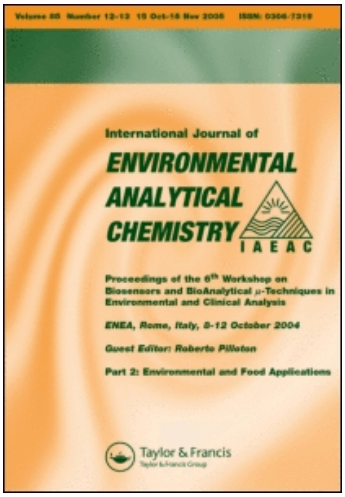

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Dynamic Vapour Generation and Head Space Determination of *m*-XYLENE for Pharmacokinetic Studies

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DYNAMIC VAPOUR GENERATION AND HEAD SPACE DETERMINATION OF *m*-XYLENE FOR PHARMACOKINETIC STUDIES

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A custom designed vapour generation-exposure chamber assembly and head space analysis method were evaluated for use in pharmacokinetic investigations. The predicted *m*-xylene concentration was within 1% of the average value measured by gas chromatography-flame ionization detection (GC-FID) in the glass exposure chamber during controlled vapourization of *m*-xylene. The concentration variability was ± 13% and ± 4 %, respectively, with and without rats in the chamber. Blood and tissue samples from male, Sprague Dawley rats exposed to 1100 ppm of xylene, were analyzed by means of a static head space (HS)-GC analysis method. The average *m*-xylene level in the blood of exposed animals was ca. 5000 ng/mL. *m*-Xylene was detected in brain, kidney, skin, fat and liver. The analytical precision for duplicate samples was < ± 5% for all tissues, except liver and skin. The feasibility of detection of *m*-xylene in unhomogenized tissue by HS-GC analysis was demonstrated.

KEY WORDS: *m*-Xylene, vapour generation, exposure chamber, head space analysis, blood, tissue.

INTRODUCTION

Xylene is a widely used solvent in industrial and household products. It also occurs in vehicle emissions, tobacco smoke, and other combustion emissions, as well as naturally as a component in petroleum products[1]. Because of its widespread use and human exposure, concern has been raised over its potential health effects.

Toxic effects have been reported in rats and mice exposed to xylenes. Some neurological effects have also been reported in humans[2]. In order to accurately assess the health effects of xylene, reliable analytical methods are required for precise monitoring of animal exposure systems, and biological samples.

Investigations on the effects of xylene exposure on animals have been conducted both with static (closed chamber), and with dynamic (flow-through chamber) systems[3,4]. Although head space (HS) analysis of biological samples containing volatile organic compounds (VOC), including xylenes, has been reported[5–8], information on a thorough investigation of the methods is not available. For instance, sample preparation involving homogenization of tissue samples in 0.9% saline has been used typically, with a potential for loss of VOCs during homogenization, and thus reduced reliability of the analysis. Analyses of unhomogenized tissue samples containing VOCs, on the other hand, would avoid evaporative loss so that reliable results may be obtained.

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The objective of the present study was to develop a reliable, dynamic exposure apparatus for only a few rats, and a gas chromatographic method for the continuous monitoring of *m*-xylene concentrations in the exposure chamber. In order to establish a complete exposure-monitoring system for pharmacokinetic studies, the feasibility of a head space gas chromatographic method for analysis of unhomogenized tissues of exposed rats was examined.

