Disposition of Orally Administered 2,2-Bis(4-hydroxyphenyl)propane (Bisphenol A) in Pregnant Rats and the Placental Transfer to Fetuses

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We studied the disposition of bisphenol A (BPA) in pregnant female F344/DuCrj(Fischer) rats and its placental transfer to fetuses after a single oral administration of 1 g/kg BPA dissolved in propylene glycol. BPA in maternal blood, liver, and kidney reached maximal concentrations (14.7, 171, and 36μg/g) 20 min after the administration and gradually decreased. The levels were 2–5% of the maximum 6 hr after the administration. The maximal concentration of BPA in fetuses (9 μg/g) was also attained 20 min after the administration. BPA levels then gradually reduced in a similar manner to maternal blood. These results suggest that the absorption and distribution of BPA in maternal organs and fetuses are extremely rapid and that the placenta does not act as a barrier to BPA. *Key words*: bisphenol A, fetus, placenta, placental transfer. *Environ Health Perspect* 108:931–935 (2000). [Online 12 September 2000]
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2,2-Bis(4-hydroxyphenyl)propane (bisphenol A; BPA) has been used in the manufacture of polycarbonate and epoxy resins and other plastics (polysulfone, alkylphenolic, polyalylate, polyester-styrene, and certain polyester resins) and has also been used as a fungicide, antioxidant, flame retardant, rubber chemical, and polyvinyl chloride stabilizer. Polycarbonate resins are employed in food-contact uses as in components of food processors; microwave oven-ware; tableware; refrigerator crisper drawers; food-storage containers; returnable water, milk, and juice containers; feeding bottles for infants; and interior coatings of cans and drums. Polycarbonate and these other resins are also used as films, sheets, and laminations; reinforced pipes; floorings; watermain filters; enamels and vanishes; adhesives; artificial teeth; nail polish; compact discs; electric insulators; and as parts of automobiles, certain machines, tools, electrical appliances, and office automation instruments. The estimated average annual production of BPA in Japan for 1995 was approximately 260,000 tons (*1*), and the production of polycarbonate and epoxy resins for 1998 was approximately 317,000 and 204,000 tons, respectively (*2*). Release and migration of BPA monomer from polycarbonate tableware, baby bottles, and cans coated with epoxy or polyvinylchloride resins have been recognized (*3–5*). A small amount of BPA is detectable in tap water and river water (*6–8*).

An excess dose of BPA (5.5–6.3 g/kg/day) is nephrotoxic in mice (*9*), but BPA is neither carcinogenic nor teratogenic (*10,11*). *In vivo* estrogenic activities (400–1,000 mg/kg/day) in immature or ovariectomized rats and mice have been recognized (*12–14*). Reduction of epididymal weight, seminal vesicle weight, and sperm motility in F_0 and F_1 mice given a large dose of BPA (437–1,750 mg/kg/day) has been also observed (*15*). Recently, it has been reported that low doses of BPA *in utero* (2–400 μg/kg/day) can be toxic in reproductive organs of male offspring of mice and rats (*16–18*). Cagen et al. (*19,20*) attempted to repeat the study, but they have not been able to reproduce the results. Therefore, the toxicity of low doses of BPA on male reproductive organs remains controversial.

Knaak and Sullivan (*21*) reported that most of an orally administered dose of ^{14}C-BPA was excreted in feces and urine in rats within 24 hr. Over 8 days, 28% of the ^{14}C was excreted in urine and 56% in feces. The BPA was excreted in urine as glucuronide or in feces as free BPA, hydroxylated BPA, and a conjugate (*21*). Some preliminary reports indicate that the metabolism of BPA is different based on the route of administration (*22*). BPA is distributed in mouse fetuses (*23*) and in human umbilical cords (*24*). However, there have been few reports on disposition of BPA.

In the present study we examine the disposition of orally administered BPA in pregnant rats and the placental transfer to fetuses. The purpose of this experiment was to know whether or not BPA could be easily distributed to fetuses.

