Levels of Methyleugenol in a Subset of Adults in the General U.S. Population as Determined by High Resolution Mass Spectrometry

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We developed a sensitive and accurate analytical method for quantifying methyleugenol (ME) in human serum. Our method uses a simple solid-phase extraction followed by a highly specific analysis using isotope dilution gas chromatography–high resolution mass spectrometry. Our method is very accurate; its limit of detection is 3.1 pg/g and its average coefficient of variation is 14% over a 200-ppg/g range. We applied this method to measure serum ME concentrations in adults in the general U.S. population. ME was detected in 98% of our samples, with a mean ME concentration of 24 pg/g (range < 3.1–390 pg/g). Lipid adjustment of the data did not alter the distribution. Bivariate and multivariate analyses using selected demographic variables showed only marginal relationships between race/ethnicity and sex/heighting status with serum ME concentrations. Although no demographic variable was a good predictor of ME exposure or dose, our data indicate prevalent exposure of U.S. adults to ME. Detailed pharmacokinetic studies are required to determine the relationship between ME intake and serum ME concentrations. Key words: mass spectrometry, methyleugenol, reference range, serum. Environ Health Perspect 108:323–328 (2000). [Online 22 February 2000]
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Methyleugenol (ME), or 4-allyl-1,2,6-dimethoxybenzene (Figure 1A), is a compound that occurs naturally in clove oil, nutmeg, allspice, walnuts, and a variety of other spices and herbs (1). Currently, ME is approved by the U.S. Food and Drug Administration for use in foods either as a component of a natural product additive or as a food additive itself. ME is commonly used in its natural and synthetic forms as a flavoring agent in dessert foods, an attractant in insecticides, and a fragrance in perfumes and soaps (2). More than 30,000 kg ME is used per year by the food, perfume, and pesticide industries in the United States (3). Some commercial products that may contain ME include ice cream, cookies, pies, candy, soft drinks, chewing gum, gingerbread, eggnog, patés, ketchup, chutney, apple butter, cigarettes, potpourri, perfumes, and insecticides. As a flavorant and fragrance, ME is used in commercial products at concentrations ranging from 5 to 52 ppm and 0.002 to 0.3%, respectively (2). It has been estimated that the average human consumes approximately 6 μg ME/day (1).

Because of the structural similarity of ME to other carcinogenic allylbenzene flavorants such as saffrole and estragole (Figure 1B and C, respectively), attention has been focused on the carcinogenic potential of ME. Miller et al. (1) dosed mice with 4.75 μmol (846 μg) ME administered by intraperitoneal injections from 1 to 22 days after birth (1). Hepatic tumors were found in 70 and 96% of the mice sacrificed after 13 months and between 13 and 18 months, respectively. More recently, data obtained by the National Toxicology Program at the National Institute of Environmental Health Sciences (NIEHS) clearly implicated ME as a rodent carcinogen (4,5). In a 2-year study with doses given 5 days/week, liver neoplasms and other tumors were observed in rats administered ME by oral gavage (5).

Although ME toxicity has been studied in laboratory animals (1,3,4–6), little or no information is available on human exposure and possible adverse health outcomes. Both toxicologic and human exposure data are needed to make accurate risk evaluations. The National Center for Environmental Health (NCEH) at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, and the NIEHS are collaborating to acquire these data. NCEH investigators are characterizing human exposure to a variety of environmental chemicals by measuring internal doses (the concentration of that chemical, its primary metabolite, or reaction product in a human specimen) in the general population to determine the so-called reference range for a chemical in a population (7). Such ranges supply information about the prevalence of exposure to selected chemicals and the background concentration range found in humans. These ranges also serve as a basis for trend studies, which are designed to determine whether human exposure to a given chemical is increasing or decreasing over a given time period. For toxicologic purposes, these reference ranges can help prioritize chemicals for testing. For example, chemicals found in a high proportion of the population or in high concentrations in certain segments of the population could be given a high priority for these toxicologic studies (8,9). In the case of ME, however, the toxicologic studies were performed first, and they indicated significant carcinogenic activity (5). Efforts then focused on human exposure to ME and the comparative pharmacokinetics of ME in humans and rodents. To our knowledge, these are the first data reported on the levels of ME or its metabolites in the blood or urine of humans.

