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Distribution of ^{14}C-bisphenol A in pregnant and newborn mice

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ABSTRACT

Objectives. The purpose of the present investigation was to trace the fate of bisphenol A injected into pregnant mice, focusing on its potential accumulation in the fetus and the brain, critical targets of hormonal chemicals, using whole-body autoradiography.

Methods. Pregnant mice were injected intraperitoneally with 0.46 MBq of ^{14}C-BPA and then killed at 1 h or 1, 3, or 5 days after injection. Sections for autoradiography were prepared in a cryomicrotome and the exposed imaging plate was processed using a fluorescent/radioisotope image analyzer.

Results. Intraperitoneally injected ^{14}C-BPA was distributed throughout the body, including the fetus and the brain, within 1 h. Radioactivity faded gradually from the whole body by the fifth day, and no accumulation in any specific organ was found. However, although ^{14}C was detected in the fetuses immediately after injection, the transfer of BPA from mother to newborn was not observed.

Significance. The routes of rapid BPA discharge were confirmed, and BPA neither accumulated in the body nor was it transferred to newborn mice. No evidence was observed to suggest the existence of a blood–placenta or blood–brain barrier for BPA. This information should be taken into consideration when assessing the risks of using dental materials that contain BPA.

1. Introduction

The problem of endocrine-disrupting chemicals was first recognized in the field of dentistry when bisphenol A (BPA) was found to be leaking into the saliva from sealant applied to patients [1]. Because sealants are commonly applied in children, who are generally more sensitive to chemical exposure, this observation created great concern among dentists. Particular concern was expressed regarding the possibility that BPA may disrupt the human hormonal system. Although there is evidence to suggest that these initial estimates of BPA leakage were rather high [2], establishing the safety of BPA is difficult. Glass ionomer cement represents an alternative sealing material for pits and fissures, but its retention is unsatisfactory and therefore its benefit for patients is limited.

Despite extensive discussion of the safety of BPA [3–9] no decisive conclusion has been reached because extremely small amounts of BPA, as low as 10 pM, can have hormonal effects [10]. Previous studies indicated that BPA modulates cellular function at concentrations between 1 pM and 1 nM [11]. When high-performance liquid chromatography (HPLC) is used to detect low concentrations of BPA, the lower limit of detection is generally in the nanomolar range [12,13]; therefore, precise detection of BPA is difficult.

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More thorough investigation is required to assess the safety of BPA, especially with regard to its effect on the fetus and the brain. The fate of BPA is an important concern in the use of dental materials containing BPA. The purpose of this study was to investigate whether BPA injected into pregnant mice accumulates in a specific part of the body and whether it is transmitted to fetuses through the placenta or to the brain through the blood–brain barrier. BPA distribution was monitored via autoradiography and transfer to pups was examined both *in utero* and after delivery.

2. Materials and methods

This study used eight mice in the 13th day of pregnancy (Sankyo Labo-Service Inc., Tokyo, Japan). Pregnant mice weighing 46–48 g were injected intraperitoneally with 0.46 MBq of ^{14}C-BPA (Moravek Biochemicals, Inc., Brea, CA) and then killed at 1 h or 1, 3, or 5 days after injection. Sections for autoradiography were prepared following the methods outlined by Kawamoto and Shimizu [14] and Kawamoto [15]. Briefly, the entire mouse was frozen in hexane cooled to −94 °C immediately after sacrifice, and then immersed in 5% carboxymethyl cellulose (CMC) gel in a stainless steel container. The whole sample was immersed again in the cooled hexane and completely frozen. The embedded frozen CMC block was placed in a cryomicrotome (CM 3500; Leica Instruments, Germany) and whole-body sections of approximately 20 μm were cut along the sagittal plane. By the fifth day after injection, all mice had delivered. Four pups were selected and processed as described for adult mice.

