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THE TIME-COURSE AND SENSITIVITY OF MUCONIC ACID AS A BIOMARKER FOR HUMAN ENVIRONMENTAL EXPOSURE TO BENZENE

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Preliminary results are presented that show the effect of increased benzene exposure on the urinary elimination of *trans,trans*-muconic acid (MA) for an adult male. These results were generated from a controlled exposure experiment during which an individual was exposed to benzene during a shower with gasoline-contaminated ground water. Based on measured air and water concentrations, it is estimated that the 25 minute shower and drying-off exposure period resulted in an inhalation and dermal absorbed dose of 122 μg and 19 μg, respectively, yielding an average dose rate of 334 μg/h during the shower period. The measured background dose rate of 1.2 μg/h was exceeded by a factor of 278 during the shower exposure. The average urinary MA elimination rate increased from 3.7 μg/h during the 30 h period before the exposure to 17.9 μg/h during the 22 h period after the exposure. The post-exposure profile of muconic acid elimination (μg/h) was characterized by two minor peaks (47 and 35 μg/h) occurring within 3 h and a major peak (61 μg/h) at approximately 11 h.

Key Words: Muconic Acid, Time Course, Validation, Exposure, Dose, Biomarker, Benzene, Dermal, Inhalation.

This study was reviewed and approved by the Research Triangle Institute Committee for the Protection of Human Subjects.

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INTRODUCTION

Human exposure to benzene in community and occupational environments is common[1]. This fact, along with compelling evidence suggesting that benzene exposure causes leukemia in humans[2], gives reason for evaluating and minimizing routes of exposure.

Biomarkers can provide a powerful tool for assessing exposure and risk. The measurement of a biomarker can provide individual-based evidence that exposure has occurred *ex post facto*. A biomarker measurement establishes, in fact, a body burden that can otherwise only be estimated through external measurements of exposure. Although a biomarker measurement may in theory be a more valuable means of assessing exposure, its practical value is dependent upon the reliability and validation of the biomarker.

Various urinary biomarkers of benzene exposure have been investigated including phenol[3], S-phenyl-N-acetylcysteine[4,5] and muconic acid[6,7,8]. *trans,trans*-Muconic acid (MA) shows particular promise as a biomarker for human environmental exposure due to its specificity and its presence at detectable levels in individuals exposed to background benzene levels[7]. Furthermore, MA provides an indication of toxicological potential because it is formed from the toxic metabolic intermediate, muconaldehyde[9].

It is the aim of this research to provide further data regarding the validation of muconic acid as a biomarker of more subtle, non-occupational, benzene exposures. Research to date has generally involved highly exposed individuals, such as smokers or workers[6,7,8]. Specific research objectives include discerning low from relatively high levels of exposure through the urinary elimination of MA and to characterize the profile of MA elimination following a single acute exposure.

The exposure and muconic acid data presented herein are partial and represent two components of a multi-faceted study that also included measurements of dosimetry (respiratory and cardiac rate), blood, and breath benzene. The analysis of the full complement of data will be reported at a later date.

METHODS

The experimental design consisted of a short-term acute benzene exposure preceded and followed by periods of low-level background exposure. The short-term acute exposure was generated by an individual taking a shower using gasoline contaminated ground water. Therefore, a "bolus-like" dose of benzene was introduced by absorption through the lung and skin after a period of low level background benzene exposure. The level of contamination, and the likely resulting exposure was characterized prior to, as well as during the study[10,11]. The shower dermal and inhalation exposure was limited to 20 minutes followed by a 5 minute inhalation-only exposure period during drying off. Background exposures resulted from normal activities within ambient, office, in-transit, and home microenvironments. The identical shower exposure scenario was conducted three times in 1991 (June 11, 12, and 13) with one shower per day over three consecutive days.

