ABSTRACT

CATHERINE CHERYL BODUROW. A Modified Method for Determination of Free Styrene Glycol in Human Blood. (Under the Direction of STEPHEN M. RAPPAPORT, Ph.D.)

Partitioning of free styrene glycol in plasma and packed red blood cells was investigated in this study. Allyl benzene glycol was synthesized and used as an internal standard in the procedure. It was determined there was no significant difference in the styrene glycol concentrations in the plasma and red blood cells layers (ß=0.08, SE=1.01) of dosed blood samples. This result suggested that free styrene glycol distributes uniformly throughout whole blood. The plasma layer was chosen for analysis in this study and free styrene glycol concentrations were determined in 85 plasma samples using the developed methodology of this study. Samples and exposure data were obtained from three reinforced plastic industry companies in the state of Washington. The exposure data investigated were: eight-hour air styrene concentrations, whole blood styrene concentrations and urinary metabolite (sum of mandelic and phenylglyoxylic acids) concentrations. Companies # 1, 2 and 3 afforded the following number of plasma samples, respectively: 31, 17 and 17. Fourteen and six replicate measurements and sets of exposure data were obtained for Companies # 1 and 2, respectively, approximately one year after initial monitoring. Statistical analysis regressing exposure data and plasma styrene glycol concentrations against exposure data for single and replicate observations are presented in this work. Our findings vary depending upon the company sampled. Company # 3 produced statistically significant relationships in every regression. Companies # 1 and 2 did not.

ACKNOWLEDGEMENTS

This work is dedicated to Mathukutty whose love and devotion have unselfishly encouraged my graduate studies. I thank the Lord each day for bringing him into my life.

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ABG	allyl benzene glycol
ABG-PFB	pentafluorobenzoyl ester of allyl benzene glycol
ANOVA	Analysis of Variance
conc	concentration
ECD	electron capture detector
GC	gas chromatograph
marker	biological marker
MS	mass spectrometer
PFB	pentafluorobenzoyl chloride
SG	styrene glycol
SG-PFB	pentafluorobenzoyl ester of styrene glycol

I. INTRODUCTION

1. Biological Monitoring

Occupational hygienists have traditionally monitored air concentrations to evaluate human exposure to chemicals in the workplace. The growing necessity, however, to quantify a worker's actual body burden has enabled hygienists to make use of the emerging biological monitoring field (Fiserova-Bergerova, 1990). A basic definition of biological monitoring is the measurement of an internal exposure through the analysis of a biological specimen (Zielhuis, 1978). Wogan (1989) defines biological monitoring as measurements made on cells, tissues, or body fluids of exposed people with the intent to determine an internal or biologically effective dose on an individual basis. Several media suitable for biological monitoring are represented in Figure 1. The most common media are exhaled air, blood and urine (Hulka, 1990 and Bernard, 1986).

There are several advantages to the use of biological monitoring as a supplement to environmental monitoring. Biological monitoring can more adequately determine susceptible groups of individuals than environmental monitoring. Factors such as age, sex, body fat, intake of drugs and alcohol, and socioeconomic status will affect an individual's uptake and metabolism of a chemical (Guidotti, 1988). Secondly, it may be easier to estimate external exposure by utilizing the individual as the sampling instrument. For example, if the biological half-life of the chemical is longer than six hours, biological monitoring may be more cost effective and more precise than environmental monitoring (Zielhuis, 1978). Another point is that physical work load, work

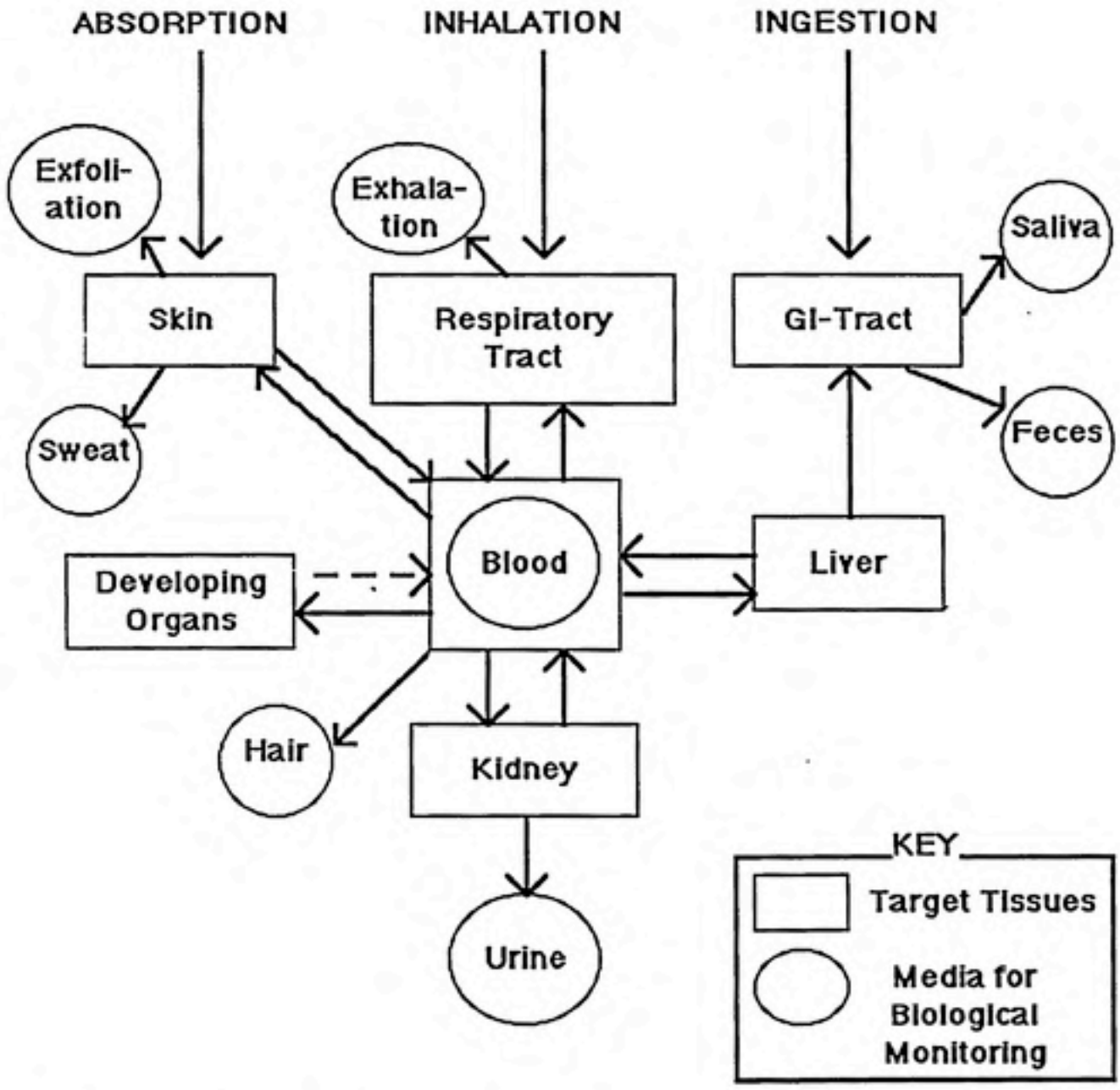

Figure 1. Sites of uptake, target tissue, and media for monitoring of xenobiotic compounds (Taken from Hulka, 1990).

practices and respiratory minute volume are not considered by environmental monitoring. These factors contribute to a worker's body burden. It should also be recognized that people are exposed to many chemicals simultaneously through different pathways. Biological monitoring allows investigation of total body burden which may aid in health risk assessment (Committee on Biological Markers of the National Research Council, 1987). Finally, and perhaps most importantly, biological monitoring offers the opportunity to consider individual variability among those exposed (Hattis, 1987).

Although several advantages in favor of biological monitoring exist, the method also possesses its disadvantages. People are the sampling instrument in biological monitoring. This may be inconvenient for some individuals and cause others unneeded anxiety. Although this field is rapidly developing, it is a fact that many biological monitoring procedures for chemicals and/or their metabolites do not yet exist. It is also important to consider that investigators are not yet capable of producing reliable data on an individual monitoring basis. Finally, biological monitoring may not be the appropriate method of evaluation when external exposures vary greatly with time and the chemical of interest exerts an acute effect on the individual (Zielhuis, 1978).

Griffith (1989) defines a biological marker as "any measurable biochemical, physiological, cytological, morphological, or other biological parameter obtainable from human tissues, fluids, or expired gases, that is associated (directly or indirectly) with exposure to an environmental pollutant." The process of choosing a biological marker (hereafter referred to as "marker") must be undertaken with great care. When chemicals undergo metabolic changes in the body before excretion, a marker of the active metabolite may yield a more appropriate estimate of the internal dose. In instances of endogenously produced chemicals, baseline levels of these chemicals must be considered

before a marker is chosen (Schulte, 1987). This will determine the validity of the use of a marker at low exposure levels. Adducts of endogenous macromolecules, such as DNA or proteins, may also indicate if damage and/or repair have occurred due to chemical exposure (Perera, 1987). Another form of markers of exposure or effect are cellular changes (Hulka, 1990). Cellular changes may in fact be a link to carcinogenesis found in individuals exposed to certain chemicals (Garner, 1985). Finally, the amount of time before a marker appears and the persistance of the marker are important considerations. The half-lives of chemicals may vary dramatically in different "compartments" of the body. Many decisions in biological monitoring are indeed based upon biological half-lives of chemicals and markers (Droz, 1989 and Monster, 1991). Regardless of the choice of marker, Schulte (1987, 1989 and 1991) emphasizes the importance of validation studies, including determinations of sensitivity, specificity, and predictive value, before a marker is used in an epidemiologic study.

Biological monitoring is not a new concept. Hints of biological monitoring date back to the early 20th century when methods were developed more actively in Europe than in the United States (Lowry, 1986). The past two decades have yielded many advances worldwide. Of course, the appropriate use of biological monitoring is as a complement to environmental monitoring and rigorous epidemiologic designs (Brown, 1989). The field will continue to grow and may act as the bridge between laboratory and epidemiologic assessment studies in the century to come (Vainio, 1985).

2. Styrene Exposure in the Reinforced Plastic Industry

Chemical and Engineering News (1992) recently reported that styrene monomer production has reached an all-time annual high of 9 billion lbs in the United States . It also reported that combined quantities of polystyrene, styrene-acrylonitrile and acrylonitrile-butadiene-styrene thermoplastic resins amounted to approximately 7.3 billion lbs in 1991 production.

Although the reinforced plastic industry consumes less than ten percent of the world's styrene production, the industry experiences the greatest exposures to styrene. The primary raw material used in the reinforced plastic industry is unsaturated polyester resin dissolved in styrene. The fabrication process adds organic peroxides to the resins to initiate polymerization between the unsaturated polyester resin and styrene. This produces the hard polymer which is essential for many durable items, such as boats and automobiles (Tossavainen, 1978).

In the reinforced plastic industry, styrene primarily enters the body through the respiratory system (Malek, 1986). Dermal exposure may be avoided by strict use of protective clothing and gloves. Brooks (1980) showed that skin protection reduced the risk of dermatitis from styrene. In addition, Brooks also found that "percutaneous absorption of styrene was not significant and indeed did not significantly contribute to the body burden of styrene of workers engaged in hand lay-up operations." Berode (1985) and Wieczorek (1985) reported similar results in studies of percutaneous absorption of styrene. It is for this reason that styrene exposure is normally assessed by measuring air concentrations.

Mortality studies performed by Okun (1985) and Wong (1990) of styrene-exposed workers in the reinforced plastics industry did not yield a significant excess of cause- specific mortality for the investigated cohorts.

3. The Metabolism of Styrene

The uptake of styrene vapor from the lungs has been investigated and is reported to be 63% of the inspired quantity (Engström, 1978). Ramsey (1978) found approximately 97% of the absorbed styrene was cleared from the body by metabolism, and Teramoto (1979) showed styrene's biological half-life in the human body to be 40 minutes in the rapid phase and 180 minutes in the slow phase.

The metabolism of styrene has been extensively studied and is summarized in Figure 2 (Löf, 1983). The first step of the proposed metabolic pathway is the formation of styrene-7,8-oxide (phenyloxirane) from catalysis by microsomal cytochrome P-450 (Watabe, 1978). The epoxidation of styrene may also be catalyzed by blood erythrocytes and lymphocytes (Norppa, 1983). Styrene-7,8-oxide may then spontaneously hydrate to styrene glycol (1-phenyl-1,2-ethanediol) or be converted by microsomal epoxide hydratase (Dansette, 1978). Next, styrene glycol may either be conjugated with ß-glucuronic acid (Pantarotto, 1978) or oxidized to mandelic acid which can be further oxidized to phenylglyoxylic acid (Bardodej, 1966). Mandelic acid may also oxidize to benzoic acid which has been shown to conjugate with glycine (Leibman, 1975).

Another metabolic pathway primarily found in animals acts as a minor pathway in humans. Styrene-7,8-oxide conjugates with glutathione in the presence of glutathione S-transferase (Boyland, 1965). These conjugates will generally degrade to mercapturic acids (James, 1967).

A minor amount of ring hydroxylation may occur at the 1,2- or 3,4-position of styrene and small quantities of phenyl ethanols and phenylacetaldehyde are also produced (Pantarotto, 1978). Several other metabolites have been identified in rat urine: phenaceturic acid (Delbressine, 1980) which most likely forms from

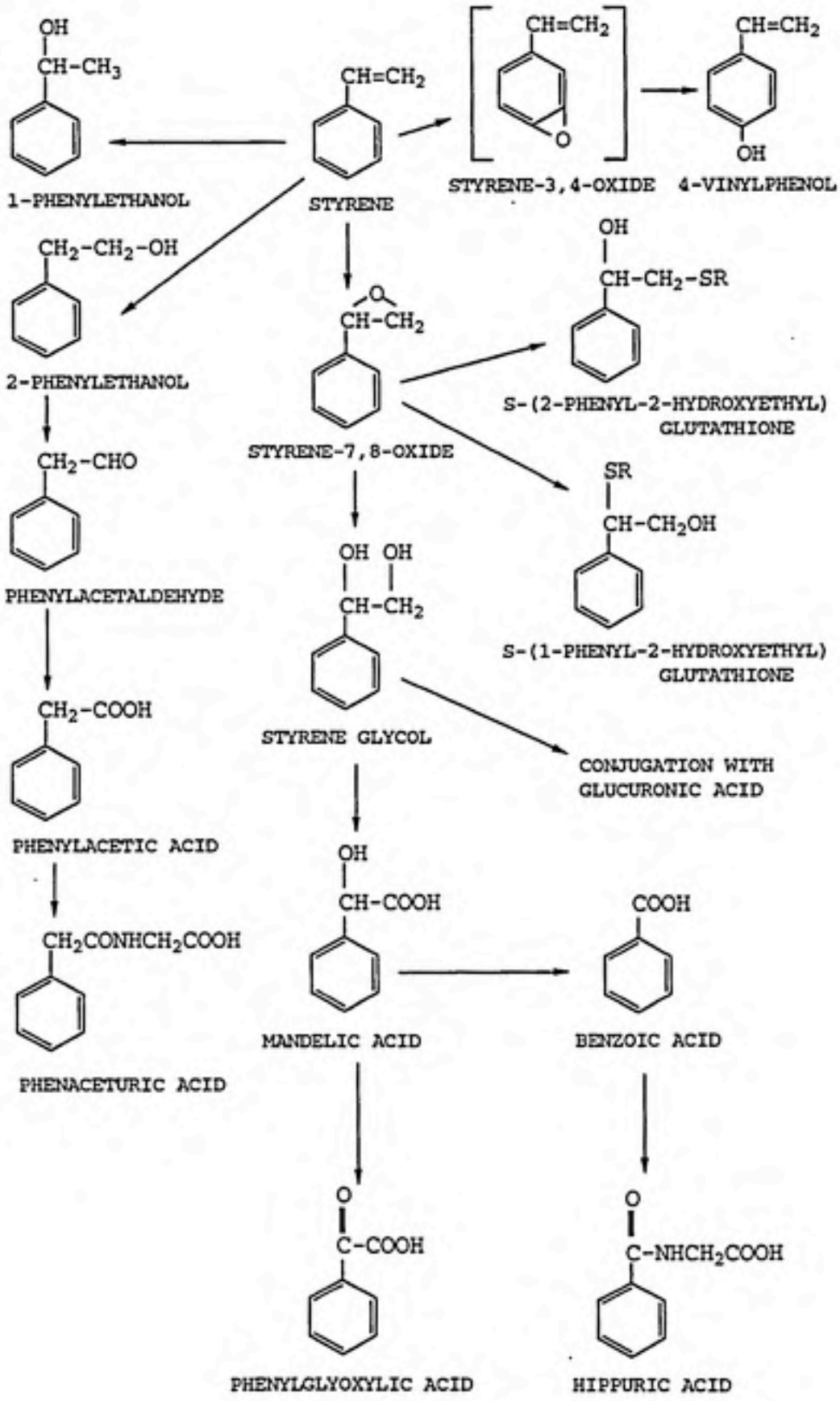

Figure 2. Proposed Metabolic Pathways of Styrene (Taken from Löf, 1986).

2-phenylethanol, p-hydroxymandelic acid, p-hydroxybenzoic acid, and p-hydroxyhippuric acid (Pantarotto, 1978).

Although approximately 85% of absorbed styrene is eliminated as mandelic acid and 10% as phenylglyoxylic acid in the urine (Guillemin, 1979), a small quantity of unchanged styrene may also be excreted in the urine (Dolara, 1984). In addition, Engström (1978) reported that the remainder of absorbed styrene, approximately 2%, was exhaled unchanged.

4. Purpose of Study

It is clear that several media--exhaled air, blood and urine--may be used in the biological monitoring of styrene-exposed workers. Löf has developed several methods for the assessment of styrene exposure in workers (1983, 1986). Of particular interest is a method for determination of free styrene glycol in whole blood which has been used in correlations with air styrene concentrations (Löf, 1986). It was our intention to streamline this procedure so that styrene glycol could be determined in human plasma or packed red blood cells rather than in whole blood.

The specific goals of this study were: a) to synthesize an appropriate internal standard for the procedure; b) to develop methodology for the determination of free styrene glycol in human plasma and packed red blood cells; c) to test the new method in a styrene-exposed population; and d) to compare plasma styrene glycol concentrations with styrene in air and blood and with urinary metabolite concentrations.

II. METHODS AND MATERIALS

1. Plasma Samples and Exposure Data

Plasma samples and exposure data were obtained from a group at the University of Washington which conducted a study of styrene exposure in the reinforced plastic industry. Blood samples and exposure data were collected at three companies in the state of Washington over a two year period. Immediately after collection, the blood samples were separated into plasma and red blood cells layers and stored at -80 ^{o}C prior to analysis. Exposure to styrene was determined by personal monitoring over the eight-hour work shift. Styrene was also measured in whole blood, and mandelic and phenylglyoxylic acid were measured in the urine.

The number of workers monitored at Companies #1, #2 and #3, respectively, were: 31, 17 and 17. Replicate measurements and blood samples were obtained from selected workers approximately one year after initial monitoring at Companies #1 and #2. Company #1 yielded fourteen replicates and Company #2 six replicates. The total number of plasma samples was 85.

2. Experimental

2.1. Materials and Instrumentation

Reagents and chemicals. All chemicals were of reagent grade and used without further purification. Formic acid, hydrogen peroxide, allyl benzene, sodium hydroxide, hexanes, pentafluorobenzoyl chloride, methanol and styrene glycol were obtained from Aldrich Chemical Company, Inc. Ethyl acetate was obtained from EM Science. Pyridine was obtained from Pierce.

Apparatus and conditions. Gas chromatography: A Varian model 3740 Gas Chromatograph equipped with ^{63}Ni electron-capture detector was employed. A J & W Scientific DB5 fused silica column (internal diameter = 0.25 mm, thickness = 0.25 µm, length = 30 meters) was used. The operating conditions were: column temperature, 240 °C; injector temperature, 250 °C; detector temperature, 320 °C; carrier gas, He at a flow rate of 1 mL/min; backflush, 0.5 minutes; length of run, 10 minutes.

Mass spectrometry: A Hewlett-Packard model 5890 Series II Gas Chromatograph / 5971A Mass Selective Detector was employed under the above operating conditions.

Nuclear Magnetic Resonance: Proton NMR spectra were obtained in deuterated methanol on a Varian XL 400 MHz NMR fitted with Varian's 6.1E Database.