The frozen CMC block was placed in the cryomicrotome and trimmed to the area of interest. The exposed surface was covered with an adhesive film (Cryofilm Type 2C, FINETEC Co. Ltd., Tokyo, Japan). For histological staining and autoradiography, 7- and 20-μm-thick sections, respectively, were cut along the sagittal plane and lined with adhesive film. The sections for autoradiography were completely freeze-dried in the cryochamber and stored in a desiccator containing silica gel inside the cryochamber. Sections covered with a 2-μm-thick plastic film were placed on the imaging plate (BAS-MS2040, Fuji-film Inc., Tokyo, Japan) and exposed for 4 days. The exposed imaging plate was processed using a fluorescent/radioisotope image analyzer (FLA-3000, Fuji-film Inc., Tokyo, Japan) and autoradiograms were obtained and analyzed. Freeze-dried sections were quickly thawed, fixed with 4% paraformaldehyde, stained with hematoxylin and eosin (H-E), and mounted on a slide glass under an adhesive film using 30% glycerol as an adhesive. To quantitatively assess the data obtained from the fluorescent/radioisotope image analyzer, several areas were cut out from sections and analyzed using a liquid scintillation counter. Data from the imaging plate were converted to radioactivity and expressed in Bq per mm^3.

All experimental procedures followed the guidelines for animal studies established by Tsurumi University and Iwate Medical University.

3. Results

Fig. 1 shows an H-E section and the corresponding ^{14}C-BPA distribution of a whole mouse at 1 h after intraperitoneal

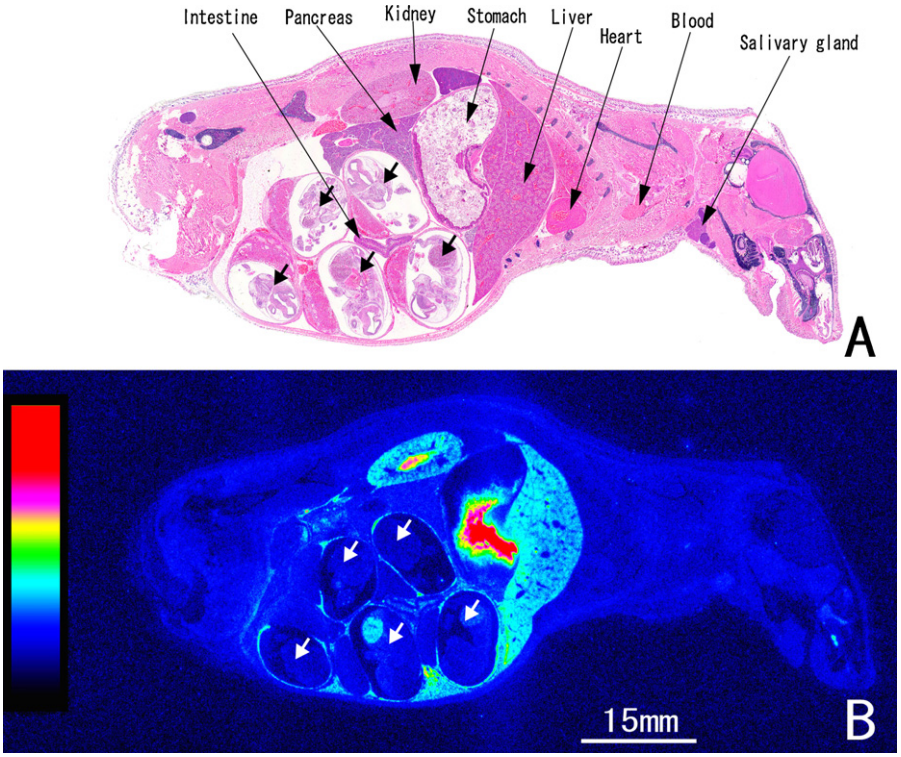

Fig. 1 – Whole-body section of a pregnant mouse stained with hematoxylin and eosin and its corresponding autoradiogram 1 h after ^{14}C-bisphenol A injection. The small arrows indicate fetuses. The red color in the autoradiogram indicates the highest region of radioactivity.