MATERIALS AND METHODS

Materials

All chemicals were of ≥ 99% purity. *m*-Xylene, ethylbenzene and chlorobenzene were obtained from Aldrich Chemicals Company Inc. (Milwaukee, WI); *o*-xylene and *p*-xylene were purchased from Eastman Kodak Company (Rochester, NY). The sodium salt of ethylene diamine tetraacetic acid (EDTA) was of ACS reagent grade purity, and was purchased from Sigma Chemical Company (St Louis, MO). Glass distilled acetone was supplied by Caledon Laboratories Ltd., (Georgetown, Ont). Somnotol (sodium pentobarbital) was obtained from Canada Packers Inc. (Cambridge, Ont). Deionized water was obtained from a Super-Q Plus High Purity Water System (Millipore Corporation, Bedford, MA), and was degassed by use of nitrogen gas. All gases were supplied by Matheson Gas Products, Canada (Whitby, Ont.). The compressed air was zero grade, and helium, hydrogen, nitrogen and oxygen were ultra high purity grade quality. The standard gas mixture (10 ppm, of each *m*-, *o*-, *p*-xylenes and ethylbenzene in air) was custom made by Matheson Gas Products. Amber glass vials (40, 14 and 1.5 mL) , the open top screw caps, Tuf-Bond Teflon/Silicone (TBTS) discs, and the Hamilton gas tight syringes were purchased from Chromatographic Specialties Inc., (Brockville, Ont.). The discs for the open top screw caps of 1. 5 mL autosampler vials were purchased from Chromacol Ltd. (Montreal, Quebec).

A composite stock solution of *m*-, *o*-, *p*-xylene isomers and ethylbenzene (ca. 62 mg/mL, each) was made by transferring 2.0 mL of each of the compounds into 20.0 mL of acetone containing the internal standard, chlorobenzene (55.4, µg/mL). Composite working standard solutions were prepared in 40 mL vials by serial dilution of measured aliquots of the stock solution with acetone spiked with chlorobenzene (55.4 µg/mL) to contain each of the target compounds at concentrations of 0.04, 0.22, 1.3, 8.0, 48, and 290 ng/µL. The vials were sealed with open top screw caps and TBTS discs, and then with Teflon tape.

Apparatus

Gas chromatograph (GC)

All GC analyses were carried out with a Hewlett-Packard (HP; Avondale, PA) Model 5890 gas chromatograph equipped with a HP 20 M capillary column (Carbowax-20M; 0.53 mm, I.D.; 30 m, length; 1.33 µm, film thickness), and a flame ionization detector (FID). The instrument was operated using the HP 3365 Chemstation software installed on an Olivetti Model 300 personal computer. Aliquots of working standard solutions were injected into the GC by means of an autosampler (HP model 7672A), and the head

space vapours in vials were sampled (1 mL) with an automated head space sampler (DANI, HP model 19395A) connected to the GC by a heated (70°C) transfer line (300–257–DAN). The exposure chamber air was sampled (0.5 mL) by means of a Carle mini MK II valve connected to the GC.

The GC injection port temperature was 200°C, and optimized gas flows to the FID (220°C) were 36, 56 and 343 mL/min, respectively, for hydrogen, auxiliary gas (He) and air. The column oven temperature was initially set at 50°C, increased at a rate of 5°C/min to 80°C, held for 3.75 min, increased further to 100°C at the rate of 20°C/min, and was held at 100°C for 3 min. The carrier gas (He) flow during the analysis of working standard solutions was 6.17 mL/min, and injections were made in a splitless mode.

The GC carrier gas flow during head space analyses was 6.25 mL/min. Optimized head space sampler carrier, and auxiliary helium gas flows, respectively, were 10.2 and 8.8 mL/min. The servo air pressure that causes the sample carousel to advance, was set at ca. 3.5 bar. A pressurization time of 5 sec, a venting time of 2 sec, and an injection time of 2 min were chosen for optimum performance of head space sampling. All samples were equilibrated at a head space sampler bath temperature of 55°C for 60 min prior to analysis. GC conditions for the exposure chamber air analyses were the same as mentioned above except that the GC carrier gas flow was 9.9 mL/min, and the oven temperature was held at 80°C during analyses.

Vapour generating system

The vapour generating system (Figure 1) was custom designed (Concord Scientific Corporation, Downsview, Ont.) and consisted of the following components.

Solvent delivery system. A 5 mL Hamilton gas tight syringe (#1005) with a Teflon luer lock adaptor, a Sage model 341A syringe pump (Orion Research Inc., Cambridge, MA), and a Teflon transfer tube that extended from the tip of the gas tight syringe. The Teflon transfer tube was connected to a needle which was inserted into a heated block injector.

