Metabolism and Kinetics of Bisphenol A in Humans at Low Doses Following Oral Administration

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Bisphenol A is a widely used industrial chemical with many potential sources of human exposure. Bisphenol A is a weak estrogen and has been implicated as an "endocrine disruptor". This term is used for a variety of chemicals encountered in the environment which have estrogenic activity. It has been postulated that human exposure to these chemicals may elicit unwanted estrogenic effects in humans such as reduced fertility, altered development and cancer. Up to now the body burden of bisphenol A in humans is unknown. Therefore, we investigated the metabolism and toxicokinetics of bisphenol A in humans exposed to low doses since systemic bioavailability has a major influence on possible estrogenic effects in vivo. Human subjects (three males and three females, and four males for detailed description of blood kinetics) were administered d_{16}-bisphenol A (5 mg). Blood and urine samples were taken in intervals (up to 96 h), metabolites formed were identified by GC/MS and LC-MS/MS and quantified by GC/MS-NCI and LC-MS/MS. d_{16}-Bisphenol A glucuronide was the only metabolite of d_{16}-bisphenol A detected in urine and blood samples, and concentrations of free d_{16}-bisphenol A were below the limit of detection both in urine (6 nM) and blood samples (10 nM). d_{16}-Bisphenol A glucuronide was cleared from human blood and excreted with urine with terminal half-lives of less than 6 h; the applied doses were completely recovered in urine as d_{16}-bisphenol A glucuronide. Maximum blood levels of d_{16}-bisphenol A glucuronide (∼800 nM) were measured 80 min after oral administration of d_{16}-bisphenol A (5 mg). The obtained data indicate major species differences in the disposition of bisphenol A. Enterohepatic circulation of bisphenol A glucuronide in rats results in a slow rate of excretion, whereas bisphenol A is rapidly conjugated and excreted by humans due to the absence of enterohepatic circulation. The efficient glucuronidation of bisphenol A and the rapid excretion of the formed glucuronide result in a low body burden of the estrogenic bisphenol A in humans following oral absorption of low doses.

Introduction

Bisphenol A is a component of polycarbonate plastics and used for a variety of applications such as dental composite fillings and food-can linings. In rats, the weak estrogenicity of bisphenol A, as measured by special protocols, has been known for decades (*1*, *2*). Recently, scientific and public attention has been focused on chemicals which may mimic endogenous hormone action and thus interfere with endocrine function (*3*−*6*). These chemicals, which include certain pesticides and plasticizers, are designated as "endocrine disruptors" and human exposure to these chemicals from the environment is sometimes postulated to alter normal hormonal function in humans and wildlife (*5*, *7*). Suspected effects of modified endocrine functions may be reduced fertility, altered development, and cancer in estrogen-sensitive tissues. The interest in endocrine disruptors has led to intensified studies on estrogenic effects of bisphenol A due to widespread potential human exposure. In vitro, bisphenol A exhibits weak estrogenic activity (compared to 17β-estradiol) by binding and activating estrogen receptors (*8*, *9*). In rats, high oral doses of bisphenol A (50−1 000 mg/kg) are required to induce estrogen dependent responses (*1*, *2*, *10*, *11*). However, the estrogenicity of bisphenol A in vivo is dependent on the route of administration. Subcutaneous administration of bisphenol A resulted in effects at substantially lower doses as compared to oral or intraperitoneal administration, suggesting that differences in bioavailability may be responsible for the different responses (*12*, *13*). Indeed, significant differences in bioavailability and biotransformation of bisphenol A in rats, as shown by various studies, are dependent on the route of administration. Oral bioavailability of bisphenol A is low and intensive biotransformation occurs in the liver resulting in the formation of bisphenol A glucuronide, which is devoid of estrogenicity (*14*−*16*).

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Table 1. Characterization of Human Subjects Participating in the Study

subject	gender	age	height (cm)	body weight (kg)	administration of
A	female	29	165	57	d_{16}-bisphenol A
B	female	24	175	63	d_{16}-bisphenol A
C	female	31	162	62	d_{16}-bisphenol A
E	male	29	184	82	d_{16}-bisphenol A
F	male	30	175	73	d_{16}-bisphenol A
G	male	28	176	63	d_{16}-bisphenol A
M	male	47	188	83	d_{16}-bisphenol A
N	male	54	182	92	d_{16}-bisphenol A
O	male	39	179	74	d_{16}-bisphenol A

In this study, we have investigated the toxicokinetics and biotransformation of d_{16}-bisphenol A in humans after administration of low doses to obtain data on bioavailability and biotransformation in humans for integration into the risk assessment process for bisphenol A. d_{16}-Bisphenol A was used to avoid interferences with background concentrations of bisphenol A due to dietary exposures as well as contamination of reagents and materials with bisphenol A (*17*−*20*).