2.2. Synthesis of 3-Phenyl-1,2-propanediol (Allyl benzene glycol)

Method adapted from Duverger-VanBogaert (1978)

Formic acid (96%) (30 mL, 0.80 mole) was placed in a 250 mL three-neck round bottom flask equipped with a mechanical stirrer, thermometer and dropping funnel. Hydrogen peroxide (30%) (7 mL, 0.2 mole) was then added dropwise at room temperature. Dropwise addition of allyl benzene (98%) (6.7 mL, 51 mmole) occurred thereafter. The reaction flask was next placed in an ice bath and the reaction mixture was stirred one hour. During this time, the temperature did not

exceed 35 ^{o}C . The reaction was left stirring overnight at room temperature. Excess reagent was removed by evaporation and addition of 7.5 mL saturated sodium hydroxide hydrolysed the formyl esters of allyl benzene glycol. The mixture was then extracted with 5 X 20 mL ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate and were concentrated *in vacuo* to approximately 15 mL. After cooling at 0 ^{o}C, the clear oil (5.2g) was recovered by filtration and distilled under vacuum. ^{1}H-NMR and Mass Spectrum confirmation are found in Appendix A as Figure 1 and 2, respectively.

2.3. Styrene Glycol Partitioning in Human Plasma and Red Blood Cells

Derivatization method adapated from Duverger-Van Bogaert (1978)

Thirty mL of whole blood from a volunteer in our laboratory were drawn into three ten mL heparinized vacutainers. The following procedure began within one hour of the blood drawing. Twenty whole blood samples (1mL) were placed in 4 mL capped glass vials. Ten µL of 1-Phenyl-1,2-ethanediol (styrene glycol) solution in physiological saline (0.9% NaCl, by weight) were injected into each whole blood sample, in duplicate, yielding the following levels: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 ng styrene glycol/mL blood. The samples were gently inverted several times and then placed in a warm bath at 37 ^{o}C for 2 hours. The samples were next centrifuged at 2500 rpm for 8 minutes to separate the plasma and red blood cells layers. Injection of 10 µL of a solution of 100 ng/µL 3-Phenyl-1,2-propanediol (allyl benzene glycol) in saline (0.9% NaCl, by weight) afforded 1000 ng allyl benzene glycol as an internal standard in each sample. The samples were extracted 3 times with 1mL of ethyl acetate. If emulsions occurred during the extractions, the samples were frozen in an acetone/dry ice bath, warmed to room temperature and centrifuged at 1500 rpm for 4 minutes. This procedure was repeated until separation occurred. The

combined organic layers were dried over anhydrous sodium sulfate and concentrated under nitrogen to a few drops. One mL of hexane was added to each sample. Then 2 µL pyridine and pentafluorobenzoyl chloride (1 µL, 7 µmole) were added to convert the glycols to the corresponding pentafluorobenzoyl esters. The samples were mixed and placed in a heating block at 50 °C for 20 minutes. The samples were then dried under nitrogen flow. In order to trap excess pentafluorobenzoyl chloride, 0.5 mL of an 85% solution of methanol in water was added. Then the methanol/water layer was extracted with one mL of hexane. Hexane standards containing 0, 30, 100, 300 and 1000 ng styrene glycol/mL hexane and 1000 ng allyl benzene glycol/mL hexane in each sample were derivatized as above. GC/MS confirmed the presence of the PFB derivatives of styrene glycol and allyl benzene glycol. Two µl aliquots were then injected into the GC/ECD (in triplicate). Three gas chromatograms were obtained for each sample. A typical chromatogram is shown as Figure 3 in Appendix A. The ratio of SG peak area and ABG peak area were compared to a standard curve in order to determine the concentration of SG present in 1 mL hexane.

2.4. Analysis of Styrene Glycol in Workers' Plasma Samples

Each plasma sample (0.5mL) was gradually warmed to room temperature and placed in a 4 mL capped glass vial. Injection of 10 µL of a 100 ng/µL 3-phenyl-1,2-propanediol (allyl benzene glycol) solution in physiological saline (0.9% NaCl, by weight) afforded 1000 ng allyl benzene glycol as an internal standard in each sample. The samples were gently inverted several times and then placed in a warm bath at 37 °C for 2 hours. The samples were extracted with 3 times 1mL of ethyl acetate and further processed as described in Section 2.3. GC/MS confirmed the presence of styrene glycol and allyl benzene glycol derivatives. Two µl aliquots of each sample were then injected into the GC/ECD

(in duplicate). Ten samples were analyzed per experiment. A typical gas chromatogram is shown as Figure 4 (Appendix A). The ratio of SG peak area and ABG peak area were compared to a standard curve in order to determine the concentration of SG present in 1 mL hexane.

3. Statistical Analysis

3.1. Regression Analysis for Partitioning Experiment

Statistical analysis of the partitioning experiment was conducted with SAS software (SAS Institute, Cary, NC). Data were organized as shown in Table 1. The variables considered in the regressions were: type (red blood cells or plasma), initial styrene glycol concentration (100-1000 ng/mL), repeat (replicates were run) and injection (1,2 or 3). These variables were regressed against the final styrene concentration in different combinations as models.

3.2. Regression Analysis for Workers' Plasma Samples

The exposure data received from the University of Washington were regressed against one another and also against the workers' plasma styrene glycol concentrations via Lotus 123 software. Simple F-tests were also performed in Systat Version 3.0.

III. RESULTS AND DISCUSSION

1. Synthesis of 3-Phenyl-1,2-propanediol (Allyl benzene glycol)

Allyl benzene glycol was used as the internal standard for our procedure. Spectral verification of the structure of ABG may be found in Appendix A. One interesting observation was the loss of water when ABG-PFB was injected into the GC/MS. This is in agreement with the findings of Duverger-Van Boagaert (1978). It appears that 1,2-elimination, liberating one water molecule, occurs for both styrene glycol and allyl benzene glycol derivatives. Therefore, observation of a true molecular ion peak in either case does not occur. Instead a peak with the mass of the derivative of interest less 18 (MW of H_2O) is observed. Only monoderivatization was found in both styrene glycol and allyl benzene glycol samples.

Figure 3 shows the proposed mechanism of allyl benzene glycol formation. A somewhat perplexing step in the verification of our internal standard was determining the proper positional isomer for use in our study. Figure 2 (Appendix A) depicts a typical mass spectrum of ABG-PFB. The peak used as an internal standard by most investigators in styrene glycol determination appears at 7.8 minutes. This is the terminally derivatized ABG. The alpha-derivatized ABG appears at 3.7 minutes.

The proper internal standard is crucial to any experiment in which minute amounts of material are being analyzed. An internal standard accounts for experimental losses due to transfer, human error or acts of God, as its disappearance should be directly related to that of the chemical of interest. The

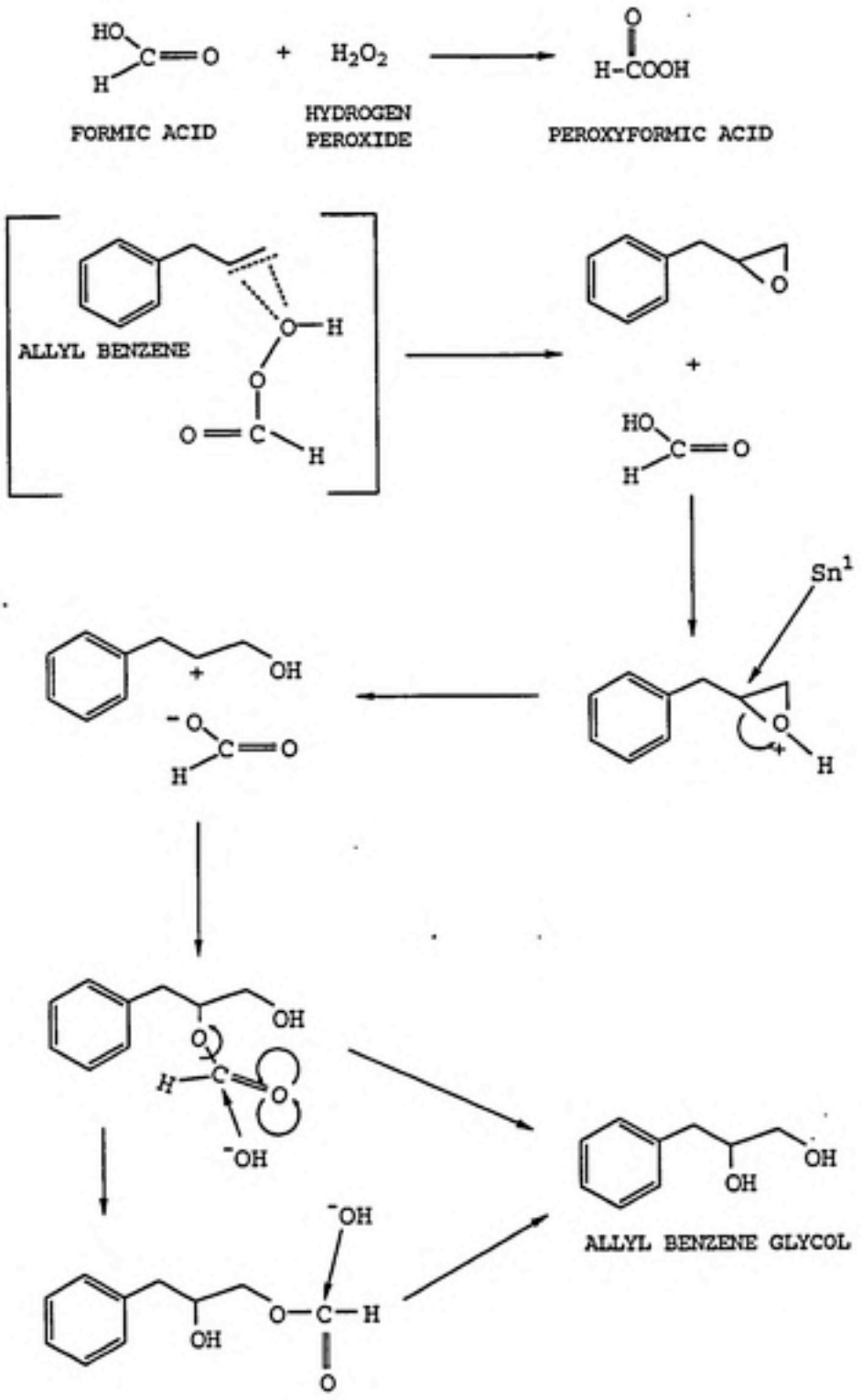

Figure 3. Proposed Mechanism for Allyl Benzene Glycol Formation

extraction and derivatization efficiencies of an internal standard will determine if it is an appropriate comparison to the chemical being quantified. In addition, the gas chromatographic retention time of the internal standard in relation to the chemical sought is important. Normally, the ratio of the gas chromatographic peak areas of the chemical of interest to the internal standard are used to determine concentrations of the chemical of interest. This was the method used in our study.

Allyl benzene glycol has proven to be the most appropriate internal standard to date for the analysis of styrene glycol concentrations. Three other possible internal standards were evaluated (DL-2-phenyl-1,2-propanediol, 4-methyl styrene glycol and 3-(4-hydroxyphenyl)-1-propanol); however, none of these compounds extracted and/or derivatized as well as allyl benzene glycol.

2. Styrene Glycol Partitioning in Human Plasma and Red Blood Cells

2.1. Experimental Analysis

It was our initial hypothesis that the concentrations of free styrene glycol in the plasma and red blood cells layers of whole blood would be equal. Regardless, this experiment was crucial in proving that analysis of either plasma or red blood cells layers may be used to predict the amount of styrene glycol in the other layer or whole blood.

The experiment consisted of twenty human whole blood samples which were dosed in increasing levels with styrene glycol and then divided into plasma and red blood cells layers. Both plasma and red blood cells layers were run in duplicate (i.e. 20 plasma samples at 10 different concentrations). In this manner,

we could recognize differences in duplicates, levels and between the plasma and red blood cells layers.

It was observed during the separation of red blood cells and plasma that minimal breakage of red blood cells occurred. This is an important consideration in subsequent statistical analysis, as red blood cell breakage will bias one's results. The extractions were performed with great ease due to development of an unpublished freeze/centrifuge procedure in our laboratory (Jin, 1990). Typically, some samples of plasma and red blood cells emulse when extracted with ethyl acetate. This procedure yields optimal separation of the organic and aqueous layers.

2.2. Statistical Analysis

Relevant data for the statistical analysis of the partitioning experiment may be found in Table 1. The variables investigated in the regression analyses were: type (red blood cells or plasma), initial styrene glycol concentration (100-1000 ng/mL), replicate of type (1 or 2) and injection (1,2 or 3). Table 3 summarizes the results of the regression analyses of variables against the final styrene glycol concentrations obtained. Table 2 describes the various models presented in Table3. A two-way nested ANOVA model indicates a total of 5.7% error related to experimental procedure (2.0% due to the duplicate observations and 3.7% due to the injections).

As shown in Table 3, the differences in the regresson coefficients for Models 1-3 and 4-6 are not significant. In fact, the regression coefficients of Models 7 and 8 demonstrate that there is, essentially, no difference in the regression coefficients for each injection. By grouping all of the data points together, we see that the difference in the regression coefficients for plasma and red blood cells is not significant. In fact, the difference in the slopes of these two

lines is not significant (ß = 0.08, SE = 1.01). This is a powerful and logical result, as it suggests that styrene glycol distributes uniformly throughout whole blood. Thus, the concentration of styrene glycol may be predicted in one layer (red blood cells or plasma) by determination of the other.

Table 4 presents the plasma, red blood cells and whole blood styrene glycol concentrations found in the partitioning exeriment. Figure 4 depicts the direct relationship between styrene glycol concentrations in the plasma and whole blood.

By analyzing styrene glycol concentration in plasma, one may obtain the whole blood concentration by multiplying by 3.08. That is:

$$[SG]_{blood} = [SG]_{plasma} \times [SG]_{blood}/[SG]_{plasma}$$
$$= [SG]_{plasma} \times 3.08.$$

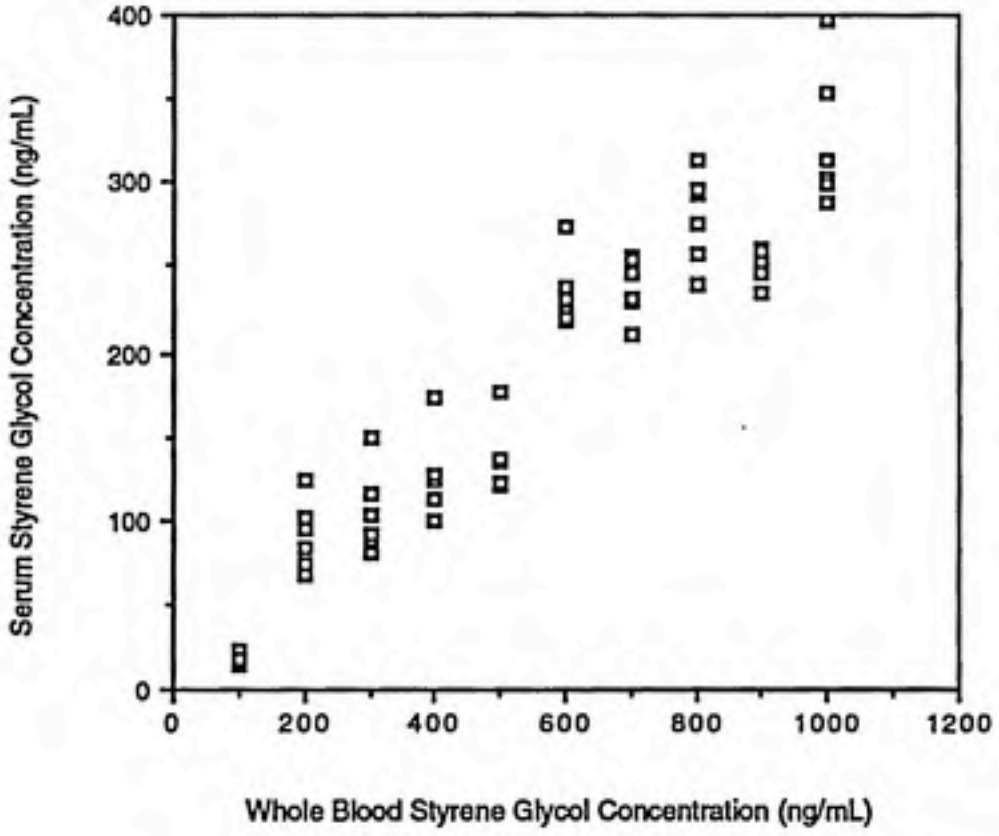

Figure 4. Whole Blood Styrene Glycol Concentration VS Plasma Styrene Glycol Concentration

Table 1. Plasma and Red Blood Cells Partitioning Experiment Data

Type	Initial Styrene Glycol Concentration (whole blood)	Repeat	Injection	Recovered Styrene Glycol Concentration (ng/mL)
RBC	100	1	1	23
RBC	100	1	2	34
RBC	100	1	3	24
RBC	200	1	1	56
RBC	200	1	2	62
RBC	200	1	3	65
RBC	300	1	1	61
RBC	300	1	2	73
RBC	300	1	3	67
RBC	400	1	1	196
RBC	400	1	2	180
RBC	400	1	3	173
RBC	500	1	1	158
RBC	500	1	2	161
RBC	500	1	3	133
RBC	600	1	1	186
RBC	600	1	2	171
RBC	600	1	3	198
RBC	700	1	1	287
RBC	700	1	2	249
RBC	700	1	3	322
RBC	800	1	1	245
RBC	800	1	2	263
RBC	800	1	3	253
RBC	900	1	1	285
RBC	900	1	2	262
RBC	900	1	3	245
RBC	1000	1	1	384
RBC	1000	1	2	359
RBC	1000	1	3	336
RBC	100	2	1	48
RBC	100	2	2	45
RBC	100	2	3	50
RBC	200	2	1	87
RBC	200	2	2	84
RBC	200	2	3	76
RBC	300	2	1	34
RBC	300	2	2	32
RBC	300	2	3	35
RBC	400	2	1	144
RBC	400	2	2	150
RBC	400	2	3	148
RBC	500	2	1	168
RBC	500	2	2	151
RBC	500	2	3	148
RBC	600	2	1	176
RBC	600	2	2	190
RBC	600	2	3	191

Table 1. (continued)

Type	Initial Styrene Glycol Concentration (whole blood)	Repeat	Injection	Recovered Styrene Glycol Concentration (ng/mL)
RBC	700	2	1	225
RBC	700	2	2	235
RBC	700	2	3	219
RBC	800	2	1	205
RBC	800	2	2	198
RBC	800	2	3	191
RBC	900	2	1	285
RBC	900	2	2	258
RBC	900	2	3	225
RBC	1000	2	1	401
RBC	1000	2	2	374
RBC	1000	2	3	380
PLASMA	100	1	1	18
PLASMA	100	1	2	20
PLASMA	100	1	3	15
PLASMA	200	1	1	102
PLASMA	200	1	2	68
PLASMA	200	1	3	84
PLASMA	300	1	1	89
PLASMA	300	1	2	103
PLASMA	300	1	3	81
PLASMA	400	1	1	99
PLASMA	400	1	2	124
PLASMA	400	1	3	100
PLASMA	500	1	1	122
PLASMA	500	1	2	120
PLASMA	500	1	3	135
PLASMA	600	1	1	224
PLASMA	600	1	2	273
PLASMA	600	1	3	219
PLASMA	700	1	1	245
PLASMA	700	1	2	230
PLASMA	700	1	3	256
PLASMA	800	1	1	274
PLASMA	800	1	2	292
PLASMA	800	1	3	240
PLASMA	900	1	1	260
PLASMA	900	1	2	252
PLASMA	900	1	3	261
PLASMA	1000	1	1	298
PLASMA	1000	1	2	353
PLASMA	1000	1	3	302
PLASMA	100	2	1	22
PLASMA	100	2	2	17
PLASMA	100	2	3	22
PLASMA	200	2	1	94
PLASMA	200	2	2	124
PLASMA	200	2	3	74
PLASMA	300	2	1	149
PLASMA	300	2	2	91
PLASMA	300	2	3	116

Table 1. (continued)

Type	Initial Styrene Glycol Concentration (whole blood)	Repeat	Injection	Recovered Styrene Glycol Concentration (ng/mL)
PLASMA	400	2	1	127
PLASMA	400	2	2	113
PLASMA	400	2	3	174
PLASMA	500	2	1	136
PLASMA	500	2	2	177
PLASMA	500	2	3	122
PLASMA	600	2	1	238
PLASMA	600	2	2	232
PLASMA	600	2	3	220
PLASMA	700	2	1	211
PLASMA	700	2	2	254
PLASMA	700	2	3	232
PLASMA	800	2	1	314
PLASMA	800	2	2	296
PLASMA	800	2	3	257
PLASMA	900	2	1	245
PLASMA	900	2	2	258
PLASMA	900	2	3	235
PLASMA	1000	2	1	397
PLASMA	1000	2	2	287
PLASMA	1000	2	3	314

Table 2. Model Definitions for Partitioning Experiment

Model	Description
1	Plasma SG Conc = ß x Whole Blood SG Conc (injection=1, no intercept)
2	Plasma SG Conc = ß x Whole Blood SG Conc (injection=2, no intercept)
3	Plasma SG Conc = ß x Whole Blood SG Conc (injection=3, no intercept)
4	RBC SG Conc = ß x Whole Blood SG Conc (injection=1, no intercept)
5	RBC SG Conc = ß x Whole Blood SG Conc (injection=2, no intercept)
6	RBC SG Conc = ß x Whole Blood SG Conc (injection=3, no intercept)
7	Plasma SG Conc = ß x Whole Blood SG Conc (injection=1,2,3, no inter)
8	RBC SG Conc = ß x Whole Blood SG Conc (injection=1,2,3, no intercept)
9	All Conc = ß x Whole Blood SG Conc (injection=1,2,3, no intercept)

Legend: ß - regression coefficient of styrene glycol concentration (ng/mL) [dependent variable] on whole blood concentration (ng/mL).

