Bisphenol A diglycidyl ether (BADGE) is a member of the glycidyl ether group. Glycidyl ethers have been widely used as basic components of epoxy resins since the late 1940s. BADGE and its oligomers are major components of epoxy resins. Occupational exposure to BADGE occurs in its production, epoxy resin production, and various uses of epoxy resins. 

Animal experiments have shown effects of bisphenol A on the body, the endogenous bisphenol A may affect endocrinological function. In mice orally exposed to BADGE, generated from BADGE through a metabolic transformation of testosterone in male workers, we conducted a cross sectional study of epoxy resin workers who sprayed BADGE with mixed organic solvents in plastic plants. Their urinary bisphenol A and plasma hormones (including luteinizing hormone (LH), follicle stimulating hormone (FSH), and free testosterone) were examined.

**SUBJECTS AND METHODS**

**Subjects and sample collection**

Forty two male workers, whose work was to spray BADGE with mixed organic solvents as an epoxy resin hardening agent in a machine plant and two related plants located in Aichi Prefecture, served as the exposed workers in this study. They included all workers using BADGE in these plants. According to the product specification sheets of seven hardening agents used, they contained 10–30% BADGE. The product sheets indicated that these agents also contained toluene (0–30%), xylene (0–20%), 2-ethoxyethanol (0–20%), 2-butoxyethanol (0–20%), and methyl isobutyl ketone (0–30%). The workers used the above agents in spray rooms which were not completely airtight. They were in charge of the spraying at least three hours a day, and wore protection devices during the spraying, but their workplace atmosphere was polluted.

To select control subjects, 82 male workers whose age was similar to that of the epoxy resin workers, were randomly selected from 1202 assembly workers who did not use BADGE in the same plants. None refused to be recruited for this study. Forty two workers were finally selected as the controls to be matched to each epoxy resin worker by age (±3) and number of cigarettes/day.

**Main messages**

- BADGE may generate bisphenol A endogenously.
- Bisphenol A may disrupt secretion of gonadotrophic hormones in men.

**Policy implication**

- Clinical significance of endocrine disrupting effects by bisphenol A should be further investigated in male workers exposed to bisphenol A.

**Abbreviations:** BADGE, bisphenol A diglycidyl ether; FSH, follicle stimulating hormone; LH, luteinising hormone; HPLC, high performance liquid chromatography; ECD, electrochemical detector; BDL, below detection limit
This study was conducted according to the Declaration of Helsinki. All subjects participated in the study as volunteers, and gave their written informed consent.

Mean age (37±9, 38±10), percentage of current smokers (86%, 86%), and number of cigarettes/day among smokers (21±7, 21±6) were comparable between the epoxy resin sprayers and the controls. The percentage of alcohol drinkers was significantly lower in the epoxy resin sprayers (43%) than in the controls (57%) (p = 0.031).

Urine and blood samples were collected at periodic health examinations conducted in June and July, 1999, but not after a night shift. Urine samples were collected in the morning (10–12 am), but not on the first day of the week. Peripheral blood was collected in an EDTA-2Na tube at 10–12 am on the same day as urine sampling. Plasma was collected by centrifugation. A simple questionnaire was used to obtain lifestyle information, including smoking and alcohol drinking habits.