The methods of collecting and analyzing water and microenvironmental air benzene levels are described in a microenvironmental measurements/intersampler comparison investigation conducted in conjunction with this biomarker validation experiment[12]. Integrated and grab samples were collected throughout the study using Summa™ canisters, Tenax GC™, and glass gas-tight syringes. Low-flow personal sampling was conducted using the sorbent Tenax GC™ to measure the shower and background

personal exposures. The pump (DuPont P4000) was operated at 10 cc/minute during the approximately 20 h background period which preceded and followed each shower exposure. Flow calibration was conducted at the beginning and end of each sampling period. Personal sampling was delayed for approximately 2 h following the shower exposure to minimize contamination of the Tenax GC™ with the elevated levels of exhaled benzene.

All urine passed during each of the three days of the shower plus approximately two days of background samples was collected. Voids were collected at 1-1½ and 4 h intervals during day/evening and night periods, respectively. Each void was collected separately in polypropylene (500 ml) or polyethylene (100 ml) screw-cap bottle with exact time and date of collection recorded on the bottle label. Each sample was immediately placed into a freezer or dry-ice cooler. Samples were transferred to a -20°C laboratory freezer within 1-2 days after collection.

Air samples collected on Tenax GC™ were thermally desorbed and analyzed by gas chromatography/mass spectroscopy (GC/MS). Water samples were similarly analyzed by GC/MS using a purge and trap technique. Grab air samples were collected with syringes and analyzed on-site by GC/PID (photo-ionization detection). Urinary muconic acid was quantified by GC/MS (single ion monitoring) after the addition of biosynthesized muconic Acid-^{13}C internal standard and liquid extraction (ethyl ether) according to methods described by Bechtold *et al.*[13]

RESULTS

Dose Estimates

MA data are currently available only for the June 13th shower exposure. Air and water benzene concentrations and quality assurance results are reported by Lindstrom *et al.*[12] The relevant data required for the biomarker assessment are specified here.

Personal sampling yielded air concentrations of 1-2 $\mu g/m^3$ during the background periods. The benzene air concentration during the 20 minute shower and 5 minute dry-off period was 525 and 398 $\mu g/m^3$ as determined from the integrated Tenax GC™ and grab syringe samples (20 and 25.5 minute), respectively. From these measurements, dose was estimated using equation 1.

$$D_{inhal} = \sum (C_i \times t_i) \times MV \times F \qquad (1)$$

Where: D_{inhal} is the inhaled absorbed dose (μg); C_i is the benzene concentration in microenvironment i ($\mu g/m^3$); MV is the minute ventilation rate (0.014 m^3/minute)[14]; t_i is the duration of exposure in microenvironment i (minute); and F is the fraction of inhaled benzene that is available for gas exchange (70%)[15]. An inhaled dose of 29 μg is estimated over the 24 h background period giving a dose rate of 1.2 $\mu g/h$. The inhaled dose during the 25 minute shower exposure was calculated to be 122 μg yielding a dose rate of 334 $\mu g/h$.

The dose delivered by dermal absorption is estimated from equation 2 to be 19 μg based on a mean water concentration at the shower head of 247 $\mu g/L$ (185 and 309 $\mu g/L$, at times 5 and 18 minutes into the shower, respectively).

$$D_{Derm} = CW \times SA \times K_p \times t \times U \qquad (2)$$

Where: D_{Derm} is the dose absorbed through the skin (μg); CW is the concentration of benzene in water (μg/L); SA is the surface area of a 180 lb, 6'4" male (2090 cm^2); K_p is the dermal permeability constant for benzene in an aqueous solution (0.111 cm/h)[16]; t is the duration of the exposure (0.33 h); and U is the units conversion factor (1 L/1000 cm^3).

Therefore, it is estimated that a total benzene dose of 141 μg (13% dermal absorption, 87% inhalation) resulted from the 25 minute exposure on June 13th giving an hourly dose rate of 334 μg/h. This dose rate exceeded the background dose rate of approximately 1.2 μg/h by a factor of 278.