Table 3. Summary Statistics for Partitioning Experiment

Model	Type	n	Injection	ß	SE	R^2
1	Plasma	20	1	0.331	0.0128	0.97
2	Plasma	20	2	0.332	0.0121	0.98
3	Plasma	20	3	0.311	0.0093	0.98
4	RBC	20	1	0.337	0.0134	0.97
5	RBC	20	2	0.322	0.0114	0.98
6	RBC	20	3	0.317	0.0148	0.96
7	Plasma	60	all	0.325	0.0066	0.98
8	RBC	60	all	0.326	0.0076	0.97
9	Both	120	all	0.325	0.0050	0.97

Legend: ß-regression coefficient of styrene glycol concentration (ng/mL) [dependent variable] on whole blood concentration (ng/mL); SE - standard error of ß.

Table 4. Plasma, Red Blood Cells and Whole Blood Concentrations

Repeat	Injection	RBC Styrene Glycol Concentration (ng/mL)	Plasma Styrene Glycol Concentration (ng/mL)	Whole Blood Styrene Glycol Concentration (ng/mL)
1	1	23	18	100
1	2	34	20	100
1	3	24	15	100
1	1	56	102	200
1	2	62	68	200
1	3	65	84	200
1	1	61	89	300
1	2	73	103	300
1	3	67	81	300
1	1	196	99	400
1	2	180	124	400
1	3	173	100	400
1	1	158	122	500
1	2	161	120	500
1	3	133	135	500
1	1	186	224	600
1	2	171	273	600
1	3	198	219	600
1	1	287	245	700
1	2	249	230	700
1	3	322	256	700
1	1	245	274	800
1	2	263	292	800
1	3	253	240	800
1	1	285	260	900
1	2	262	252	900
1	3	245	261	900
1	1	384	298	1000
1	2	359	353	1000
1	3	336	302	1000
2	1	48	22	100
2	2	45	17	100
2	3	50	22	100
2	1	87	94	200
2	2	84	124	200
2	3	76	74	200
2	1	34	149	300
2	2	32	91	300
2	3	35	116	300
2	1	144	127	400
2	2	150	113	400
2	3	148	174	400
2	1	168	136	500
2	2	151	177	500
2	3	148	122	500
2	1	176	238	600
2	2	190	232	600
2	3	191	220	600

Table 4. (continued)

Repeat	Injection	RBC Styrene Glycol Concentration (ng/mL)	Plasma Styrene Glycol Concentration (ng/mL)	Whole Blood Styrene Glycol Concentration (ng/mL)
2	1	225	211	700
2	2	235	254	700
2	3	219	232	700
2	1	205	314	800
2	2	198	296	800
2	3	191	257	800
2	1	285	245	900
2	2	258	258	900
2	3	225	235	900
2	1	401	397	1000
2	2	374	287	1000
2	3	380	314	1000

3. Analaysis of Workers' Plasma Samples

3.1. Experimental Analysis

Two important findings may be concluded from the partitioning experiment. Firstly, there is essentially no difference in the partitioning of dosed human whole blood samples into plasma and red blood cells layers. Secondly, there is minimal variability in injections at one concentration level. These considerations afforded a more streamlined analysis of the workers' samples.

There were two reasons that plasma was chosen as the layer of human whole blood for analysis in this study. The first reason was that the plasma layer yielded slightly lower standard errors than the red blood cells layer in the regression analysis of the partitioning experiment. The second reason was that emulsions occur less frequently during extractions in plasma than red blood cells because the plasma layer of whole blood contains less protein than the red blood cells layer.

The analysis of the workers' plasma samples occurred more than one year after collection of the blood. For the purposes of this study, we have assumed the degradation of styrene glycol over time will not affect our analysis. If this were not the case, it would be crucial to investigate how styrene glycol degrades over time.

Ten plasma samples were analyzed per experiment and new hexane standards of styrene glycol and allyl benzene glycol were prepared for each experiment. As the samples had been frozen for a long time, it was critical to warm the samples slowly from -80 ^{o}C. They were warmed in the following manner: -20 ^{o}C freezer, 0 ^{o}C freezer, 4 ^{o}C refrigerator, room temperature and warm water bath.

3.2. Statistical Analysis of Exposure Data

The exposure data received from the University of Washington are found in Table 5. The data were evaluated by Company # and all pertinent regression information may be found below the appropriate scatter diagram for each of the described one-way Analysis of Variance (ANOVA) models (at the end of this section). Although the regression information in Table 7 of this section is summarized by model, the following results will be discussed by Company #. Table 6 describes the regression models presented in Table 7. It is important to note that the urinary metabolite concentration is the sum of mandelic acid and phenylglyoxylic acid concentrations. All replicate measurements for Companies #1 and 2 are treated separately in the analyses to ensure the independence of observations.

Company #1 exposure data (obtained from the University of Washington) are graphically represented in Figures 5-7. These are Models 1, 2 and 3, respectively, in Table 6. Figure 5 plots air styrene concentration against blood styrene concentration and yields a correlation coefficient of 0.48 (F-ratio = 12.401, p-value = 0.001, R^2 = 0.23). Figure 6 depicts air styrene concentration against urinary metabolite concentration. The correlation coefficient of 0.46 (F-ratio = 11.131, p-value = 0.002, R^2 = 0.21) is similar to that in Model 1. Finally, Figure 7 graphs blood styrene concentration against urinary metabolite concentration. These variables correlate poorly, yielding a R-value of 0.20 (F-ratio = 1.649, p-value = 0.206, R^2 = 0.04). In summary, air styrene concentration for Company #1 correlates acceptably with blood and urinary metabolite concentrations. Each p-value is significant. Blood and urinary metabolite concentrations, however, do not correlate well.

Figures 8-10 graphically represent exposure data collected at Company #2. These are Models 1, 2 and 3, respectively, in Table 8. Figure 4 depicts the correlation of air styrene concentration and blood styrene concentration (R = 0.36, F-ratio = 2.980, p-value = 0.100, R^2 = 0.13). A correlation coefficient of 0.61 (F-ratio = 11.559, p-value = 0.003, R^2 = 0.37) is obtained when air styrene and urinary metabolite concentrations are regressed (Figure 9). Regression of blood styrene and urinary metabolite concentrations (Figure 10) yields a correlation coefficient of 0.41 (F-ratio = 4.154, p-value = 0.055, R^2 = 0.17). Summarizing, Company #2 afforded p-values which are not significant when air styrene concentration is regressed against blood styrene concentration and when blood styrene and urinary metabolite concentrations are regressed. Regression of air styrene and urinary metabolite concentrations yielded a significant p-value.

Company #3 exposure data are graphically presented in Figures 11-13. These are Models 1, 2 and 3, respectively, in Table 6. Figure 11 shows air styrene concentration plotted against blood styrene concentration. These variables correlate well, yielding a R-value of 0.77 (F-ratio = 22.466, p-value = 0.000, R^2 = 0.60). A correlation coefficient of 0.89 (F-ratio = 57.470, p-value = 0.000, R^2 = 0.79) is found when air styrene and urinary metabolite concentrations are regressed (Figure 12). Figure 13 depicts the relationship between blood styrene and urinary metabolite concentrations. The variables have a strong correlation coefficient of 0.87 (F-ratio = 48.578, p-value = 0.000, R^2 = 0.76). In summary, Company #3's exposure data correlates well with each other.

Company #3 possesses the best correlated data among the three companies. It is the only company which yields significant p-values in all

Table 5. Workers' Styrene Glycol Concentrations and Exposure Data

Company #-Worker ID-Blood Drawing	Injection	Plasma Styrene Glycol (ng/mL)	Average Plasma Styrene Glycol (ng/mL)	Air Styrene Concentration (ppm)	Blood Styrene Concentration (µg/mL)	Urine Metabolite Concentration (mg/mL)
1-1-1	1	12.18	11.16	20.66	0.049	0.067
	2	10.13				
1-2-1	1	16.85	17.33	8.34	0.224	0.156
	2	17.80				
1-3-1	1	14.91	15.40	4.35	0.122	0.006
	2	15.90				
1-4-1	1	9.38	9.40	14.17	0.091	0.021
	2	9.43				
1-4-2	1	0	0	3.07	0	0.039
	2	0				
1-5-1	1	32.41	32.23	63.72	0.564	0.202
	2	32.09				
1-6-1	1	22.37	19.24	15.18	0.481	0.087
	2	16.10				
1-6-2	1	0	0	3.23	0	0.183
	2	0				
1-7-1	1	4.55	5.09	3.28	0.052	0.060
	2	5.62				
1-8-1	1	13.73	14.75	12.15	0.130	0.047
	2	15.77				
1-9-1	1	11.33	11.85	12.97	0.163	0.079
	2	12.37				
1-9-2	1	0	0	NA	0	0.041
	2	0				
1-10-1	1	17.68	17.10	43.39	0.142	0.285
	2	16.51				
1-10-2	1	2.28	2.40	2.53	0.017	0.082
	2	2.52				
1-11-1	1	14.55	14.46	35.79	0.493	0.116
	2	14.37				
1-12-1	1	5.41	5.16	4.21	0.092	0.042
	2	4.91				
1-12-2	1	13.51	14.63	7.91	0.133	0.750
	2	15.74				
1-13-1	1	12.12	11.58	3.75	0.156	0.524
	2	11.03				
1-13-2	1	156.15	155.43	19.84	1.362	0.079
	2	154.71				
1-14-1	1	5.46	5.15	3.45	0.057	0.055
	2	4.84				
1-15-1	1	20.58	19.96	15.49	0.443	0.294
	2	19.35				
1-15-2	1	14.44	17.10	18.14	0.291	0.180
	2	19.77				
1-17-1	1	12.04	12.74	47.50	0.350	0.057
	2	13.42				
1-18-1	1	132.81	128.60	82.02	0.506	0.555
	2	124.40				

NA represents not available

Table 5. (continued)

Company #- Worker ID- Blood Drawing	Injection	Plasma Styrene Glycol (ng/mL)	Average Plasma Styrene Glycol (ng/mL)	Air Styrene Concentration (ppm)	Blood Styrene Concentration (µg/mL)	Urine Metabolite Concentration (mg/mL)
1-18-2	1	1.60	1.40	3.26	0.030	0.241
	2	1.19				
1-19-1	1	5.31	4.88	8.10	0.064	0.039
	2	4.46				
1-19-2	1	0	0	4.67	0	0.020
	2	0				
1-20-1	1	3.78	3.56	3.72	0.072	0.008
	2	3.34				
1-20-2	1	8.76	8.70	46.53	0.130	0.350
	2	8.64				
1-21-1	1	7.33	7.39	4.32	0.088	0.024
	2	7.45				
1-21-2	1	23.87	20.66	37.06	0.155	1.272
	2	17.45				
1-22-1	1	61.48	65.71	63.97	0.719	0.975
	2	69.93				
1-23-1	1	6.83	8.86	1.97	0.043	0.024
	2	10.89				
1-23-2	1	0.69	0.66	3.89	0	0.166
	2	0.62				
1-24-1	1	46.09	48.64	37.51	0.699	0.110
	2	51.20				
1-24-2	1	22.79	26.17	24.60	0.660	0.125
	2	29.56				
1-25-1	1	132.04	138.74	1.12	0.904	0.318
	2	145.44				
1-26-1	1	7.31	8.56	4.51	0.058	0.044
	2	9.80				
1-26-2	1	3.92	4.26	3.56	0	0.051
	2	4.61				
1-27-1	1	30.27	26.25	19.56	0.299	0.192
	2	22.23				
1-28-1	1	7.78	11.50	6.65	0.091	0.159
	2	15.21				
2-32-1	1	105.61	108.01	51.87	0.135	1.749
	2	110.40				
2-33-1	1	121.19	127.67	70.83	0.015	2.287
	2	134.15				
2-34-1	1	193.66	195.09	64.21	0.124	0.324
	2	196.52				
2-34-2	1	16.20	19.58	85.15	0.017	0.192
	2	22.97				
2-35-1	1	107.18	113.66	51.38	0.325	0.886
	2	120.14				
2-36-1	1	144.52	139.62	12.48	0.120	0.299
	2	134.72				
2-37-1	1	7.70	8.09	18.77	0.132	0.084
	2	8.48				

NA represents not available

Table 5. (continued)

Company #-Worker ID-Blood Drawing	Injection	Plasma Styrene Glycol (ng/mL)	Average Plasma Styrene Glycol (ng/mL)	Air Styrene Concentration (ppm)	Blood Styrene Concentration (µg/mL)	Urine Metabolite Concentration (mg/mL)
2-37-2	1	74.32	75.62	10.61	NA	0.133
	2	76.91				
2-38-1	1	26.19	26.68	9.85	0.176	0.326
	2	27.16				
2-38-2	1	485.41	482.00	6.98	0.150	0.109
	2	478.59				
2-39-1	1	31.32	35.15	19.29	0.410	0.580
	2	38.97				
2-40-1	1	4.38	4.90	0.55	0.001	0.005
	2	5.41				
2-41-1	1	5.55	5.83	2.57	0.016	0.066
	2	6.11				
2-42-1	1	31.98	27.76	0.96	0.020	0.002
	2	23.54				
2-43-1	1	12.17	10.70	1.86	0.031	0.004
	2	9.22				
2-44-1	1	3.37	3.63	0.49	0.009	0.005
	2	3.89				
2-44-2	1	27.75	27.24	1.12	0.001	0.005
	2	26.73				
2-47-1	1	14.32	13.79	13.90	0.047	0.319
	2	13.26				
2-48-1	1	14.76	14.58	56.83	0.125	0.212
	2	14.39				
2-48-2	1	437.35	439.11	61.31	0.052	0.251
	2	440.88				
2-49-1	1	37.24	37.17	43.53	0.054	0.419
	2	37.09				
2-49-2	1	150.86	157.25	76.38	0.747	1.456
	2	163.64				
3-52-1	1	6.43	9.91	3.33	0.027	0.399
	2	13.40				
3-53-1	1	30.09	28.48	22.46	0.153	0.572
	2	26.88				
3-54-1	1	4.91	4.67	4.21	0.054	0.083
	2	4.43				
3-55-1	1	100.70	103.45	42.95	0.504	1.779
	2	106.20				
3-56-1	1	0	0	1.36	0	0.046
	2	0				
3-57-1	1	5.81	6.54	1.78	0.020	0.059
	2	7.27				
3-58-1	1	17.22	18.92	160.90	0.351	2.645
	2	20.62				
3-59-1	1	171.21	167.25	134.90	0.582	2.001
	2	163.28				
3-60-1	1	0	0	4.40	0.003	0.021
	2	0				

NA represents not available

Table 5. (continued)

Company #-Worker ID-Blood Drawing	Injection	Plasma Styrene Glycol (ng/mL)	Average Plasma Styrene Glycol (ng/mL)	Air Styrene Concentration (ppm)	Blood Styrene Concentration (µg/mL)	Urine Metabolite Concentration (mg/mL)
3-61-1	1	2.00	1.77	2.95	0.008	0.029
	2	1.54				
3-62-1	1	16.43	15.68	53.24	0.022	0.359
	2	14.94				
3-63-1	1	72.28	74.19	37.55	0.196	0.105
	2	76.10				
3-64-1	1	12.03	12.75	2.31	0.010	0.008
	2	13.48				
3-65-1	1	15.16	14.80	4.87	0.056	0.414
	2	14.44				
3-66-1	1	21.10	20.12	25.63	0.165	0.939
	2	19.15				
3-67-1	1	37.82	40.69	29.22	0.123	0.524
	2	43.56				
3-68-1	1	0	0	1.06	0.021	0.009
	2	0				
1-99-2	1	3.22	2.90	3.24	0	0.116
	2	2.59				
1-100-2	1	26.41	28.06	11.60	0.076	0.235
	2	29.70				
1-101-2	1	20.69	21.06	7.76	0.148	0.436
	2	21.43				
1-102-2	1	74.86	81.56	40.47	0.530	0.472
	2	88.26				
2-121-2	1	0	0	0.78	0.001	0.007
	2	0				

NA represents not available

Table 6. Model Definitions for Exposure Data

Model	Description
1	Blood Styrene Conc = Constant + ß x Air Styrene Conc
2	Urinary Metabolite Conc = Constant + ß x Air Styrene Conc
3	Urinary Metabolite Conc = Constant +ß x Blood Styrene Conc

Legend: ß - regression coefficient of dependent variable on the independent variable.

Table 7. Summary Statistics for Exposure Data

Model	Company	n	F-ratio	p-value	R^2	R
1	1	44	12.401	0.001	0.23	0.48
1	2	22	2.980	0.100	0.13	0.36
1	3	17	22.466	0.000	0.60	0.77
2	1	44	11.131	0.002	0.21	0.46
2	2	22	11.559	0.003	0.37	0.61
2	3	17	57.470	0.000	0.79	0.89
3	1	44	1.649	0.206	0.04	0.20
3	2	22	4.154	0.055	0.17	0.41
3	3	17	48.578	0.000	0.76	0.87

regression models. Company #1 correlates acceptably when air styrene concentration is regressed against blood styrene and urinary metabolite concentrations; however, blood styrene and urinary metabolite concentrations do not correlate well. Company #2's exposure data produce p-values which are not significant in two instances. However, a significant p-value is obtained when air styrene concentration is regressed against urinary metabolite concentration.