Materials and Methods

Chemicals. We purchased BPA (> 95.0% pure) and propylene glycol (practical grade) from Wako Pure Chemical Company (Osaka, Japan). Acetone, *n*-hexane (for pesticide residue analysis), methanol, and acetonitrile (for HPLC) were obtained from Kanto Chemicals Co., Inc. (Tokyo, Japan).

Animals and administration. Pregnant female F344/DuCrj (Fischer) rats, 10 weeks old, were purchased from Japan Charles River (Yokohama, Japan) on day 4 of gestation; the day vaginal plugs were observed was considered day 0 of gestation. Rats were individually housed in stainless steel suspended cages and fed a standard diet (CLEA CE-2; CLEA Japan Inc., Tokyo, Japan) in an air-conditioned room at 25±1°C with a relative humidity of 55±5%. F344 rats are widely used for many toxicology studies and carcinogenesis bioassays (*10*); because they are thought to be estrogen-sensitive (*25*), we chose this strain for the present study.

On day 18 of gestation, rats were orally administered BPA dissolved in propylene glycol (25%, wt/v) at a dose of 1 g/kg. This dose is approximately one-fourth the median lethal dose (LD_{50}) for rats (*26*).

We conducted the experiment under ethical consideration for experimental animals from the standpoint of animal welfare according to the "Guiding Principles for the Care and Use of Laboratory Animals," approved by the Japanese Pharmacological Society (*27*) and by the Tokyo Metropolitan Research Laboratory of Public Health (*28*). This study was approved by the Committee of Animal Experiments in the Tokyo Metropolitan Research Laboratory of Public Health.

BPA analysis. Rats were killed by decapitation 10, 20, 30, and 40 min and 1, 2, 4, 6, 12, 24, and 48 hr after administration. After collection of blood, fetuses were removed and then maternal kidneys and livers were dissected out. The study included two to six mothers per time point (three animals for 10, 20, and 40 min and for 6 and 12 hr; two animals for 30 min and 1, 2, and 4 hr; six animals for 24 hr; and four animals for 48 hr). We used 8–12 fetuses from two to three mothers per time point (12 fetuses for 10 and 20 min and 12 and 24 hr; 10 fetuses for 40 min; and 8 fetuses for 1, 2, 4, 6, and 48 hr). Generally, we used only fetuses attached to the ovarian and cervical ends of both right and left uterine horns of a mother (4 fetuses) for analyses.

We used a Polytron homogenizer (Kinematica GmbH, Lucerne, Switzerland)

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and acetone (1 g tissue/20 mL acetone) to homogenize maternal organs and whole fetuses. After centrifugation, the acetone extracts were dried at 40°C under a stream of nitrogen. Because these acetone extracts contained many contaminants to disturb the analysis of BPA, we added 1 volume of water and then 1 or 2 volumes *n*-hexane to the extracts and vigorously mixed them to exclude the contaminants into the water layer. After centrifugation, the hexane extracts were dried under a stream of nitrogen and dissolved in methanol because of the solubility of BPA in methanol and the miscibility of methanol solution to the mobile phase. We analyzed BPA in methanol solution by HPLC using a Mightysil RP-18 (150 × 4.6mm) column (Lot 98M1019; Kanto Chemical Co., Inc., Tokyo) eluted with acetonitrile:water (35:65) at a flow rate of 1.0 mL/min by a Toso CCPE pump (Toyo Soda, Tokyo, Japan) and detected by a Shimadzu SPD-6AV UV-VIS spectrophotometric detector (Shimadzu Corp., Kyoto, Japan) or by a Toso UV-8020 detector (Toyo Soda, Tokyo, Japan) at a wavelength of 227 nm. We determined BPA using an external standard. We evaluated the accuracy and validity of this method using > 95% pure BPA. The retention time of BPA was 16.6–16.7 min or 17.1–17.2 min [when another Mightysil RP-18 column (Lot 99M0212) and another pump and detector were used]. The calibration curve was linear from 0.005 to 54 µg/g.