Urinary Muconic Acid Elimination

Muconic acid elimination results are shown in Figure 1 as a frequency distribution of elimination rates for the background and post-shower exposure periods. Muconic acid elimination is reported as a rate (μg/h) based on the assay results (ng/ml), the void volume (ml), and the time between collections (h). The post-shower mean elimination of 17.9 μg/h exceeded the mean background elimination rate of 3.7 μg/h by a factor of 4.8. Duplicate analysis (n=21) yielded an average deviation from the mean of 8% (range: 1-53%).

The time course of muconic acid elimination relative to the background and shower exposure periods is illustrated in Figure 2. Relative to the background period, the shower exposure appears to be associated with two minor peaks in MA elimination at post-shower times of 1.3 h and 2.8 h with the most substantial peak at 11 h. Since the subject was exposed to two showers, one and two days prior to the day for which these results are reported, this time course may in part reflect more than one exposure.

CONCLUSIONS

Urinary MA elimination resulting from a relatively high short-term dermal and respiratory exposure shows two minor peaks occurring within the first three hours and a dominant peak approximately 11 hours following the exposure. Interpretations of this time course will be made based on these results, and the confirming results from the two previous exposures when they become available.

These data demonstrate the relationship between benzene exposure and MA elimination for a single individual during one of three repeated controlled exposure experiments. Data from the two unreported experiments will be used to confirm these findings and to further investigate the validity of MA as an exposure biomarker.

The increased rate of MA elimination corresponded to an increased benzene exposure suggesting that MA has some capacity as a biomarker for non-occupational exposures. Although the time metric by which dose and MA elimination are reported are not directly comparable, it is noted that the benzene dose increased 280 fold while MA elimination increased four-fold. This suggests that large changes in exposure are reflected by relatively small changes in MA elimination. Additional studies characterizing MA response to varying levels of benzene exposures are required to more fully assess this relationship and the sensitivity of MA resulting from benzene exposures.

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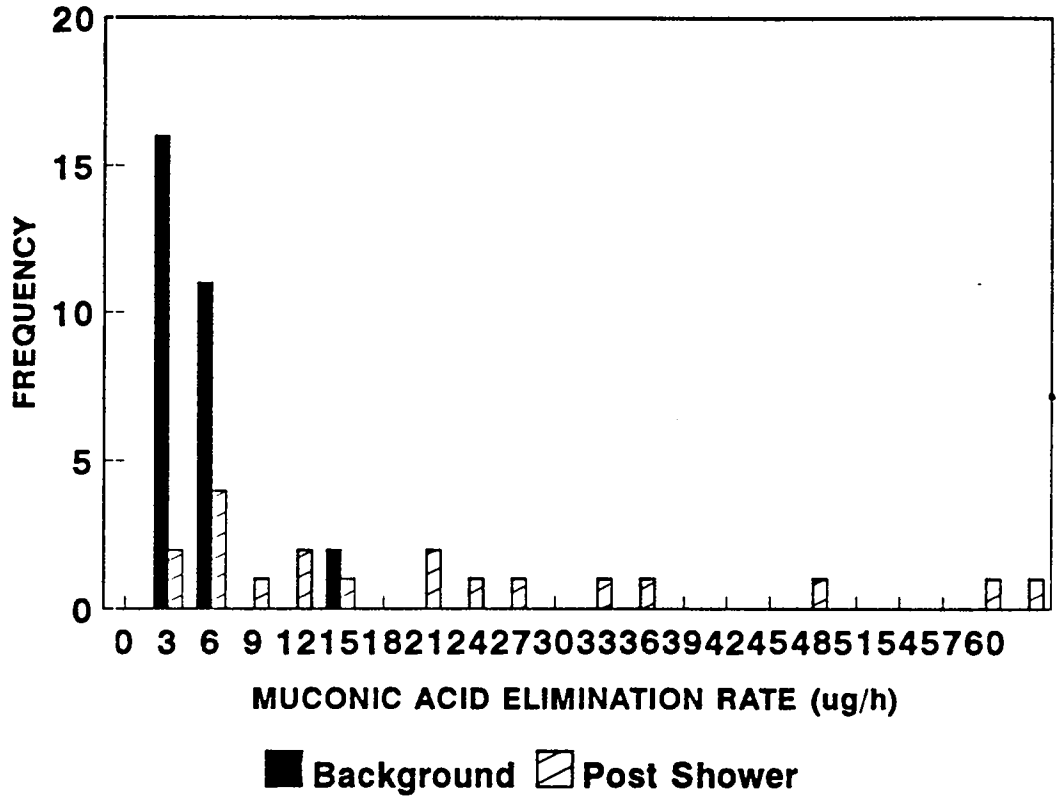

