well-illustrated brochures. Inhalant abuse should be prevented through similar methodology. Appropriate modules in the elementary "scientific awakening" curriculum may be an effective prevention strategy. In addition, campaigns with the help of well-informed social educators, particularly in quarters where inhalant abuse is prevalent, may help to make significant changes in the rising rate of inhalant abuse.

Conclusion

Tunisia is concerned about the practice of inhalant abuse. The analysis of solvents metabolites, particularly those of toluene (HA and/or *o*-Cr) and hexane (HD) enable easy detection and evaluation of commonly abused products such as glues, paints, and thinners. Nevertheless, the time of sampling in relation to the time elapsed after exposure with regard to metabolites half-lives must always be considered for a correct interpretation of the results.

When using our technique, a systematic 10-fold dilution of samples before HA analysis is recommended so as to meet linearity interval, avoid contamination, and enhance column lifetime.

At the same time, sample dilution is not necessary for HD and *o*-Cr as the linearity range for both analytes was large enough. The method described in this paper allows a unique sample preparation for both analyses. Concurrent sample preparation, high sensitivity, and large linearity range favors the use of these techniques for the detection and evaluation of inhalation abuse.

Because solvent addiction may cause significant health and social problems, a prevention strategy based on education and social worker intervention should be adopted to curtail its use.

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Additional information and reprint requests:
Radhouane Chakroun, M.Sc.
Laboratoires de Biologie et de Toxicologie Professionnelle
Institut de Santé et de Sécurité au Travail
5 Boulevard M. Khaznadar
1007 Tunis
Tunisia
E-mail: r_chakroun@yahoo.fr