Analytical methods

Urinary bisphenol A was measured by high performance liquid chromatography (HPLC) with an electrochemical detector (ECD, Model 5600A CoulArray Detector, ESA, Inc., Chelmsford, MA) using modifications of methods reported previously.10,11 Briefly, 50 µl of β glucuronidase (Wako Chemicals, Osaka, Japan) and 200 µl of 0.1 M sodium acetate buffer (pH 5.0, 0.1% ascorbic acid, 0.01% EDTA) were added to 200 µl of urine. Dimethylbutylidene bisphenol (Wako Chemicals) was added to this solution as an internal standard. After incubation at 37°C for three hours, 1.2 ml ethanol was added to the solution. One ml of supernatant was obtained by centrifugation at 12 000 g for 15 minutes, and was evaporated by a vacuum evaporator. Residue was resolved by 0.3 ml of 50% methanol. After filtration, 40 µl of the solution was injected into the HPLC system. The analysis conditions of the HPLC-ECD system were as follows: octadecyl column (ODS250, 250×4.6 mm, MC Medical, Inc., Tokyo, Japan); mobile phase (A), acetonitrile–water–phosphoric acid (20:79:8.0); mobile phase (B), acetonitrile–water–phosphoric acid (80:18.0:2.0); linear gradient programme, 25%B (0–28 min), 25%B–100%B (28–37 min); ECD detector voltages, 360, 400, 440, 480, 520, 560, 600, and 640 mV. The peak of bisphenol A was confirmed using profiles of reactions in these eight channels. The bisphenol A detection limit in this solution as an internal standard. After incubation at 37°C for 15 minutes, and was evaporated by a vacuum evaporator. Residue was resolved by 0.3 ml of 50% methanol. After filtration, 40 µl of the solution was injected into the HPLC system. The analysis conditions of the HPLC-ECD system were as follows: octadecyl column (ODS250, 250×4.6 mm, MC Medical, Inc., Tokyo, Japan); mobile phase (A), acetonitrile–water–phosphoric acid (20:79:8.0); mobile phase (B), acetonitrile–water–phosphoric acid (80:18.0:2.0); linear gradient programme, 25%B (0–28 min), 25%B–100%B (28–37 min); ECD detector voltages, 360, 400, 440, 480, 520, 560, 600, and 640 mV. The peak of bisphenol A was confirmed using profiles of reactions in these eight channels. The bisphenol A detection limit in this solution was approximately 10 pg (0.05 pmol). The bisphenol A concentration was determined by a linear regression line of standard bisphenol A (Tokyo Kasei Co., Tokyo, Japan), and was adjusted by a recovery rate of the internal standard. The recovery rates of bisphenol A and the internal standard were approximately 100%. The coefficient of variation of the measure was <10%. A blank sample containing water (Milli-Q SP VOC, Millipore Co., MA) instead of urine was treated using the same method, and analysed. The value of the blank sample (0.5 pmol/ml) was subtracted from the values of the samples. If the value of a sample was below the blank level, the sample level was considered “not detected”. The water was confirmed to contain no bisphenol A. The bisphenol A concentration was adjusted by urinary creatinine concentration.

We analysed the concentrations of the following urinary metabolites: o,p′-cresol, o,p-,m-,p′-,p-,p′-,o-,m′-,p″-,2,3-dimethylphenol, methylhippuric acids, 2,3-dimethyl-2,6-dimethoxyphenol, and methyl isobutyl ketone.

Urinary o-cresol was measured using the method of Taguchi and colleagues.12 Briefly, the hydrolysed sample was extracted with ethyl acetate. The extract was measured using a high performance liquid chromatograph (HPLC, Model L-7000, Hitachi Ltd, Ibaraki, Japan) equipped with a fluorescence spectrophotometer (Ex270/Em290 nm, Model F-1050, Hitachi Ltd).

The methylhippuric acid concentration was determined according to Hasegawa and colleagues.11 Briefly, a diluted urine sample was measured using an HPLC (Model L-7000, Hitachi Ltd) with a fluorescence detector (230 nm). The detection limit was <5%. The coefficients of variation for these measures were <5%.

Statistical analysis

Values, including urinary bisphenol A, metabolites, and plasma hormones, were log transformed for statistical analysis because their distribution was not normal. Differences between the two groups (BADGE workers and controls) were tested by paired t test. Pearson’s correlation coefficient was calculated to assess the degrees of relations between the metabolites and plasma hormones. To adjust for age and alcohol drinking habits, possible confounders, a multiple linear regression analysis was used to assess relations between the metabolites and the hormones. Differences in proportions concerning basic characteristics between BADGE workers and controls were tested by the χ² test. A p value less than 0.05 (two tailed) was considered significant.

RESULTS

Urinary metabolites of organic solvents were detected more frequently in the epoxy resin workers compared with the controls: o-cresol (81%, 33%), methylhippuric acids (62%, 12%), and 2-butoxyacetic acid (62%, 0%). Median concentrations (range) were as follows: o-cresol, 35 (below detection limit (BDL) to 816) µmol/mol creatinine; methylhippuric acids, 1.7 (BDL to 23) µmol/mol creatinine, and 2-butoxyacetic acid, 0.6 (BDL to 70) µmol/mol creatinine in the epoxy resin sprayers. In controls, o-cresol was BDL (BDL to 467) µmol/mol creatinine and methylhippuric acids were BDL (BDL to 4) µmol/mol creatinine. Concentrations of urinary 2-butoxyacetic acid in the controls were below the detection limit. Urinary metabolite concentrations were all higher in the epoxy resin workers compared with the controls. Urinary 2-ethoxyacetic acid and MIBK were not detected in either group.

