Food Sci. Technol. Res., **13** (4), 305–309, 2007

Dietary Psyllium Protects Immature Rats from Estrogenic Activity of Bisphenol A

Makoto SUZUKI and Keisuke TSUJI*

School of Human Science and Environment, University of Hyogo, Shinzaike-Honcho, Himeji, Hyogo 670–0092, Japan

Received September 29, 2006; Accepted March 22, 2007

This study was conducted to determine whether dietary psyllium (PSY) can protect against the estrogenic activity of Bisphenol A (BPA) in a rat uterotrophic bioassay. Fifteen immature female rats were fed a commercial diet (blank diet), a blank diet containing 0.1% BPA (control diet), or a blank diet with 0.1% BPA and 5% PSY (PSY diet) for 6 days. The uterine weight of the control group was significantly higher than of the blank group. The uterine weight and the uterine BPA levels of the PSY group were significantly lower than the control group. Serum BPA concentrations and liver BPA levels of the PSY group tended to decrease compared to the control group. However, BPA excretions in feces were significantly higher in the PSY group than in the control group ($p<0.01$). These observations indicate that dietary PSY feeding can protect against the estrogenic activity of BPA in rats.

Keywords: psyllium, bisphenol A, uterotrophic bioassay, estrogenic activity

Introduction

In recent years, "endocrine disruptors (EDs)" have been the focus of international attention. They may have the potential to disturb normal sexual differentiation and development in wildlife and humans (McLachlan, 1993; McLachlan and Korach, 1995). There are several mechanisms through which these environmental chemicals disrupt our endocrine functions. EDs mainly influence the hormone receptors in the cells. They can initiate the transcription of the estrogen receptor-regulated genes (Bulger *et al.,* 1978; Bolger *et al.,* 1998; Gould *et al.,* 1998) and stimulate estrogen-mediated physiologic responses (Gray *et al.,* 1988; Cooper and Kavlock, 1997; Gray and Ostby, 1998). It is also a significant concern that EDs have been linked to an increase in the incidence of endometrial or breast cancers (Falck *et al.,* 1992; Wolff *et al.,* 1993).

Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl)-propane, is used in the manufacture of polycarbonate plastics and epoxy resins, both of which are used in a wide variety of applications. Domestic BPA manufactures supplied approximately 580,000 tons, and the domestic consumption was approximately 430,000 tons in 2003 (Nakanishi *et al.,* 2005). It is also used for the internal coating of food cans for long-term storage of food because BPA is stable in acid and at high temperature. However, in recent years, BPA has been confirmed to have weak estrogenicity, that is, approximately 15,000 times less potent than 17 β-estradiol (Feldman and Kishnan, 1995; Olea *et al.,* 1996; Gaido *et al.,* 1997; Kuiper *et al.,* 1997; Laws *et al.,* 2000). A number of investigators have previously demonstrated the potential estrogenic activities of BPA (Dodds and Lawson, 1936; Krishman *et al.,* 1993; Soto *et al.,* 1995; Nagel *et al.,* 1997; Colerangle *et al.,* 1997; Yamasaki *et al.,* 2000).

Previous studies on EDs have mainly focused on the development of ED measurement techniques of EDs, the risk assessment approach, and the evaluation of exposure levels into the environment (Giesy *et al.,* 1994; Sumpter and Jobling, 1995; Guilltte *et al.,* 1996; Coldham *et al.,* 1997). However, there are few studies to prevent or diminish the toxicity of their estrogenic activities. The exposure to EDs via food is the major route for humans and wildlife. Thus, to prevent any toxicity from EDs, it is important to inhibit the absorption of EDs from the intestine and to increase the excretion of EDs into the feces. If these chemicals ingested via food could be captured in the digestive tract, it may be possible to protect against the estrogenic effects of EDs.

Psyllium (PSY) is a partially fermented dietary fiber from *Plantago ovata.* PSY increases the stool weights and promotes laxation by its presence in the stool and by increasing the moisture content of the stool. PSY has mainly focused on the effect of soluble dietary fiber on modulating the lipid metabolism function by stimulating the gastrointestinal tract as a result of inhibiting cholesterol absorption and bile acid reabsorption as non-digestive properties (Prynne and Southgate, 1979; Spiller *et al.,* 1979; Stevens *et al.,* 1988). We postulated that it may also be possible to inhibit the absorption of EDs by dietary PSY.