Material and Methods

Chemicals. d_{16}-Bisphenol A, bisphenol A, bis-(4-hydroxyphenyl)-propane, and *N*-methyl-bis-trifluoroacetamide were obtained from Sigma-Aldrich (Deisenhofen, Germany). All other reagents and solvents were reagent grade or better and obtained from several commercial suppliers. β-Glucuronidase/sulfatase from helix pomatia (EC. 3.2.1.31) was obtained from Sigma-Aldrich. All GC-columns were obtained from J&W scientific (Folsom, CA). d_{16}-Bisphenol A glucuronide or bisphenol A glucuronide as standards for calibration solutions were isolated from the urine of two male rats after oral administration of 100 mg/kg d_{16}-bisphenol A or 100 mg/kg bisphenol A by preparative HPLC. Urine samples were collected for 48 h after administration in 12 h intervals on ice. To isolate the glucuronides, urine samples (20 μL) were separated using a 250 mm × 4 mm i.d. steel column filled with Partisil ODS-III (Knauer, Stuttgart, Germany). Separation was performed using a linear gradient from 100% water (adjusted to pH 2 with trifluoroacetic acid) to 20% water in methanol over 40 min with a flow rate of 1 mL/min. The eluates were monitored by a HP 1040 diode array detector (Agilent, Waldbronn, Germany) at 220 nm, and the eluting glucuronides (retention times 22 min) were collected in cooled flasks. Usually, 5−10 chromatographic runs were performed to isolate sufficient amounts (about 70 μM) of the standards. From the collected material, the solvent was removed by lyophilization, and the obtained residues were dissolved in methanol. d_{16}-Bisphenol A and bisphenol A glucuronide contents of these solutions were determined by HPLC as described above using calibration curves obtained with d_{16}-bisphenol A or bisphenol A due to identical UV-absorption. In addition, d_{16}-bisphenol A and bisphenol A content was determined after glucuronidase treatment. Identities of the glucuronides were confirmed by LC-MS/MS (see below for details).

Exposure of Human Subjects to d_{16}-Bisphenol A. d_{16}-Bisphenol A (5 mg/person) was orally administered in a hard gelatine capsule (size 0, volume 0.67 mL, Pharmapohl GmbH, Dägeling, Germany) to three healthy female (A, B, C, Table 1) and three healthy male subjects (E, F, G, Table 1). Detailed kinetics of d_{16}-bisphenol A in blood were determined in four male subjects (G, M, N, O, Table 1). All subjects enlisted in the study had to refrain from alcoholic beverages and medicinal drugs 2 days before and throughout the experiment. Subjects did not abuse alcohol and were nonsmokers or occasional smokers. Subjects were healthy as judged by medical examination and clinical blood chemistry. The study was carried out according to the Declaration of Helsinki, after approval by the Regional Ethical Committee of the University of Würzburg, Germany, and after written informed consent by the volunteers.

Urine samples from the subjects were collected in 6 h intervals. After urine volume was determined, two aliquots (50 mL each) were stored at −20 °C. Blood samples (10 mL) were also taken in defined intervals by the supervising physician with heparinized syringes and immediately centrifuged for 5 min at 1500*g* to separate erythrocytes and plasma. Plasma samples were immediately transferred to a freezer set at − 20 °C and stored at −20 °C until further sample preparation.

Sample Preparation for Gas Chromatography/Mass Spectrometry. Plasma (1 mL) or urine (250 μL to 1 mL) were fortified with 100 pmol of bis-(4-hydroxyphenyl)-methane (dissolved in 5 μL of methanol) as internal standard, 5 mL of sodium acetate buffer (0.2 M, pH 4.7), and 2200 units of β-glucuronidase. The resulting mixture was incubated for 18 h at 37 °C and then applied to a preconditioned SPE-cartridge (Chromabond C18, Macherey-Nagel, Düren, Germany). The cartridge was eluted with 1.5 mL of methanol, the eluate was dried under reduced pressure, and the obtained residue was treated with 250 μL of *N*-methyl-bis-trifluoroacetamide. After 30 min at 80 °C, this solution was extracted twice with 250 μL of *n*-hexane. The *n*-hexane phases were combined and 2 μL from the combined phases were injected into the GC/MS.

Gas Chromatography/Mass Spectrometry. GC-MS analysis was performed on a MD 800 mass spectrometer equipped with a Carlo Erba 8000 series GC (Fisons Instruments, Mainz, Germany). For separations, DB-5MS (J & W Scientific, Folsom, CA) fused silica capillary columns (30 m, 0.25 mm i.d., 0.25 μm film thickness) and helium (average linear velocity 35 cm/min) as carrier gas were used. Splitless injections were made (valve time 1.0 min), and a temperature gradient starting at an oven temperature of 80−300 °C with a heating rate of 20 °C/min was used for separation. The transfer line was kept at 290 °C. Injector temperature was 280 °C, and the electron source of the mass spectrometer was adjusted to 170° in the chemical ionization mode with methane (1 Torr) as reactant gas. Solvent delay was 8 min. For all quantitations in the selected ion mode, dwell times of 0.12 s, an interchannel delay of 0.01 s, and a span of one mass unit were used. During the separation, ions *m*/*z* 337 (representative of bis-trifluoroacetyl d_{16}-bisphenol A) and 295 (representative of bis-trifluoroacetyl-4,4′-dihydroxyphenyl methane) were monitored. Calibration curves were constructed from plasma or urine samples collected from the subjects before the exposure after addition of known amounts of d_{16}-bisphenol A (0−200 nM for plasma and 125−3000 nM for urine samples) and internal standard. Calibration curves were recorded before every serial sample analysis. Curves were calculated from seven (urine) or five (plasma) data points using Microsoft Excel spreadsheets ($r^2 > 0.99$). Intraassay variability was below 20%.