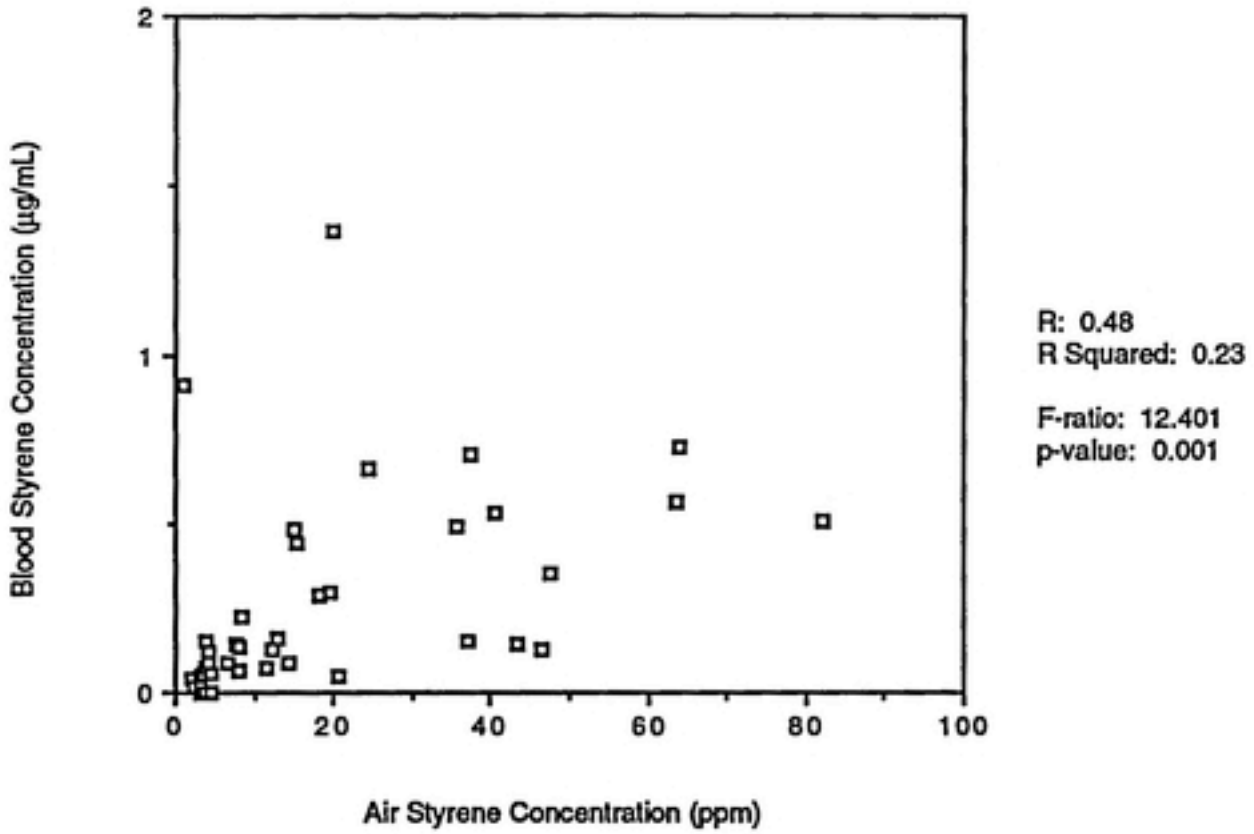

Figure 5. Air Styrene Concentration VS Blood Styrene Concentration (Company # 1)

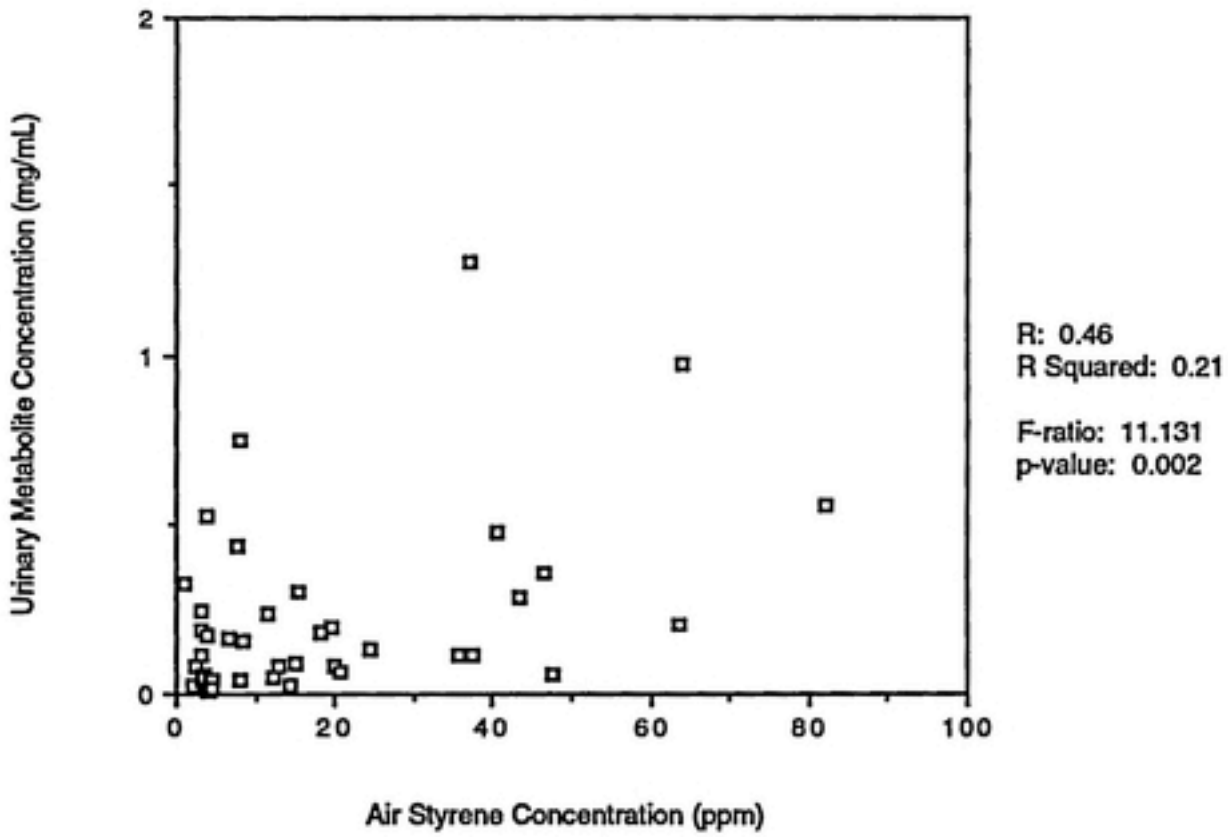

Figure 6. Air Styrene Concentration VS Urinary Metabolite Concentration (Company # 1)

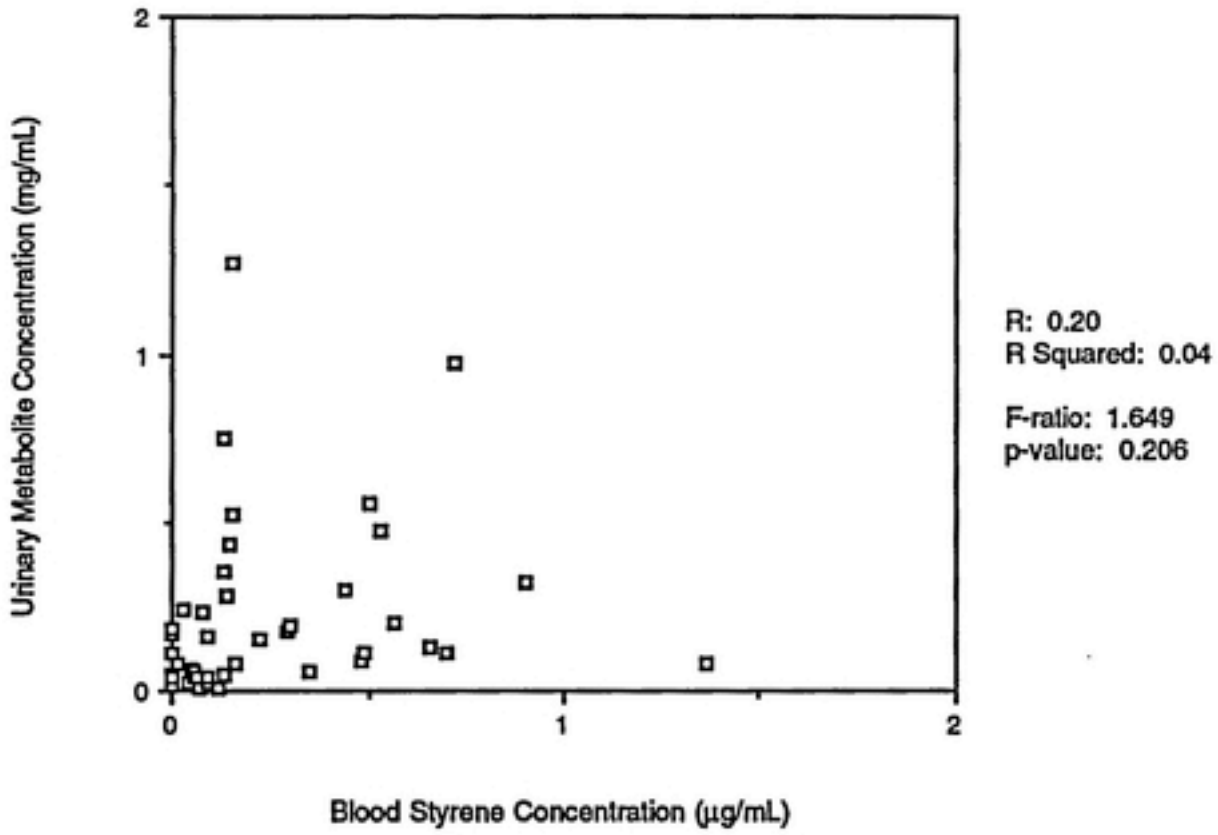

Figure 7. Blood Styrene Concentration VS Urinary Metabolite Concentration (Company # 1)

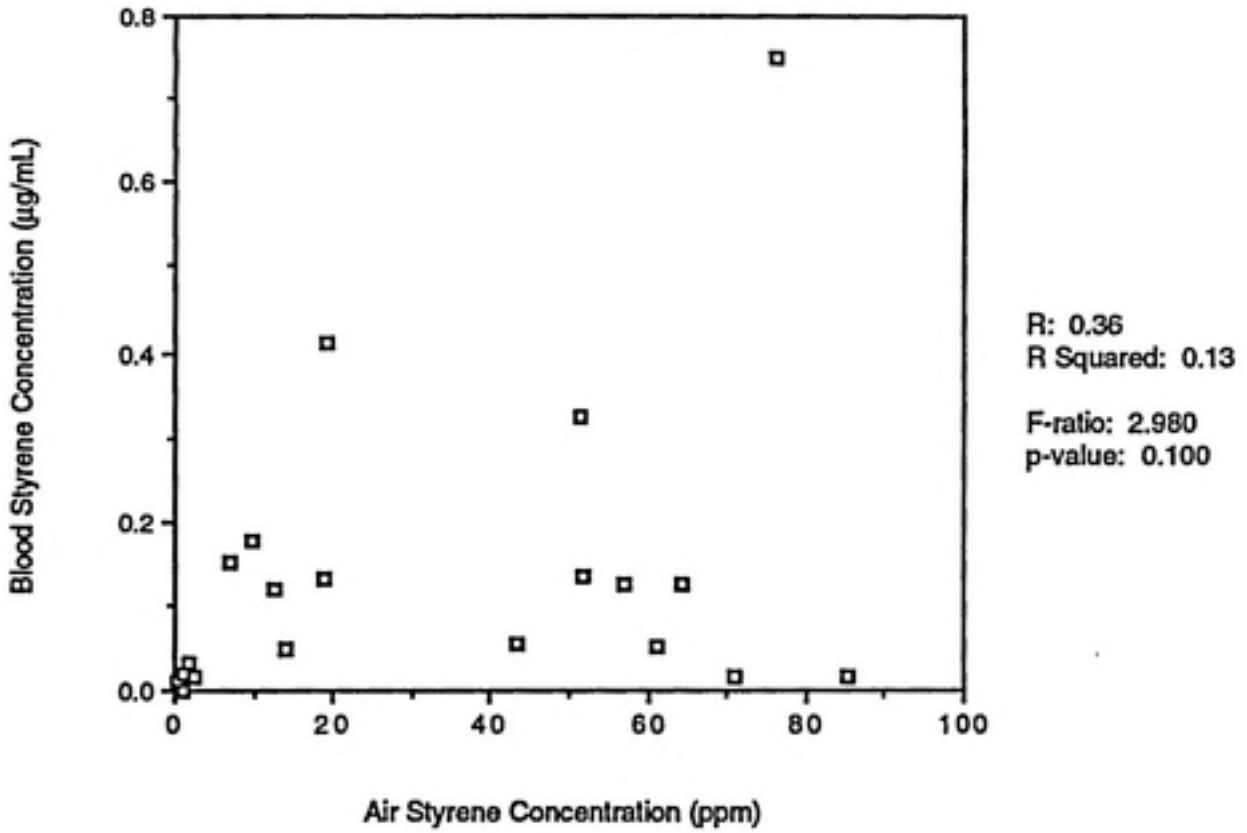

Figure 8. Air Styrene Concentration VS Blood Styrene Concentration (Company # 2)

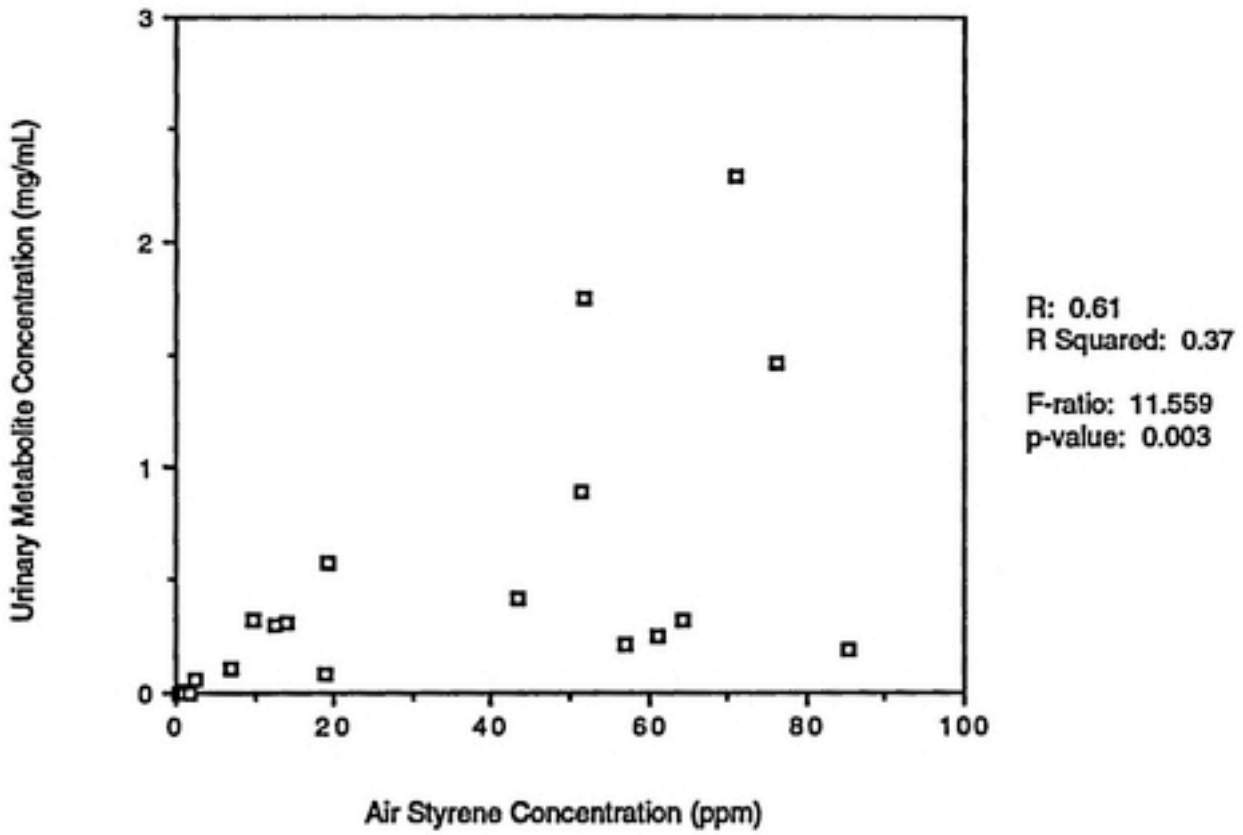

Figure 9. Air Styrene Concentration VS Urinary Metabolite Concentration (Company # 2)

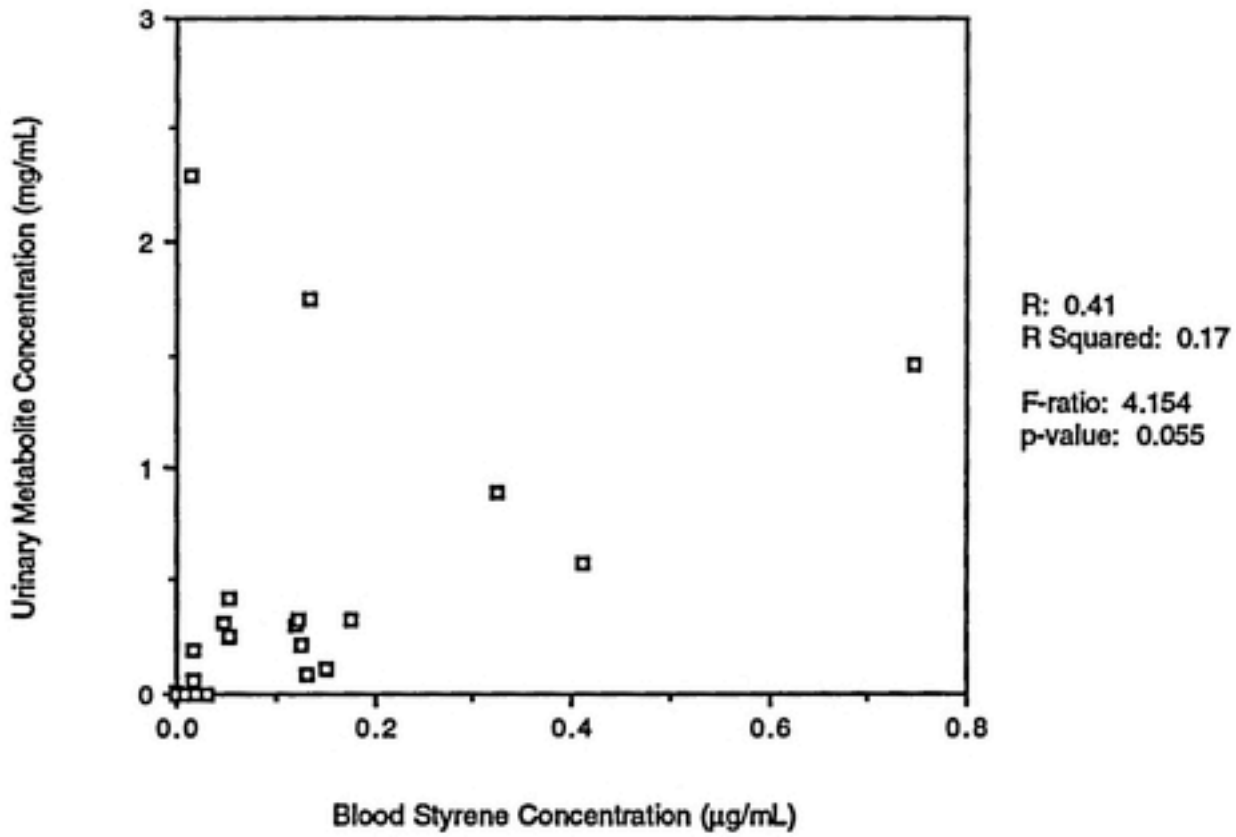

Figure 10. Blood Styrene Concentration VS Urinary Metabolite Concentration (Company # 2)

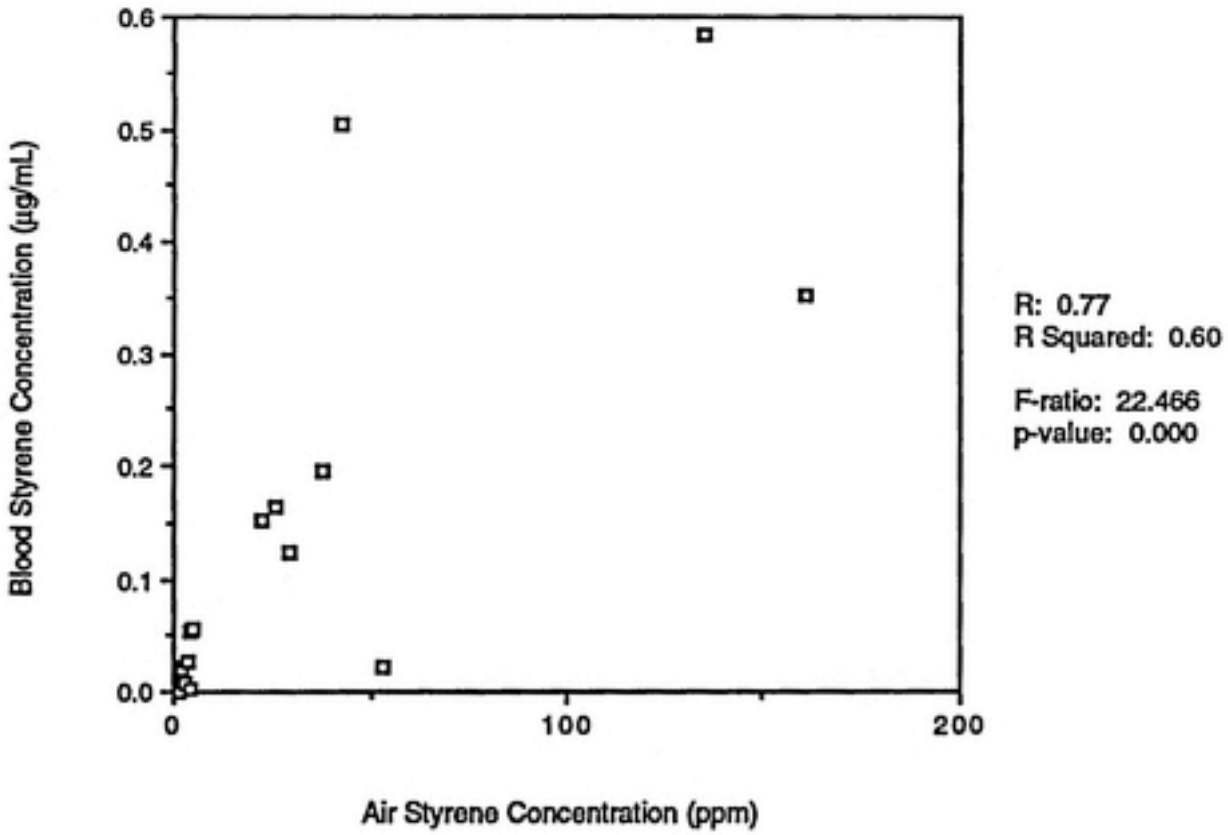

Figure 11. Air Styrene Concentration VS Blood Styrene Concentration (Company # 3)