The aim of the present study was to determine whether dietary PSY feeding can protect from the estrogenic activity of BPA in rats. The uterotrophic bioassay was proposed by the Organization for Economic Cooperation and Development (OECD) as one of the screening methods for detecting the estrogenic properties of some chemicals *in vivo* (OECD, 1999). It was elucidated that some estrogenic chemicals possess the ability to increase the uterine weights of female immature rats or ovariecto-

* To whom correspondence should be addressed.
E-mail: ktsuji@shse.u-hyogo.ac.jp

mized adult rats. In the present study, the rat uterotrophic bioassay using BPA was conducted to determine whether dietary PSY can protect against the estrogenic activity of BPA.

Materials and Methods

Materials and reagents We purchased Bisphenol A (BPA) (>99.9%) from Kanto Chemical Co., Tokyo, Japan, and psyllium seed gum (Healthy gum; Dainippon Pharmaceutical Co., Ltd, Osaka, Japan) was from Nisshin Food Products Co., Ltd, Osaka, Japan. All other chemicals were of the highest quality available.

Animals, diets and study design Fifteen female immature Sprague-Dawley rats (Nihon Clea, Inc., Tokyo, Japan), 15 days old, were individually housed in stainless steel wire cages in a room maintained at 22–24℃ with a controlled 12-hour light (07 : 00–19 : 00)/dark cycle and free access to food and water. After a 3-day acclimatization, the rats were randomly divided into 3 groups of 5 animals each. Water and feed were consumed *ad libitum*. Rats received the experimental diets shown in Table 1 for 6 days. The diets were based on the commercial powder diet, CE-2 (moisture; 8.6%, crude protein; 24.9%, crude fat; 4.6%, crude fiber; 3.7%, crude ash; 6.7%; NFE, nitrogen free extracts, 51.4%) from Nihon Clea. Rats were fed a blank diet, a blank diet containing 0.1% BPA (control diet), or a blank diet with 0.1% BPA and 5% PSY (PSY diet). The food intake was measured by subtracting the remaining feed from the amount offered every day. The body weight gains of the rats were monitored daily. After 6 days of the experimental feeding program, the rats were killed by decapitation and the blood was withdrawn from the cervical aorta under diethyl ether anesthesia. The wet liver, uterine, and other tissues of each rat were extracted and immediately weighed. The feces of the rats were collected every 2 days during the experimental period.

All animals were cared for according to the principles outlined in the guide for animal experimentation prepared by the Japanese Association for Laboratory Animal Science.

Analysis of BPA The serum of the rats were separated by centrifugation at 3,000 rpm for analysis of the BPA and stored at −20℃. The serum (2 mL) was added to 3 mL of cold acetone, and the mixture was thoroughly vortexed and centrifuged at 12,000 rpm and 4℃ for 3 min. The supernatant (500 μL) was added to 1.5 mL of distilled water, and diluted with 20% acetone. The liver and uterine tissues were homogenized with 25 mL of chloroform/methanol (2 : 1) using a homogenizer, and 1 mL of the homogenate was centrifuged at 12,000 rpm and 4℃ for 3 min. The supernatant was removed and evaporated to dryness, and the residue was then solubilized in 20% acetone. The fecal samples from each rat were homogenized after drying for 7 days, and then quantitatively extracted with 60% cold acetone. The mixture was thoroughly vortexed and centrifuged at 12,000 rpm and 4℃ for 3 min. The supernatant (500 μL) was added to 1.5 mL of distilled water, and diluted with 20% acetone. The concentrations of BPA in the serum, liver, uterine, and feces were determined by competitive enzyme-linked immunosorbent assay (ELISA) using commercial BPA ELISA kits (Takeda Chemical Industrial, Ltd., Tokyo, Japan). Our procedures were followed as per the manufacturer's instructions.

Apparent absorption rates of BPA The apparent absorption rates of the BPA were calculated as follows: absorption rate (%)=(intake of BPA)/(excretion of BPA) ×100.

Statistical analysis A stati stical analysis was performed using the SPSS ver.8.0 software package (SPSS Japan, Inc., Tokyo, Japan). The results were expressed as mean values with their standard errors for the 5 rats in each group. One-way ANOVA was used to determine the dietary effects. When a significant diet effect was detected, differences among the diet groups were identified using Tukey's multiple range tests. The differences were considered statistically significant at p-values <0.05 or <0.01. Student's t tests were used to compare the BPA levels in the serum, liver, and uterine and BPA excretion into the feces within the control group and the PSY group.