Liquid Chromatography/Mass Spectrometry. To quantify d_{16}-bisphenol A glucuronide, 250 μL of plasma samples from the subjects, 5 μL of a solution of the internal standard (5.6 μg/mL bisphenol A glucuronide in MeOH), and 250 μL of MeOH were mixed thoroughly, and the samples were centrifuged for 4 min at 15000*g*. The supernatants were placed on ice for 30 min, 200 μL of acetonitrile were added followed by centrifugation for 4 min at 15000*g*. The obtained supernatants were then directly injected into the LC-MS/MS system. Urine samples (10 μL) were diluted with 990 μL of water and internal standard (5 μL of methanol containing 5.6 μg/mL bisphenol A glucuronide) was added.

To record mass spectra, 10 μL of the samples were injected on a Nucleosil RP18 HPLC column (2.0 mm × 150 mm; 5 μm, 100 A; Phenomenex, Aschaffenburg, Germany) using an Agilent 1100 autosampler and an Agilent 1100 HPLC-pump (Agilent, Waldbronn, Germany). The samples were separated by gradient elution with water (solvent A) and MeOH and 2 mM NH_4Ac (solvent B) using the following conditions: 70% A, isocratic for 2.5 min, linear increase to 50% A within 4.5 min, then linear increase to 70% A within 1 min at a flow rate of 200 μL/min.

The HPLC system was directly coupled to a triple stage quadrupole mass spectrometer (API 3000, Applied Biosystems, Langen, Germany) equipped with a TurboIonSpray source. Analytes were detected in the negative-ion mode at a vaporizer temperature of 400 °C and a TurboIonSpray voltage of −4.0 kV. Declustering potential was −36 V and collision energy was −24 V for both transitions. Spectral data were recorded with N_2 as collision gas (CAD = 4) in the multiple reaction monitoring (MRM) mode monitoring the following MS-MS ion-transitions: 417.2 to 113.0 (d_{16}-bisphenol A glucuronide) and 403.2 to 113.2 (BPA-glucuronide as internal standard). Quantitation of d_{16}-bisphenol A glucuronide was based on calibration curves obtained after addition of known amounts of d_{16}-bisphenol A glucuronide (0−0.7 mM) to urine and plasma samples from the subjects collected before the exposure. Calibration curves were constructed from seven data points using Microsoft Excel ($r^2 > 0.99$). Intra assay variability was below 20%.

For identification of the potential metabolites 4-hydroxy-d_{15}-bisphenol A glucuronide, d_{16}-bisphenol A sulfate, and hydroxy-d_{15}-bisphenol A sulfate, MS-MS ion transitions were adapted from Elsby et al. (*21*). After adjustment for deuterium content, MS-MS ion transitions 432.2 to 113.2 (hydroxy-d_{16}-bisphenol A glucuronide), 336.0 to 256.0 (d_{16}-bisphenol A sulfate), and 321.1 to 241.1 (hydroxy-d_{16}-bisphenol A sulfate) were monitored during separation of urine and blood samples.

For detection of d_{16}-bisphenol A and d_{16}-bisphenol A glucuronide by LC-MS/MS within a single separation, the following HPLC gradient formed with solvent A (H_2O) and B (MeOH) was used: 80% A linear to 30% A within 15 min, isocratic for 5 min, then linear to 80% A within 2 min, and isocratic for 3 min with a flow rate of 250 μL. The sample workup and the parameters for data aquisition were identical to those described above. The MS-MS ion-transitions 417.2 to 113.0 (d_{16}-bisphenol A glucuronide) and 241.1 to 223.3 (d_{16}-bisphenol A) were monitored in the MRM mode.

