Appendix: Evaluation of individual chemicals and future examination

This committee mainly discussed future fundamental lines and examined, among the substances suspected of being endocrine disruptors, some substances used as materials for plastic food containers. Substances being eluted from food containers have high potential for intake with foods, and therefore are regulated by the Food Sanitation Law to prevent the occurrence of hygienic problems. The substances were discussed at the Joint Committee of Toxicity and Apparatus, Containers and Packages under the Food Sanitation Council, which met on March 13, 1998. Current information about these substances, and the basic line of future research, can be summarized as follows.

We refer to data that have not been published as papers. These data should be examined in detail after the official publication of papers.

1. Polycarbonate

Owing to its high transparency and hardness, polycarbonate is used in food containers, compact discs, car light covers and OA instruments. This plastic is generally formed by repeated polycondensation using bisphenol A and carbonylchloride or diphenylcarbonate as materials. Bisphenol A is suspected of being an endocrine disruptor.

- Annual food-related usage (investigated by Japan Hyginic Olefine and Styrene Plastics Association, unit: 1,000 ton)
  Production: 251; export: 121; domestic sales: 130, including food-related use: 4

(1) Elution of bisphenol A

Elution of bisphenol A from tableware was studied at the National Institute of Hygienic Sciences and other facilities. As shown in Table 1, its elution volumes were approximately below 100 ppb, and many were below the detection limit[*91*92*93*94*95*96].

N-heptan (mimicing solvent for oils, fats and liposoluble foods), 4% of acetic acid (mimicing solvent for water-soluble and acid foods), ethanol (mimicing solvent for

alcoholic beverages) and water (other foods) were used as elution solvents. The elution volumes from n-heptan were relatively high, but some were below the detection limit. It seems that elution volumes are influenced by many factors, such as temperature, extent of deterioration in tableware, conditions of tableware use and kind of solvent.

(2) Safety of bisphenol A

① Screening

・ In vitro study

Binding capacity of bisphenol A to hormone receptor, and gene activation resulting from such binding, can be determined by in vitro study.

It has been pointed out that bisphenol A has estrogenic (female sex hormone) activity; there are reports showing the extent of such action:

1) In a study to examine binding antagonism with ^{3}H-estradiol (E_2) and rat uterine cytosolic fraction, binding affinity of bisphenol A to estrogen receptor (ER) was 0.012 assuming that of E_2 as 100[*97].
2) Bisphenol A that eluted from a polycarbonate flask showed estrogenic action and its binding capacity to ER was 1/2,000 that of E_2[*98].
3) In a study to examine binding affinity with ER using MCF-7 (human mammary carcinoma cell line), binding affinities of bisphenol A in the medium with or without adult human male serum were 0.01 and 0.006, respectively, assuming the binding affinity of E_2 as 100[*99].

・ In vivo study

Bisphenol A (1.0 - 1,000 mg/kg, once a day for 4 days) was given subcutaneously to ovariectomiyed B6C3F1 mice. Increase in uterine weight and relevant histological changes were found in parallel to the induction of heat-shock protein[*100].

(Reference data)

At present, a quick, simple and representative in vivo method for evaluating estrogenic action of chemicals is to administer chemicals to rodents such as rats and weigh the increase in uterine weight. Subcutaneous administration of bisphenol A at a dose of 400 mg/kg once daily for 3 days to immature rats resulted in increase in uterine weight[*101].

② Repeat dose toxicity study

The two-generation reproductive study has been considered suitable for comprehensively evaluating endocrine disrupting influence on reproductive function and investigating influence on subsequent generations. In a two-generation reproductive study, a certain chemical is administered over two generations and, its influence on reproductive functions such as estrus, mating, conception, delivery and nursing, and neonatal growth after delivery and weaning are examined.

As regards bisphenol A, though the data from two-generation reproductive studies are not sufficient, several animal experiments have been reported:

1) bisphenol A was given to pregnant mice at doses of 2 and 20 μg/kg/day, and the prostate weight of 6 month-old male offsprings increased by 30 to 35%[*99].
2) In a 103 week chronic toxicity study in F344 rats fed a mixed diet (1,000 and 2,000 ppm), rats in all subject groups lost weight, but carcinogenicity was not observed[*102].
3) In a 103-week chronic toxicity study in B6C3F1 mice fed a mixed diet (male: 1,000 and 5,000 ppm; female: 5,000 and 10,000 ppm), mice in groups with the 5,000 ppm and more lost weight, but carcinogenicity was not observed[*102].
4) In a subacute toxicity study using dogs, rats and mice, only the dogs in the highest (9,000 ppm) group showed increase in the liver weight[*102].
5) After rat dams (SD) were given drinking water containing bisphenol A (0.005, 0.05, 0.5, 5 and 50 mg/L) over the period from 2 days after pregnancy to 21 days after parturition, bisphenol A was detected in the dam's milk in the 5 and 50 mg/L groups. There was no estrogenic signs in the pregnant and nursing dams, although there was an increase in dams' relative kidney weight in the 50 mg/L group as a sign of general toxicity[*103]. In the same study, bisphenol A did not affect sexual maturation of female offsprings, esterous cycle, and pituitary and hypothalamic regulation on progesterone[*104].
6) In studies using SD rats fed a mixed diet (1,000, 3,000 and 9,000 ppm; equivalent to approx. 50, 150 and 450 mg/kg/day, respectively) for 100 days in F0 and 90 days in F1, dose-related decrease in body weight gain was observed in dam and F1 infant groups given 3,000 ppm or more, but

bisphenol A did not influence on indices relevant to litter size at birth, postnatal mortality and fertility[*105].