Results

Growth performance and BPA intake The cumulative food consumption and the body weight (bw) gain during the feeding period did not vary among the experimental diet groups (Table 2). The BPA intake was 237±4 mg/kg bw/day and 243±7 mg/kg bw/day in the control group and the PSY group, respectively.

Uterine and other tissue weights The wet uterine

Table 1. Composition of the experimental diets.

	Blank	Control	Psyllium
Commercial diet*	100.0	99.9	94.9
Bisphenol A	–	0.1	0.1
Psyllium	–	–	5.0
Total	100.0	100.0	100.0

Eighteenth day old female rats of SD were divided into 3 groups.
* CLEA rodent diet, CE-2 (Nihon Clea Inc.).

Table 2. Effects of psyllium feeding on growth and food intake in SD rats fed diets supplemented 0.1% bisphenol A.

	Blank	Control	Psyllium
Body weight			
Initial (g)	48.6 ± 1.7	49.8 ± 2.1	47.2 ± 2.3
Final (g)	87.2 ± 4.1	89.8 ± 2.6	86.5 ± 1.9
Body weight gain (g/day)	6.43 ± 0.42	6.67 ± 0.11	6.56 ± 0.48
Food intake (g/day)	16.1 ± 0.4	16.5 ± 0.3	16.2 ± 0.3
Food efficiency*	39.8 ± 1.5	40.4 ± 0.4	40.5 ± 3.0

Data are expressed as mean±SE (n=5).
* (g body weight gain)/(g food intake)×100.

weight was significantly heavier in rats fed the control diet compared to the rats fed the blank diet ($p<0.01$) (Fig. 1). Conversely, the wet uterine weight of rats fed the PSY diet was significantly lower than that of rats fed the control diet. In other tissue weights, no significant differences were observed in the carcass, liver, heart, lungs, kidneys, spleen, thymus, mesenteric adipose, and perirenal adipose. However, the digestive tract, i.e., stomach, small intestine and cecum, weights of the rats in the PSY group were significantly higher than that of the control group (data not shown).

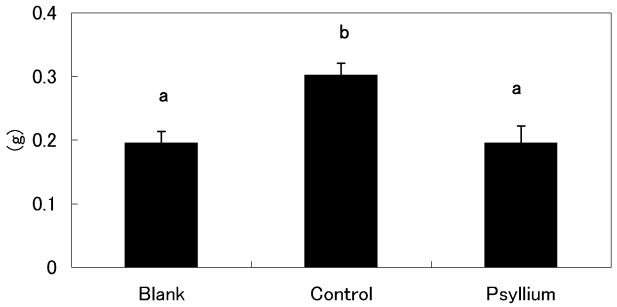

Fig. 1. Effects of psyllium feeding on wet uterine weights of SD rats fed diets supplemented with 0.1% bisphenol A.
Data are expressed as mean±S E ($n=5$). Values in each column not followed by the same alphabetical letter are significantly different ($p<0.01$).

Table 3. Effects of psyllium feeding on levels of serum, liver, and uterine bisphenol A concentration in SD rats fed diets supplemented 0.1% bisphenol A.

	Blank	Control	Psyllium
Bisphenol A			
Serum (ng/ml)	0	298 ± 16	190 ± 8**
Liver (μg/Liver)	0	153.5 ± 21.6	96.7 ± 15.5*
Uterine (μg/Uterine)	0	11.86 ± 2.16	4.39 ± 0.95**

Data are expressed as mean±SE ($n=5$).
bw: body weight.
** Significantly different from control group ($p<0.01$).
* Significantly different from control group ($p<0.05$).

Serum, liver, uterine, and fecal BPA levels PSY feeding affected the BPA levels in the serum, liver, uterus, and feces of the rats. The concentrations of BPA in the serum, liver and uterine were significantly lower in the PSY group compared to the control group ($p<0.05$) (Table. 3). The excretion amount in feces and the fecal BPA levels of the rats fed the PSY diet were significantly higher than those of the rats fed the control diet during the experimental period (Fig. 2). The fecal excretion of BPA in the PSY group was also significantly higher than that of the control group, and as much as 3 times higher by the end of the experiment (days 5–6).