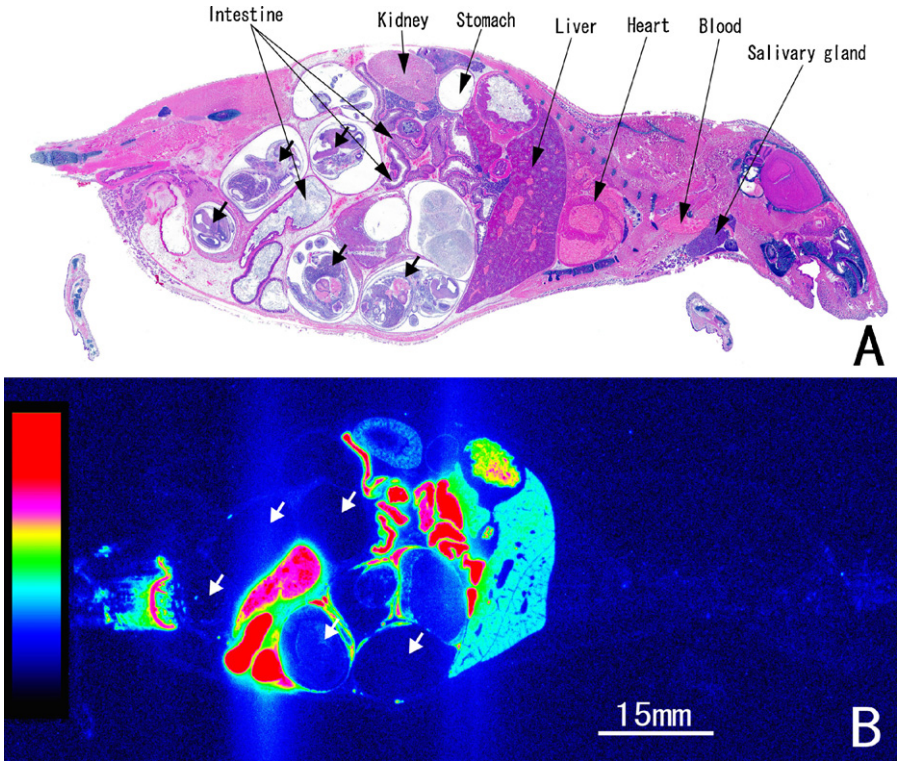

Fig. 2 – Whole-body section of a pregnant mouse and its corresponding autoradiogram 1 day after ^{14}C-bisphenol A injection. The small arrows indicate fetuses.

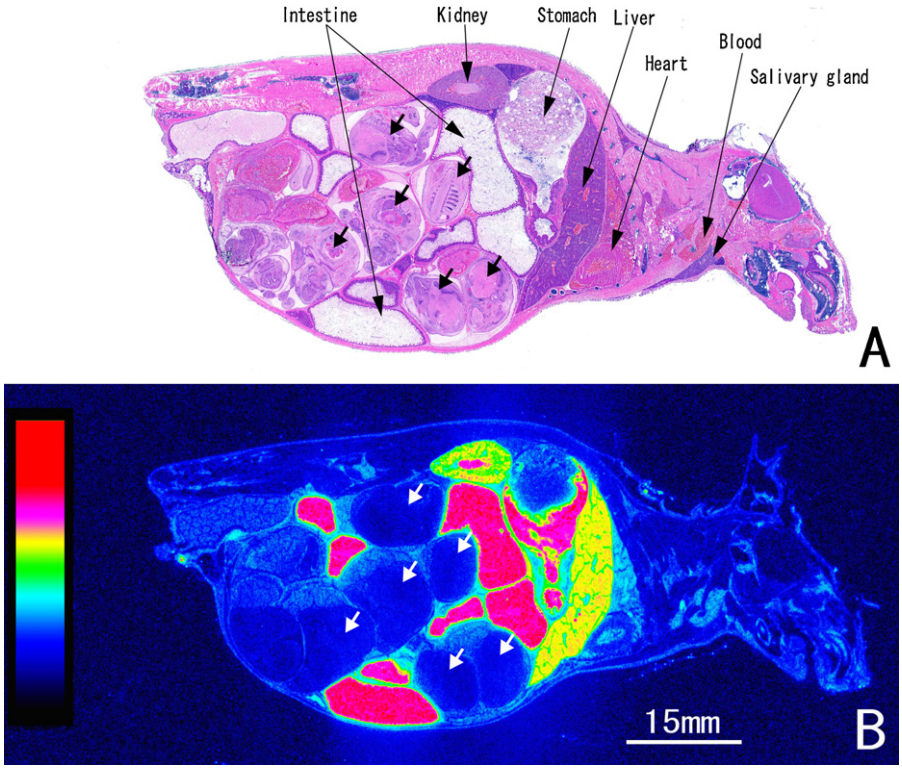

Fig. 3 – Whole-body section of a pregnant mouse and its corresponding autoradiogram 3 days after ^{14}C-bisphenol A injection. The small arrows indicate fetuses.

