Effects of Sodium Alginate and Fish Oil to Reduce Trichloroethylene Accumulation in Rats

Sachie IKEGAMI1,* and Keizo UMEGAKI2
1 Division of Food Science and 2 Division of Applied Food Research, National Institute of Health and Nutrition, Shinjuku-ku, Tokyo 162-8636, Japan
(Received September 28, 1998)

Summary This study was conducted to examine the effect of dietary sodium alginate and fish oil on bodily accumulated trichloroethylene (TCE), which has been widely used as a halogenated solvent and is metabolized at a high rate. Each of three groups of rats was fed on either of diets containing cellulose-soybean oil (control), Na-alginate-soybean oil or cellulose-fish oil for 3 wk, and thereafter given a single oral dose of TCE (100mg (0.76mmol)/rat). TCE levels in the blood were monitored for 10h after the administration of TCE. The peak concentrations of TCE in the blood tended to be higher in the alginate and fish oil groups as compared with those in the cellulose-soybean oil group, but not to a significant extent. TCE concentrations in the liver, kidney, brain and the three fat tissues (epididymal, perirenal and subcutaneous) were significantly lower in the alginate and fish oil groups than in the cellulose-soybean oil group. Fat tissue weights were also lower in the alginate group and fish oil group. The hepatic drug metabolizing enzymes could not account for the remarkable decreases of residual TCE contents in the alginate and fish oil groups. These findings indicate that the metabolism and excretion of TCE might be accelerated in animals with reduced fat tissue mass.

Key Words trichloroethylene, Na-alginate, fish oil, accumulation, fat tissue

In previous studies, we have observed that the metabolism and excretion of lipophilic and relatively metabolizable pentachlorobenzene (PECB) were markedly increased in rats when they were fed a restricted diet (1, 2), viscous dietary fibers (3, 4) or fish oil (5, 6). We have also reported that the feeding of fish oil (7) or guar gum (8) to rats enhanced the metabolism and excretion of hexachlorobenzene (HCB),

*To whom correspondence should be addressed.
Abbreviations: TCE, trichloroethylene; PECB, penta-chlorobenzene; HCB, hexachlorobenzene.
which is a ubiquitous contaminant found in food (9) and milk (10) and is metabolized at a slow rate. The enhanced metabolism and excretion of PECB and HCB were not due to the increased activity of hepatic drug-metabolizing enzymes per se, but due to the reduced amounts of fat tissue that resulted from those treatments. The mechanism was as follows: with the decrease in fat tissue mass that accumulates PECB and HCB, concentrations of PECB and HCB in the blood and liver increased, and the amounts of metabolites formed therefore increased. The role of fat tissue mass is uncertain in the metabolism of rapidly metabolized lipophilic pollutants.

1,1,2-Trichloroethylene (TCE) has been widely used as a halogenated solvent. Occupational and environmental exposure of TCE to the human population has been reported in industrialized areas (11). Recently, high levels of TCE pollution have been found in electronic factories and their surrounding areas. Long-term carcinogenicity studies in rodents demonstrate that exposure to high doses of TCE results in the induction of liver, lung, kidney and testes tumors (12). Nakajima et al (13) reported that pretreatments with ethanol and phenobarbital enhanced the metabolism of TCE. TCE is metabolized at a high rate by the catalytic action of cytochrome P-450 and converted to trichloroethanol and trichloroacetic acid (14). The induction of cytochrome P-450 is undoubtedly responsible for the enhanced metabolism of TCE brought about by the treatment with ethanol and phenobarbital. However, the effects of dietary components on the metabolism and excretion of TCE have not yet been reported. If certain dietary components enhance TCE excretion, they could be used to minimize TCE effects in humans.

TCE was rapidly metabolized within a few hours after its ingestion (15), while the PECB and HCB used in our previous studies had a half-life of 42 h (2) and 3 mo (16), respectively. Unmetabolized TCE can be retained in fat tissues because of its high liposolubility (17). Therefore, the ingestion of dietary components that reduce fat tissue mass might decrease the amounts of TCE residue in the body as has been observed for PECB and HCB. In this study, we have determined whether the feeding of sodium alginate and fish oil to rats enhances the disappearance of TCE from the body in a short period of time, as has been reported for PECB and HCB.