To recover BPA from organs, we conducted experiments using liver, which possibly contained biologic materials that can interfere with analysis. The average recovery of BPA from liver in these procedures was 87.45 ± 5.73% (mean ± SD). There was no peak of biologic materials that interfered with the determination of BPA. The variance of measurement was within approximately 5%. The injection volume was 1.0–10.0 µL. To determine BPA in organs, we compared the peak area with that of 95% pure BPA, which was freshly prepared daily and frequently used for calibration. The detection limit was 0.005 µg/g. No BPA was detected in organs of rats administered propylene glycol (vehicle) at a dose of only 4 mL/kg. Figure 1 shows a chromatogram of BPA in fetuses.

Data analysis. Data are expressed as mean ± SD. We used model-independent analysis to determine the toxicokinetic parameters (*29*). The area under the tissue concentration-time curve (AUC) was calculated by the trapezoidal method. We calculated mean residence time (MRT) and variance of residence time (VRT) from AUC values (*30*). Biologic half-lives of organs were estimated by a least-squares fit from the descending portion of the semilogarithmic plot of concentration versus time. We read the multiple linear descending portions, so we calculated two or three different biologic half-lives by this method.

Results

Changes in BPA concentrations in maternal blood, liver, and kidney. BPA (2.89 µg/g) was already in maternal blood 10 min after dosing (Figure 2), and reached a maximal concentration (14.7 µg/g) 20 min after dosing. The maximal level was 0.007% of the administered dose per gram of blood. This was followed by a gradual decrease such that the concentrations fell by approximately 100-fold over a period of 10 hr. The concentration was 2% of maximum after 6 hr.

The high concentration of BPA was also detected in maternal liver 10 min after administration (Figure 2). The maximal hepatic concentration (171 µg/g; 0.083% of the dose per gram of liver) was obtained at 20 min, and then decreased by approximately 20-fold over a period of 10 hr. The concentration after 6 hr was 5% of maximum. The concentration of BPA in liver was 10–100 times higher than in blood.

The peak BPA concentration (36.2 µg/g; 0.017% of the dose per gram of tissue) in kidney was also attained 20 min after gavage (Figure 2), and then decreased by approximately 50-fold over 10 hr. The concentration after 6 hr was 5% of maximum. The concentration of BPA in kidney was 10 times higher than in blood.

Changes in BPA concentrations in whole fetuses. In fetuses we found 2.00 µg/g BPA 10 min after administration (Figure 3). BPA reached the maximal concentration (9.22 µg/g) after 20 min, and then gradually decreased by approximately 50-fold over 10 hr. The fetal maximal level of BPA was 0.004% of the dose per gram of fetus. The concentration after 6 hr was 5% of maximum. The concentration of BPA in fetuses decreased in almost the same manner as that in maternal blood.

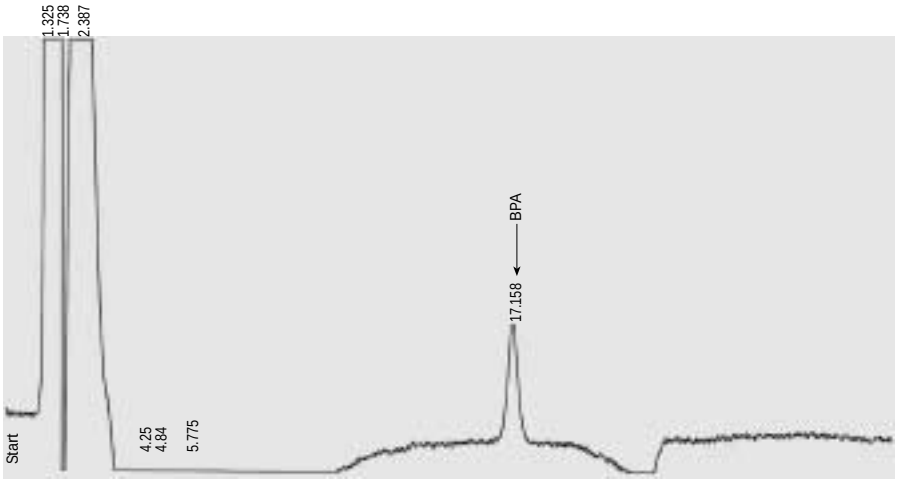

Figure 1. HPLC chromatogram of BPA from a fetus 10 min after a single oral dose of 1 g/kg BPA was administered to a pregnant rat. The fetus was homogenized with 20 mL acetone. The final solution for injection was 10-times concentrated extracts, and the injection volume was 5 µL. Absorbance of a full scale is 0.01.