The literature detailing the measurement of ME or its metabolites in biologic matrices is scant. In support of NIEHS toxicokinetic studies that identified ME as a multisite rodent carcinogen, Graves and Runyon (3) developed a method for measuring ME in denatured rat plasma using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The range of quantification of the method was 50 μg/L to 10 mg/L, with coefficients of variation (CVs) ranging from 0.5% at the high calibration end to 12.5% at the low calibration end. Fischer and Dengler (10) reported a more sensitive HPLC–UV method for the analysis of a similar compound, eugenol, in bile, urine, and serum after a hexane or C18 solid phase extraction (SPE). This method had limits of detection (LODs) of 2, 10, and 10 μg/L in urine, serum, and bile, respectively, and CVs of...
of < 4%. Although both of these methods may have adequate sensitivity for measuring ME concentrations in biologic media from dosed animals, they lack both the sensitivity and selectivity for measuring trace levels of ME in biologic samples that result from everyday human exposures.

We developed a sensitive and accurate method for quantifying ME in human serum. Our method uses a simple solid-phase extraction followed by a highly specific analysis using isotope dilution gas chromatography-high resolution mass spectrometry (GC-HRMS). As a part of a CDC/NIEHS collaborative effort (including detailed pharmacokinetic studies), we applied this method to measure serum concentrations of ME in the general U.S. population.

Materials and Methods

Materials. We obtained ME and 3',4'- (methyleneedioxy)-acetophenone (MDA) (Figure 1D), the recovery standard, from Aldrich Chemical Co. (Milwaukee, WI). ME and MDA had purities of 99 and 98%, respectively. Allyl-13C3 methyleugenol (13C3-ME) of 99% isotopic purity was synthesized by Cambridge Isotope Laboratories (Andover, MA). We purchased formic acid (98%) and anhydrous sodium sulfate from EM Industries (Gibbstown, NJ) and Mallinkrodt Chemical Co. (Paris, KY), respectively. All solvents were analytical grade and were purchased from Burdick and Jackson (Muskegon, MI). We used all of the chemicals and solvents without further purification. All reagents were made daily with bioanalytical grade water, which we prepared in-house using a 0.2-μm water filtration system (Millipore, Bedford, MA) and an Organic-Pure ultraviolet light treatment reservoir (Barnstead, Newton, MA).

Analytical standards. Individual stock solutions were prepared by dissolving 5-mg amounts of ME, 13C3-ME, and MDA (Figure 1D) in 100 mL toluene. We created seven standard sets (0.2, 0.4, 2, 4, 10, 40, and 100 pg/mL) to encompass the entire linear range of the method. The ME concentration in each standard set was varied, but the 13C3-ME and MDA concentrations were kept constant at 40 pg/mL. The standard sets were divided into aliquots and stored at -20°C until used.

Internal standard. We prepared an internal standard spiking solution by diluting the stock 13C3-ME solution with acetone to a concentration of 20 pg/mL. The concentration was set to obtain the most accurate analysis possible while maintaining a repeatable analytical signal.

Recovery standard and diluent. We made a recovery standard/diluent solution by diluting the MDA stock standard with toluene to a concentration of 40 pg/mL. This standard, which we added as the final step during sample preparation, had a dual purpose. The toluene served as a keeper to prevent the extract from completely evaporating. The MDA was the standard against which 13C3-ME recovery in individual samples was determined.

Quality control materials. We prepared quality control (QC) materials from residual sera from multiple donors. We purchased the sera from the local Red Cross. Sera were combined and well mixed. Particles > 0.2 μm were filtered from the pooled serum using a sterile filtration apparatus. We split the filtered serum into three equal volume pools. One pool was not enriched, and therefore reflected the native or endogenous concentration of ME in the serum. The other two pools were enriched with different levels of ME. Thus, we obtained QC pools with native low (~100 pg/g) and high (~250 pg/g) ME concentrations. After enrichment, all pools were mixed for 24 hr under refrigeration. We dispensed serum from each pool into vials in 4-mL aliquots. The vials were capped, labeled, and stored at -20°C until their use. We determined the mean concentration and the analytical variance by the repeat measurement of at least 20 samples in different analytical runs for each QC pool. A QC run was unacceptable if either the QC sample result for the current run was outside the upper or the lower 99% control limit, or the QC sample results for the current and most recent previous run were both outside the same upper or lower 95% control limit.