Figure 1. Frequency distribution of MA elimination rate during background and post-exposure periods.

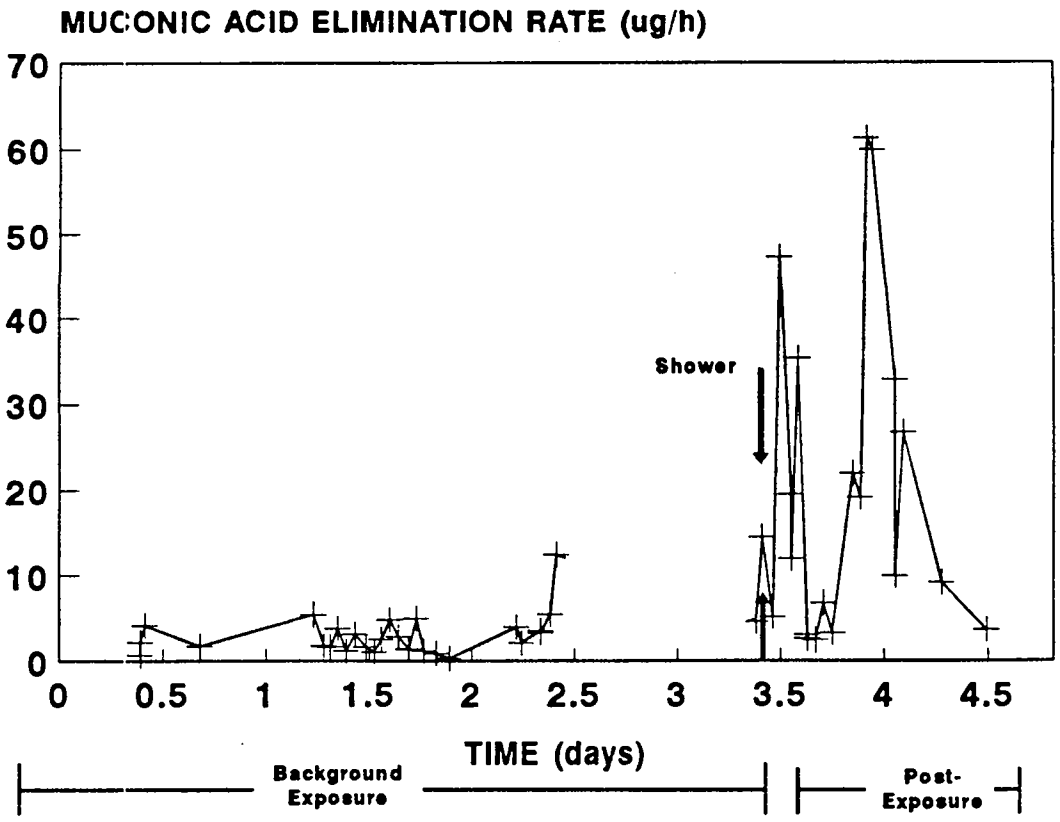

Figure 2. Time course of MA elimination during background and exposure periods.

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