7) In studies using SD rats fed a mixed diet (100, 250, 750 and 1,000 ppm; equivalent to approx. 5, 12.5, 37.5 and 50 mg/kg/day, respectively) for 90 days, bisphenol A had no influence other than low body weight in the male parent at 1,000 ppm group; bisphenol A did not influence female estrous cycle and the above reproductive indices[*105].

8) In a multi-generation reproductive study in ICR mice fed a mixed diet (2,500, 5,000 and 10,000 ppm; equivalent to approx. 473.5, 875 and 1,750 mg/kg/day, respectively), decrease in body weight gain, increase in the liver and kidney weight, prolonged gestation period, low values of male accessory reproductive organs, and reduction in sperm motility etc. were observed in F0 group at 10,000 ppm; and decreased in food intake, litter-size at birth, and numbers of survived pups per litter were observed in the groups at 5,000 ppm or more. As for F1 groups, increase in mortality was observed at 10,000 ppm group; increase in the liver and kidney weight, and decrease in male reproductive organ weight were seen in all groups given 2,500 ppm or more, but decrease in litter-size at birth was not observed in any group. Some adverse influence on reproduction was observed in such high-dose groups, that showed systemic toxicosis[*106].

9) In a teratogenic study in SD rats injected intra peritoneally for 15 days from gestation day (GD) 1 to 15 (85 and 125 mg/kg/day, corn oil), maternal toxicities such as decrease in pregnancy ratio etc. were observed at 125 mg/kg group; fetal toxicity such as ossification failure was seen in the groups at 85 mg/kg or more[*107].

10) In a teratogenic study in ICR mice orally administered for 10 days from GD 6 to 15 (500, 750, 1,000 and 1,250 mg/kg/day), increase in dam mortality was observed in all groups; and decrease in relative liver weight was seen in groups at 500 mg/kg or more. In 1,250 mg/kg group, decrease in uterine weight and numbers of corpora lutea, and increase in numbers of dead and absorbed fetuses and of fetuses with defects were observed, with associated severe toxicosis in dams[*108].

11) In a teratogenic study in SD rats orally administered for 10 days from GD 6 to 15 (160, 320, 640 and 1,280 mg/kg/day), increase in dam mortality was observed at 1,280 mg/kg group; decrease in body weight gain was seen in groups given 160 mg/kg or more. Fetal toxicity and teratogenesis were not

observed in groups given 640 mg/kg or less. Because many dams at 1,280 mg/kg group died, teratological evaluation could not be done[*108].

③ Pharmacodynamics

Studies on chemical accumulation and metabolite influence would provide information whether the chemical effect in vivo was caused by the parent compound itself or by the metabolites, as well as cues to clarify the causes of in vivo effects. Particularly in case of endocrine disruptors with suspected concerns of adverse effects over generations, information on the bio-accumulation has a vital importance. In view of the influence on the fetus, chemical concentration in the umbilical cord blood would provide one of the useful indices.

There are not many reports on in vivo dynamics of bisphenol A. In a single oral dose (800 mg/kg) study on ^{14}C bisphenol A in male rats, 28% and 56% of ^{14}C that has been administrated were excreted into the urine and the stool, respectively, within 8 days after administration. It was also reported that residual ^{14}C was not detected in the body at 8 days after administration[*109].

(3) Conclusion

Bisphenol A in vitro has approx. 10^{-5} to 10^{-4} times of ER binding capacity, compared to that of estradiol (E_2). In vitro study results suggest that bisphenol A might have a similar action in vivo as well, however, in vivo, multiple factors such as metabolism, biological process including excretion, endocrine feedback mechanisms and influence of blood component etc. would complicate the situation. Therefore, it is necessary to evaluate the actual in vivo effect after careful assessment of the in vivo testing.

In vivo studies on prostate weight and carcinogenicity have been carried out, but there are no data showing in vivo toxicity of bisphenol A at the actual exposure level, by the following reasons: 1) prostate weight is not a general assessment item, and the mechanism of its weight gain is unknown; 2) carcinogenicity and two-generation reproductive studies were performed at the relatively high doses of 1,000 ppm and more and 100 ppm and more, respectively. The US Environmental Protection Agency has settled the RfD (equivalent to acceptable

daily intake (ADI)) of bisphenol A as 0.05 mg/kg/day[*102].

As indicated above, there is no scientific information that bisphenol A, at the levels eluting from polycarbonate, seriously affects human health; therefore, it would not be necessary to prohibit the use of polycarbonate at present. However, endocrine disruptors arise a new problem; some researchers claim that even small amounts of bisphenol A can have an effect especially in infants, whose endocrine systems are still immature, even if it is not toxic to adults, whose endocrine feedback systems has already been established. We should therefore conduct further research such as two-generation reproductive studies.

(Reference data)
Recently, exposure to drinking water with very low concentrations of bisphenol A, during gestation and lactation was conducted; it was reported that bisphenol A has no influence on the offsprings. However, this report was presented at an academic meeting, and should be reviewed in detail after published as a paper[*110].

(4) Future research

- A two-generation reproductive study in rats, and a Pharmaco-dynamic study in rats (bio-accumulation, transfer to the fetus and milk) etc.
- Bisphenol A concentration research in human umbilical cord blood and breast milk, and research for organ distribution
- Research for elution volumes from tableware and cans