Vapourizing system. A Miller-Nelson (Dublin, CA) heated block injector was connected to a UE SPACEPAK temperature control/read out unit (Johns Scientific Inc., Toronto, Ont.) which in turn was equipped with a thermocouple probe that was inserted into the heated block injector.

Gas supply. Carrier (Nitrogen), diluent (air) and make-up gas (oxygen) lines were stainless steel, and were linked as depicted in Figure 1. All gas lines were connected to calibrated Scott rotameters.

Mixing chamber. A 2 L capacity glass jar was equipped with an inlet for the gas mixture from the heated block injector and an outlet to transfer the air/*m*-xylene mixture through a Teflon tube into the exposure chamber.

Exposure chamber and monitoring system

The exposure chamber had a volume of 27.5 L, an inlet from the mixing chamber, and two outlets. One of the outlets was connected in series to a Carle valve, a Gilian LFS 113D pump (Gilian Instruments Corp., Wayne, NJ; pump 3 in Figure 1), and the vent.

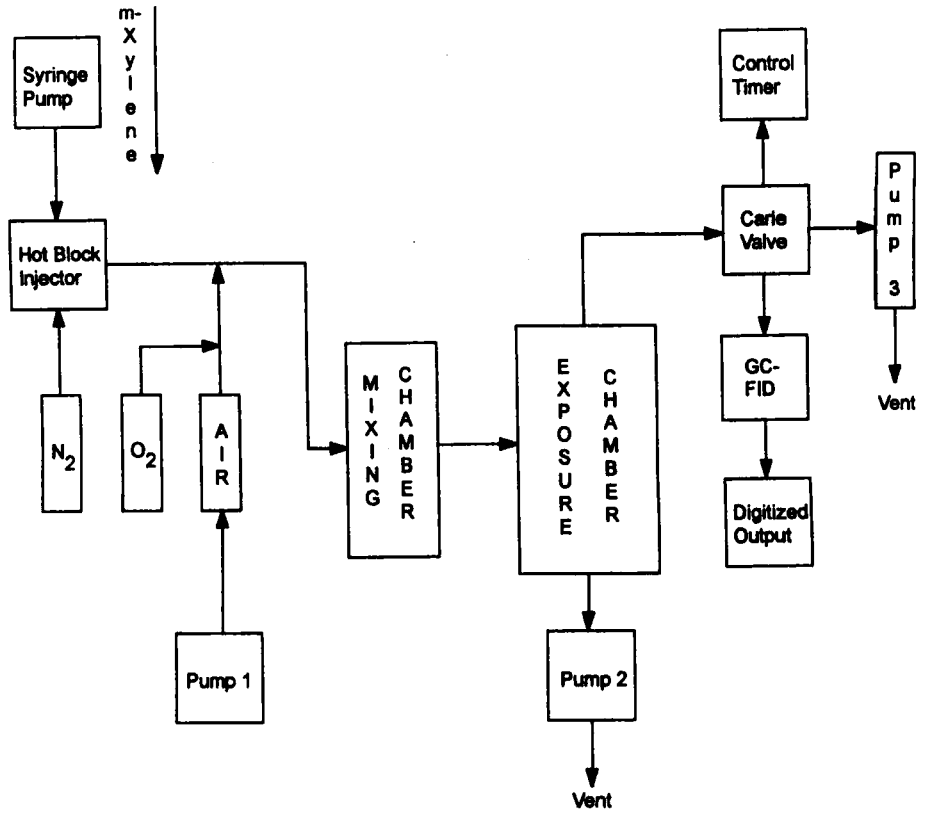

Figure 1 A schematic siagram of the *m*-Xylene vapour generating system, exposure chamber and the monitoring system assembly.

The other outlet was connected to a Gilian HFS 113A pump as shown in Figure 1 (pump 2). Flow rates through the Gilian pumps were measured by means of a Gilibrator flow calibrator (Gilian Instruments Corp., Wayne, NJ).