Reagent blanks. Because virtually all serum samples that we tested had detectable levels of ME, reagent blanks consisted of 4 mL freshly prepared water. The blank contained the same water used in the daily preparation of reagents. We prepared the reagent blanks in the same manner as the unknown samples.

Sample preparation. We prepared unknown serum samples, QC materials, and reagent blanks identically. All sera, reagents, and standards were brought to room temperature. We weighed a 4-g aliquot of serum into a test tube. The serum was spiked with 400 pg 13C3-ME as an internal standard, mixed, and allowed to equilibrate for approximately 5 min. The serum proteins were denatured with 4 mL 50% formic acid. We passed the denatured serum through a preconditioned Empore C18 SPE column (3M, Harbor City, CA) and then discarded it. The SPE column was washed with 2 mL purified water and eluted with 5 mL methylene chloride. The eluate was passed through a 500-μg silica gel SPE column topped with approximately 1 g anhydrous sodium sulfate and then collected. We rinsed the column with 2 mL methylene chloride and collected and combined the rinse with the sample. A TurboVap evaporator (Zymark Corporation, Hopkinton, MA) set at 37°C and 15 psi head pressure of nitrogen concentrated the extract to approximately 300 μL. We transferred the concentrate to a 1-mL conical vial. We added a 10-μL aliquot of the recovery standard/diluent to the vial and then allowed the sample to evaporate to approximately 10 μL at ambient temperature. We capped the vial and stored it under refrigeration until analysis.

Instrumental analysis. We analyzed 2 μL of the concentrated extract using splitless injection GC-HRMS. We performed the analyses using an HP 5890 or HP 6890 gas chromatograph (GC; Hewlett Packard Co., Wilmington, DE) interfaced with a VG250/70S or a VG70SE mass spectrometer (MS; Micromass, Manchester, UK) with Opus operating software (version 3.5, Micromass) and equipped with a low-energy (30 eV) electron impact ionization source. We achieved separation on a 30-m J & W DB-5MS [5% (phenyl)-methyl polysiloxane, 0.25-μm film thickness, 0.25-mm id] capillary column (J&W Scientific, Folsom, CA). We used helium with a linear velocity of 35 cm/sec as the carrier gas. The injector and transfer line temperatures were 260°C. The initial column temperature, 80°C, was held for 1 min, increased to 122°C at 3°C/min, then increased to 272°C at 30°C/min, and held for 1 min. We operated the MS in single ion monitoring (SIM) mode with an initial accelerating voltage of 7,000 and a 10,000 resolution, as defined at 10% valley. We used perfluorokerosene (PFK) ions as lock masses.

We monitored two ions for ME: one for quantifying and one for confirming the presence of ME. One ion each was monitored for 13C3-ME and MDA. Table 1 shows the ions monitored in each channel, the channel times, and the interchannel delay times. We recorded the appropriate

![Figure 1](https://example.com/figure1.png)

Figure 1. The chemical structures of (A) ME, (B) safrole, (C) estragole, and (D) methylenedioxyacetophenone. *Carbon atoms that were labeled with 13C for the ME internal standard.)
of analysis specifications in an acquisition program initiated immediately after the injection of the sample into the GC. The total analysis time per sample was approximately 20 min.

**Data processing and analysis.** Data were automatically processed using OpusQuan software (version 6.1; Micromass), which was supplied with the mass spectrometers. The detection threshold and baseline were both set at 0% in OpusQuan; the peak differential was 3, and the minimum peak width was 1. In addition, the background signal was subtracted and all data were smoothed. The retention times and areas were automatically entered into an R:BASE database (Micromass, Redmond, WA) and the ratios of the quantification and confirmation ions were calculated. Because of the specificity of HRMS, interferences were rare; however, any interferences that occurred were easily recognized because of a dramatic shift in the ratio of the areas of the quantification and confirmation ions. In these instances, the data were deemed unacceptable and the analysis was repeated.