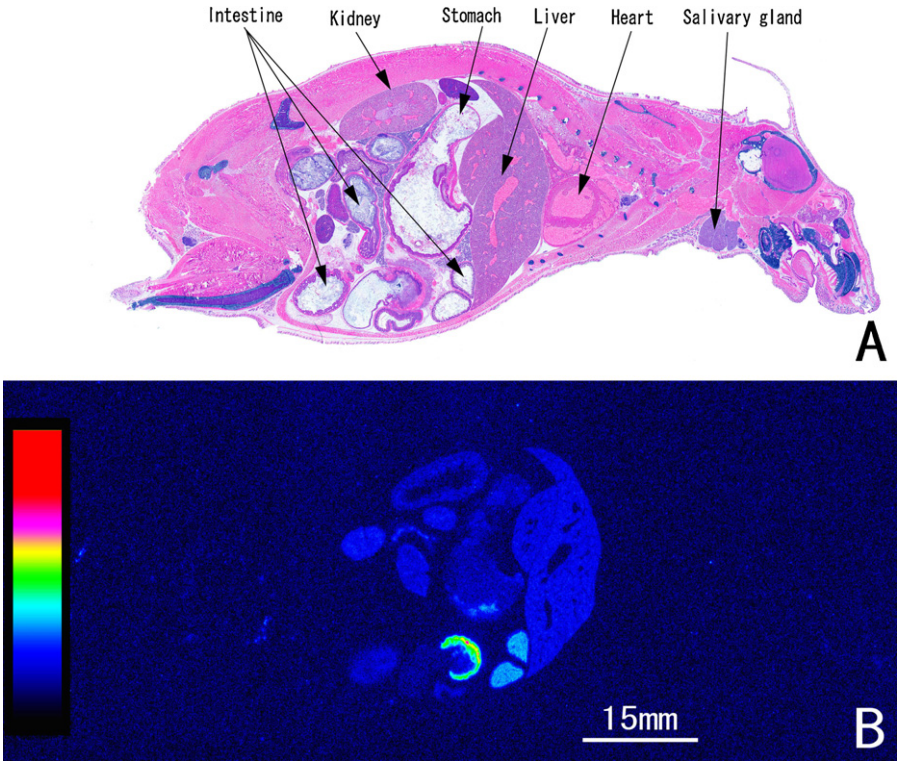

Fig. 4 – Whole-body section of a mouse that had delivered within 1 day and its corresponding autoradiogram 5 days after ^{14}C-bisphenol A injection.

injection. ^{14}C-BPA was distributed throughout the body, including the fetus and brain. High amounts of ^{14}C were detected in the kidney, liver, lower part of the stomach, and fetal liver, and to a lesser extent in the brain. The highly radioactive area in the stomach area appears to be derived from the liver rather than the duodenum. The white arrows in the autoradiogram indicate the same position in the H-E section. Another mouse showed strong radioactivity in the upper intestine and the area of the submandibular gland 1 h after injection. In the kidney, a high concentration of ^{14}C was detected and a difference in distribution was observed between the cortex and the medulla. Blood did not exhibit strong radioactivity, as evidenced by the relative lack of radiation in the heart. The fetal liver displayed higher levels of ^{14}C than other parts of the fetus.

The results obtained from mice at 1, 3, and 5 days after injection, are shown in Figs. 2–4, respectively. Fig. 5 shows the change in the average radioactivity of each area over time. As there was considerable variation between animals even within the same areas, several typical points were selected and their radioactivity was measured, and the resulting average values were plotted. At 1 day, high levels of ^{14}C were detected in the intestine and, to a lesser extent, in the stomach, liver, and kidney, but radioactivity was very low in the brain and fetus. These trends were similar at 3 days, and the injected BPA appeared to be excreted to the intestine through the liver, and to the urine through the kidneys. The thick contents of the intestine appeared to dilute the ^{14}C label per mm^3 in the 3-day animals. By the fifth day, all mice had delivered and whole-body radioactivity was lower. Activity was limited to

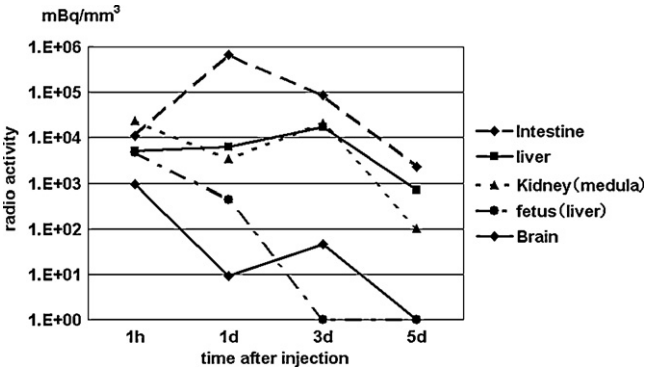

Fig. 5 – Change in average radioactivity in each area over time.

the intestine and, to a lesser extent, the liver, stomach, and kidney. Almost no radioactivity was detected in the brain. ^{14}C activity was not detectable in the pups, which were born on the fourth day after injection (Fig. 6).