The Carle mini MK II six port gas sampling valve (model 5621, EG&G Chandler Engineering, Carle Chromatography, Tulsa, OK), with a 0.5 mL loop was housed in a valve oven held at 100°C. The Carle valve was connected to a ChronTrol timer by means of two cables which electrically triggered the rotation of the valve to "fill" and "inject" positions as required. Timed events for exposure chamber air sampling were controlled by the ChronTrol timer (Lindberg Enterprises Inc., San Diego, CA). The timer was programmed to initially maintain the Carle valve in the "fill" position. At a set time, the valve was rotated to the "inject" position, loop contents were transferred to the GC (30 sec), and the valve was then reset to its "fill" state.

Procedures

Calibration of the GC-FID was done by analysis of 1 μL aliquots of working standard solutions of xylene isomers and ethyl benzene in acetone, and manually injected 0.5 mL

and 1.0 mL samples of a standard gas mixture of xylenes and ethylbenzene in air (10 ppm of each VOC). Manual injections of the standard gas mixture were done by means of a warm Hamilton gas tight syringe with a side-port needle. The syringe was flushed with the gas mixture prior to injection of a sample of the mixture.

In order to calibrate the Carle gas valve performance, the inlet of the valve was connected by means of Teflon tubing through a T-junction to the standard gas mixture cylinder. The flow rate through the Gilian LFS pump was adjusted to 27.6 mL/min to flush the sample loop at least three times with the standard gas mixture prior to the injection. The head space GC-FID (HS-GC) method was calibrated for xylenes and ethylbenzene in blood by means of automated injections (1 ml) of head space vapour above spiked blood samples containing individual VOC at concentrations of 0.001, 0.006, 0.04, 0.24, 1.4, 8.6 and 50 μg/mL.

A preliminary inhalation exposure experiment was carried out as follows. Initially, the solvent delivery system was tested to confirm delivery rates. The syringe pump was then set to deliver *m*-xylene at a rate of 1.2 mL/h. The vapour generating system carrier gas (nitrogen, 800 mL/min) swept the xylene vapour from the heated block injector into the mixing chamber. The diluent air was drawn through a Gilian Aircon 520 AC air sampling pump (Gilian Instruments Corp., Wayne, NJ) as shown in Figure 1 (pump 1), and then through a cartridge packed with activated charcoal and molecular sieve in series with a filter cassette containing a particle filter (Gelman Sciences) in a 47 mm Teflon holder. The air flow was adjusted at the pump to ca. 3.5 L/min. Compensation for the addition of nitrogen was achieved by setting the make-up gas oxygen flow at 200 mL/min. The heated block injector potentiometer was set at 68% of maximum voltage output, and the temperature controller was set at 160°C. The observed temperature readings ranged from 165–167°C. The Gilian LFS pump at the outlet of the Carle valve was set at a flow rate of 100 mL/min (the sample loop was flushed ca. 10 times with the exposure chamber air prior to injection). The Gilian HFS pump connected to the outlet of the exposure chamber was set at a flow rate of 3.5 L/min, and the timer was programmed so that the inject/fill cycle was repeated every 5 min for 3 h.

All connections and the exposure chamber were checked for leaks. The Hamilton gas tight syringe containing ca. 6 mL of *m*-xylene was fitted to the syringe pump. The timer and GC were started as the syringe plunger movement started. Male Sprague Dawley rats (300–350 g) were placed in the inhalation chamber when the *m*-xylene levels reached ca. 95% of the desired level.