Figure 1 shows the concentrations of bisphenol A in urine. A significant difference in bisphenol A concentrations was observed between the epoxy resin sprayers (median 1.0; range: not detected to 11.2 µmol/mol creatinine) and the controls (median 0.52; range: not detected to 11.0 µmol/mol creatinine) (p = 0.002, average difference = 2.5; 95% confidence interval (CI) 1.4 to 4.7). We could not detect bisphenol A for three epoxy resin sprayers and one control.

Table 1 shows the average plasma hormone concentrations. Free testosterone concentrations did not differ between the epoxy resin sprayers and controls (p = 0.74, average difference = 1.0; 95% CI –1.1 to 1.1). No difference in LH concentrations between the two groups was observed (p = 0.41, average difference = 1.1; 95% CI –1.1 to 1.3). However, we observed a significant difference in FSH concentrations between the sprayers (median 5.3 mIU/ml) and the controls (median 7.6 mIU/ml) (p = 0.022, average difference = 1.1; 95% CI –1.1 to 1.1).

FSH showed a mild correlation with urinary bisphenol A (correlation coefficient = 0.20, p = 0.071, fig 2), but not with colleagues.14 Briefly, a urine sample was extracted by methyl-ene chloride. The methylene chloride layer was injected into a gas chromatograph (GC) equipped with a flame ionisation detector (Model G-3000, Hitachi Ltd).

Methyl isobutyl ketone in urine was analysed by the head space method. Briefly, urine was heated, then the head space air inside the vial was injected into a GC equipped with a flame ionisation detector (Hitachi Model 263–50, Hitachi Ltd).

Detection limits were as follows: cresol 4 ng/ml; methylhippuric acid 1 µg/ml; 2-methoxyacetic acid 2 µg/ml; and 2-butoxyacetic acid 1 µg/ml.

Luteinising hormone (LH), follicle stimulating hormone (FSH), and free testosterone in plasma were measured by radio immunosolvent assay in a commercial laboratory (SRL Inc., Tokyo, Japan). The reference values for these determinations provided by the laboratory were 1.8–5.2 IU/ml, 2.9–8.2 IU/ml, and 14–40 pg/ml, respectively. The coefficients of variation for these measures were <5%.
the metabolites of organic solvents. The multivariate analysis showed a statistically significant relation between FSH and bisphenol A, adjusted for alcohol drinking habits (p = 0.045). No significant correlations were observed between other hormones and urinary metabolites (table 2).

DISCUSSION

In this cross sectional study, we observed an increased excretion of bisphenol A and decreased plasma FSH in the male workers who sprayed epoxy resin hardening agents containing BADGE compared to the matched controls.

A previous in vivo study reported many types of urinary metabolites in mice which had orally ingested BADGE. According to the study, bisphenol A was one of the minor metabolites, and the amount was reported to be small. The experiment by Climie et al used radiolabelled BADGE, so the results of their study seemed to be generally reliable. Nevertheless, we observed an increased mean concentration of bisphenol A in the exposed sprayers.

In this study, we could not analyse major metabolites of BADGE, for example, bis-diol of BADGE, because their standard reagents were not available. Thus, we could not directly confirm exposure to BADGE in the sprayers. However, increased metabolites of some organic solvents in the epoxy resin hardening agents supported the hypothesis that BADGE also entered their body during work. The vapour pressure of BADGE is unknown, but as it is generally used as a liquid, workers can intake the mist. Consumption of canned beverages, a suspected potential source of bisphenol A exposure in daily life, was comparable between the epoxy resin sprayers and the matched controls, but few subjects consumed them in either group. Urinary bisphenol A was detected in most of the controls, therefore other unknown sources of bisphenol A intake cannot be excluded. However, no specific source contributed to bisphenol other than in BADGE in the sprayers, because a study of urinary bisphenol A concentrations in rural residents in Japan has shown that most of the general population have concentrations similar to those of the controls in the present study (manuscript in preparation). It is reasonable to consider that bisphenol A was generated endogenously by occupational exposure to BADGE.

One control showed a high concentration of bisphenol A as shown in fig 1. One possibility is that he was exposed to BADGE passively near the spraying works. Similar phenomena were observed in urinary metabolites of organic solvents. Use of protective devices and passive exposure might cause a selection bias in this survey.