MATERIALS AND METHODS

Chemicals. TCE and sodium alginate were purchased from Wako Pure Chemical Industries, Osaka, Japan. Fish oil (sardine oil) was kindly provided by Nihon-Yushi, Tokyo, Japan, and soybean oil was purchased from Kosakai Pharmaceutical, Tokyo, Japan. Major fatty acid components were shown in a previous paper (7). Fish oil was stored in several small bottles under a N₂ atmosphere at −40°C until use. Other dietary components were purchased from Oriental Yeast Co., Tokyo, Japan. n-Hexane, of a grade for determining residual pesticides, was purchased from Wako Pure Chemical Industries, Osaka, Japan, and other chemicals were of analytical grade.
**Animals and diets.** Male weanling Sprague-Dawley rats (Clea Japan, Tokyo) were obtained at the age of 4 wk and housed individually in stainless steel wire-bottomed cages, in a room with a constant temperature of 23 ± 1°C and a 12-h light : dark cycle. Rats were given free access to food and water. Each rat was fed one of the three experimental diets (cellulose-soybean oil as control diet, sodium alginate-soybean oil or cellulose-fish oil) for 3 wk. The compositions of the diets were: 45% sucrose, 20% casein, 0.3% dl-methionine, 15% cornstarch, 10% soybean oil or fish oil, 5% cellulose or sodium alginate, 3.5% mineral mixture (AIN-76), 1% vitamin mixture (AIN-76) and 0.2% choline bitartrate. To avoid the oxidation of oil, the food for each group was freshly prepared every day.

After 3 wk of consuming an experimental diet, rats were orally given a single dose of TCE 100 mg (0.76 mmol) dissolved in soybean oil at 7 a.m. At that time, food was removed and all feeding was discontinued. Blood (25–500 μL) was taken from the tail vein at 1, 2, 4, 6 and 10 h after the administration of TCE within 1 min under consciousness. Rats were anesthetized with ether and blood was taken by heart puncture after taking the last blood sample. After that, the fat tissues and organs were removed and weighed. All procedures were in accordance with the National Institute of Health and Nutrition Guidelines for the Care and Use of Laboratory Animals.

**Analytical methods.** Blood was mixed with 1 mL distilled water. Organs were homogenized with 4 volumes of water. To extract TCE, n-hexane was added to the blood mixture and the homogenate. Fat tissue was directly homogenized and extracted with n-hexane. Mixing was done by a vortex or homogenizer, and the upper layer was obtained by centrifugation at 600 × g for 5 min. The extracted solution was cleaned up by a florisil column and diluted if necessary. TCE was analyzed on a Hitachi 663-30 gas chromatograph with an electron capture detector. The column (3 mm × 3 m), packed with 20% Silicon DC-550 coated on Uniport HP, was used at 100°C of column temperature, with 50 mL/min N2 as the carrier gas.

Drug-metabolizing enzyme concentrations and activities in the liver were assayed as previously reported (7). Cytochrome P-450 was measured by the method of Omura and Sato (18), UDP-glucuronosyltransferase activity by the method of Kuno (19) and glutathionetransferase activity by the method of Habig and Jakoby (20). Protein was determined according to the method of Lowry et al (21).

**Statistical analysis.** The Yukmus computer program (Yukmus, Tokyo, Japan) was used for statistical analysis of the data. All results were subjected to ANOVA. Data are presented as means ± SE. Differences in mean values between groups were tested by using Duncan’s multiple comparison test and considered significant at p < 0.05.
Table 1. Body weight and weights of organs and fat tissues.

|                     | Cellulose-soybean oil (g) | Na-alginate-soybean oil (g) | Cellulose-fish oil (g) |
|---------------------|---------------------------|-----------------------------|------------------------|
| Body weight         | 284 ± 9                   | 277 ± 7                     | 270 ± 9                |

(g/100 g body weight)

|                |                |                |                |
|----------------|----------------|----------------|----------------|
| Liver          | 3.60 ± 0.17    | 3.81 ± 0.22    | 3.56 ± 0.19    |
| Kidney         | 0.74 ± 0.01    | 0.78 ± 0.02    | 0.78 ± 0.01    |
| Brain          | 0.66 ± 0.02    | 0.69 ± 0.03    | 0.71 ± 0.02    |
| Epididymal fat | 1.04 ± 0.05a   | 0.78 ± 0.05ab  | 0.81 ± 0.06b   |
| Perirenal fat  | 1.54 ± 0.10a   | 0.74 ± 0.09b   | 0.93 ± 0.11ab  |

Values are means ± SE for 6 rats.
Values in the same line not sharing a common superscript letter are significant at p < 0.05.

RESULTS

The effect of Na-alginate and fish oil on body weight and the relative weights of organ and fat tissue in rats are shown in Table 1. Body weight and the relative weights of liver, kidneys and brain were not different among the three diet groups. However, the relative weights of fat tissue were lower in the alginate and fish oil groups than in the control group.