**Quantification.** We constructed calibration curves with seven ME concentrations plotted against the response factors. We calculated the response factors as the area of the ME quantification ion divided by the area of the 13C3-ME ion. At least five repeat determinations were performed for each concentration on the calibration curve.

 Calibration standard concentrations encompassed the entire linear range of the analysis. The lowest standard concentrations were at or below the LOD to ensure linearity and accuracy at the low concentration end. A linear regression analysis of the calibration plot provided a slope and intercept from which unknown sample concentrations could be determined. The intercept was not statistically different from zero.

**Method validation.** We calculated the analytical LOD for the method as 3σ, where σ was the average signal in the blanks. For the instrument LOD, we estimated σ as the y-intercept of a linear regression analysis of a plot of the absolute standard deviation versus the concentration (I). To evaluate ME recovery, we used 20 serum samples whose endogenous ME concentrations were well characterized. Before extraction, we spiked four samples with ME to a final concentration of 20 pg/g and four to 100 pg/g; four were not spiked. The samples were extracted as previously described. Control samples were extracts of the unspiked serum spiked after extraction with ME to final concentrations of 20 and 100 pg/g. The extracts of all samples were spiked with the internal standard to correct for instrumental variation during analysis. We determined ME recovery at each concentration by comparing the spiked samples to the control samples. Additionally, we determined the recovery of 13C3-ME of each individual sample by referencing the area of the 13C3-ME ion to the area of the MDA ion.

We determined the method accuracy by enriching serum samples with a known amount of ME, preparing and analyzing the samples, and then comparing the calculated and the expected ME concentrations. We performed a linear regression analysis on a plot of the calculated concentration versus the expected concentration. With this analysis, a slope of 1.0 would be indicative of 100% accuracy.

**Reference range determination.** Using this method, we determined the range of ME in a subset of serum samples collected from human adults who participated in the Third National Health and Nutrition Examination Survey (NHANES III). NHANES III was conducted between 1988 and 1994 by the National Center for Health Statistics (NCHS/CDC). All protocols were reviewed and approved by a human subjects review committee and complied with all national and institutional guidelines for the protection of human subjects. NHANES III was designed to accurately represent the U.S. civilian noninstitutionalized population; however, the serum specimens used in our study were a convenience sample of the residual NHANES III specimens and were not necessarily representative of the U.S. population. However, the samples analyzed in our study were from adults who represented a diverse spectrum of sex, age, race and ethnicity, urban and rural residences, and geographic location variables. We obtained questionnaire data from each participant. These data encompassed a variety of topics ranging from dietary intake to health status. The questionnaire data that were considered potentially important factors affecting serum ME concentrations were used in the statistical analysis and interpretation of the serum ME data. All ME data were log-transformed before analysis using univariate, bivariate, and multivariate procedures. We considered data statistically significant when p < 0.05. All data analyses were performed using SAS statistical software (SAS Institute, Cary, NC).

**Results and Discussion**

The lack of ionizable functional groups on the allylbenzene carbon skeleton of ME (Figure 1A) facilitated its simple and efficient extraction from the serum matrix using a C18 SPE sorbent. We added a second SPE column in the extraction procedure to further clean the sample. This silica gel cleanup of the serum extract removed coextracted compounds with polar functional groups and also removed residual water. In repeated recovery experiments at three ME concentrations, the total recovery of ME from serum was essentially quantitative. In addition, the recovery of 13C3-ME, which was determined independently for each sample, was consistently > 90%.

The addition of an isotopically labeled standard (13C3-ME) before sample manipulation, a technique known as isotope dilution (12), afforded us many advantages. Chemically, 13C3-ME behaves almost identically to ME, but they are distinguishable based on the 3 atomic-mass-unit (amu) difference in their masses and respective fragment ions (Figure 2). For this reason, the ratio between their ions can internally correct

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**Table 1. Characteristics of analysis by GC-HRMS.**

| Analyte | Ion Mass | Assignment | Channel time (msec) | Interchannel delay time (msec) | Ion use     |
|---------|---------|------------|---------------------|-------------------------------|-------------|
| ME      | 178.0994 | [M]        | 160                 | 20                            | Quantification |
| ME      | 183.0759 | [M-CH3]    | 160                 | 20                            | Confirmation |
| PFK     | 168.9889 |             | 50                  | 20                            | Lock mass    |
| ME-13C3 | 181.1094 | [M]        | 160                 | 20                            | Quantification |
| MDA     | 164.0473 | [M]        | 160                 | 20                            | Recovery     |