Effects of bisphenol A on the endocrinological system have been reported. Its oestrogen receptor binding capacity has been observed in earlier reports. Concerning genital function in males, vom Saal et al observed decreased sperm production, although a negative result has also been reported, and Takao et

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**Table 1** Median (interquartile range) of plasma hormone levels in workers using bisphenol A diglycidyl ether and control workers

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Exposed sprayers (n=42)</th>
<th>Control workers (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH [mIU/ml]</td>
<td>4.0 (4.0–5.0)</td>
<td>4.0 (3.0–6.0)</td>
</tr>
<tr>
<td>FSH [mIU/ml]</td>
<td>5.3 (4.0–8.3)</td>
<td>7.6 (5.4–11.0)</td>
</tr>
<tr>
<td>Free testosterone [pg/ml]</td>
<td>15.2 (12.7–18.7)</td>
<td>15.1 (12.5–17.1)</td>
</tr>
</tbody>
</table>

**Table 2** Standardised partial regression coefficients between selected serum hormones and urinary metabolites in spray workers and controls

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>LH [mIU/ml]</th>
<th>FSH [mIU/ml]</th>
<th>Free testosterone [pg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisphenol A [µmol/mol creatinine]</td>
<td>-0.05 0.65</td>
<td>-0.23 0.045</td>
<td>-0.15 0.17</td>
</tr>
<tr>
<td>o-Cresol [µmol/mol creatinine]</td>
<td>-0.05 0.64</td>
<td>-0.03 0.82</td>
<td>-0.13 0.23</td>
</tr>
<tr>
<td>Methylhippuric acids [µmol/mol creatinine]</td>
<td>-0.01 0.90</td>
<td>-0.07 0.56</td>
<td>0.19 0.08</td>
</tr>
<tr>
<td>2-butoxyacetic acid [mmol/mol creatinine]</td>
<td>-0.02 0.88</td>
<td>-0.18 0.12</td>
<td>-0.06 0.61</td>
</tr>
</tbody>
</table>

Log translated values were used for metabolites and hormone levels. Standardised partial regression coefficients were adjusted for age and alcohol drinking habits.

*Half the detection limit was used for a subject whose urinary metabolite was not detected.
observed decreased free testosterone and LH in male mice exposed to bisphenol A. Furthermore, an experiment by Gupta et al. showed that bisphenol A decreased epididymal weight in male offspring following maternal exposure.1 In men, LH and FSH are involved in sperm production and androgen synthesis. In the present study, LH and free testosterone were not different between the exposed sprayers and the controls, but we observed decreased FSH in the former. The controls in this study were clinically healthy persons confirmed by a periodical health examination. The reference values for FSH determination also supported the fact that they were clinically normal although some showed relatively high concentrations.

In endocrinological studies, timing of blood sampling in the daytime is an important factor for estimation of hormone concentrations. A "cross sectional" evaluation is a major limitation of our study. However, in this study, the timing of blood sampling was almost similar, and was not after the night shift, for all subjects; a previous study observed that diurnal rhythm for FSH was less notable than that for LH and testosterone in men.12 This may explain why there was no observable relation between LH and bisphenol A.

We speculate that endogenous bisphenol A bound to oestrogen receptors in the pituitary body, and FSH secretion was directly suppressed. Oestrogen receptors have been found in the human pituitary body,23 and Finkelstein et al. showed that oestradiol directly inhibited gonadotrophin secretion at the pituitary level in men.24 On the other hand, absence of a feedback effect of testosterone on FSH in men has been reported,25 a finding that may also support our speculation.

Effects of chemical exposure on male reproduction have been a concern from the late 1800s in occupational medicine. Some male fertility have been discussed elsewhere.26 Vents were relatively low. It cannot reasonably be assumed that one possibility is that mixed organic solvents contained in the one was observed in mice,27 observation, serum gonadotrophic hormones may be a sensitive feedback effect of testosterone on FSH in men.28 FSH concentrations in male workers exposed to toluene.29 The pituitary level in men.30 FSH concentrations. A "cross sectional" evaluation is a major limitation of our study. However, in this study, the timing of blood sampling was almost similar, and was not after the night shift, for all subjects; a previous study observed that diurnal rhythm for FSH was less notable than that for LH and testosterone in men.12 This may explain why there was no observable relation between LH and bisphenol A.

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