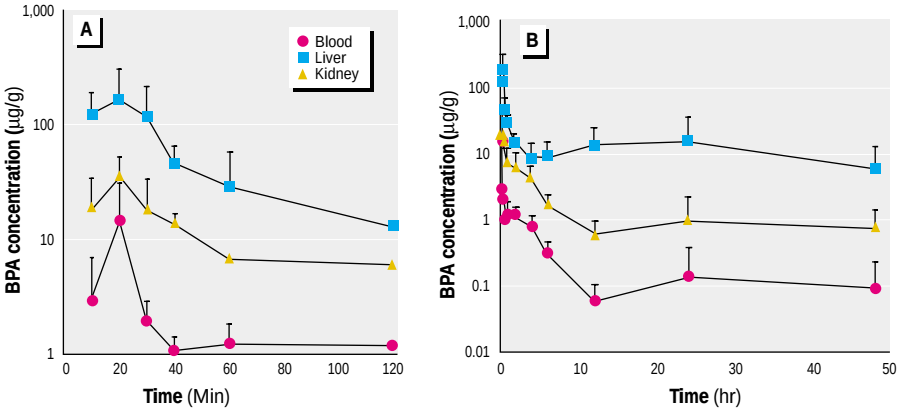

Figure 2. Changes in bisphenol A concentrations in maternal blood, liver, and kidney over 10–120 min and 0–48 hr after a single oral administration of 1 g/kg bisphenol A to pregnant rats. Points indicate mean ± SD.

Tokicokinetic parameters. We calculated toxicokinetic parameters using the results shown above (Table 1). Hepatic AUC was highest. Fetal AUC was larger than that for maternal blood. The MRT of maternal blood was 11 hr, whereas that of fetuses was 20 hr. Fetal VRT was also larger than that for maternal blood. The initial biologic half-lives of BPA in maternal organs were extremely rapid (5.7–14.7 min). Terminal half-lives in those organs were approximately 2–5 hr. In fetuses, the initial half-life was rapid (33 min), whereas the terminal half-life was slightly longer (173 hr).

Discussion

The results suggest that the absorption and distribution of BPA in maternal organs and in fetuses are extremely rapid and that BPA can easily pass through the placenta. The placenta is not a barrier to BPA. We investigated a high dose of 1 g/kg BPA, approximately one-fourth of the LD_{50} for rats, to more accurately determine fetal concentrations by HPLC, especially when concentrations decreased afterward. In the case of a lower dose, for example, 0.1 g/kg, BPA concentrations could be determined accurately only near peak levels. No toxicologic signs were observed after the administration of BPA at 1 g/kg. Gross findings of internal organs of rats given BPA were similar to those of the nontreated control rats. The choice of such a high dose may raise doubts about the relevance of the biodisposition data in relation to lower and less toxic doses. The extremely rapid absorption and clearance are consistent with other reported results (*21–23*) using lower doses (100–800 mg/kg), so the disposition of this dose was thought to be representative of lower and less toxic doses. For substances absorbed by passive diffusion, the fraction of the dose absorbed and the kinetics (i.e., the absorption rate constant) are generally independent of the administered dose (*31*). If carrier-mediated absorption, delayed gastric emptying, or gastrointestinal mucosal damage can occur, higher doses may reduce the efficiency and rate of absorption. In the present experiment, a high dose of BPA was readily absorbed, so these problems did not occur. Unconjugated metabolites can increase in the circulation when chemicals are metabolized and cleared in the intestines or liver during absorption by the first-pass clearance mechanism. It is possible that plasma concentrations of unconjugated BPA are small when low doses of BPA are administered. As reported by Iguchi (*23*), when mice were administered 100 mg/kg BPA, a considerable amount of unconjugated BPA was detected in maternal and fetal plasma and organs. Saillenfait et al. (*32*) reported that the distribution profile of metabolites in tissues of pregnant rats was not affected by increasing the dose of di-*n*-butyl phthalate from 0.5 to 1.5 g/kg, whereas the time course of tissue concentrations was dose-dependent. The dose-dependent effect was caused by the saturation of esterase converting di-*n*-butyl phthalate into mono-*n*-butyl phthalate but not glucuronidase (*32*). In a study of the disposition of thiabendazole, Yoneyhama et al. (*33*) also used a large dose (1 g/kg).