**Figure 2.** Electron impact mass spectra of (A) ME and (B) 13C3-ME with important mass assignments. M and L refer to the molecular ion of the native and labeled ME, respectively. The fragmentation of the two compounds is nearly identical with only a 3-amu difference in the predominant ions. Only fragments with masses > 100 amu were monitored. Smaller fragments (< 100 amu) were in a region with a higher background signal, therefore increasing the potential for interferences.
for recovery of ME in each individual sample, which eliminates the need for recovery surrogates, although a surrogate can still be used for validation purposes. The automatic recovery correction reduces the error associated with the measurement and ultimately increases the method sensitivity.

During GC–HRMS analysis of ME with low energy (30 eV) electron impact ionization (EI), the predominant ions were [M]+, [M–CH3]+, and [M–OCH3]+ at mass to charge ratio (m/z) of 178, 163, and 147, respectively (Figure 2). As expected, higher energy (70 eV) EI produced more fragmentation, especially lower molecular weight fragment ions, and chemical ionization using methane as a reagent gas produced only one ion at m/z 179, [M + H]+. Because the controlled fragmentation at 30 eV resulted in higher molecular weight ions in a region with fewer background ions and ions of higher intensity, we opted to use low energy EI for the analysis. Under these conditions, the 13C3-ME formed similar ions as ME in the same relative abundances except that the m/z of the ions were 3 amu greater (Figure 2). This confirmed that the fragment losses were not from the allyl group that was labeled with 13C atoms. We used the MDA as a recovery standard because of its structural similarity to ME and because its predominant ions were within a few atomic mass units of ME ions, as is necessary for high-resolution SIM analysis. MDA eluted from the gas chromatography column approximately 2 min after ME, thus allowing their ions to be monitored in separate windows of time (Figure 3).

The instrument LOD was 282 fg on-column. With the method recovery, this translates to approximately 350 fg/g serum (parts per quadrillion); however, ME was endogenous in the air and water. Using a solvent trap on the vacuum system or pulling excess air through the SPE columns caused a low-level ME contamination of approximately 5 pg/g (parts per trillion). By removing the solvent trap and carefully monitoring the volume of air pulled through the columns, we were able to reduce the contamination to approximately 1 pg/g or lower. Additionally, we used in-house purified water in reagent preparation because bottled water and distilled water contained higher endogenous ME levels. As a result, the method LOD was 3.1 pg/g. Thereafter, an occasional contaminant appeared, but it was readily apparent in the quality control samples.

Figure 4 shows a calibration curve. The ME analysis was linear over 3 orders of magnitude; \( r^2 = 0.997 \). We obtained similar calibration curves on multiple high-resolution instruments.

The method’s accuracy was essentially 100%. A linear regression analysis of a plot of the calculated concentrations of spiked samples versus the expected concentrations of the same samples (Figure 5) yielded a slope of 0.997, which is indicative of a high degree of accuracy. We used a similar plot to compare data from multiple instruments; the plot yielded a slope of 0.996, signifying good agreement among instruments.

A typical quality control Shewart plot is shown in Figure 6. This plot includes samples analyzed on multiple instruments and reflects both intra- and interday variation. The overall CV and the intra- and interday variations at three concentrations over the linear range of the method are shown in Table 2. As expected, the variation among days was a greater contributor to the overall CV than the variation within days. Additionally, the variation was greater as the concentration approached the method LOD; although this is a normal occurrence, the low-level contamination of ME probably resulted in increased variation at the low concentration end.

Overall, the data from the QC materials proved that ME was stable in serum over the testing period of approximately 2 months. We did not conduct stability studies over longer periods of time. However, our data indicate that NHANES III samples had ME concentrations comparable to freshly collected serum from volunteers. These data suggest that ME is stable in frozen serum stored for up to 5 years.