Changes in the concentration of TCE in the blood up to 10 h after TCE administration are shown in Fig. 1. Peak blood concentration of TCE was reached at 2 to 4 h. The peak concentrations of the alginate and fish oil groups were higher and the concentrations at 6 and 10 h were lower as compared with those in the control group. However, the differences between the two groups and the control group were not significant because of large individual differences.

TCE concentrations in the organs and the concentrations and contents in the fat tissues are shown in Tables 2 and 3, respectively. TCE concentrations in the liver, kidneys, brain and three fat tissues were significantly lower in the alginate and fish oil groups than in the control group. Furthermore, due to the changes in the fat tissue mass, the total amounts of TCE in the epididymal and perirenal fat tissues were remarkably decreased in the alginate and fish oil groups as shown in Table 3. As shown in Fig. 2, marked positive correlation was noted between the hepatic contents of TCE and perirenal fat weight in each rat. Similar positive correlations between TCE contents in the other organs and fat tissue weights were also observed.

To examine the contribution of hepatic drug-metabolizing enzymes on the reduced amounts of TCE in the various tissues in rats fed alginate and fish oil, cytochrome P-450 content and the activities of UDP-glucuronosyltransferase and...
Alginate and Fish Oil Reduce Residual TCE

Fig. 1. Changes in TCE concentration in the blood. Rats were orally administered TCE 100 mg (0.76 mmol)/rat after being fed experimental diets containing dietary fiber and fat for 3 wk. Each point and vertical bar indicate the mean and SE for 6 rats per group.

Table 2. Concentrations of TCE in the liver, kidneys and brain.

|                  | Cellulose-soybean oil | Na-alginate-soybean oil | Cellulose-fish oil |
|------------------|-----------------------|-------------------------|--------------------|
|                  | (nmol/g)              |                         |                    |
| Liver            | 3.30 ± 0.91<sup>a</sup> | 0.96 ± 0.42<sup>b</sup> | 1.38 ± 0.55<sup>b</sup> |
| Kidney           | 5.69 ± 1.61<sup>a</sup> | 1.81 ± 0.91<sup>b</sup> | 1.74 ± 0.52<sup>b</sup> |
| Brain            | 6.85 ± 1.02<sup>a</sup> | 4.43 ± 0.98<sup>b</sup> | 3.84 ± 0.62<sup>b</sup> |

Values are means ± SE for 6 rats. Values in the same line not sharing a common superscript are significantly different at $p < 0.05$. 

Vol 45, No 2, 1999
Table 3. Concentrations and total amounts of TCE in the fat tissues.

|                     | Cellulose-soybean oil | Na-alginate-soybean oil | Cellulose-fish oil |
|---------------------|-----------------------|-------------------------|--------------------|
| Epididymal fat       |                       |                         |                    |
| µmol/g               | 1.18 ± 0.25<sup>a</sup> | 0.35 ± 0.15<sup>b</sup> | 0.35 ± 0.19<sup>b</sup> |
| µmol/tissue          | 3.32 ± 0.69<sup>a</sup> | 1.07 ± 0.35<sup>b</sup> | 0.70 ± 0.42<sup>b</sup> |
| Perirenal fat        |                       |                         |                    |
| µmol/g               | 1.22 ± 0.18<sup>a</sup> | 0.18 ± 0.06<sup>b</sup> | 0.48 ± 0.21<sup>b</sup> |
| µmol/tissue          | 5.34 ± 0.94<sup>a</sup> | 0.38 ± 0.13<sup>b</sup> | 1.14 ± 0.52<sup>b</sup> |
| Subcutaneous fat     |                       |                         |                    |
| µmol/g               | 0.81 ± 0.28<sup>a</sup> | 0.21 ± 0.07<sup>b</sup> | 0.29 ± 0.09<sup>b</sup> |

Values are means ± SE for 6 rats. Values in the same line not sharing a common superscript letter are significantly different at p < 0.05.

Fig. 2. Correlation between hepatic contents of TCE and epididymal fat weight. Rats were orally administered TCE 100 mg (0.76 mmol)/rat after being fed experimental diets containing dietary fiber and fat for 3 wk. Each point represents the data of one rat.