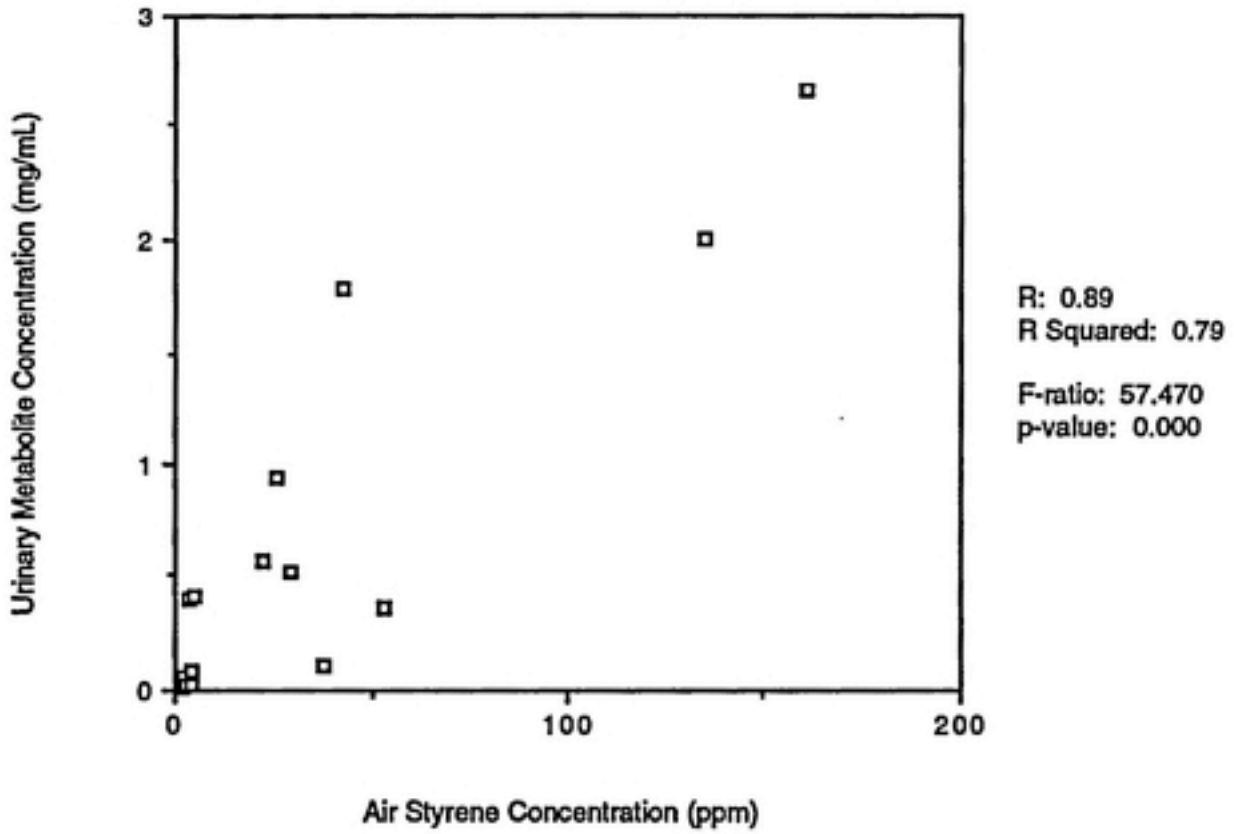

Figure 12. Air Styrene Concentration VS Urinary Metabolite Concentration (Company # 3)

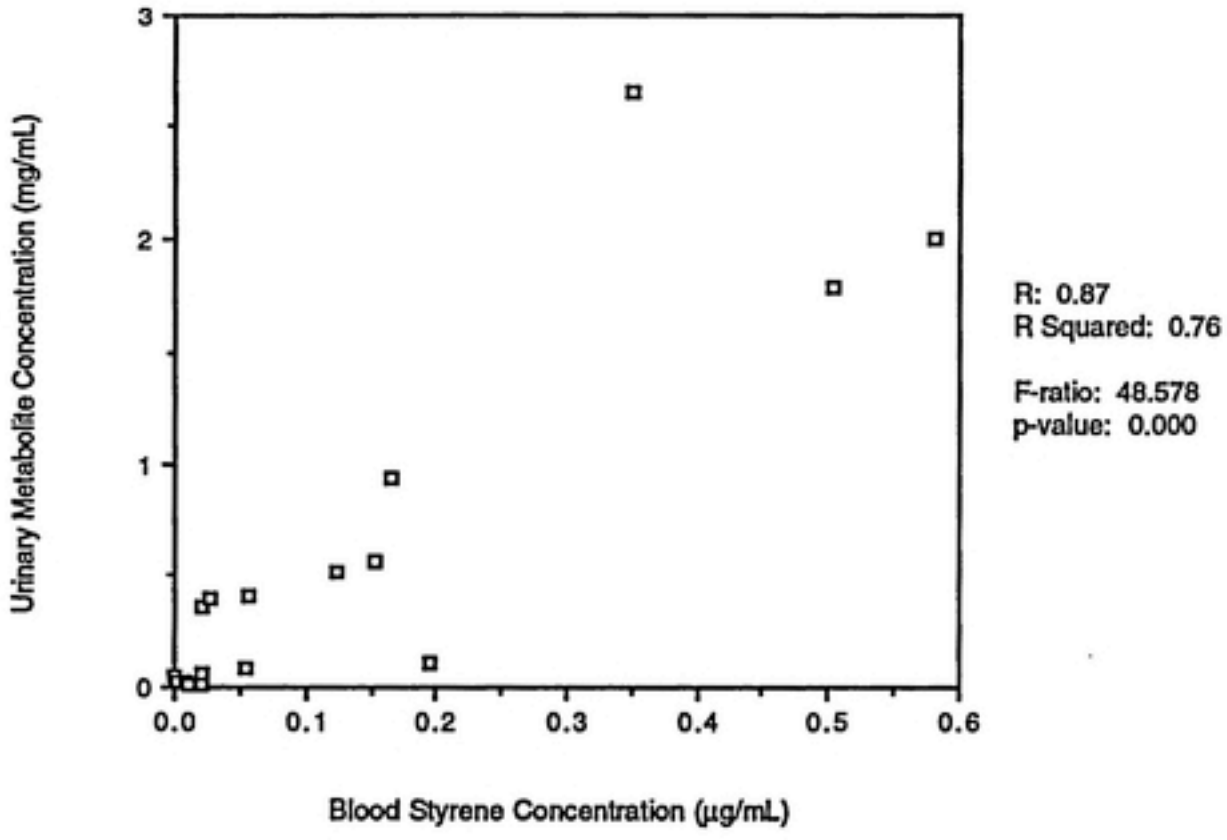

Figure 13. Blood Styrene Concentration VS Urinary Metabolite Concentration (Company # 3)

3.3. Statistical Analysis of Workers' Plasma Samples

Experimentally determined plasma styrene glycol concentrations from workers in the reinforced plastic industry may be found in Table 5. The data were evaluated by Company # and all pertinent regression information may be found below the appropriate scatter diagram for each of the described one-way ANOVA models (at the end of this section). Although regression information in Table 9 is summarized by model, the following results will be discussed by Company #. Table 8 describes the regression models presented in Table 9. All replicate measurements for Companies #1 and 2 are treated separately in the analysis to ensure the independence of observations. The plasma styrene glycol concentrations are represented as the average concentration of the two injections.

Company #1 data are depicted in Figures 14-16. These are Models 1, 2 and 3, respectively, in Table 8. Figure 14 plots air styrene concentration against average plasma styrene glycol concentration. The correlation coefficient is 0.48 (F-ratio = 9.815, p-value = 0.003, R^2 = 0.23). Blood styrene concentration is correlated with average plasma styrene glycol concentration in Figure 15. Here a relatively good correlation coefficient of 0.60 (F-ratio = 101.790, p-value = 0.000, R^2 = 0.60) is observed. Figure 16 depicts the correlation of urinary metabolite concentration and average plasma styrene glycol concentration. The correlation is weak, yielding a R-value of 0.28 (F-ratio = 3.691, p-value = 0.062, R^2 = 0.08). Summarizing, Company #1 air and blood styrene concentrations appear to regress relatively well against the average plasma styrene glycol concentration. However, urinary metabolite concentration does not regress as well against average plasma styrene glycol concentration. In fact, the p-value of 0.062 signifies only marginal significance.

Table 8. Model Definitions for Workers' Plasma Samples

Model	Description
1	Plasma SG Conc = Constant + ß x Air Styrene Conc
2	Plasma SG Conc = Constant + ß x Blood Styrene Conc
3	Plasma SG Conc = Constant + ß x Urinary Metabolite Conc

Legend: ß - regression coefficient of dependent variable on the independent variable.

Table 9. Summary Statistics for Workers' Plasma Samples

Model	Company	n	F-ratio	p-value	R^2	R
1	1	44	9.815	0.003	0.23	0.48
1	2	22	1.740	0.202	0.08	0.28
1	3	17	7.878	0.013	0.34	0.58
2	1	44	101.790	0.000	0.60	0.77
2	2	22	0.654	0.428	0.03	0.17
2	3	17	56.913	0.000	0.79	0.89
3	1	44	3.691	0.062	0.08	0.28
3	2	22	0.476	0.498	0.02	0.14
3	3	17	8.351	0.011	0.36	0.60

Company # 2 data are presented in Figures 17, 18 and 19. These figures are Models 1,2 and 3, respectively, in Table 8. Figure 17 shows air styrene concentration plotted against average plasma styrene glycol concentration. A weak correlation coefficient of 0.28 is obtained (F-ratio = 1.740, p-value = 0.202, R^2 = 0.08). Figure 18 presents the correlation of blood styrene concentration with average plasma styrene glycol concentration. A R-value of 0.17 (F-ratio = 0.654, p-value = 0.428, R^2 = 0.03) demonstrates a very weak correlation. The regression of urinary metabolite concentration and average plasma styrene glycol concentration is shown in Figure 19. Another weak correlation coefficent, R = 0.14 (F-ratio = 0.476, p-value = 0.498, R^2 = 0.02), is observed. In summary, Company #2's exposure data do not correlate well with average plasma styrene glycol concentration.

Figures 20-22 represent Models 1, 2 and 3, respectively, in Table 8. These models investigate Plant #3's relationship to average plasma styrene glycol concentration. Figure 20 depicts the regression of air styrene concentration against average plasma styrene glycol concentration. A marginal correlation coefficient of 0.58 (F-ratio = 7.878, p-value = 0.013, R^2 = 0.34) was found. Blood styrene concentration and average plasma styrene glycol concentration are plotted in Figure 21. An exceptional R-value of 0.89 is obtained (F-ratio = 56.913, p-value = 0.000, R^2 = 0.79). Figure 22 plots urinary metabolite concentration against average plasma styrene glycol concentration, yielding a marginal coefficient of 0.60 (F-ratio = 8.351, p-value = 0.011, R^2 = 0.36). Company #3's exposure data regresses relatively well with the average plasma styrene glycol concentraiton. Each p-value is significant.

Company #3 provides the best overall correlations for air styrene, blood styrene and urinary metabolite concentrations with average plasma styrene glycol concentration. Each p-value is significant. Company #1's air and blood

styrene concentrations correlate well with average plasma styrene glycol concentration, yielding significant p-values. However, the correlation of urinary metabolite concentration with average plasma styrene glycol concentration for Company #1 yields only a marginally significant p-value. Company #2 does not correlate well with average plasma styrene glycol concentration, affording non-significant p-values in each regression.

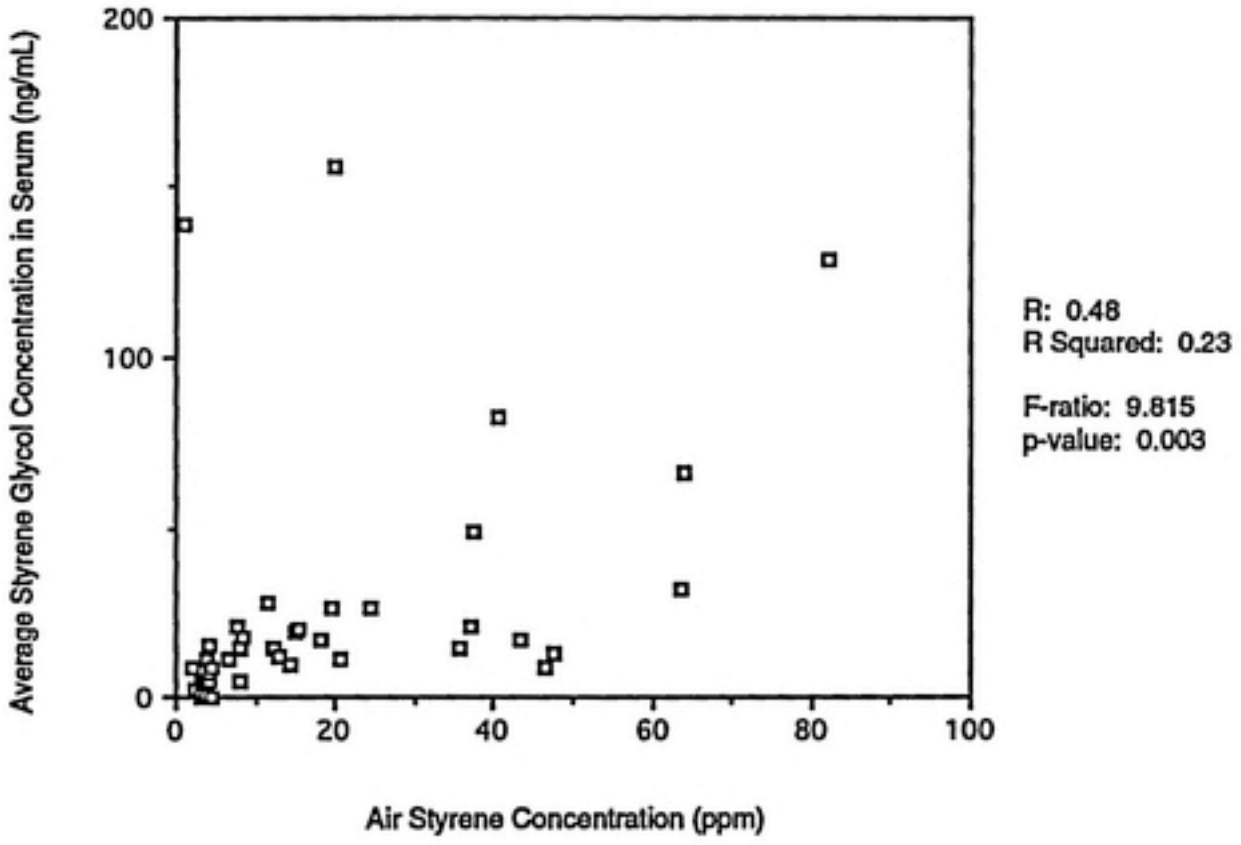

Figure 14. Air Styrene Concentration VS Average Plasma Styrene Glycol Concentration (Company # 1)

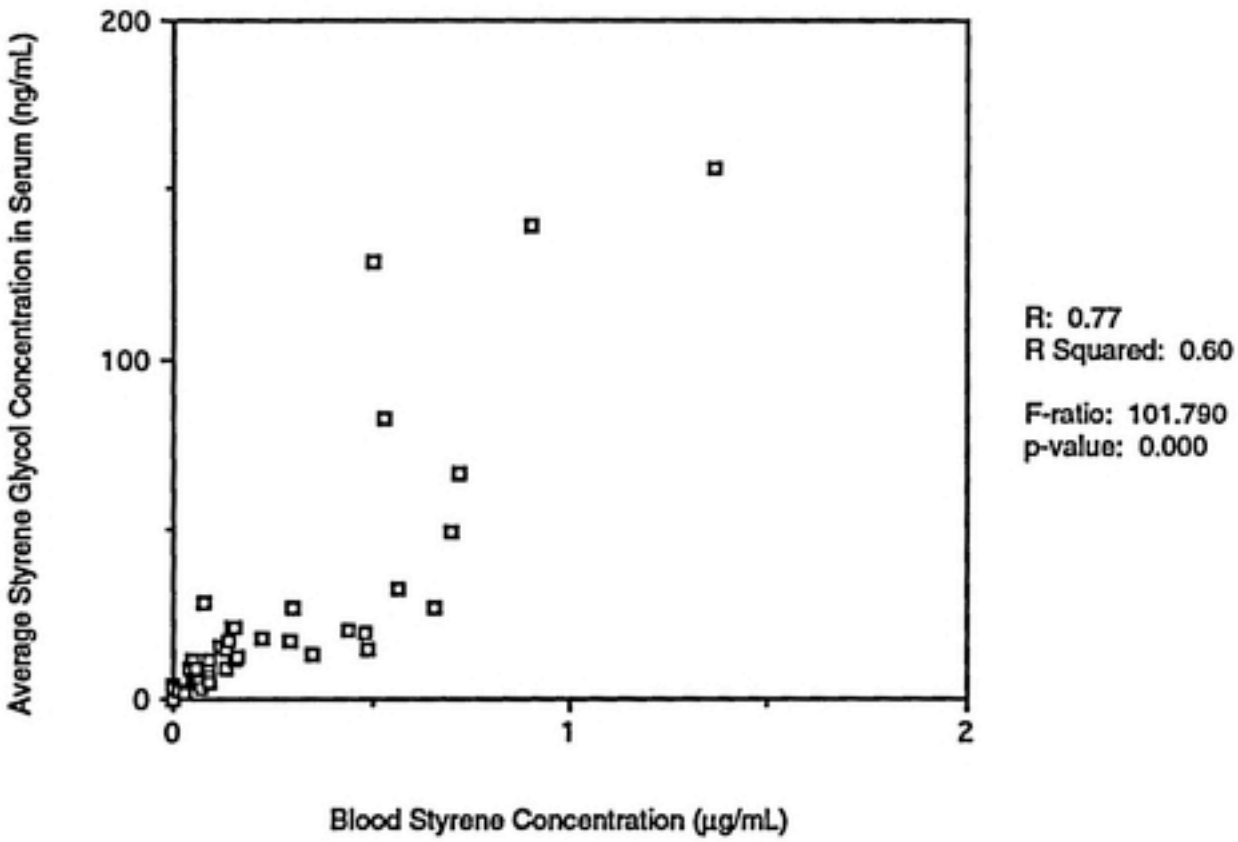

Figure 15. Blood Styrene Concentration VS Average Plasma Styrene Glycol Concentration (Company # 1)

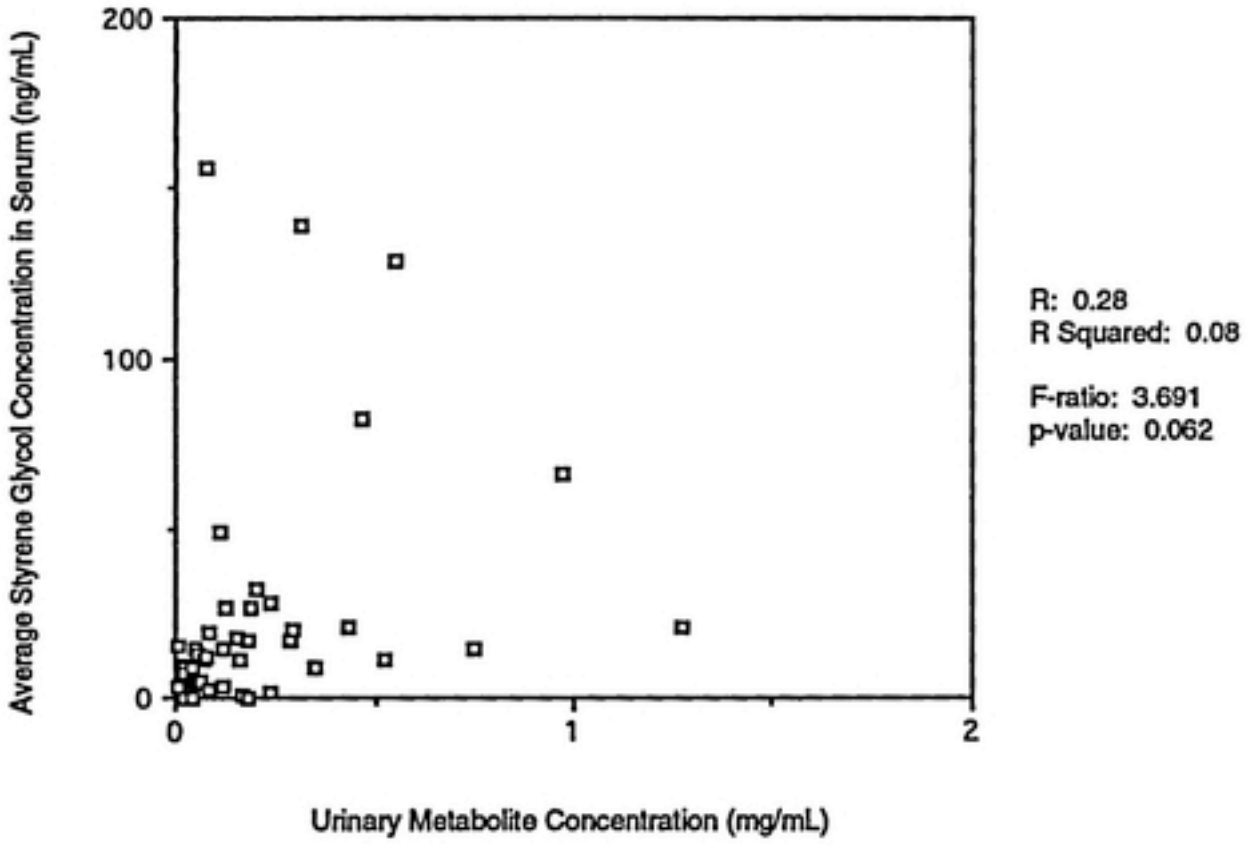

Figure 16. Urinary Metabolite Concentration VS Average Plasma Styrene Glycol Concentration (Company # 1)