At the end of 2 h of exposure, the rats were removed from the inhalation chamber, and were anaesthetized by intraperitoneal injections of Somnotol (0.3 mL/rat). Injected rats were immediately placed back in the exposure chamber until sedation. Rats were removed from the chamber after sedation, and dissected immediately. Blood was withdrawn from the abdominal aorta, and was transferred into amber glass vials containing EDTA (2.3 mg/mL blood), and the vials were manually agitated. Brain, liver, kidney, fat, and skin were then harvested in a random order. As one analyst harvested different tissue types, the other analyst immediately transferred measured amounts (1 ± 0.5 g) of tissue samples into 14 mL amber glass vials. Control blood specimens were also obtained from unexposed rats. Measured aliquots of blood (1 mL) and portions of tissue samples in vials were spiked with 5 μL of acetone containing chlorobenzene (55.4 μg/mL). Aliquots (1 mL) of control blood samples were spiked with 5 μL of two working standard solutions (1433 ng/mL and 8600 ng/mL, of *m*-xylene), and were analyzed along with the exposed samples using the HS-GC method. Blank blood and water samples were prepared by spiking 1 mL each of control blood and degassed Super-Q water with 5 μL aliquots of acetone. The head space above exposed tissue and blood

samples was analyzed by GC-FID at 4 h, 1 d and 2 d after the vials containing samples were sealed.

RESULTS AND DISCUSSION

A dynamic atmosphere was used in these trials so that exposures could be done under controlled, constant, and reproducible conditions. In the case of static conditions in sealed chambers, animals can only be exposed to the target contaminant for short time periods since the atmospheric conditions cannot be maintained constant and would not be suitable for controlled studies. In static systems, there would be a decrease in airborne target contaminant (e.g. *m*-xylene) and oxygen concentrations during exposure due to metabolic activities of the animals. There would also be an increase in concentrations of substances exhaled (e.g. carbon dioxide, water) and otherwise released by the animals (e.g. NH_3). These changes can lead to oxygen deprivation, excess humidity, heat, and the presence of excessive amounts of other contaminants which can lead to discomfort, and abnormal physiological conditions and behaviour of the exposed animals. A dynamic exposure system, such as that used in these studies, allows maintenance of a constant and desirable environment.

Performance of equipment

Exploratory tests showed that adequate resolution and sensitivity was achieved with a carbowax column and GC-FID detection for *m*-, *o*-, *p*- xylenes and ethylbenzene which often occur as co-contaminants in environmental samples. Consequently, it was decided to monitor *m*-xylene occurrence both in the inhalation chamber air, and static head space above enclosed tissue and blood samples by this analytical approach. A linear (r^2 = 0.99999) FID response was observed for concentrations ranging from 0.22 to 290 ng/μL of *m*-xylene, and similarly for the other xylene isomers, based on the GC-FID analysis of working standard solutions. Normalization of *m*-xylene peak areas to a representative internal standard (chlorobenzene) peak area resulted in good precision values for replicate measurements. The precision of measurements for triplicate samples was better than 5% relative standard deviation (RSD) after normalization, for *m*-xylene concentrations at and above 0.2 ng/μL.

The GC-FID response was also calibrated with the certified standard gas mixture containing xylenes and ethylbenzene (10 ppm, of each compound) in air by means of manual sampling and injection. The analytical precision of measurements for triplicate samples was better than 2% RSD. The performance of the Carle valve used in exposure chamber air sampling was calibrated by means of the same standard gas mixture. The volume of gas sampled and injected by the automated Carle valve system was determined to be 0.5 mL by comparison of analytical results with those obtained by the manual (0.5 mL) injection method. The average *m*-xylene peak area value for manual injections was within ± 3% of that obtained with Carle valve injection.

Exposure experiments were conducted to assess the custom designed vapour generation-exposure chamber apparatus performance in producing constant concentrations of *m*-xylene in air, and to obtain biological samples containing *m*-xylene from exposed animals. Figure 2 illustrates that the GC-FID response to airborne *m*-xylene in the exposure chamber was relatively constant during the time of rat exposure.