Apparent absorption rates of BPA The apparent absorption rates of BPA were significantly lower in rats fed the PSY diet than in rats fed the control diet (Fig. 3). Although the absorption rate on days 3–4 and days 5–6 in the rats fed the control diet were 86 % and 80%, the

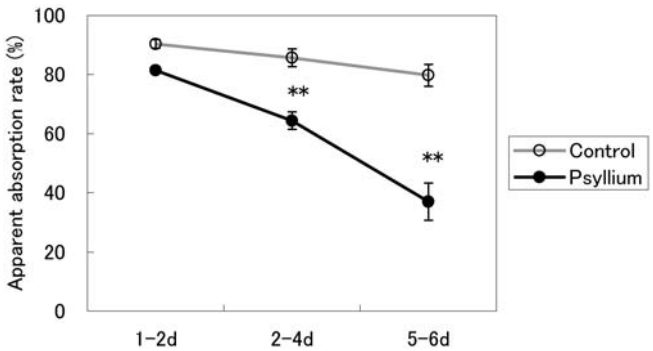

Fig. 3. Effects of psyllium feeding on apparent absorption of bisphenol A in SD rats fed diets supplemented with 0.1% bisphenol A.
Data are expressed as mean±SE ($n=5$).
** Significantly different from control group ($p<0.01$).

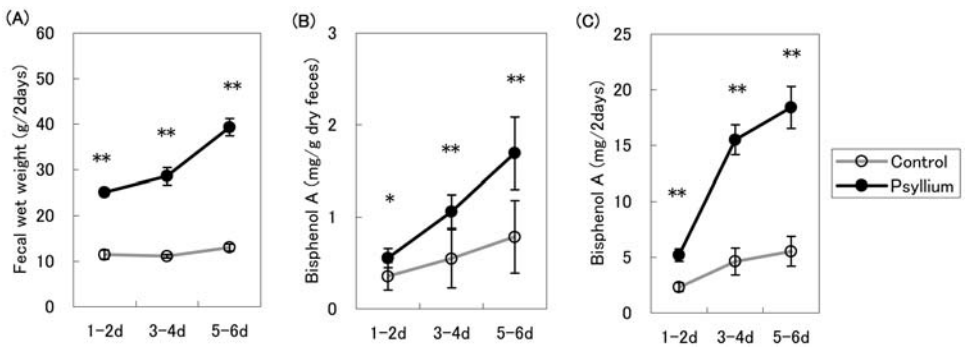

Fig. 2. Effects of psyllium feeding on excretion of feces and fecal bisphenol A excretion in SD rats fed diets supplemented with 0.1% bisphenol A.
Feces were gathered every 2 days in experimental period.
(A) Fecal wet weight, (B) Bisphenol A concentration and (C) Bisphenol A excretion into feces.
Data are expressed as mean±SE ($n=5$).
** Significantly different from control group ($p<0.01$).
* Significantly different from control group ($p<0.05$).

absorption rates in the rats fed the PSY diets were reduced to 64% and 37%, respectively.

Discussion

Although there are concerns that certain chemicals like BPA have the capability to disrupt endocrine functions of animals and humans, there have been few studied on preventing the harmful effect of these chemicals. To prevent damage by BPA in humans, it is important that the BPA ingested via food should be captured in the digestive tract and its absorption decreased. The present study demonstrated that dietary PSY in rats can negate the estrogenic activity of BPA by resisting BPA absorption and increasing BPA excretion into the feces. The wet uterine weights of immature rats have been used as a sensitive parameter for detecting estrogenic activity. The BPA administration level in this study was based on the results of uterotrophic assay by Yamasaki *et al.* (2000), which demonstrated that the wet uterine weight of immature female rats was increased by oral BPA doses of more than 160 mg/kg bw/day. In our study, the BPA intake levels were 237±4 mg/kg bw/day and 243±7 mg/kg bw/day in the control group and the PSY group, respectively.

The wet uterine weight of rats in the PSY group was significantly lower than that of the control group. This observation indicates that dietary PSY may minimize the estrogenic activity of BPA by inhibiting BPA absorption in the small intestine (Fig. 3). Indeed, the lower uterine weight in the PSY group could be due to the significantly higher fecal BPA excretion. These results may be related to the gel forming ability of PSY. Morita *et al.* (1997) reported the effects of several dietary fibers extracted from cereals and vegetables on the fecal excretion of polychlorinated dibenzo-*p*-dioxins congeners in rats. According to this report, although the fecal excretion of 1,2,3,6,7,8,-HxCDD (HxCDD) in the group fed a non-fiber diet was 21% of the dose, the fecal excretion in the group fed 10% rice-bran fiber and spinach fiber was 44% and 45%, respectively. Additionally, Aozasa *et al.* (2001) investigated the effects of 16 dietary fibers on the fecal excretion of dioxin isomer in mice administered a dioxin mixture. Although the fecal excretion of HxCDD in the group fed the non-fiber diet was 22% of the dose, the fecal excretion in the group fed 10% guar gum, locust gum, chitin, pectin, and cellulose was promoted to 57%, 45%, 45%, 43%, and 33%, respectively. Moreover, the fat excretions into the feces of the group fed several dietary fibers were also greater than that of the group fed a non-fiber diet. The enhanced fecal excretion rate of HxCDD by dietary fiber was closely related to fecal fat content. These observations indicated that enhancement of the fecal dioxin isomer excretion by dietary fiber is associated with lipophilic compositions such as lipids or lipophilic vitamins. In our study, PSY could also promote the fecal excretion of BPA in rats. It is well known that PSY has a potential water holding capacity and modulates the lipid metabolism function by a greater excretion of bile acid and total steroids leading to an up-regulation of the bile acid and biosynthesis (Buhman *et al.*, 1998). We speculated that the gel forming ability of PSY may result in a greater viscosity of the intestinal contents, thus reducing the absorption of BPA from the small intestine (unpublished data).