Results

As a preparation for the study, a highly sensitive GC/MS method was developed to determine bisphenol A in urine and blood samples in very low concentrations. When this method was used to quantify bisphenol A content from "unexposed" individuals, a small background of bisphenol A (up to 50 nM) was detected in all urine and blood samples analyzed. Identical background concentrations as seen in the human blood and urine samples were also observed when water samples purified by a Millipore Water purification system were subjected to the workup procedures. These observations indicate that small concentrations of bisphenol A may leach from plastic materials used in the extraction process, from GC septa or may be present in some of the reagents used. Similar background concentrations of bisphenol A were also detected in plasma, urine and water samples using LC-MS/MS. Therefore, to avoid an interference with quantitation in controlled human exposure studies, d_{16}-bisphenol A was used to study biotransformation and toxicokinetics in human subjects. A kinetic isotope effect was considered unlikely since oxidative metabolism of bisphenol A with cleavage of a carbon−hydrogen (respectively carbon−deuterium) bond has not been reported as a significant metabolic step for bisphenol A in rat liver microsomes, rat hepatocytes and in human liver microsomes (*14*, *15*, *21*).

Metabolite Identification. To identify possible urinary metabolites of d_{16}-bisphenol A, urine samples were analyzed by LC-MS/MS using multiple reaction monitoring and by GC/MS with chemical ionization. In LC-MS/MS, the only metabolite detected showed a full scan mass

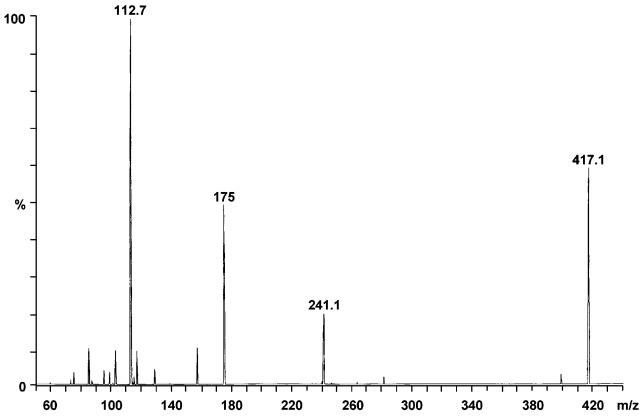

Figure 1. Product ion spectrum obtained from d_{16}-bisphenol A glucuronide (*m/z* 417; $[M - H]^-$) after collision with N_2.

spectrum (Figure 1) with an intense deprotonated molecular ion $[M - H]^-$ (*m/z* 417) consistent with the molecular ion of d_{16}-bisphenol A glucuronide. Moreover, collision induced fragmentation resulted in the formation of characteristic fragments with *m/z* 241 (M − H glucuronic acid moiety), 175 (M − H-d_{16} bisphenol A moiety), and 113 (M − H d_{16}-bisphenol A-CO_2−H_2O moiety) from the molecular ion (Figure 1). The observed fragmentation pattern is consistent with this metabolite being d_{16}-bisphenol A glucuronide. Analyzing urine or blood samples in the MRM mode using previously published MS/MS spectra (after correction for deuterium content) for reported minor metabolites (*21*) formed from bisphenol A in rat hepatocytes (d_{16}-bisphenol A sulfate, hydroxy-d_{15}-bisphenol A sulfate) did not result in the detection of any of these compounds in urine or plasma samples from individuals administered d_{16}-bisphenol A. Using the MRM-mode to detect the presence of d_{16}-bisphenol A glucuronide in urine and blood samples from the individuals clearly showed the presence of intense signals for d_{16}-bisphenol A glucuronide in urine (Figure 2A) and blood samples (Figure 2B) taken after giving d_{16}-bisphenol A. Peaks indicating the presence of d_{16}-bisphenol A glucuronide were not present in blood samples taken before administration of d_{16}-bisphenol A (data not shown).

GC/MS analyses of urine and blood samples for free d_{16}-bisphenol A did not give consistent results when identical samples were repeatedly analyzed and only small amounts of d_{16}-bisphenol A were found in samples analyzed without prior glucuronidase treatment. Concentrations of d_{16}-bisphenol A were significantly increased and could be reproducibly quantified in the samples only when these were treated with β-glucuronidase/sulfatase. Likely, some of the d_{16}-bisphenol A glucuronide decomposed during sample processing or in the heated injector of the GC. Separation of extracts from urine and blood samples after glucuronidase treatment and derivatization for GC/MS clearly showed the presence of characteristic fragments of O,O′-bis-trifluoroacetyl-d_{16}-bisphenol A in urine (Figure 3A) and plasma (Figure 3B) samples taken after administration of d_{16}-bisphenol A. These peaks were not observed in urine and plasma samples collected before administration of d_{16}-bisphenol A (data not shown).