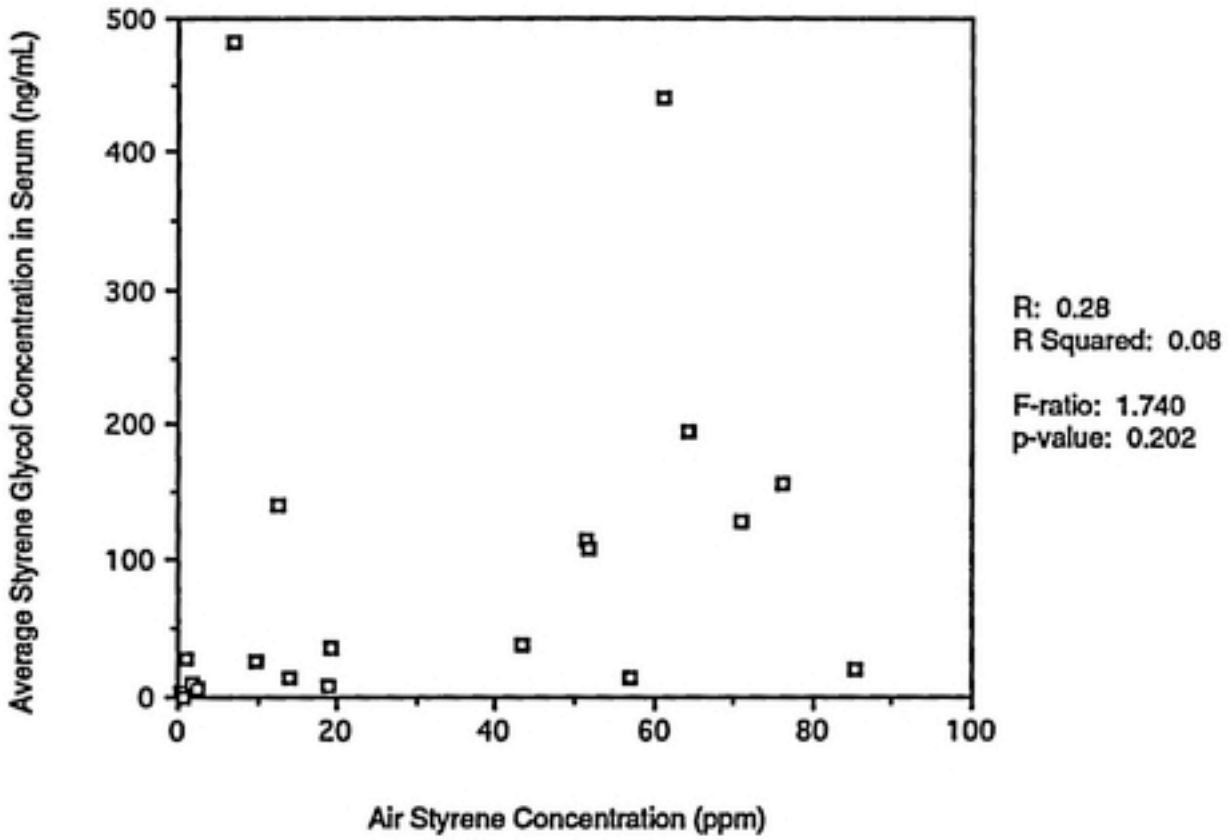

Figure 17. Air Styrene Concentration VS Average Plasma Styrene Glycol Concentration (Company # 2)

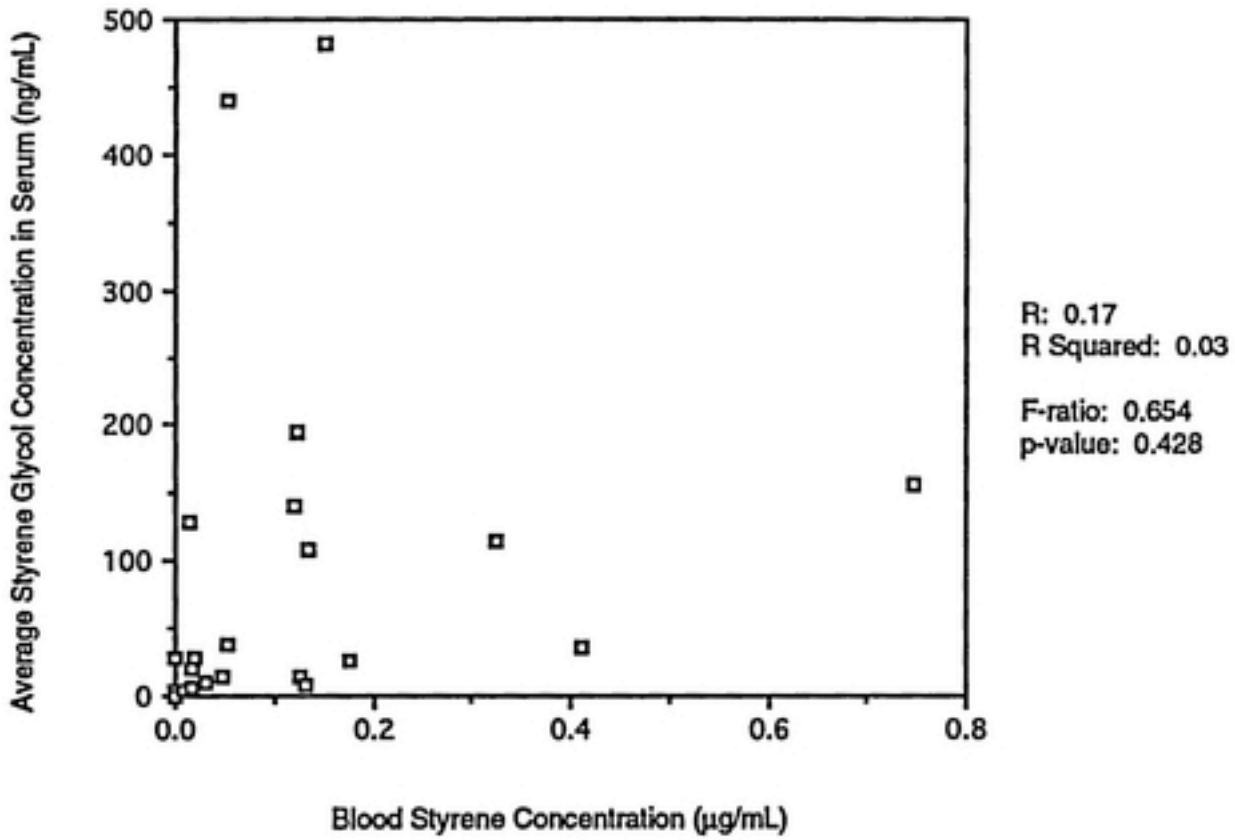

Figure 18. Blood Styrene Concentration VS Average Plasma Styrene Glycol Concentration (Company # 2)

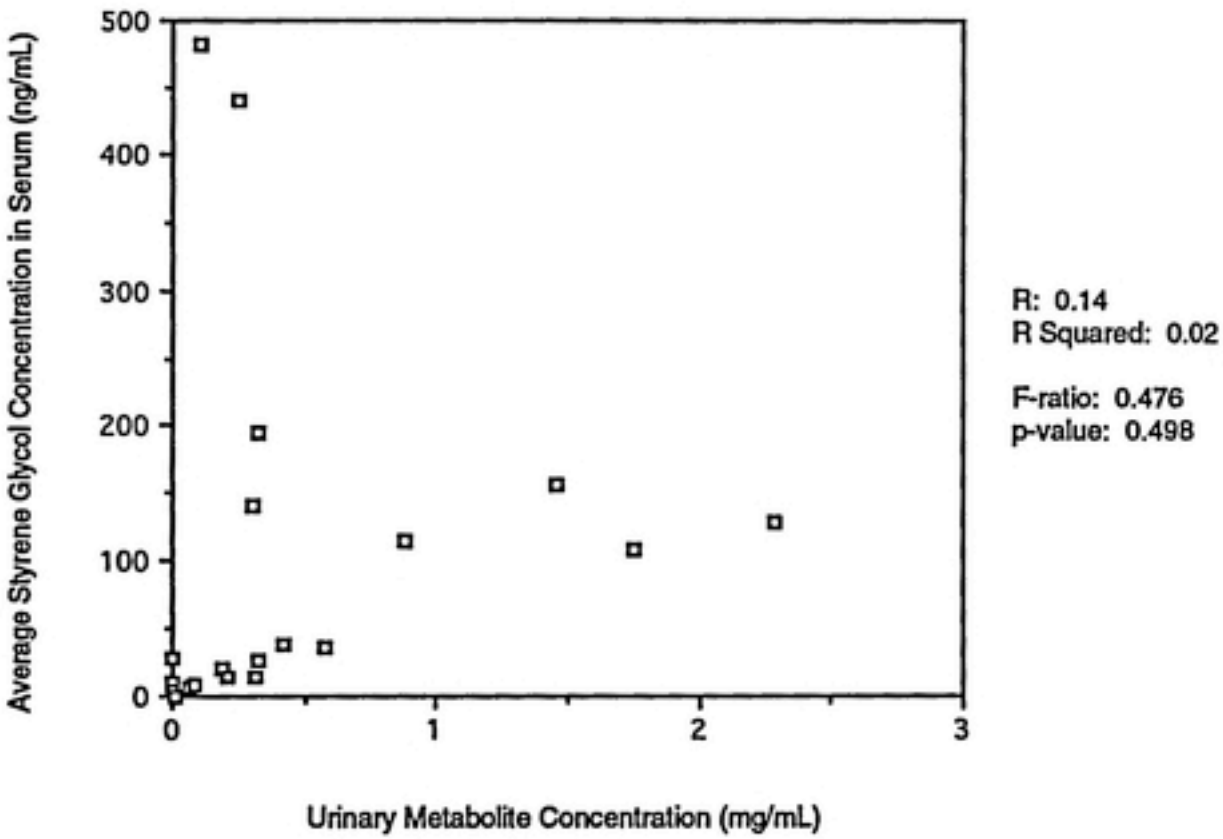

Figure 19. Urinary Metabolite Concentration VS Average Plasma Styrene Glycol Concentration (Company # 2)

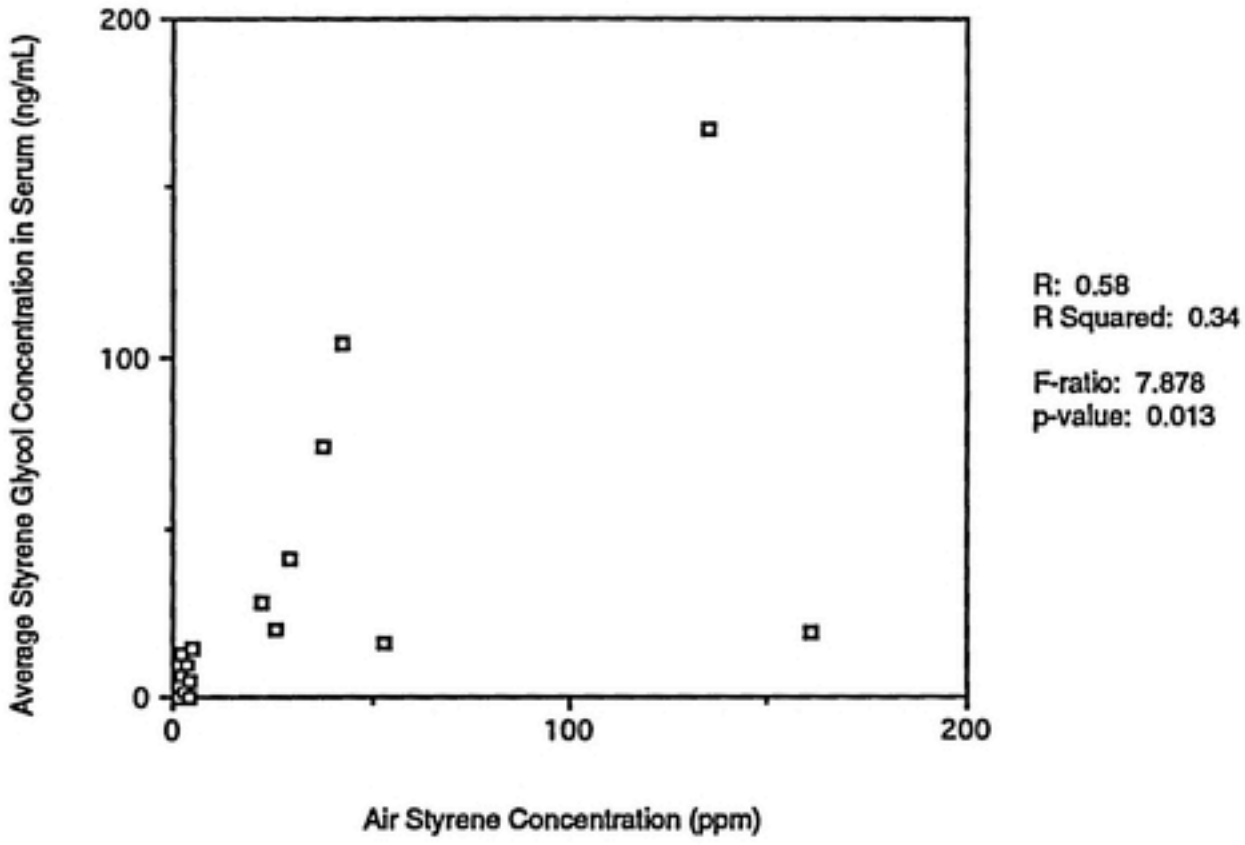

Figure 20. Air Styrene Concentration VS Average Plasma Styrene Glycol Concentration (Company # 3)

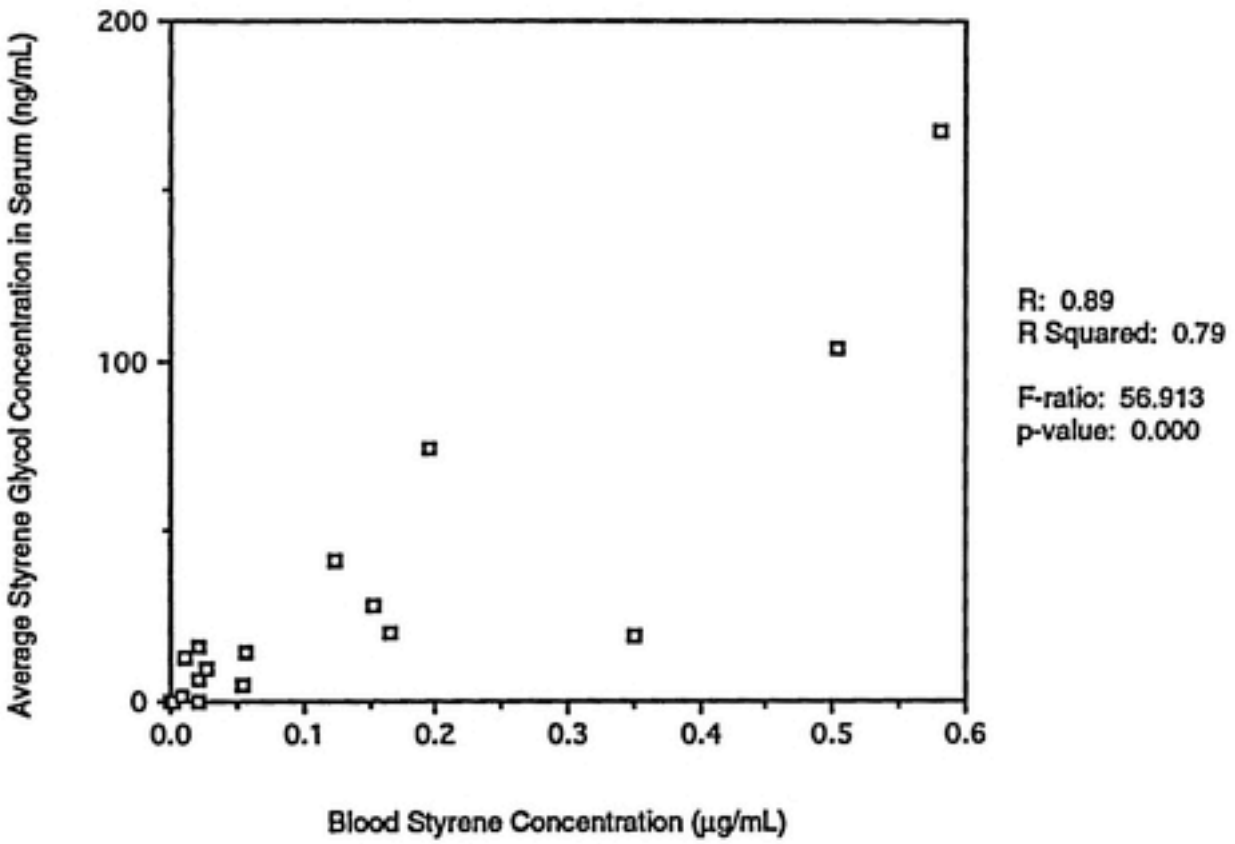

Figure 21. Blood Styrene Concentration VS Average Plasma Styrene Glycol Concentration (Company # 3)

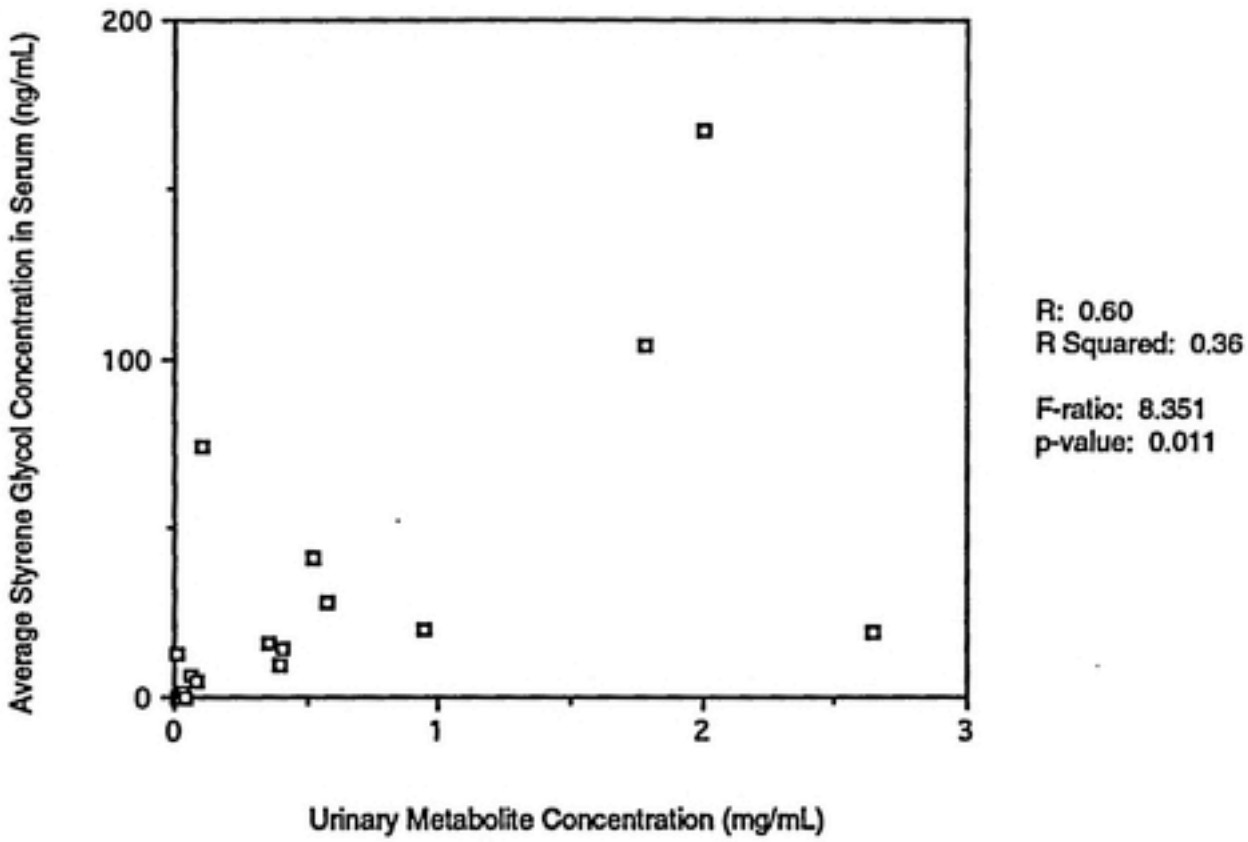

Figure 22. Urinary Metabolite Concentration VS Average Plasma Styrene Glycol Concentration (Company # 3)

3.4. Statistical Analysis of Replicate Exposure Data and Workers' Plasma Samples

Approximately one year after initial collection, replicate measurements for air and blood styrene and urinary metabolite concentrations were collected for Companies # 1 and # 2. Blood samples were also obtained at this time. Thirteen sets of data came from Company #1 and five sets from Company #2. There were no replicates measured for Company #3. For the purposes of our analysis, the data from Companies #1 and 2 have been combined and the replicate measurements have been averaged. The data are found in Table 10. All pertinent regression information may be found below the appropriate scatter diagram for each of the described one-way ANOVA models (at end of this section).

