Determination of Tetramethylsuccinonitrile in Food Containers and Packaging Made of Plastics

Hajimu Ishiwata,* Takiko Inoue, Miyako Yamamoto, and Kunitoshi Yoshihira

Tetramethylsuccinonitrile (TMSN), a decomposition product of 2,2′-azobisisobutyronitrile which is used as a catalyst for polymerization of monomers, was determined in plastic products in contact with food. TMSN was extracted from plastic products with dichloromethane, steam-distilled, extracted from the distillate with dichloromethane, and determined by gas chromatography using a nitrogen–phosphorus detector. TMSN was detected in 10 samples (six kinds of resin) from 76 plastic products (18 kinds of resin). The resins in which TMSN was detected were acrylonitrile-butadiene-styrene resin, methyl methacrylate-acrylonitrile-butadiene-styrene resin (MABS), poly(methyl methacrylate) (PMMA), poly(acrylonitrile) (PAN), polystyrene foam, and poly(vinyl chloride). The highest frequency of detection of TMSN was observed in PMMA, three out of four samples, and the highest concentration was observed in MABS (134.4 μg/g). The presence of TMSN in PAN, MABS, and PMMA was confirmed by gas chromatography–mass spectrometry.

Aliphatic and aromatic nitriles are widely used in the manufacture of plastics and synthetic intermediates. Among these nitrile compounds, 2,2′-azobisisobutyronitrile (AIBN) is used as a catalyst for polymerization in the production of many kinds of resins (Lefaux, 1972; Japan Hygienic Olefin and Styrene Plastic Association, 1979) such as poly(vinyl chloride), polystyrene, and acrylonitrile copolymers. AIBN is known to decompose to tetramethylsuccinonitrile (TMSN) at 100–107 °C, releasing nitrogen gas that originates from the azo group (Waki and Yamashita, 1969). The toxicity of TMSN was evaluated by NIOSH (1978). Although chronic toxicity of TMSN is not well-known, the compound is known to have a potent acute toxicity. The LD_{50} is 0.13 mmol ip/kg body weight in mice (Doherty et al., 1982), and the lethal effect in hamsters is considered to be central neurotoxicity (Doherty et al., 1983).

The maximum amount of AIBN that can be used, or the limit of its decomposition products, is usually regulated at less than 2 or 2.5%. Nevertheless, few analytical studies of residual TMSN in plastics in contact with food have been reported. Mandik and Culkova (1982) reported up to 85 μg/g of TMSN in impact-resistant polystyrene. The present authors have previously reported a new analytical method for TMSN in poly(vinyl chloride) (Ishiwata et al., 1986), and the concentration of TMSN and the effects of temperature and time on the release of TMSN in poly(vinyl chloride) into food-simulating solvents have also been investigated (Ishiwata et al., 1987). The present paper describes the results of determinations of residual concentrations of TMSN in plastic products made of various resins used for food containers and packaging.

EXPERIMENTAL SECTION

Apparatus. A Hewlett-Packard Model 5710A gas chromatograph, equipped with a nitrogen–phosphorus detector (NPD) and a flame ionization detector (FID), was used with a Shimadzu Chromatopac C-R1A integrator. The gas chromatograph was equipped with a 6-ft-long coiled glass column (2-mm i.d.) packed with 80–100-mesh Chromosorb W (AW-DMCS) coated with 5% Thermon 1000 plus 0.5% phosphoric acid. A high-performance liquid chromatograph (HPLC), Model L-2000 (Yanagimoto Co., Ltd.), equipped with an ODS-A column (4.6-mm i.d., 250-mm length) and a gas chromatograph–mass spectrometer (GC–MS), Model JMS-DX 300 (JEOL Co., Ltd.), equipped with a column (0.530-mm i.d., 10-m length, Carbowax 20M) were used for the cleanup process and for the confirmation of TMSN, respectively. A Biotron BT10 20 3500 was used for homogenization of samples.

Samples. Food containers and packaging made of plastics were obtained from markets and plastics manufacturers in Tokyo, Japan. In samples used for packaging of foods, the foods were removed and the containers and packaging were rinsed with water. The resin materials in plastic products were discriminated by reference to the Household Goods Quality Labeling Law (Ministry of Health and Welfare, 1962) (the material name of most plastic products is printed in a label or stamped with a die on the body) and by the methods described by Yoda et al. (1985) (for example, combustion test and hardness test).