A separate quantitation of bisphenol A glucuronide and bisphenol A is considered necessary for use in the risk assessment process. Bisphenol A glucuronide does not exert hormonal activity (*15*, *16*), and only unconjugated

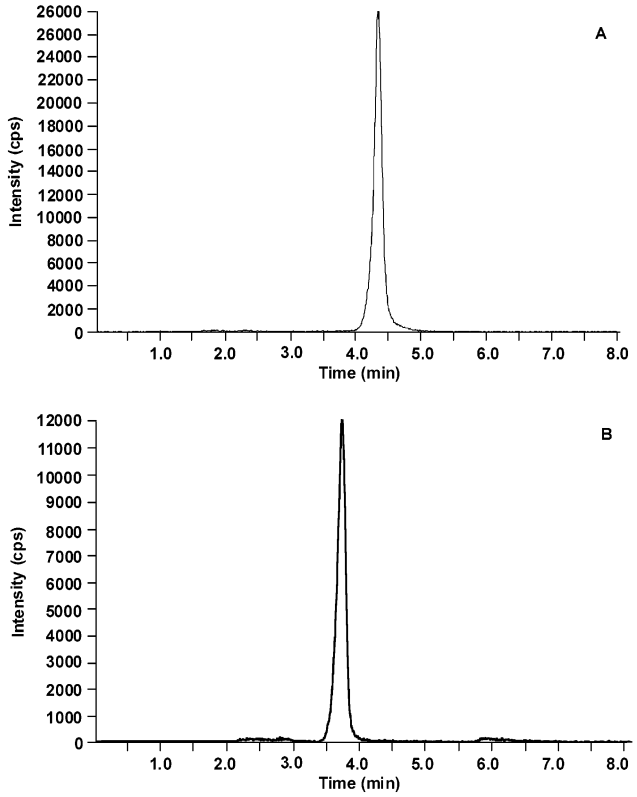

Figure 2. Intensity of MRM 417 to 113 in an urine (A) and a plasma (B) sample of a human subject 6 h after oral application of 5 mg d_{16}-bisphenol A. The peaks in both panels were not present in urine or plasma samples taken before administration of d_{16}-bisphenol A.

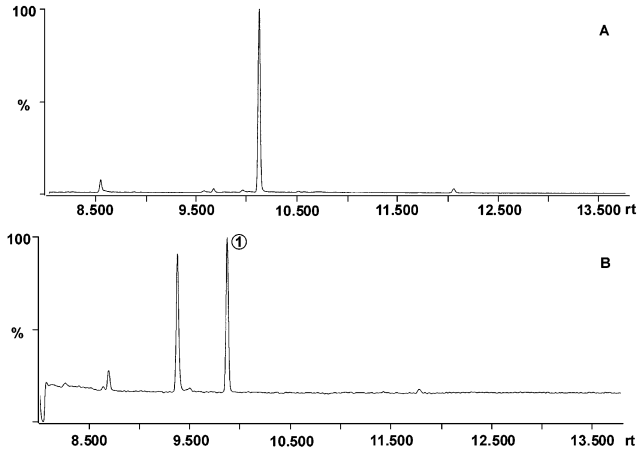

Figure 3. GC/MS separation of d_{16}-bisphenol A (*m*/*z* 337) after glucuronidase cleavage and derivatization with *N*-methyl-bis-trifluoroacetamide, in an urine (A) and a plasma sample (B) from a human subject after oral application of 5 mg of d_{16}-bisphenol A. The identity of the peak at 9.3 min in the chromatogram of the urine sample is not known; however, this peak was also present in urine samples taken before administration of d_{16}-bisphenol A. The peaks representing d_{16}-bisphenol A (retention times 9.4−9.6 min) were not present in urine and plasma samples taken before the exposures.

bisphenol A is therefore expected to interact with the estrogen receptor and possibly cause hormonal effects. Therefore, methods were developed to detect and quantify free d_{16}-bisphenol A in the presence of d_{16}-bisphenol A glucuronide in urine and blood samples without cleaving the glucuronide. For this purpose, only liquid chromatography could be used since a minor extent of decomposition of d_{16}-bisphenol A glucuronide to d_{16}-bisphenol