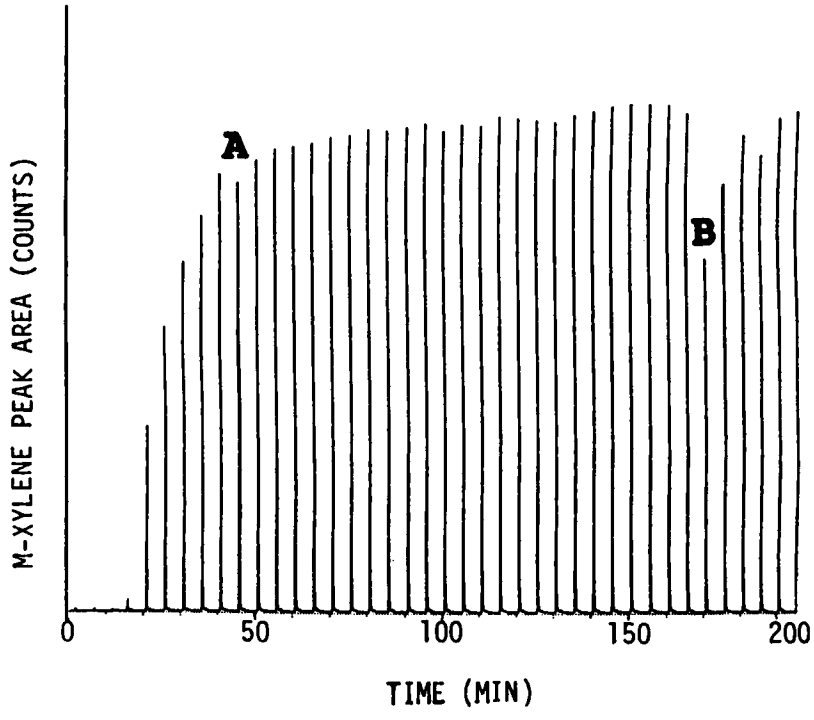

Figure 2 *m*-Xylene profile in the exposure chamber during exposure. Lid removed at 45 (A) and 170 min (B).

A low sampling rate (100 mL/min) at the Carle valve outlet was chosen to minimize any alteration in the chamber *m*-xylene concentration that could arise due to sampling. Although the time to attain 95% of the steady state *m*-xylene concentration in the chamber was calculated to be ca. 25 min according to McFarland[9], a period of about 45 min (Figure 2) was required to achieve steady state conditions, probably due to a deviation from the ideal (100% effectiveness) mixing of generated vapours with the air already in the chamber. The slight drop in the *m*-xylene concentration observed at about 45 min was due to the temporary removal of the lid during placement of rats into the chamber. The dip in the *m*-xylene concentration at ca. 170 min was caused by the removal of rats for anaesthetization. Once the lid was replaced the concentration returned to the previous level in ca. 20 min.

The average *m*-xylene concentration during the period of exposure was 1100 ppm based on the calibrated GC-FID analysis results. This value was in good agreement with the estimated value of 1098 ppm, which was based on the *m*-xylene delivery rate of 1.2 mL/h and the air flow rate of 3.5 L/min. Two analysts who conducted independent tests at different times achieved a *m*-xylene concentration of 1100 ± 10 ppm in the chamber by using the same operating conditions and equipment. The variability in *m*-xylene peak areas was within ± 13% and ± 4%, respectively, of the average value, with and without rats in the exposure chamber. The presence of rats in the chamber appeared to increase the variability in *m*-xylene concentrations compared to a system without rats. If animal activities influence contaminant concentrations to such an extent in a dynamic system, then the decay rates for concentrations in static systems would be expected to be equally or more variable.

Static head space analyses

A linear calibration curve ($r^2 = 0.99999$) was obtained for a plot of GC-FID response and *m*-xylene concentrations (six levels) up to 50 μg/mL in blood by the automated static head space (HS) analysis method. The method detection limit (MDL) was 0.006 μg/mL for blood samples. The precision of measurements for duplicate samples was < ± 4% at, and above, 0.24 μg/mL when *m*-xylene peak areas were normalized to the internal standard response. Blank water samples were typically analyzed after every four head space samples to assess cross contamination and to reduce the use of blood samples serving as blanks.