The plastic product materials used in the present study were as follows: styrene-acrylonitrile resin (SA), acrylonitrile-butadiene-styrene resin (ABS), polycarbonate (PC), poly(methyl methacrylate) (PMMA), polyphenylene ether resin (PPE), poly(methylpentene) (PMP), methyl methacrylate-acrylonitrile-butadiene-styrene resin (MABS), polyamide-6 (PA-6), poly(ethylene terephthalate) (PET), poly(vinyl alcohol) (PVAL), poly(acrylonitrile) (PAN), polystyrene (PS), polystyrene foam (PSF), polypropylene (PP), polyethylene (PE), poly(vinyl chloride) (PVC), and unsaturated polyester resin (UP). Some other plastic products and laminated films, the contents of which were unknown, were also examined.

Preparation of Test Solution. The method previously described (Ishiwata et al., 1986) was used with some modification.

Dichloromethane-Insoluble Plastics. Twenty milliliters of dichloromethane was added to 1 g of the sample in small pieces, and the mixture was homogenized with a homogenizer for 30 s. Twenty milliliters of methanol was added to the homogenate, and the mixture was centrifuged at 2000 rpm for 5 min. The resulting pellet in the centrifuge tube was treated in the same manner as described above. The supernatant was combined with the former extract, and the solvent was evaporated to 5–7 mL under reduced pressure at 30–35 °C. The concentrate was transferred to a distillation flask with a small portion of dichloromethane, and 10 mL of water and 10 g of sodium chloride were added.

Dichloromethane-Soluble Plastics. One gram of the sample in small pieces was added to a steam distillation

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National Institute of Hygienic Sciences, 18-1, Kamiyoga 1-chome, Setagaya-ku, Tokyo 158, Japan.

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Table I. Recovery Test of TMSN[a]

matl	content, µg/g	spiked, µg	rec, %
PE	nd	1	100.2 ± 1.5
		10	95.9 ± 2.5
		100	99.5 ± 1.8
PSF	5.6	5	88.5 ± 3.1
PAN	19.4	20	103.9 ± 11.0
PMMA	21.5	20	101.8 ± 14.9
MABS	134.3	100	102.1 ± 5.7

[a] Each quantity of TMSN in 1 mL of dichloromethane was added to 1 g of sample pieces of plastic.

flask, and 10 mL of dichloromethane was added. After the sample had been dissolved in dichloromethane, 10 mL of water and 10 g of sodium chloride were added. Although PS and PSF were soluble in dichloromethane, the samples made of these materials were treated in the same way the dichloromethane-insoluble plastics because PS and PSF dissolved in dichloromethane were precipitated by the addition of 20 mL of methanol.

Preparation of Test Solution for GC. The mixture was steam-distilled until 100 mL of distillate including organic solvents had been collected. A definite amount of nitrobenzene was added to the distillate as an internal standard, and the mixture was shaken with 50 mL of dichloromethane. The extraction was repeated twice with 30 mL of dichloromethane, and the solvent layers were combined, dried with 5 g of anhydrous sodium sulfate, and concentrated to 1 mL or to a definite volume.

Determination and Confirmation of TMSN. TMSN was determined by GC-NPD under the following conditions: nitrogen carrier gas, 45 mL/min; hydrogen, 3 mL/min; air, 100 mL/min; injection port temperature, 150 °C; column temperature, 125 °C; detector temperature, 250 °C. Experiments were carried out in triplicate, and the results are presented as means ± S.D.

TMSN in some samples was confirmed by GC–MS after purification by HPLC. The conditions of GC–MS and HPLC were as follows: ionization voltage, 70 eV; helium carrier gas, 15 mL/min; injection port, 150 °C; column temperature, 70 °C for 4 min, subsequently raised to 130 °C at 4 °C/min for GC–MS; pressure, 80 kg/cm^2; flow rate, 0.73 mL/min for HPLC.

Recovery Test. A specified amount of TMSN–dichloromethane solution was added to the sample suspension in dichloromethane or to the dichloromethane solution. The recoveries of TMSN are shown in Table I.

RESULTS AND DISCUSSION

Applicability of the Determination Method to Plastic Products. The method developed for PVC was applicable to the determination of TMSN in all samples examined in the present experiments at a level of 0.1 µg/g of plastic except for two samples of PPE products. The recoveries of TMSN were between 95.9 and 100.2% from PE, which was one of the dichloromethane-insoluble samples and in which no TMSN was detected, when 1, 10, or 100 µg of TMSN was added. In this case recoveries do not necessarily indicate the extraction ability of TMSN from PE. The recoveries were between 88.5 and 103.9% from PSF, PMMA, and MABS, which were soluble in dichloromethane and contained TMSN, and from PAN, which was insoluble and contained TMSN, when almost the same amount of TMSN as the content level was added. These results were almost the same as that obtained for PVC (Ishiwata et al., 1987). A typical gas chromatogram of the test solution obtained from a PSF product is shown in Figure 1. During steam distillation, dichloromethane was distilled first, and then the polymer dissolved in dichloromethane precipitated resultingly in the distillation flask. However, recoveries of TMSN from dichloromethane-soluble plastics such as PSF, PMMA, and MABS were not affected by the preciptation of polymers, which is showing that TMSN was not retained in precipitated polymers. No peak was observed at the time corresponding to the retention time of nitrobenzene used as an internal standard substance in the chromatograms of the test solutions without nitrobenzene.