Exposure data for replicate observations in Companies #1 and 2 are graphically represented in Figures 23-25. These figures correspond to Models 1, 2 and 3, respectively, in Table 11. Figure 23 plots average air styrene concentration against average blood styrene concentration. A weak correlation coefficient of 0.14 (F-ratio = 0.347, p-value = .0.564, R^2 = 0.21) is obtained. Figure 24 depicts average air styrene concentration against average urinary metabolite concentration. These variables correlate relatively well, yielding a R-value of 0.50 (F-ratio = 5.271, p-value = 0.036, R^2 = 0.25). The regression of average blood styrene concentration against urinary metabolite concentration is represented in Figure 25. A weak correlation coefficient of 0.30 (F-ratio = 1.511, p-value = 0.237, R^2 = 0.09) is obtained. In summary, when the averaged exposure concentrations are regressed against one another, the only model which yields a significant p-value is average air styrene concentration against average urinary metabolite concentration.

Table 10. Replicate Workers' Styrene Glycol Concentrations and Exposure Data

Company #-Worker ID-	Average Plasma Styrene Glycol (ng/mL)	Average Air Styrene Concentration (ppm)	Average Blood Styrene Concentration (µg/mL)	Average Urine Metabolite Concentration (mg/mL)
1-4	4.70	8.62	0.046	0.030
1-6	9.62	9.20	0.241	0.135
1-10	9.75	22.96	0.079	0.184
1-12	9.89	6.06	0.113	0.396
1-13	83.50	11.80	0.759	0.302
1-15	18.53	16.82	0.367	0.237
1-18	65.00	42.64	0.268	0.398
1-19	2.44	6.38	0.032	0.029
1-20	6.13	25.12	0.101	0.179
1-21	14.02	20.69	0.122	0.648
1-23	4.76	2.93	0.021	0.095
1-24	37.41	31.06	0.679	0.117
1-26	6.41	4.04	0.029	0.048
2-34	107.34	74.68	0.070	0.258
2-38	254.34	8.42	0.163	0.217
2-44	15.43	0.80	0.005	0.005
2-48	226.84	59.07	0.089	0.232
2-49	97.21	59.96	0.401	0.938

Table 11. Model Definitions for Exposure Data

Model	Description
1	Blood Styrene Conc = Constant + ß x Air Styrene Conc
2	Urinary Metabolite Conc = Constant + ß x Air Styrene Conc
3	Urinary Metabolite Conc = Constant +ß x Blood Styrene Conc

Legend: ß - regression coefficient of dependent variable on the independent variable.

Table 12. Summary Statistics for Replicates' Exposure Data

Model	Company	n	F-ratio	p-value	R^2	R
1	1+2	18	0.347	0.564	0.02	0.14
2	1+2	18	5.271	0.036	0.25	0.50
3	1+2	18	1.511	0.237	0.09	0.30

Plasma styrene glycol concentrations determined for replicate observations in Companies # 1 and 2 are graphically represented in Figures 26-28. These figures correspond to Models 1, 2 and 3, respectively, in Table 13. Figure 26 plots average air styrene concentration against average plasma styrene glycol concentration. A correlation coefficient of 0.46 (F-ratio = 4.372, p-value = 0.053, $R^2 = 0.21$) is obtained. Figure 27 depicts average blood styrene concentration against average plasma styrene glycol concentration, yielding a very weak correlation coefficient of 0.10 (F-ratio = 0.234, p-value = 0.635, $R^2 = 0.01$). The regression of average urinary metabolite concentration against average plasma styrene glycol concentration is graphically represented in Figure 28. A weak correlation coefficient of 0.20 (F-ratio = 0.748, p-value = 0.400, $R^2 = 0.04$) is obtained. Summarizing, it appears there is very little significance in the correlations of average plasma styrene glycol concentration and blood styrene and urinary metabolite concentrations. Marginal significance is seen in the relationship of average plasma styrene glycol concentration and average air styrene concentration.

Table 13. Model Definitions for Workers' Plasma Samples

Model	Description
1	Plasma SG Conc = Constant + ß x Air Styrene Conc
2	Plasma SG Conc = Constant + ß x Blood Styrene Conc
3	Plasma SG Conc = Constant + ß x Urinary Metabolite Conc

Legend: ß - regression coefficient of dependent variable on the independent variable.

Table 14. Summary Statistics for Replicate Workers' Plasma Samples

Model	Company	n	F-ratio	p-value	R^2	R
1	1+2	18	4.372	0.053	0.21	0.46
2	1+2	18	0.234	0.635	0.01	0.10
3	1+2	18	0.748	0.400	0.04	0.20

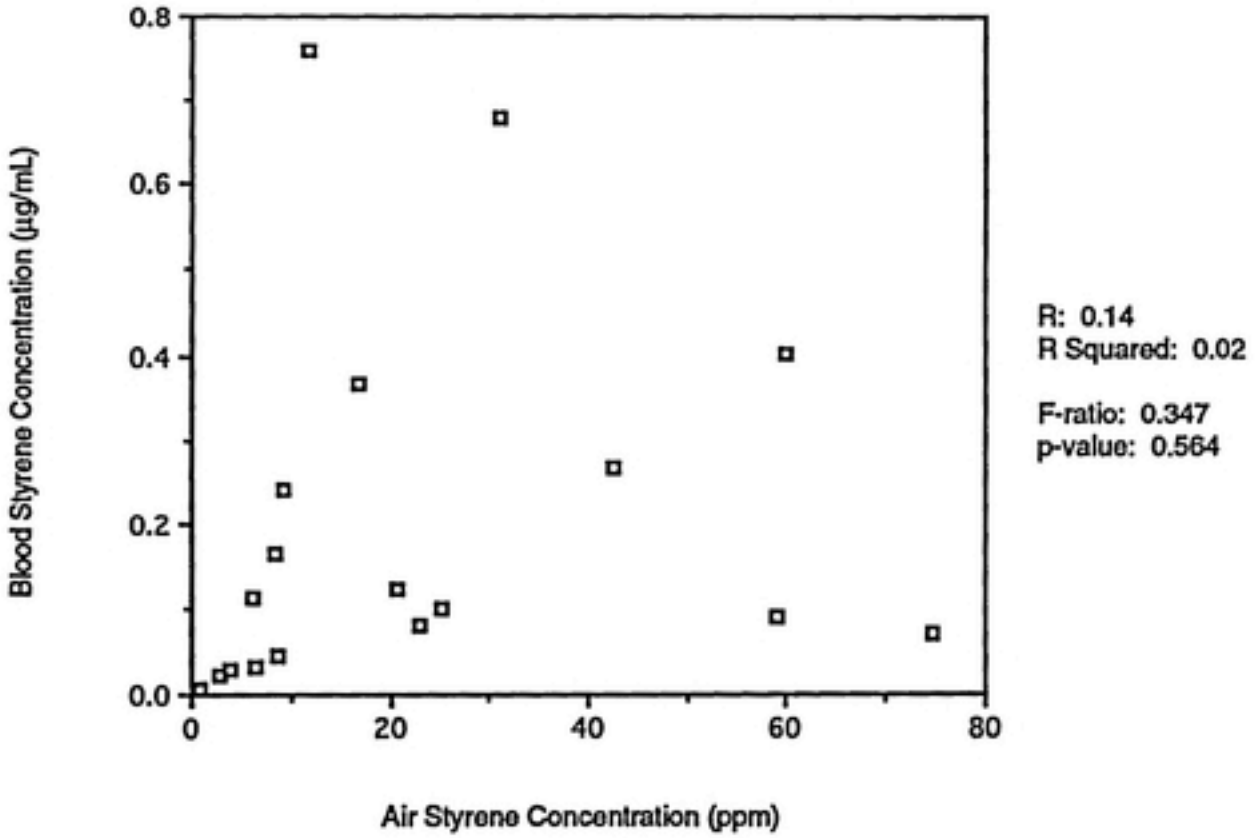

Figure 23. Air Styrene Concentration VS Blood Styrene Concentration (Companies # 1 and 2 Replicates)

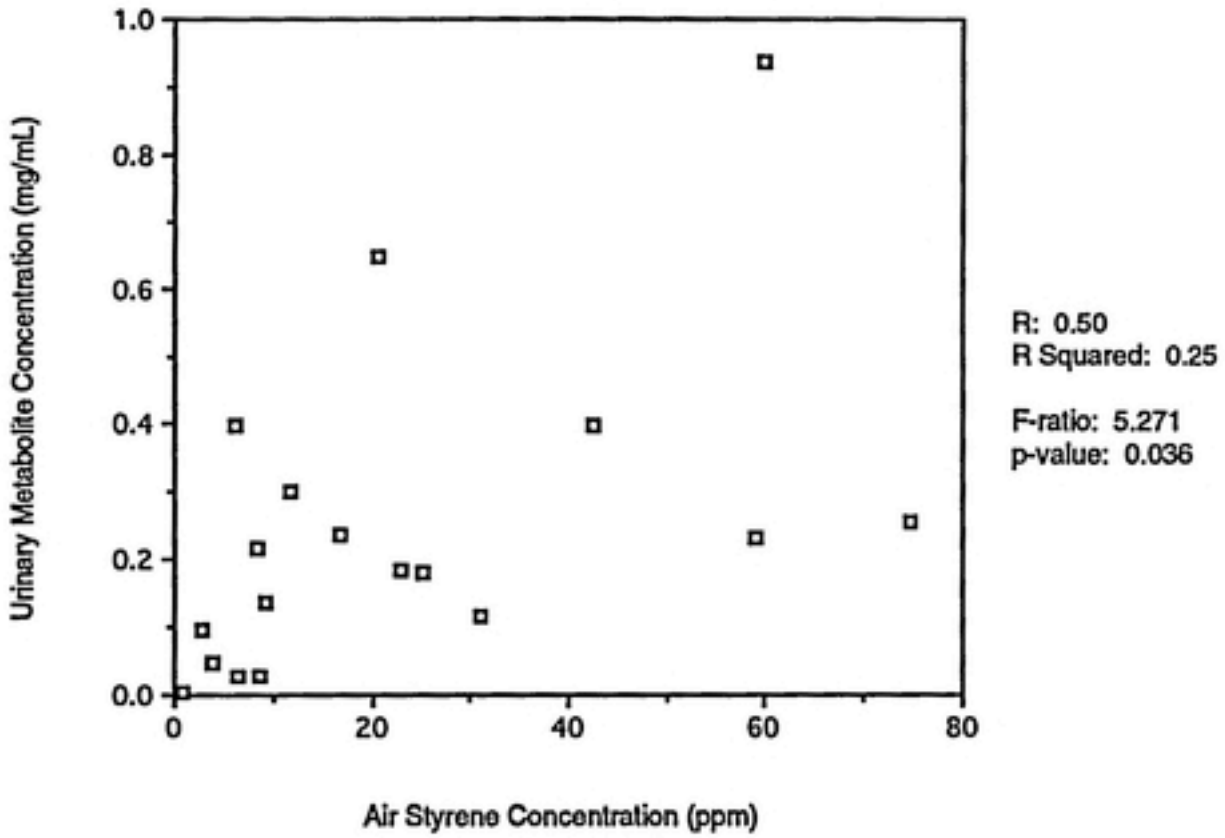

Figure 24. Air Styrene Concentration VS Urinary Metabolite Concentration (Companies # 1 and 2 Replicates)

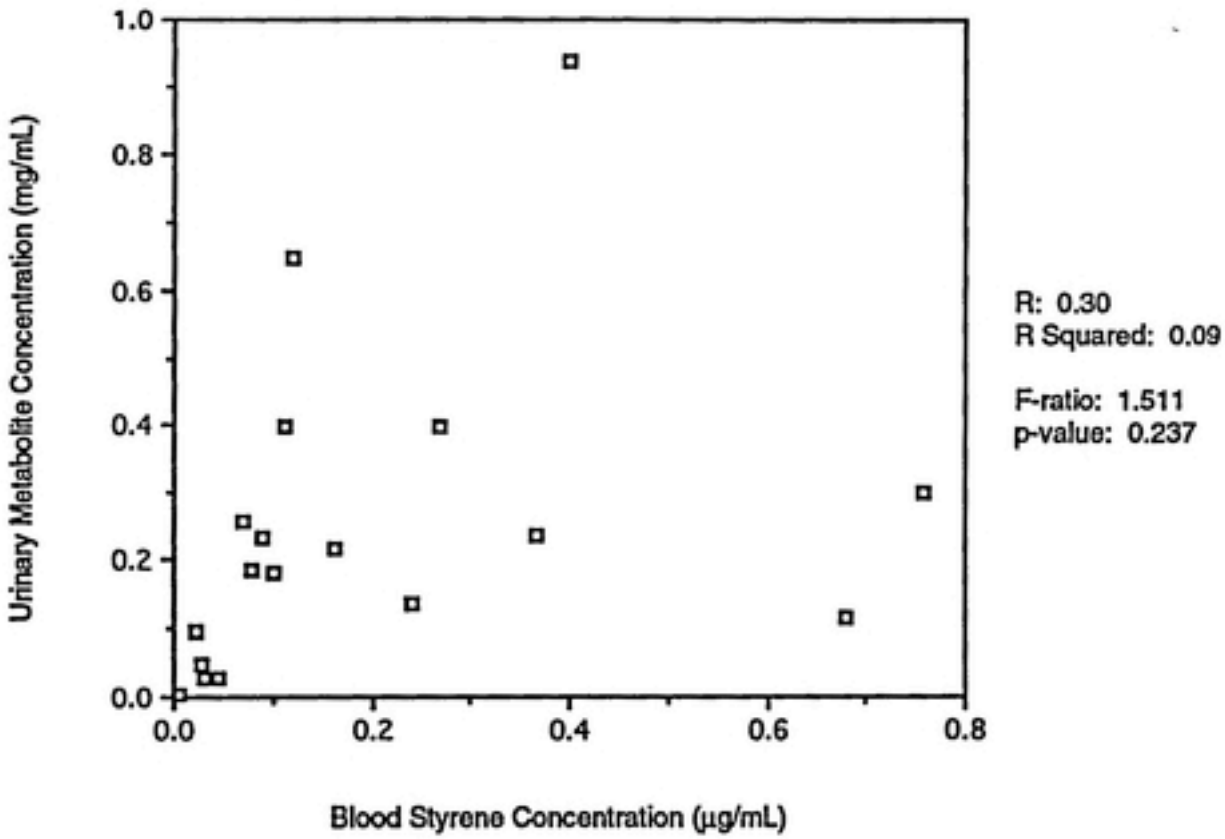

Figure 25. Blood Styrene Concentration VS Urinary Metabolite Concentration (Companies # 1 and 2 Replicates)

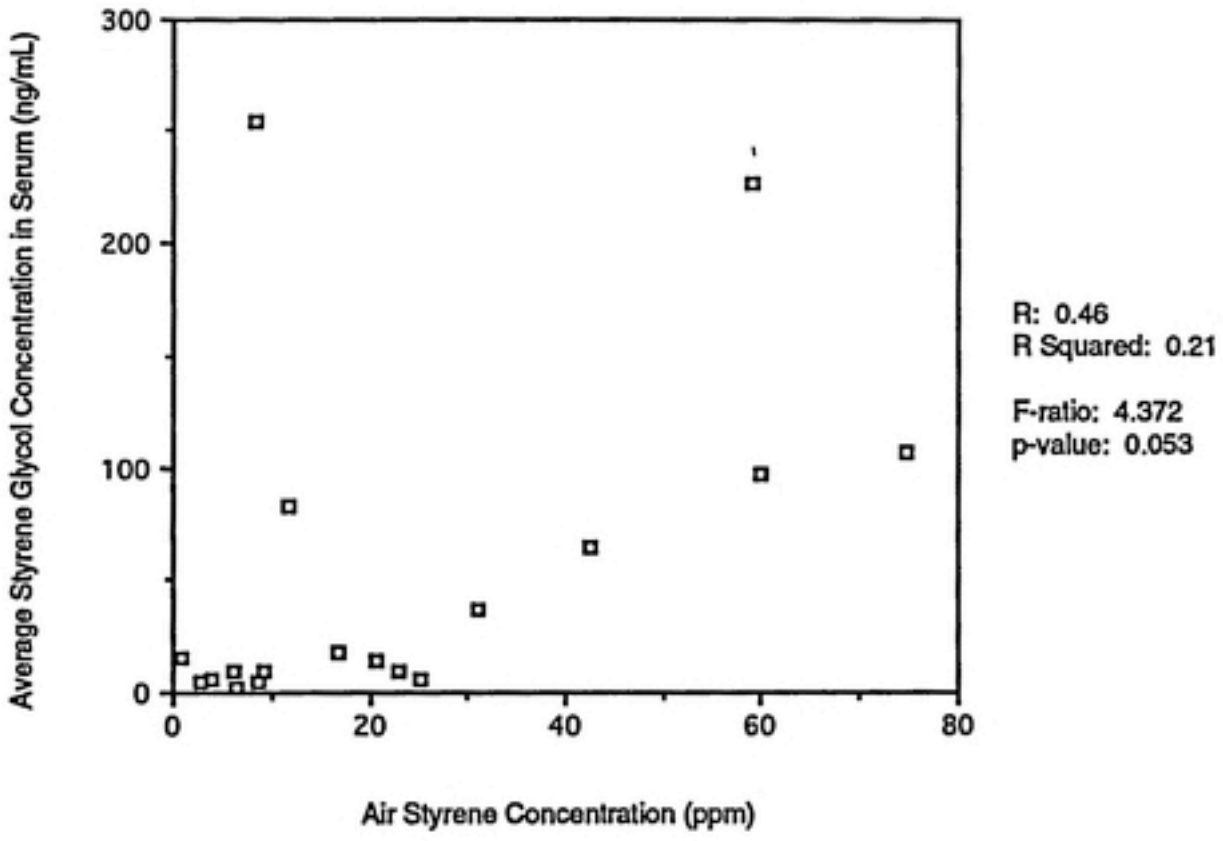

Figure 26. Air Styrene Concentration VS Average Plasma Styrene Glycol Concentration (Companies # 1 and 2 Replicates)