DISCUSSION

TCE is a ubiquitous environmental pollutant in industrialized areas. It easily crosses the gastrointestinal wall and rapidly disappears from the blood, being ex-
Alginate and Fish Oil Reduce Residual TCE

Table 4. Concentrations and activities of hepatic drug-metabolizing enzymes.

|                        | Cellulose-soybean oil | Na-alginate-soybean oil | Cellulose-fish oil |
|------------------------|-----------------------|------------------------|-------------------|
| Cytochrome P-450        | 0.441 ± 0.051         | 0.493 ± 0.047          | 0.499 ± 0.018     |
| (nmol/mg protein)       |                       |                        |                   |
| UDP-glucuronosyltransferase | 17.7 ± 2.8           | 28.4 ± 2.1             | 23.7 ± 2.1        |
| (nmol/mg protein/min)   |                       |                        |                   |
| Glutathionetransferase  | 349 ± 27             | 397 ± 20               | 245 ± 33          |
| (nmol/mg protein/min)   |                       |                        |                   |

Values are means ± SE for 6 rats.
Values in the same line not sharing a common superscript are significantly different at
p < 0.05.

inhaled unchanged or accumulated in tissues via the blood stream and metabolism
(17, 22, 23). TCE is more rapidly metabolized than PECB or HCB, which were used
in our previous studies (3–7). In those studies, we found that PECB residues in the
body were much lower in amount in rats fed viscous indigestible polysaccharides
(3, 4) and fish oil (5, 6). In addition, we have reported that feeding fish oil to rats
enhanced the metabolism of HCB, which is metabolized at a low rate (7). However,
it has not been reported whether the effects of dietary components on the metabolism
of rapidly metabolized pollutants were observed. In this study, we investigated
whether the same beneficial effect of alginate and fish oil feeding would be observed
with respect to TCE. As shown in the results, we found that the amounts of TCE
residue in the liver, kidneys, brain and fat tissues were remarkably lower in rats
fed sodium alginate-soybean oil or cellulose-fish oil diets than in those fed a
cellulose-soybean oil diet.

As shown in Table 1, the amounts of epididymal and perirenal fat tissue in
the alginate and fish oil groups were lower than those in the control group. The
results are consistent with our previous reports that feeding dietary fiber and fish
oil to rats reduces the total content of fat in the body (3–7). It is widely recognized
that fat tissue has an important role in the storage of drugs or xenobiotics in the
body. Restricted feeding has also been reported to eliminate lipophilic xenobiotics
from the tissues (2). However, there has been little critical in-depth discussion of
the specific role of fat tissue in the metabolism of such compounds. Our results
suggest that the metabolism and excretion of lipophilic xenobiotics, such as TCE,
PECB and HCB, might be accelerated in animals with reduced fat tissue mass. The
possible mechanism is as follows. The distribution of lipophilic xenobiotics in the
body is lower with low amounts of fat tissue mass than with high amounts. As a
result, the concentrations of the xenobiotics in the blood and liver increase, and
metabolism by the hepatic drug-metabolizing enzymes is accelerated.

In this study, TCE concentrations in the blood at 2 h after TCE administration
did not differ among the three groups. This suggests that the absorption of TCE did not differ among the groups. However, we could not ascertain that the concentration of TCE in the blood was significantly high at its peak values in the alginate and fish oil groups as compared with that in the control group as observed with PECB (4). It is likely that the rapid disappearance of TCE from the blood due to the metabolizable nature of TCE and the fluctuation in time response among the individual rats might mask the effect of diets on the changes of concentration in the blood after TCE administration. Hamdan and Stacey (15) have reported that the peak concentrations of TCE in blood and liver were reached at 4 h after intraperitoneal injection to rats. The peak concentrations of the main metabolite, trichloroethanol, appeared later at 8 h both in the blood and liver. By 16 h, TCE disappeared from the blood and liver. In contrast, the concentration of trichloroethanol remained high even after 16 h. Their report has shown that the concentrations of TCE and its metabolites in the blood were directly related to those in the liver. Therefore, their result supports our contention that a high concentration of TCE in the blood led to acceleration of the metabolism of TCE in the liver in rats with small fat mass. Further detailed studies concerning the time response in blood will be needed to ascertain the mechanism of the reduced accumulation of TCE observed in our experiment.

On the other hand, the decrease of TCE accumulation in rats fed alginate or fish oil could not be explained only by changes regarding hepatic drug-metabolizing enzymes. In this study, we did not determine the amounts of TCE absorbed from the gastrointestinal wall. Several papers have revealed the high degree to which TCE was absorbed (14–17, 22). However, there is a necessity to consider the effect of alginate and fish oil on the amount of TCE absorbed from the digestive organs. Further studies will be needed on this subject.

This work is supported by the Environmental Agency, Japan.

REFERENCES

1) Umegaki K, Ichikawa T. 1990. Involvement of adipose tissue in metabolism and in accumulation of pentachlorobenzene in rats: Comparison with young adult rats. *Shokuhin Eiseigaku Zasshi (J Food Hyg Soc Jpn)* 31: 485–490 (in Japanese).