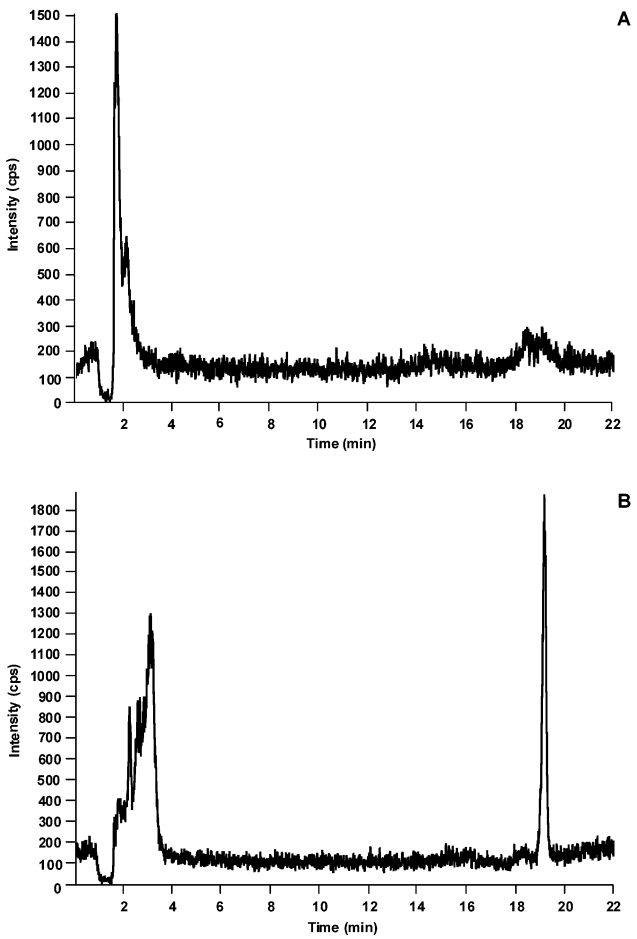

Figure 4. Intensity of MRM 241−223 representing d_{16}-bisphenol A in a plasma sample of a human volunteer obtained 80 min after oral application of 5 mg of d_{16}-bisphenol A (A). (B) Same plasma sample spiked with d_{16}-bisphenol A (68 nM).

A was always observed when derivatization procedures for gas chromatography were applied to mixtures of d_{16}-bisphenol A glucuronide and d_{16}-bisphenol A. A highly sensitive LC-MS/MS method was developed to detect free d_{16}-bisphenol A in urine and plasma. The detection limit of the method was 10 nM d_{16}-bisphenol A in plasma and 6 nM in urine. However, this method was unable to detect free d_{16}-bisphenol A in plasma or urine samples after oral administration of d_{16}-bisphenol A even in samples containing very high concentrations of d_{16}-bisphenol A glucuronide (Figure 4A), but clearly showed the presence of free d_{16}-bisphenol A when a plasma sample was spiked with low concentrations of d_{16}-bisphenol A (Figure 4B).

Metabolite Quantitation. Samples were taken after oral administration of 5 mg of d_{16}-bisphenol A to determine kinetics of elimination from blood and with urine to assess recovery in three male and three female subjects. The time courses of blood and urine concentrations of d_{16}-bisphenol A glucuronide in these subjects are shown in Figure 5. d_{16}-Bisphenol A glucuronide was rapidly cleared from blood with a terminal half-life of 5.3 h in both male and female subjects after oral administration of 5 mg of d_{16}-bisphenol A; concentrations of d_{16}-bisphenol A glucuronide in plasma and urine reached the limit of detection 24−34 h after d_{16}-bisphenol A administration. In the subjects investigated, the administered dose of d_{16}-bisphenol A was completely (118 ± 21%) recovered in urine; half-life of urinary elimination was 5.4 h.

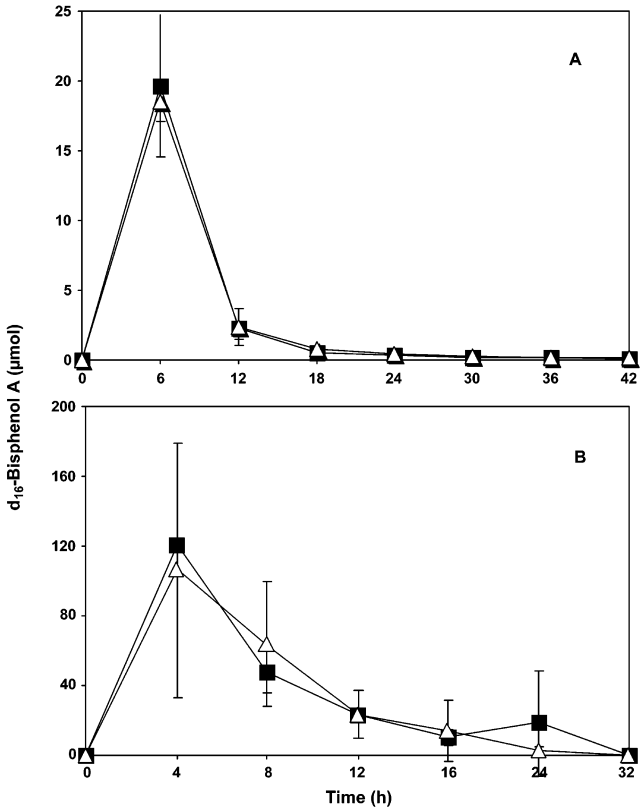

Figure 5. Urinary excretion of d_{16}-bisphenol A glucuronide (A) and elimination of d_{16}-bisphenol A glucuronide from blood (B) in three male (■) and three female (△) human subjects after oral administration of 5 mg of d_{16}-bisphenol A.