4. Discussion

This experiment provides insight into the fate of intraperitoneally injected BPA in mice. The routes of rapid BPA discharge were confirmed, and BPA neither accumulated in the body nor was it transferred to newborn mice. No evidence was observed to suggest the existence of a blood–placenta or

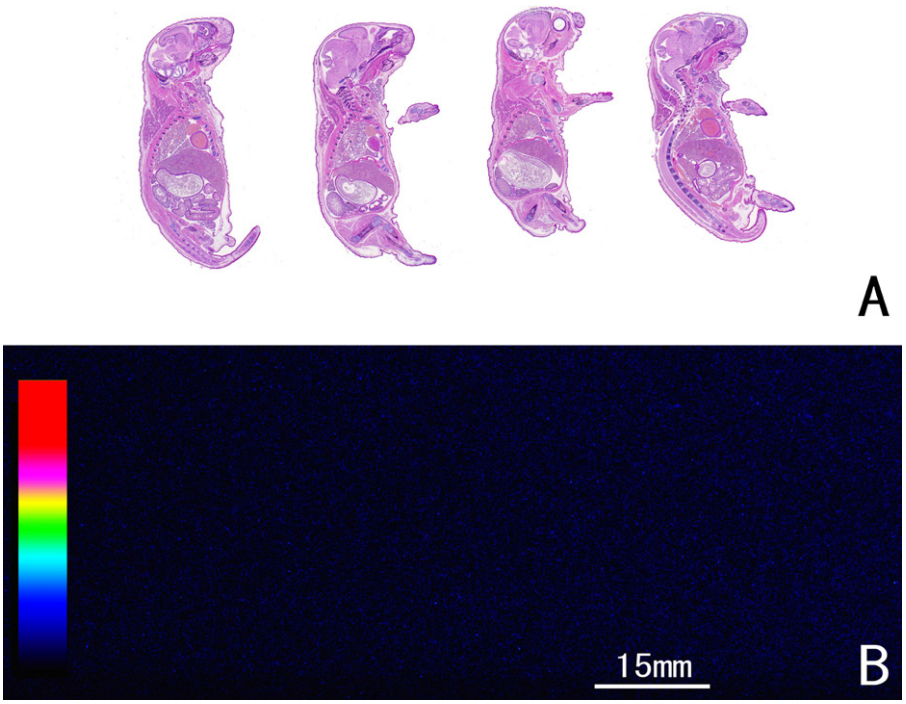

Fig. 6 – Whole-body sections of newborn mice and their corresponding radiograms. No radioactivity was detected in newborn mice.

blood–brain barrier for BPA. This information should be taken into consideration when assessing the risks of using dental materials that contain BPA.

A review article concerning the effect of BPA from dental materials [16] concluded that the short-term risk of estrogenic effects from dental treatments using BPA-based resins was insignificant because of the small dosages used, although BPA can induce changes in estrogen-sensitive organs or cells in animal or cell-culture experiments. On the other hand, among 115 published papers concerning the low-dose effects of BPA, 94 reported significant estrogenic effects [10] and disruption of cellular function was shown even at doses as low as 1 pM or 0.23 ppt [17]. The definition of "small dosage" is therefore problematic, and decisive judgment seems almost impossible. The field of dentistry should remain aware that BPA may lead to hormonal disruption in human beings.

4.1. *Effect of BPA on fetuses*

Moors et al. studied the disposition and transplacental transfer of intravenously administered BPA in pregnant rats using gas chromatography–mass spectrometry [18]. The authors noted that BPA was distributed rapidly, and peak concentrations for total BPA were attained 20–30 min after intravenous administration, with high mean values in the maternal liver, kidneys, and uterus, and lower mean values in the placenta and fetal liver. In all tissues, BPA levels declined in parallel with those in maternal blood. The authors concluded that BPA is readily transferred across the placenta of rats to the fetus, based on the similar concentrations in placenta and fetal liver.