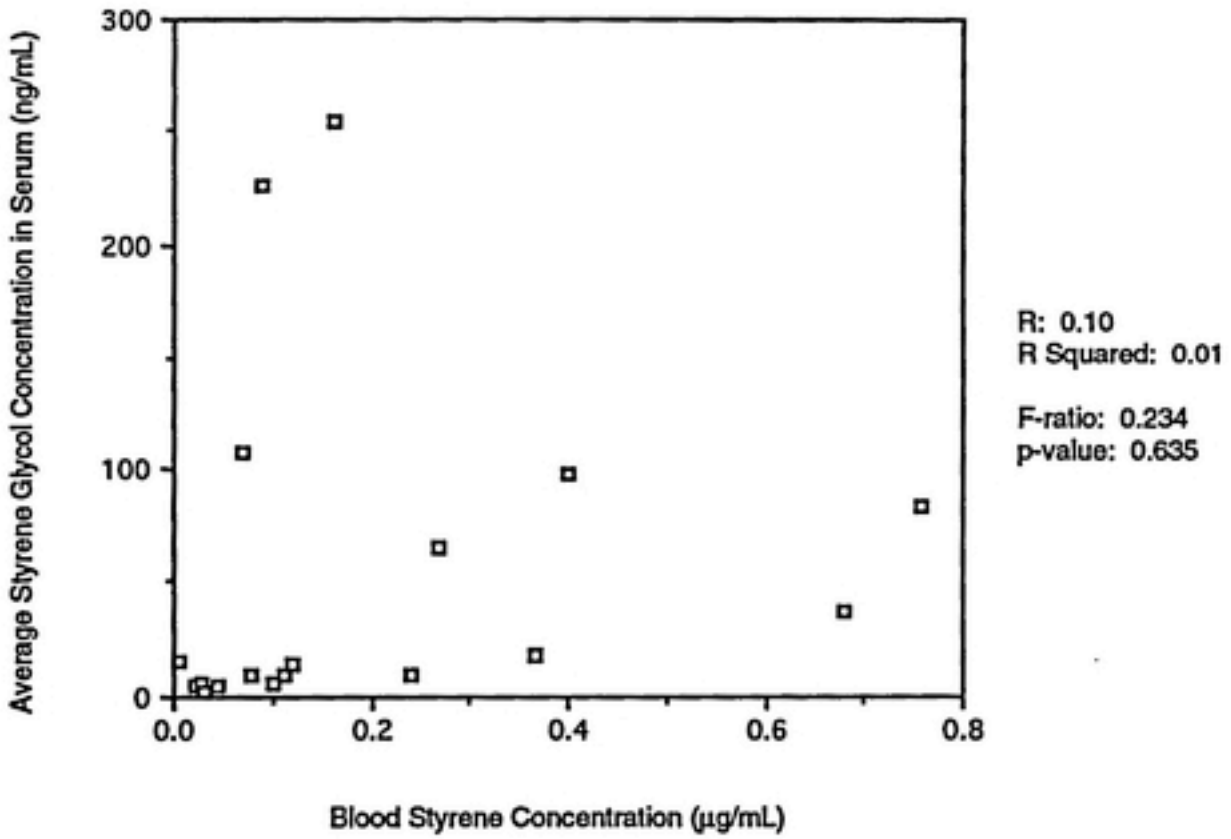

Figure 27. Blood Styrene Concentration VS Average Plasma Styrene Glycol Concentration (Companies # 1 and 2 Replicates)

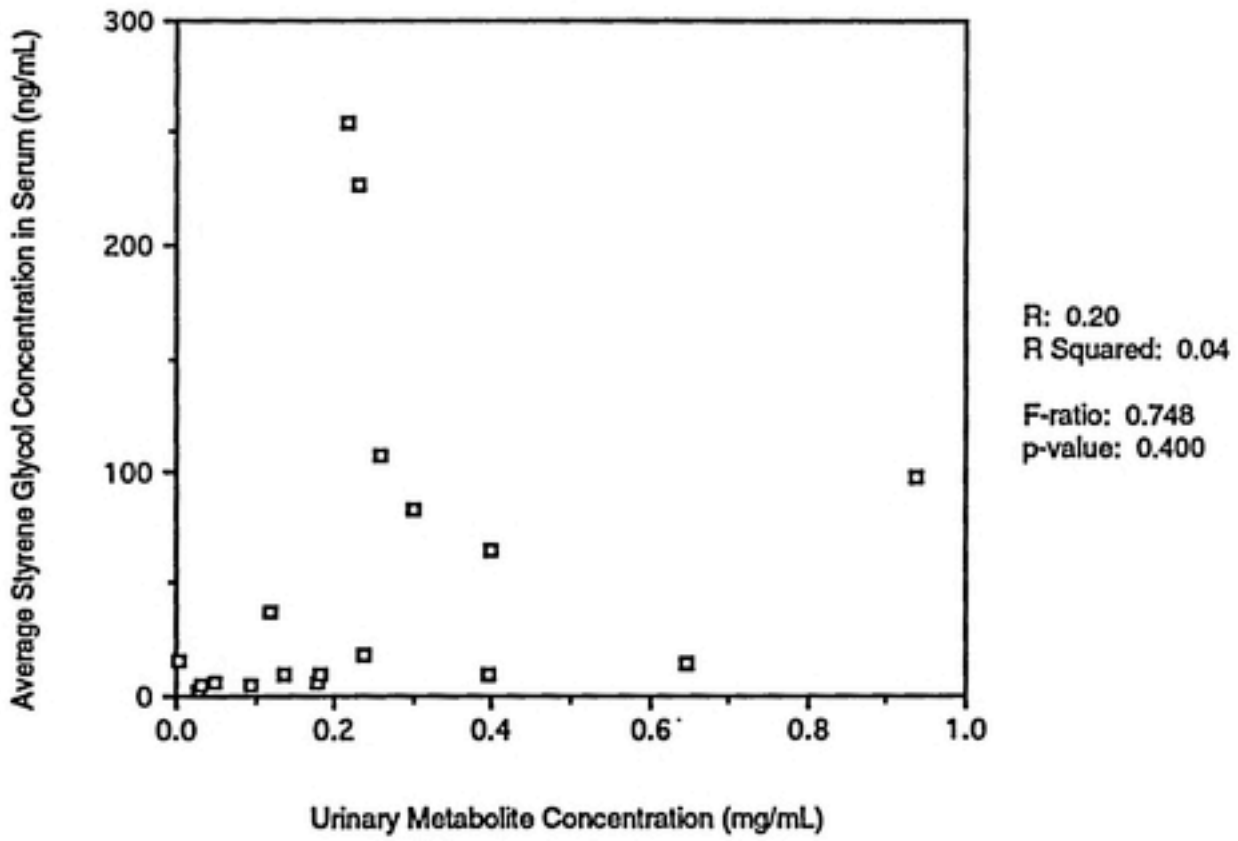

Figure 28. Urinary Metabolite Concentration VS Average Plasma Styrene Glycol Concentration (Companies # 1 and 2 Replicates)

4. General Discussion

Our statistical analysis indicates there are differences between Companies # 1, 2 and 3. Company # 3 was the only company which produced significant p-values in every regression model of the single observation analysis. The results from Companies # 1 and 2 varied.

Several hypotheses result from our findings. First, styrene exposure may not have been uniform during the time of sampling at Companies # 1 and 2. If this were the case, it would have been critical to note any changes which occurred (i.e. air concentrations and work practices) and to have performed blood and urine collections at the exact same time of day and day of the week at each Company. Second, as Companies # 1 and 2 do not produce exposure data which correlate well with themselves or experimental data but Company # 3 does, an explanation may be that improper sampling procedures were employed at Companies # 1 and 2. Another hypothesis is that several different individuals collected the data. This could possibly incorporate more error into the data due to various calibration techniques, various sampling procedures and human experimental error. Finally, it may be possible that the exposure data simply should not correlate well with each other. There is, to date, no large-scale documented study which demonstrates it should.

IV. CONCLUSIONS

Human exposure assessment via biological monitoring has gained considerable respect the past twenty years. It is now more commonplace to see regulatory agencies incorporating human exposure data into the lawmaking process. Therefore, it is essential that the greatest care be taken in the development of new biological monitoring methods.

This study supports the findings of Löf (1986), to some extent. Air and blood styrene and urinary metabolite concentrations may be correlated with free styrene glycol concentrations in human blood. Unfortunately, our study is limited by the variation observed in the exposure data obtained from Companies # 1, 2 and 3. Company # 3's exposure data correlated well with each other and with plasma styrene glycol concentration. Companies # 1 and 2 did not, in general, correlate well with each other or the experimentally obtained plasma styrene glycol concentration.

The successful development of methodology for the determination of free styrene glycol in plasma may prove to be a useful tool in future styrene exposure assessment. This method, however, should be re-evaluated in styrene-exposed populations in which the greatest care has been taken during the collection of exposure data. Only then may this method be considered valid for human exposure assessment.

IV. RECOMMENDATIONS

It is recommended our methodolgy be tested in another large-scale styrene-exposed population. It is vital that great care be taken during the collection of exposure data. In particular, if random replicate observations are made, they should be made in equal numbers at all companies investigated. Other suggestions would be: uniform calibration of instruments and in cases which are possible, the same investigators should conduct sampling and perform urinary metabolite analyses to reduce procedural error. Sampling should be well documented and any changes which occur during sample collection should be noted. Uniformity is critical.

If analysis of the plasma samples will not occur a short time after collection, it would be worthwhile to dose styrene-unexposed human plasma at known concentrations of styrene glycol on the day the sampling occurs, and then store the samples until the collected samples will be analysed. Analysis of the spiked styrene glycol samples would demonstrate the effect of time delay on the analysis of the collected samples.

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APPENDIX A

OLD COUNCIL TAPE
BOND
100% COTTON PAPER

Figure A-1. NMR of 3-phenyl-1,2-propanediol (allyl benzene glycol)

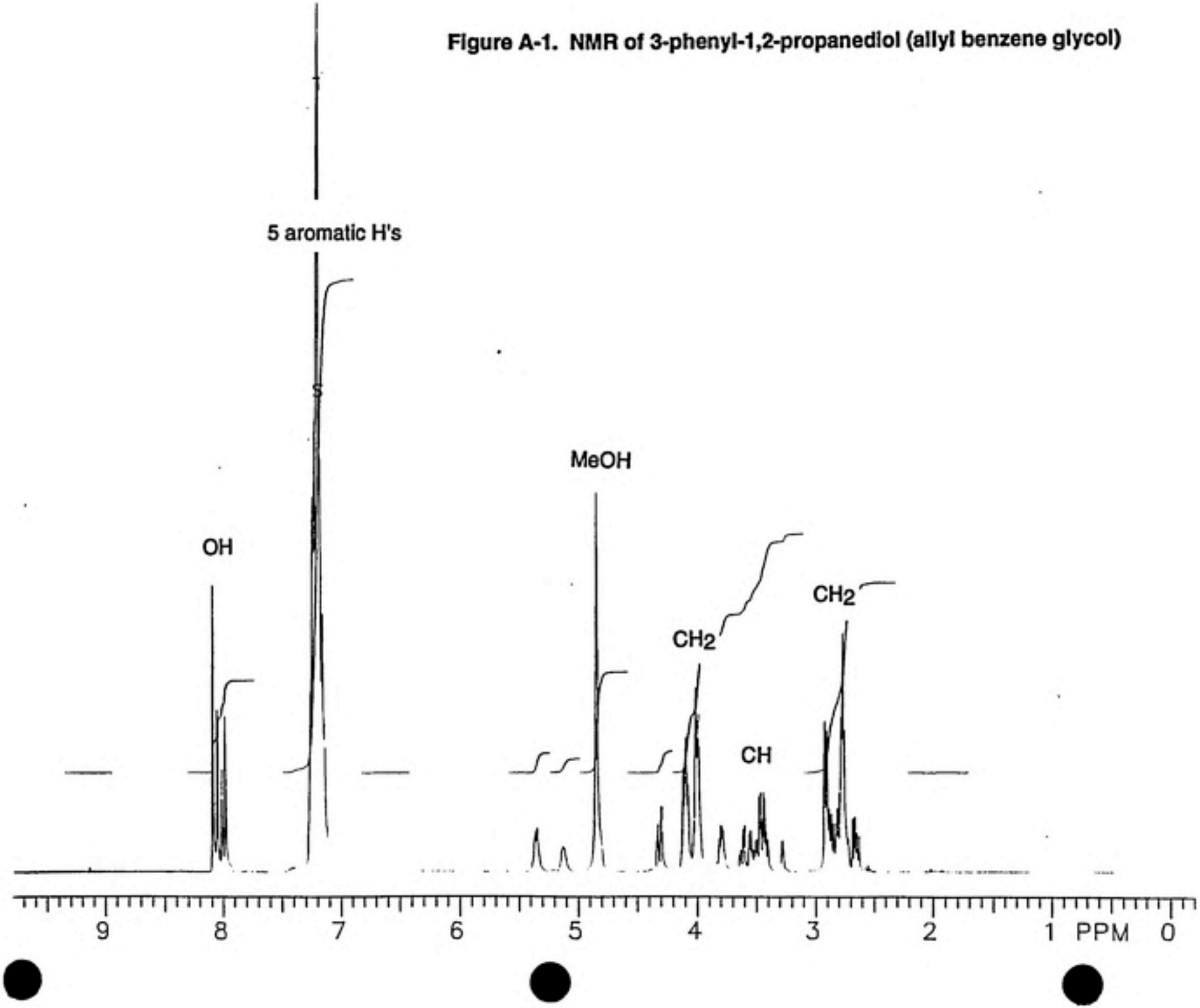

Figure A-2. Mass Spectrum of allyl benzene glycol derivative (ABG-PFB)

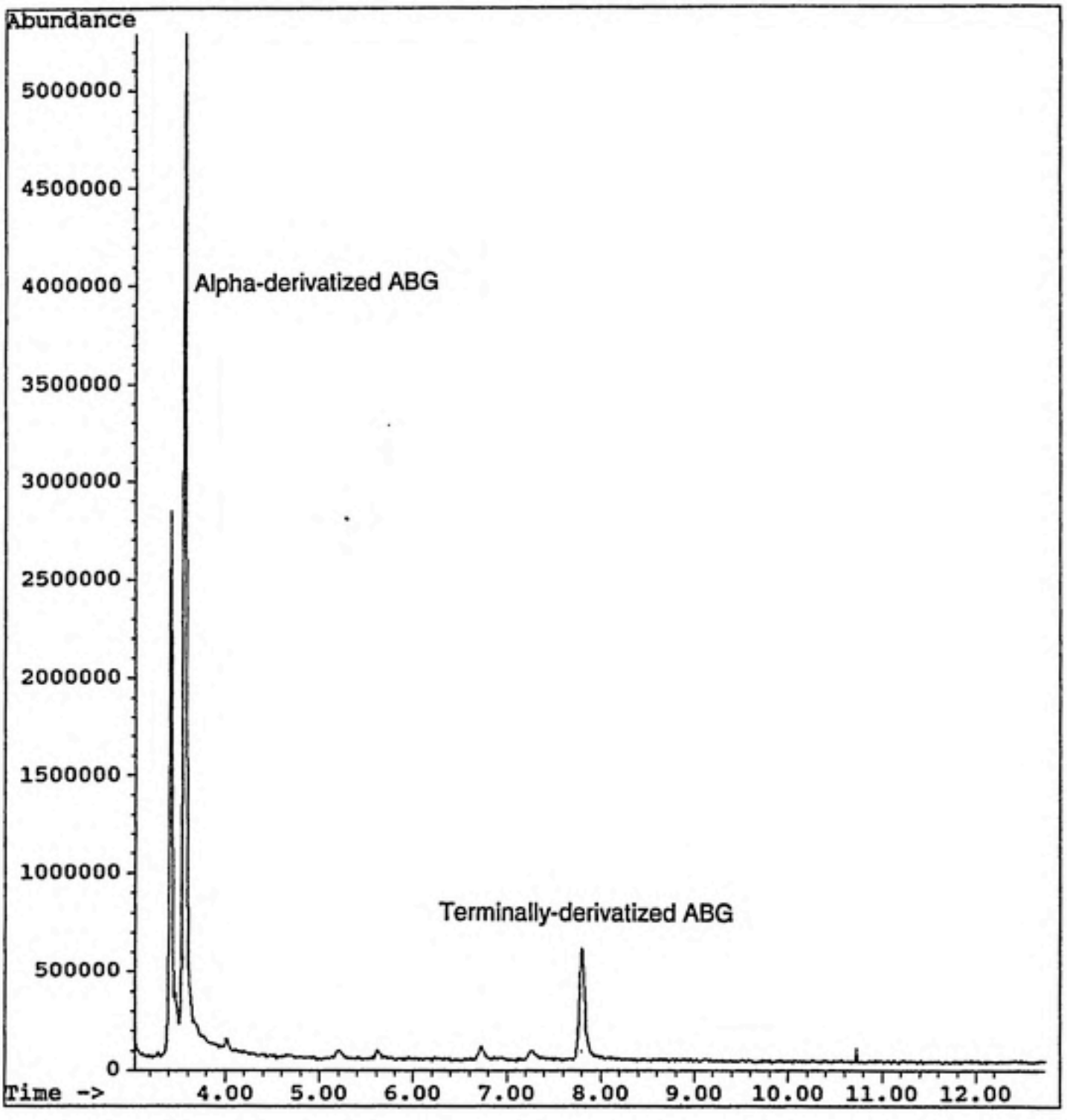

Figure A-3. Gas Chromatogram of Typical Plasma Sample

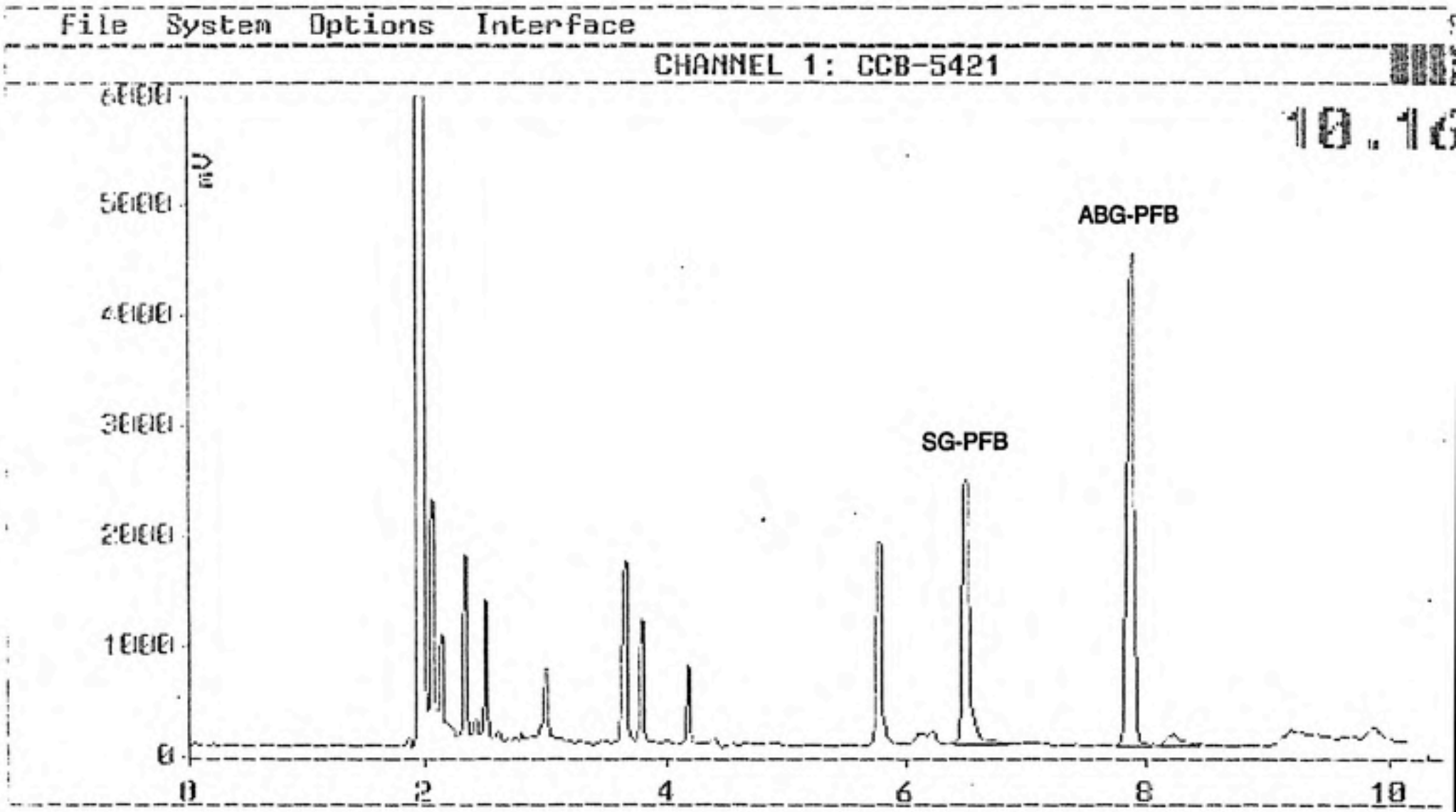

Figure A-4. Gas Chromatogram of Typical Reinforced Plastic Industry Worker Plasma Sample