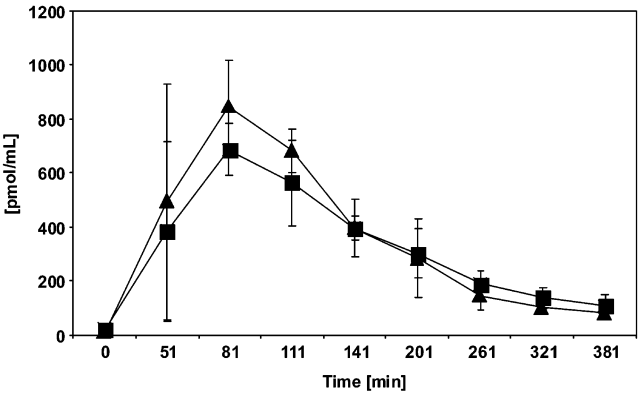

Figure 6. Blood concentrations of d_{16}-bisphenol A glucuronide [▲, determined by LC-MS/MS (mean ± SD)] and total d_{16}-bisphenol A (■, determined by GC/MS after glucuronidase treatment) in four male subjects after oral adminstration of 5 mg of d_{16}-bisphenol A.

For a better definition of the invasion and distribution kinetics of d_{16}-bisphenol A after oral administration, a second experiment was performed in four male subjects taking blood samples in short intervals. d_{16}-Bisphenol A glucuronide in these samples was quantified by LC-MS/MS and total d_{16}-bisphenol A was quantified by GC/MS after glucuronidase treatment. The results from this study show that maximal plasma concentrations of d_{16}-bisphenol A were reached approximately 80 min after oral administration; afterward, plasma concentrations rapidly declined with an initial half-life of 89 min (Figure 6). Free d_{16}-bisphenol A was not detected in any of the plasma samples. The content of d_{16}-bisphenol A after glucuronidase treatment determined by GC/MS and the content of d_{16}-bisphenol A glucuronide determined by LC-MS/MS was practically identical further confirming the precision of the two methods used and the presence of only very low concentrations of free d_{16}-bisphenol A (Figure 6).

Discussion

This study investigated the toxicokinetics and biotransformation of d_{16}-bisphenol A in human subjects after administration of low doses. The selected doses (5 mg/person or 54−90 µg/kg bodyweight) are 10-fold higher than worst case estimates for daily indirect human exposures to bisphenol A (estimated as up to 0.6 mg/day). This exposure is due to the consumption of wine (bisphenol A concentrations estimated as up to 650 ppb) and canned food (up to 100 ppb) contaminated with bisphenol A by migration of bisphenol A from epoxy resins used in food and wine storage containers (*17*, *19*, *22*). The obtained results show that, after administration of low doses, bisphenol A is rapidly absorbed from the gastrointestinal tract in humans. Conjugation with glucuronic acid in the liver in an efficient first-pass-metabolism is practically complete and the formed glucuronide is rapidly cleared from blood by elimination with urine. Elimination of orally administered bisphenol A is essentially complete within 24 h after administration.

Bisphenol A glucuronide is also the major metabolite of bisphenol A formed in rodents in vivo, in the isolated perfused rat liver and in human liver microsomes (*14*, *15*, *21*, *23*). The other minor metabolites of bisphenol A described in the literature are only formed when higher doses or concentrations of bisphenol A were applied likely resulting in a saturation of the glucuronidation pathway. The results of this study show that after oral application of low and environmentally relevant doses of d_{16}-bisphenol A in humans, only a very small percentage of the dose of bisphenol A is available for other biotransformation pathways, due to the rapid glucuronidation.

The human toxicokinetics of bisphenol A described in this paper are different from bisphenol A toxicokinetics in rats observed at 150−1500-fold higher doses. In rats, bisphenol A undergoes enterohepatic circulation resulting in a retention and possibly in higher concentrations of free bisphenol A in blood over time (*14*). In humans, the rapid and complete excretion of bisphenol A as the glucuronide with urine suggests that enterohepatic circulation does not occur. The absence of enterohepatic circulation of bisphenol A glucuronide is most likely due to a higher threshold for biliary elimination in humans as compared to rats (*24*, *25*). The molecular weight of bisphenol A glucuronide is 404 Da (d_{16}-bisphenol A glucuronide, 418 Da), and thus above the threshold for biliary elimination (350 Da) in rats, but below the threshold for biliary elimination in humans (550 Da). Therefore, bisphenol A glucuronide formed in the liver is delivered to the blood stream in humans to reach the kidney. Due to high water solubility, the glucuronide is rapidly excreted by the kidneys with urine. In rats, bisphenol A glucuronide formed in the liver is transported into bile and may thus undergo enterohepatic circulation resulting in delayed elimination.

On the basis of the glucuronidation rates measured in microsomes (*21*), a more than 10-fold faster glucuronidation in rats than in humans may be expected. However, following oral administration of bisphenol A, the rate of