It is difficult to maintain an estrogenic chemical-free environment because many of their properties have commercially important values. In this study, we found that rats fed 5% PSY diets had a significantly lower BPA-induced uterotrophy and BPA accumulation in their bodies, and also significantly promoted BPA excretion into the feces. The mechanisms by which PSY inhibits BPA absorption remain unclear, and there may be other several chemicals similar to PSY. The influence of PSY on BPA absorption needs to be further explored. In conclusion, PSY feeding can minimize the estrogenic activity of BPA in rats by preventing its intestinal absorption and increasing the fecal excretion of BPA.

References

Aozasa, O., Ohta, S., Nakao, T. and Nomura, T. (2001). Enhancement in fecal excretion of dioxin isomer in mice by several dietary fibers. *Chemosphere.,* **45**, 195–200.

Bolger, R., Wiese, T.E., Ervine, K., Nestich, S. and Checovich, W. (1998). Rapid screening of environmental chemicals for estrogen receptor binding capacity. *Environ. Health. Perspect.,* **106**, 551–557.

Buhman, K.K., Furumoto, E.J., Donkin, S.S. and Story, J.A. (1998). Dietary psyllium increases fecal bile acid excretion, total steroid excretion and bile acid biosynthesis in rats. *J. Nutr.,* **128**, 1199–1203.

Bulger, W.H., Muccitelli, R.M. and Kupfer, D. (1978). Interactions of methoxychlor, methoxychlor-base soluble contaminant, and 2,2,-bis(p-hydroxyphenyl)- 1,1,1-trichloroethane with rat uterine estrogen receptor. *Toxical. Environ. Health.,* **4**, 881–893.

Colerangle, J.B. and Roy, D. (1997). Profound effects of the weak environmental estrogen-like chemical bisphenol A on the growth of the mammary gland of Noble rats. *J. Steroid. Biochem. Mol. Biol.,* **60**, 153–160.

Coldham, N.G., Dave, M., Sivapathasundaram, S., McDonnell, D.P., Connor, C. and Sauer, M.J. (1997). Evaluation of a recombinant yeast cell estrogen screening assay. *Environmen. Health. Perspect.,* **105**, 734–742.

Cooper, R.L. and Kavlock, R.J. (1997). Endocrine disruptors and reproductive development: a weight-of-evidence overview. *J. Endocrinol.,* **152**, 159–166.

Dodds, E.C. and Lawson, W. (1936). Synthetic oestrogenic agents without the phenanthrene nucleus. *Nature.* **139**, 996.

Falck, F., Ricci, A.J., Wolff, M.S., Godbold, J. and Deckers, P. (1992). Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch. Environ. Health.,* **47**, 143–146.

Feldman, D. and Kishnan, A. (1995). Estrogens in unexpected places: possible implications for researchers and consumers. *Environ. Health. Perspect.,* **103**, 129–133.

Gaido, K.W., Leonard. L.S., Lovell. S., Gould. L.C., Babai, D., Protier, C.J. and McDonnell, D.P. (1997). Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. *Toxicol. Appl. Pharmacol.,* **143**, 205–212.

Giesy, J.P., Ludwing, J.P. and Tillitt, D.E. (1994). Deformities of birds in the Great Lakes region: assigning causality. *Environ. Sci. Technol.,* **28**, 128–135.

Gould, J., Leonard, L.S., Maness, S.C., Wagner, B.L., Conner, K., Zacharewski, T., Safe, S., McDonnell, D.P. and Gaido, K.W.