After exposure, anaesthetized rats were put back in the inhalation chamber until dissection to reduce the loss of xylene. Also, dissection and preparation of head space samples were done within a short time period (ca. 10 min/rat), to minimize any loss of xylene to air. Tissues were excised in a random order to minimize the bias in *m*-xylene results which might have occurred due to a time dependent analyte loss from the samples if they were excised in a fixed order.

The average *m*-xylene concentration in blood from rats exposed to 1100 ppm *m*-xylene was ca. 5000 ng/mL based on the analysis of spiked blood samples by the HS-GC method. The analytical precision of duplicate measurements was better than ± 4%. With the method detection limit at ca. 6 ng/mL and a linear calibration curve for xylene in blood, it was judged that the static head space analysis method should be suitable for investigations requiring exposures well below 100 ppm*h (i.e. 100 ppm for 1 h), as well as exposures above 2000 ppm*h.

Although no calibration curves were generated for tissues in these exploratory investigations, the results from head space analyses of ca. 1 g samples provided important information about the feasibility of this analytical approach. Exposed tissue samples spiked with acetone containing internal standard were equilibrated in sealed vials at room temperature for 4 h, 1 d, and 2 d to assess the effect of equilibration time on head space analyte concentration, and thus on the sensitivity of analysis. The head space *m*-xylene concentration was greater for all tissue samples equilibrated for 1 and 2 d at room temperature (21 ± 1°C) than for samples equilibrated for 4 h. An equilibration time of 1 d was judged most suitable since it provided earlier analyses and a better guarantee of sample integrity than 2 d of storage. Improved analytical precision values for duplicate samples were obtained at the longer equilibration periods. For example, *m*-xylene peak areas in FID response units and % RSD values for duplicate measurements for two tissues were: brain, 4 h – 73281 ± 33%; brain, 1 d – 113076 ± 4%; fat, 4 h – 20523 ± 27%; fat, 1 d – 21860 ± 0.7%. Precision values for replicate measurements of liver, kidney, and skin after 1 d of equilibration were < ± 8%.

It also was possible to draw some conclusions about the sensitivity of the head space analysis approach. The lowest GC-FID response was observed for *m*-xylene in the head space above fat from the rats exposed to 1100 ppm of the analyte. Based on the analytical results for all tissue samples, it was anticipated that even at considerably lower exposure levels of *m*-xylene (i.e. < 100 ppm*h) there would be sufficient analyte concentrations in the head space above all tissue samples for detection by means of the head space analysis approach. The use of unhomogenized tissues in combination with the head space technique minimizes the sample handling time compared to the commonly used homogenization procedure[6]. Extensive sample handling and homogenization can lead to loss of VOCs, and reduce analytical sensitivity and reliability.

Although an inverse relationship was observed between tissue weights and head space *m*-xylene concentrations in some instances, it was not clear if this was a true relationship.

Head space analyte concentrations, in principle, are dependent on the initial tissue analyte concentrations, the volumes of tissue and head space, and on the partition coefficient for the analyte in the two phases. Results of calculations based on these factors do not indicate an inverse relationship. However, these experimental results suggest the need for a careful investigation of the factors affecting the reliability of a head space technique for quantitative determination of xylenes in tissue.

Concluding remarks

It has been demonstrated that the desired concentration (within ± 1%) of *m*-xylene in the chamber could be generated and maintained with the custom designed vapour generation-exposure chamber assembly. The results of these exploratory tests indicate that the analytical approach is promising in that excellent sensitivity (e.g. MDL for *m*-xylene in blood was 6 ng/mL) and precision (< ± 8% for all tissues) were achieved for analyses of blood and unhomogenized tissues. Furthermore, the simplicity of the sample preparation step has the potential to reduce any loss of VOCs, and thus improve the reliability of the analysis, compared to the homogenization procedure. Development and validation of analytical protocols for operation of the exposure apparatus and for the automated head space analyses procedures will be required before their routine application in quantitative pharmacokinetic studies. It is anticipated that the procedures will be applicable, with some modifications, for investigations with other volatile organic compounds with properties similar to those of *m*-xylene.

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