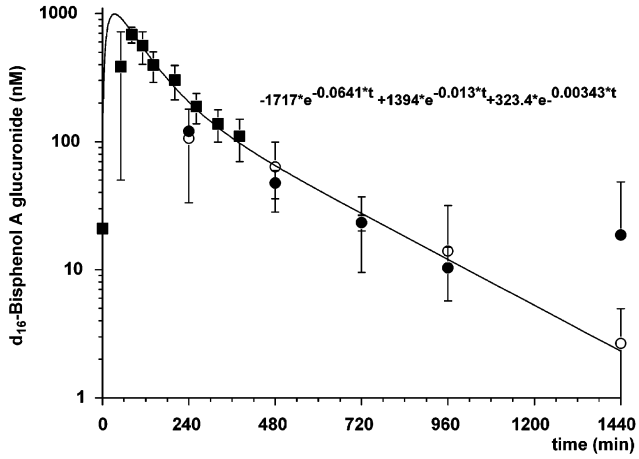

Figure 7. Concentration−time course of d_{16}-bisphenol A glucuronide in venous blood of volunteers following oral intake of 5 mg of d_{16}-bisphenol A. The data are identical with those presented in Figures 5B and 6. (■), males (from Figure 6); (●), males and (○), females (from Figure 5B); solid line, model fit to the data.

glucuronidation is limited by the rate of the transport of bisphenol A from the gastrointestinal tract into the central blood stream and the liver. Obviously, even a lower glucuronidation rate in human liver is sufficient to result in practically complete conversion of bisphenol A to its glucuronide during the first pass.

A semilogarithmic plot (Figure 7) of the combined data showing concentration−time courses of d_{16}-bisphenol A glucuronide in plasma (Figures 5 and 6) demonstrates kinetics which can be described by a two-compartment model (*26*). It consists of a central compartment representing the plasma with a volume of 3.15 L and a peripheral compartment. Elimination was allocated into the central compartment. All processes were assumed to underline first-order kinetics. The model delivers an excellent fit to the data (Figure 7). After oral uptake of d_{16}-bisphenol A, the polar glucuronide appears in blood and reaches a (predicted) maximum concentration of 990 nM after 33 min. Thereafter, the concentration−time course is approximated by two exponentials: the first term with a half-life of 89 min describes the decline after reaching the maximum. Then, the slope flattens over time approaching a terminal phase with a half-life of 202 min. Both phases are governed by the distribution together with the elimination process. The model yields half-lives of 53 min for the uptake into the central compartment (plasma) and of 17 min for the elimination from this compartment. The uptake summarizes three processes: the uptake of unmetabolized bisphenol A from the gastrointestinal tract into the liver, its glucuronidation and the delivery of this metabolite into the plasma compartment. The clearance of elimination from plasma amounts to 0.13 L/min, which approximates the creatinine clearance of a healthy adult (0.12 L/min, (*26*). Using the urinary excretion data measured between 0 and 22 h and the corresponding plasma concentrations (*27*), a value of 0.13 ± 0.04 L/min is obtained for the renal clearance. Since the clearance of elimination from plasma, the renal clearance and the creatinine clearance are almost identical—it follows that the former represents the urinary excretion of the glucuronide and that this metabolite does not bind to serum proteins. The volume of distribution related to the terminal kinetic phase is 37 L, which approximates the total body water suggesting that bisphenol A glucuronide distributes equally within the extra- and intracellular water.

The observation of an efficient glucuronidation of bisphenol A in humans after controlled low dose exposure suggests that the various attempts to measure bisphenol A in serum or urine of "unexposed" (i.e., subjects with no defined source of exposure to bisphenol A) subjects may not have selected a relevant biomarker for bisphenol A exposure. Usually, these studies determine free bisphenol A in the samples without previous treatment with glucuronidase to cleave the glucuronide (*18*, *28*−*30*). The results presented here show that after oral administration (the most relevant environmental exposure scenario), the blood and urine concentrations of free bisphenol A are very low and bisphenol A glucuronide is the major metabolite present. Therefore, the developed methods may only measure a small part of the actual bisphenol A body burden. Moreover, interpretation of the results of determinations of bisphenol A is complicated by sample contamination with bisphenol A from plastic materials used during sample preparation or other sources. Therefore, it can be assumed that the determination of bisphenol A glucuronide is much better suited as a biomarker of human bisphenol A exposure due to its expected presence in higher concentrations and to the lack of background caused by contamination. The determination of free bisphenol A may serve as a biomarker more related to potential estrogenic effects since only free bisphenol A shows weak estrogenicity.

The obtained results may have important implications for the risk assessment of human exposure to bisphenol A. Estimated exposure of the general population to bisphenol A is supposed to occur mainly from canned food and wine and may reach a body burden of up to 9 μg/kg/day (*31*). The results obtained here suggest that conversion of bisphenol A to the glucuronide in a hepatic first-pass metabolism in humans is essentially complete after exposure to these concentrations. Furthermore, in a study of bisphenol A leakage from dental sealants, an exposure route leaking to a rapid uptake of bisphenol A without first pass metabolism, no free bisphenol A could be detected in blood further supporting rapid conversion (*30*). Since bisphenol A glucuronide is devoid of hormonal activity, glucuronidation can be considered as a deactivation reaction resulting in very low levels of bisphenol A systemically available for receptor binding. Therefore, possible risks of adverse effects of bisphenol A based on hormonal activity should only partially be based on extrapolation from rodent data and integrate the differences in toxicokinetics between rodents and humans.

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