(1998). Bisphenol A interacts with the estrogen receptor alpha in distinct manner from estradiol. *Mol. Cell. Endocrinol.,* **142**, 203–214.

Gray, L.E. Jr., Ostby, J., Sigmon, R., Ferrll, J., Rehnberg, G., Linder, R., Cooper, R., Goldman, J. and Laskey, J. (1988). The development of a protocol to assess reproductive effects of toxicants in rat. *Reprod. Toxicol.,* **2**, 281–287.

Gray, L.E. Jr. and Ostby, J. (1998). Effects of pesticides and toxic substances on behavioral and morphological reproductive development: endocrine versus nonendocrine mechanisms. *Toxicol. Ins. Health.,* **14**, 159–184.

Guilltte, L.J. Jr., Pickford, D.B., Crain, D.A., Rooney, A.A. and Percival, H.F. (1996). Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. *Gen. Comp. Endocrinol.,* **101**, 32–42.

Krishman, A.V., Stathis, P., Permuth, S.F., Tokes, L. and Feldmen, D. (1993). Bisphenol-A: An estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology,* **132**, 2279–2286.

Kuiper, G.G.J., Carlsson, B., Grandienm, K., Enmark, E., Haggblad, J., Nilsson, S. and Gustafsson, J.A. (1997). Composition of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. *Endocrinology,* **138**, 863–870.

Laws, S.C., Carey, S.A., Ferrell, J.M., Bodman, G.J. and Cooper, R. L. (2000). Estrogenic activity of octylphenol, bisphenol A and methoxychlor in rats. *Toxical. Sci.,* **54**, 154–167.

McLachlan, J.A. (1993). Functional toxicology, a new approach to detect functionally active xenobiotics. *Environ. Health. Perspect.,* **101**, 386–387.

McLachlan, J.A. and Korach, K.S. (1995). Symposium on estrogens in the environment. *Environ. Health. Perspect.,* **103**, 3–4.

Morita, K., Matsueda, T. and Iida, T. (1997). Effects of dietary fiber on polychlorinated dibenzo-p-dioxins in rats. *Jpn. J. Toxixol. Environ. Health.,* **43**, 35–41.

Nagel, S.C., vom Saal, F.S., Thayer, K.A., Dhar, M.G., Boechler, M. and Welshons, M.W. (1997). Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. *Environ. Health. Perspect.,* **105**, 70–76.

Nakanishi, J., Miyamoto, K. and Kawasaki, H. (2005). "Bisphenol A risk assessment document." Maruzen.

OECD. (1999). OECD guidance and protocol for the prevalidation of the rat uterotrophic screening assay. ENJ/JM/TG/EDTA (99)2/ANN1. Paris: Organization for Economic Cooperation and Development.

Olea, N., Pulager, R., Perez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, AM. and Sonnenschein, C. (1996). Estrogenicity of resin-based composites and sealants used in dentistry. *Environ. Health. Perspect.,* **104**, 298–305.

Prynne, C.J. and Southgate, D.A.T. (1979). The effects of a supplement of dietary fiber on fecal excretion by human subjects. *Br. J. Nutr.,* **41**, 495–503.

Soto, A.M., Sonnenschein, C., Chung, K.L., Fernadez, M.F., Olea, N. and Serrano, F.O. (1995). The E-SCREEN assay as a tool to identify estrogens: An update on estrogenic environmental pollutants. *Environ. Health. Perspect.,* **103**, 113–122.

Spiller, G.A., Shipley, E.A., Chernoff, M.C. and Cooper, W.C. (1979). Bulk laxative efficacy of a psyllium seed hydrocolloid and of a mixture of cellulose and pectin. *J. Clin. Pharmacol.,* **19**, 313–320.

Stevens, J., VanSoest, P.J., Robertson, J.B. and Levisky, D.A. (1988). Comparison of the effects of psyllium and wheat bran on gastrointestinal transit time and stool characteristics. *J. Am. Diet. Assoc.,* **88**, 323–326.

Sumpter, J.P. and Jobling, S. (1995). Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environ. Health. Perspect.,* **103**, 173–178.

Wolff, M.S., Toniolo, P.G., Lee, E.W., Rivera, M. and Dubin, N. (1993). Blood levels of organochlorine residues and risk of breast cancer. *J. Natl. Cancer. Inst.,* **85**, 648–652.

Yamasaki, K., Sawaki, M. and Takatsuki, M. (2000). Immature rat uterotrophic assay of bisphenol A. *Environ. Health. Perspect.,* **108**, 1147–1150.