2) Umegaki K, Ikegami S, Ichikawa T. 1993. Effects of restricted feeding on the absorption, metabolism and accumulation of pentachlorobenzene in rats. *J Nutr Sci Vitaminol* 39: 11–22.

3) Tsuchihashi F, Ikegami S, Umegaki K, Ichikawa T. 1992. Effect of indigestible polysaccharides on accumulation of pentachlorobenzene in rats. *Shokuhin Eiseigaku Zasshi (J Food Hyg Soc Jpn)* 33: 241–245 (in Japanese).

4) Ikegami S, Umegaki K, Kawashima Y, Ichikawa T. 1994. Viscous indigestible polysaccharides reduce accumulation of pentachlorobenzene in rats. *J Nutr* 124: 754–760.

5) Ikegami S, Tsuchihashi F, Umegaki K, Ichikawa T. 1991. Effect of dietary lipids on...
Alginate and Fish Oil Reduce Residual TCE 201

accumulation of pentachlorobenzene. *Shokuhin Eiseigaku Zasshi (J Food Hyg Soc Jpn)* 32: 420–426 (in Japanese).

6) Umegaki K, Ikegami S, Ichikawa T. 1995. Fish oil enhances pentachlorobenzene metabolism and reduces its accumulation in rats. *J Nutr* 125: 147–153.

7) Umegaki K, Ikegami S. 1998. Feeding fish oil to rats accelerates the metabolism of hexachlorobenzene. *J Nutr Sci Vitaminol* 44: 301–311.

8) Nakashima Y, Ohsawa S, Umegaki K, Ikegami S. 1998. Masking of guar gum-induced acceleration of hexachlorobenzene excretion by its rapid excretion through lactation in adult female rats. *J Agr Food Chem* 46: 2241–2247.

9) Kuwabara K, Matsumoto H, Murakami Y, Hori S. 1998. Daily dietary intakes of PCBs and organochlorine pesticides during 19 years from 1977 to 1995 by adults in Osaka evaluated by total diet study method. *Shokuhin Eiseigaku Zasshi (J Food Hyg Soc Jpn)* 38: 286–295 (in Japanese).

10) Ando M, Hirano S, Itoh Y. 1985. Transfer of hexachlorobenzene (HCB) from mother to new-born baby through placenta and milk. *Arch Toxicol* 56: 195–200.

11) Environment Agency of Japan. 1992. Environmental White Paper, p 117–145. Printing Bureau, Ministry of Finance of Tokyo, Japan.

12) Crebelli R, Carere A. 1989. Genetic toxicology of 1,1,2-trichloroethylene. *Mutation Res* 221: 11–37.

13) Nakajima T, Okino T, Okuyama S, Kaneko T, Yonekura I, Sato A. 1988. Ethanol-induced enhancement of trichloroethylene metabolism and hepatotoxicity: Difference from the effect of phenobarbital. *Toxicol Appl Pharmacol* 94: 227–237.

14) Green T, Prout MS. 1985. Species differences in response to trichloroethylene II. Biotransformation in rats and mice. *Toxicol Appl Pharmacol* 79: 401–411.

15) Hamdan H, Stacey NH. 1993. Mechanism of trichloroethylene-induced elevation of individual serum bile acids. *Toxicol Appl Pharmacol* 121: 291–295.

16) Prout MS, Provan WM, Green T. 1985. Species differences in response to trichloroethylene I. Pharmacokinetics in rats and mice. *Toxicol Appl Pharmacol* 79: 389–400.

17) Monster AC. 1979. Differences in uptakes, elimination and metabolism in exposure to trichloroethylene, 1,1,1-trichloroethane and tetrachloroethylene. *Int Arch Occup Environ Health* 42: 311–317.

18) Omura T, Sato R. 1964. The carbon monoxide-binding pigment of liver microsomes. *J Biol Chem* 239: 2370–2378.

19) Kuno, Y. 1981. Methods of drug metabolism studies. In: Radioisotope Methods of Drug Metabolism Studies (Japan Radioisotope Association, ed), p 458–480. Maruzen Company, Tokyo.

20) Habig WH, Jacoby WB. 1981. Assays for differentiation of glutathione S-transferase. *Methods Enzymol* 77: 398–405.

21) Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265–275.

22) Hobara T, Kobayashi H, Kawamoto T, Iwamoto S, Sakai T. 1987. Intestinal absorption of trichloroethylene in dogs. *Toxicol Appl Pharmacol* 91: 256–265.

23) D'Souza RW, Bruckner JV, Feldman S. 1985. Oral and intravenous trichloroethylene pharmacokinetics in the rat. *J Toxicol Environ Health* 15: 587–601.