The present study demonstrates that intraperitoneally injected BPA spreads throughout the body, including fetuses, within 1 h. No evidence for the existence of a placental barrier for BPA was observed, suggesting that BPA exerts direct effects on the fetuses when introduced into the pregnant body. However, although ^{14}C was detected in fetuses immediately after injection, the transfer of BPA from mother to newborn pup was not observed, suggesting that BPA may have been excreted before the time of delivery. Although the endocrine-disrupting effects of BPA are relatively weak in adults, fetuses are generally much more sensitive to endocrine-disrupting chemicals. Because BPA may cause long term adverse effects if exposure occurs during critical periods of differentiation [19], caution should be practiced when using BPA-containing sealants or composite resins in pregnant women.

4.2. *BPA passes though the blood–brain barrier*

In a previous study, Sun et al. [20] used a HPLC-fluorescence detecting system to monitor the concentrations of BPA in rat brain and plasma for 8 h after a single i.v. or oral dose, and found that BPA was capable of penetrating the blood–brain barrier. The monitored maximum concentration of BPA in the plasma and brain after oral administration (200 mg/kg) was 1624 ppb and 20 ppb, respectively. Kim et al. [21] also showed that BPA in the rat brain increased in a dose-dependent manner after oral administration, and that the blood–brain barrier system did not limit the access of BPA to the brain. These results are consistent with the autoradiographic data presented here, in which female mice showed body-wide distribution at 1 h after intraperitoneal BPA injection.

Subcutaneous exposure to BPA in mice was reported to cause alterations in brain sexual differentiation when dams were exposed to 25 ng BPA/kg body weight per day from day 8

of pregnancy through day 16 [22]. Supposing that the rat body is 60% water [23], the 25 ng BPA/kg body weight is calculated to be 40 ppt. According to a model experiment by Imai and Komabayashi [2], the amount of BPA that leaches out from commercial Bis-GMA-based polymerized resins in the long term is negligible. However, the BPA content of unpolymerized composite resins reportedly ranges from 1.5 μg/g to 10.2 μg/g [24]. Although the simulation of BPA concentrations is impossible, it is reasonable that some dentists will think it wiser to avoid the use of Bis-GMA-based resins for pregnant women due to possible estrogenic effects in the brain.

4.3. *Route of BPA discharge*

The injected radioisotope activity faded gradually from the whole body by the fifth day, and no accumulation in any specific organ was found, indicating that a rapid route of BPA discharge must exist in mice. In an experiment using ^{14}C-labeled BPA, 28% and 56% of orally administered BPA was excreted into the urine and feces, respectively [25]. BPA was also excreted into the urine and feces in the present study. High concentrations of ^{14}C-BPA were detected in the kidney, liver, small intestine, and colon. Previous studies showed that BPA is excreted into urine through the kidney and into the feces through the intestine and colon after conjugation with glucuronic acid in rat liver microsomes [26].

Although the activity of one glucuronidation enzyme is reportedly reduced in the pregnant rat and is absent in the rat fetus [27,11], BPA levels in the liver declined fairly rapidly in this experiment by the fifth day after injection. Although the half-life of BPA is difficult to determine precisely, radioactivity in the liver declined to one-tenth within 5 days in the present study. The existence of a discharge route for BPA is important, because it indicates that BPA will be eliminated after a certain period of time.

4.4. *The safety of BPA in dental materials*

The amount of BPA leaking from dental materials such as sealants and composite resins is very small [3,8,2,6] and BPA is rapidly excreted from the body [25]. These factors support the safety of dental materials that contain BPA. However, exposure to very low concentrations does not exclude the possibility of endocrine disruption, because this possibility cannot be judged based on analytical data obtained using conventional methods. As the information concerning this subject is very limited, no decisive conclusion can be drawn at present. Although we should not underestimate the benefit that patients derive from Bis-GMA-based materials, we must continue to collect precise information and avoid unnecessary exposure in sensitive individuals. For the present, every possible effort should be made to reduce BPA levels in dental materials. Although polymerized resin is stable and the amount of BPA leaking from it is very limited, unpolymerized resin in the surface layer of sealant may mix with saliva and be absorbed after swallowing [6]. For this reason, care should be taken to remove or reduce unpolymerized resin. According to data from the authors' laboratory, brushing the sealant surface for 10–20 s was effective in reducing undesirable substances that could leach out from the cured surface of the sealant (Matsumoto et al., unpublished observations). In addition to routine practices such as this, the field of dentistry should promote the development and use of alternative dental materials.

Acknowledgments

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