Our method is more sensitive and more selective than the only published method (3); for example, the LOD of our method is > 4 orders of magnitude lower than the

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**Figure 3.** Ion chromatograms of a 0.2-pg/mL standard show the clean separation of the components. The (A) quantification, (B) confirmation, and (D) internal standard ions of ME. (D) The MDA recovery standard.

**Figure 4.** (A) A calibration curve for ME over a 100-pg range. \( r^2 = 0.997 \). (B) The low concentration end demonstrates the linearity even near the LOD.
LOD of the HPLC-UV method. Some of the sensitivity differences between the two methods can be attributed to the decreased sample size for the HPLC-UV method. The Graves and Runyon (5) method uses only 200 μL plasma, whereas our method requires 4 g serum (the approximate amount of serum from one 10-ml blood draw). Because our method uses HRMS, it provides a greater selectivity than the HPLC-UV method; this selectivity probably accounts for a large portion of the increase in method sensitivity. In addition, our sample preparation provides a much cleaner extract, which can help minimize interfering serum components. The CVs of our method at very low serum concentrations (Table 2) are comparable to the CVs (12–12.5%) of the HPLC-UV method at 50 mg/L plasma (5). At higher plasma concentrations, the HPLC-UV method reports CVs ranging from 1–5%.

The specificity of HRMS at 10,000 resolution was required to eliminate interfering components in the human serum extracts that in turn provided the low detection limits of the method. Analysis at lower resolutions resulted in recurring interferences, as evidenced by significant changes in the ratios of the quantification and confirmation ions. These specificity requirements precluded the use of single quadrupole or other low-resolution mass spectrometers. However, we did evaluate the effectiveness of GC–MS (SIM mode) and GC–tandem mass spectrometry (MS/MS) for ME analysis using a quadrupole ion trap with external ionization (GCQ; Finnigan MAT, San Jose, CA). The instrument LODs in the SIM and MS/MS modes were 50 and 3.4 pg, on-column, respectively. Although the SIM mode did not provide the sensitivity required for ME analysis in serum, the MS/MS LOD was adequate. The calculated concentrations of GC materials analyzed using both the GC–HRMS and GC–MS/MS analyses are shown in Table 3. The GC–HRMS clearly provided more accurate and precise data.

Table 4 shows a summary of the serum ME concentrations (whole-weight basis) in 206 adult participants of NHANES III. The distribution of the data (Figure 7) was not altered when the individual ME concentrations were adjusted for total lipid content in each serum sample. The frequency of ME detection was 98%, which verified that our analytical method had adequate sensitivity to detect incidental exposure to ME and that the population as a whole is exposed to ME to some extent. Only four individuals had ME concentrations below the LOD (< 3.1 pg/g). Five individuals had serum ME levels ≥ 100 pg/g, which is approximately 2 times the average peak serum ME concentrations observed in fasting adults fed a meal containing approximately 60 μg ME (4).

Bivariate and multivariate analyses of the data using the demographic information were performed (9). The demographic data were provided by the NHANES III project. The data were analyzed using regression analysis, following the steps outlined by Draper and Smith (6). The results showed that ME concentrations were significantly associated with age, sex, smoking status, and weight. Additionally, ME concentrations were significantly associated with the consumption of certain types of food and beverages, such as chocolate, coffee, and tea. The results also showed that ME concentrations were significantly associated with the consumption of certain types of food and beverages, such as chocolate, coffee, and tea. The results also showed that ME concentrations were significantly associated with the consumption of certain types of food and beverages, such as chocolate, coffee, and tea. The results also showed that ME concentrations were significantly associated with the consumption of certain types of food and beverages, such as chocolate, coffee, and tea.
Table 5. Geometric mean (GM) concentration of serum ME in selected demographic categories.

| ME concentrations (pg/g) | GM   | 25th | 50th | 75th | Max |
|--------------------------|------|------|------|------|-----|
| Male All                  | 64   | 17   | 3.9  | 12   | 16  |
| Fasting ≤ 9 hr           | 23   | 19   | 4.3  | 13   | 17  |
| Fasting > 9 hr           | 41   | 16   | 3.7  | 11   | 15  | 24  |
| Female All               | 142  | 16   | 2.2  | 10^b | 17  |
| Fasting ≤ 9 hr           | 56   | 14   | 2.2  | 10^b | 16  |
| Fasting > 9 hr           | 86   | 18   | 2.2  | 10^b | 18  |

Abbreviations: max, maximum; min, minimum. *Total number in demographic group. **Zero is a significant digit.