Most maternally administered chemicals have the potential to cross the placenta; the current question is not whether a chemical crosses the placenta, but at what rate it does so (*34*). Highly lipophilic chemicals are not necessarily well absorbed via the placenta by fetuses. Placental transfer of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated biphenyls (PCBs), hydroxylated PCBs, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (*p,p´*-DDT), 1, 1-dichloro-2,2-bis(4-chlorophenyl)ethene (*p,p´*-DDE), and pentachlorophenol, with high octanol/water partition coefficients (log P_{ow} = 5.0 to 6.0), may be limited or very slow (35 to 40), although these chemicals can be accumulated. Chemicals with lower partition coefficients (logPow=–0.9 to 5.0), such as diethylstilbestrol (DES), 1,2-diethylbenzene, mono-*n*-butyl phthalate, salicylic acid, 1,2-dichloroethane, *N,N*-dimethylformamide, and thiabendazole, move relatively easily across the placenta into fetuses, and the transfer rate may depend on the structure of each compound (*32,33,41–45*). Their maximal fetal concentrations are 30–100% of their maximal maternal plasma levels. Transplacental absorption may require both lipophilic and hydrophilic properties; therefore, most organic compounds can cross the placenta. BPA (log P_{ow} = 3.32) can also cross the placenta at a higher rate, much like thiabendazole and salicylic acid (*33,42*).

The manner in which fetal BPA levels become higher than maternal plasma levels after the maximum maternal plasma level occurs may resemble that of DES, one of the most potent nonsteroidal synthetic estrogens (*12*). BPA, 2,2-bis(4-hydroxyphenyl)propane, is somewhat structurally related to DES, 3,4-bis(4-hydroxyphenyl)-3-hexene. DES is rapidly absorbed and distributed and cleared in maternal organs, and its major site of accumulation is the liver (*45*). Maternally administered DES is also rapidly distributed to fetuses in rats, mice, hamsters, and monkeys (*45–49*); the fetal level is 2–3 times that in the maternal blood (*50*). DES deposits in fetal genital organs, which may be responsible for the transplacental reproductive toxicity (*45,47,50*). From our results, we determined that BPA is also rapidly absorbed, distributed, and cleared, and the major organ of accumulation is also the liver. After 40 min the fetal BPA concentration is higher than in maternal blood.

The concentration–time curves of maternal organs and fetuses give clear evidence of enterohepatic circulation of BPA so that

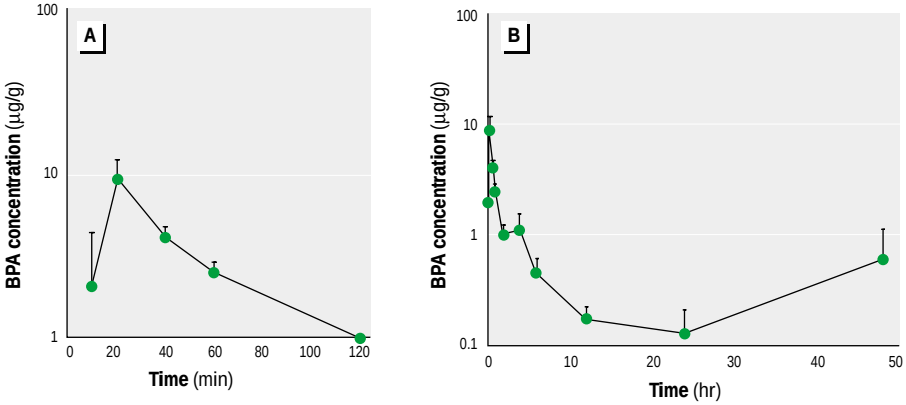

Figure 3. Changes in bisphenol A concentrations in whole fetuses over 10–120 min and 0–48 hr after a single oral administration of 1 g/kg bisphenol A to pregnant rats. Points indicate mean ± SD.