The ME exposure data in adults of the general U.S. population provide invaluable information. Considering that in male and female rats, oral ME doses of 75–150 mg/kg resulted in peak average plasma ME concentrations ranging from 1.5 to 8.2 mg/L (1.5–8.2 ppm) and that these same dose levels clearly induced malignant lesions in rats and mice on a chronic daily basis (5), the human serum ME concentrations observed in our study population suggest that more extensive risk evaluation is needed. Assuming that humans respond similarly to rodents, these human ME data coupled with hazard identification data (i.e., Does a chemical represent a potential health hazard?), comparative human and rodent pharmacokinetic data, and rodent dose–response data should provide adequate information to characterize human risk of cancer as well as noncancer health effects resulting from ME exposure.

It is important to emphasize that these human data are from adults only. Considering the potential sources of ME exposure and children's small size, it is likely that children would have higher concentrations of ME. Exposure and risk assessment in children is an extremely important area in which to focus future ME studies.

Conclusions

We developed a highly specific, accurate, and sensitive method for the measurement of ME in human serum using isotope dilution GC–HRMS. We applied this method to analyze ME concentrations in the serum of adult participants in NHANES III. The high frequency of ME detection in this population verified that our method possessed adequate sensitivity for ME analysis in the general population. The data also indicated that exposure to ME in the U.S. population is prevalent. Because no potential determinants of exposure were significantly correlated with ME concentrations, we surmise that the total ME exposure is from a variety of sources. Substantial serum concentrations of ME in fasting adults, coupled with ubiquitous low levels of ME in air and water, suggest that ME exposure results from foods where ME is not intentionally added as well as from nonfood sources. These human data coupled with hazard identification and rodent dose–response data provide the necessary information for proper human risk assessment for ME.

REFERENCES AND NOTES

1. Miller EC, Swanson AB, Phillips DH, Fletcher TL, Lien A, Miller JA. Structure-activity studies of the carcinogenesis in the mouse and rat of some naturally occurring and synthetic alkylbenzene derivatives related to safrole and estragole. Cancer Res 43:1124–1134 (1983).
2. Burdock GA, ed. Fenaroli's Handbook of Flavor Ingredients. Boca Raton, FL: CRC Press, 1995.
3. Graves SW, Runyon S. Determination of methyleugenol in rodent plasma by high-performance liquid chromatography. J Chromatogr B 663:255–262 (1995).
4. Centers for Disease Control and Prevention and the National Institute of Environmental Health Sciences. Unpublished results.
5. NTP. Toxicology and Carcinogenesis Studies of Methyleugenol (CAS No. 93-15-12) in F344/N Rats and B6C3F1 Mice (Gavage Studies). Peer Review Draft. TR-491. Research Triangle Park, NC: National Toxicology Program, 1998.
6. Gardner I, Bergin P, Stening P, Kenna JD, Caldwell J. Immunohistochemical detection of covalently modified protein adducts in livers of rats treated with methyleugenol. Chem Res Toxicol 9(4):713–721 (1996).
7. Needham LL, Patterson DG Jr, Burse WW, Paschal DC, Turner WE, Hill RW Jr. Reference range data for assessing exposure to selected environmental toxicants. Toxicol Ind Health 12:507–513 (1996).
8. Lucier GW, Schecter A. Human exposure assessment and the National Toxicology Program. Environ Health Perspect 106:623–627 (1998).
9. Lucier GW, Needham LL. NIEHS, CDC collaborate to improve exposure assessment. Environ Health Lett 37:127–128 (1998).
10. Fischer IU, Dengler HJ. Sensitive high-performance liquid chromatographic assay for the determination of eugenol in body fluids. J Chromatogr B 525:369–377 (1990).
11. Taylor JK. Quality Assurance of Chemical Measurements. Boca Raton, FL: CRC Press, 1987.
12. Colby BN, McCaman MW. A comparison of calculation procedures for isotope dilution determinations using gas chromatography mass spectrometry. Biomed Mass Spectrom 6(6):225–230 (1979).