Table 1. The AUC, MRT, VRT, and biologic half-life ($t_{1/2}$) of maternal blood, liver, and kidneys, and whole fetus after a single oral administration of bisphenol A at a dose of 1 g/kg to pregnant rats.

	Maternal blood	Maternal liver	Maternal kidney	Fetus
AUC (µg·hr/g)	13.1	700	84.0	22.6
MRT (hr)	10.6	29.3	12.0	20.0
VRT (hr^2)	203	657	227	419
$t_{1/2}$	0.0952 (20–40 min)	0.178 (20–40 min)	0.245 (20–40 min)	0.55 (20 min–2 hr)
$t_{1/2}$	2.58 (40 min–6 hr)	1.75 (40 min–6 hr)	2.98 (1–12 hr)	1.60 (20 min–6 hr)
$t_{1/2}$	4.65 (6–48 hr)	–	–	173 (4–48 hr)

The $t_{1/2}$ was calculated from each linear descending portion during the time (in parentheses).

each of the curves shows a secondary peak after 10 hr (*51*,*52*). Approximately 60% of orally administered BPA is excreted in feces, with approximately 33% being free BPA, hydroxylated BPA, or conjugates (*21*). If the liver efficiently extracts the compound from the portal blood, the enterohepatic cycling will involve only the liver and intestine, and systemic concentrations will increase very little (*53*). However, if the compound is poorly extracted from the portal blood by the liver, a significant increase in the systemic half-life can result (*20*). Most compounds subject to this cycling may be intermediate between these two extremes. BPA may also be incorporated into the cycle, and its half-life may be prolonged to some extent.

When a single dose of a chemical is administered, the MRT is the mean quantity of a molecular residence time in an organ; these values can be stochastically handled. In short, the MRT is the retention time of an administered chemical in an organ. A smaller MRT means that the chemical can pass through more rapidly. The VRT is a variance of MRT and is the distribution width of organic concentrations of the chemical. A larger VRT means that organic concentrations distribute broadly around the MRT. The AUC is the mass index of the compound incorporated into the body, the MRT is the rate index of the compound passing through the body, and the VRT is the continuation index of the compound in the body. In the present study, hepatic AUC was so high that liver could take in BPA. Because the fetal AUC was higher than that of maternal blood, fetal intake of BPA was much greater than maternal blood. The MRT of maternal blood was 11 hr, whereas the MRT of fetuses was 20 hr. The fetal VRT was also larger than that of maternal blood. Therefore, both mean and maximal retention times of BPA in fetuses are longer than in maternal blood.

BPA has been suspected of causing toxicity in male reproductive organs in mice (*16*,*17*,*20*). Transplacental exposure may enhance the toxicity in newborn mice (*17*). Iguchi (*23*) showed that BPA was readily available to fetuses via transplacental uptake by in mice. The present results agree with those in mice and clearly indicate a rapid and sufficient supply of the substance to fetuses via placenta in pregnant rats.

The estrogenic activity of BPA at levels > 200 mg/kg is clear. The toxicokinetic data after the administration of 1 g/kg BPA may be generally applied to the doses near 200 mg/kg. Howdeshell et al. (*54*) demonstrated that exposure to BPA at 2.4 µg/kg advances puberty. The problem is whether the present toxicokinetic results can be applied to this dose. One of the most important findings of our study is that the fetal BPA concentration can rapidly reach the maximum. The mechanism of estrogenicity of the lowest dose is unknown, but the rapid absorption and distribution in fetuses are certainly important for those effects. In principle, the present results may be applicable to lower and less toxic doses of BPA.

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