Figure 1. Gas chromatograms of authentic TMSN (left) and the extract from PSF (right) using NPD: peak a, TMSN; peak b, nitrobenzene (internal standard).

In the case of PPE products, many peaks appeared within 6 min after injection of the test solutions, so that it was impossible to identify the peaks of TMSN and nitrobenzene. Further cleanup or capillary GC will thus be required in order to determine TMSN in PPE products.

TMSN in Plastic Products in Contact with Food. TMSN was detected in 10 plastic products made of six kinds of resin, ABS, PMMA, MABS, PAN, PSF, and PVC, among 76 samples made of 18 kinds of resin (including six laminated films and three unknown materials and excluding two samples made of PPE) (Table II). The highest frequency of detection of TMSN was observed in PMMA, three out of four samples, and the highest concentration, 134.4 ± 2.9 µg/g, was observed in MABS. In the case of PVC, both the frequency of detection, one out of five samples, and the highest concentration, 19.4 ± 0.4 µg/g, in the present experiment were lower than those in the previous study (Ishiwata et al., 1987), 12 out of 17 samples and 523 ± 30.4 µg/g, respectively. TMSN was not detected in six laminated films and three plastic products in which the constituent materials could not be discriminated. No TMSN was detected in any of the 10 PS samples but was detected in 3 of 11 PSF samples, ranging between 0.1 and 5.6 µg/g. These concentrations in both PS and PSF were lower than those reported by Mandik and Culkova (1982), ranging between 5 and 85 µg/g in 9 of 10 PSF products. Although AIBN has a foaming function with the release of nitrogen gas from the azo group by thermal decomposition (Waki and Yamashita, 1969), it is not used as a foaming agent in Japan (Japan Hygienic Olefin and Styrene Plastic Association, 1978). The residual TMSN found in PSF may be the decomposition product

Table II. TMSN in Food Containers and Packaging Made of Plastics

matl	shape	concn of TMSN, μg/g 1	2	3	4	5	6
ABS	sheet	nd[a]	0.3 ± 0	nd			
	spoon	nd					
AS	sheet	nd	nd	nd			
	squeezer	nd					
	cup	nd					
LF[b]	bag	nd	nd	nd	nd	nd	nd
MABS	stick	nd	nd	134.4 ± 2.9[c]			
PA-6	film	nd					
	sheet	nd					
PAN	film	nd					
	sheet	19.4 ± 0.9[c]	nd				
PC	sheet	nd	nd				
	cup	nd					
PE	cup	nd					
	bottle	nd	nd	nd			
	vallum	nd					
PET	sheet	nd	nd				
PMMA	stick	nd	0.7 ± 0.1				
	bottle	21.5 ± 0.5[c]					
	cap	21.9 ± 0.9					
PMP	sheet	nd	nd				
PP	tray	nd					
	cap	nd					
	bowl	nd					
PPE	stick	*d*	*d*				
PS	box	nd	nd	nd			
	cup	nd	nd	nd	nd		
	spoon	nd	nd				
	tray	nd					
PSF	bowl	nd	nd	nd	nd	5.6 ± 0.2	
	tray	nd	2.2 ± 0.5				
	cup	nd	nd	0.1 ± 0.1	nd		
PVC	box	nd	19.4 ± 0.4				
	bottle	nd					
	cap	nd					
	bag	nd					
UK[e]	cup	nd					
	cup	nd					
	box	nd					
UP	scraper	nd					

[a] Results are shown as means ± SD of triplicate experiments. nd = less than 0.1 μg/g. [b] Laminated film, materials not known. [c] Confirmed by GC–MS. [d] Impossible to apply the present method. [e] Unknown materials.

of AIBN used as an initiator of the styrene polymerization reaction.

Migration of TMSN from PVC into olive oil has been reported to occur in accordance with the formula $\ln y = 0.08786x - 5.696$, where y is the concentration (ng/mL) of TMSN in olive oil and x is the temperature between 80 and 140 °C when a PVC product containing 523 μg/g of TMSN is used (Ishiwata et al., 1987). However, the temperature, 80 °C, is higher than the softening point of PVC. No migration of TMSN has also been reported into water, 4% acetic acid, 20% ethanol, or olive oil when the PVC product is treated at 60 °C for 30 min, but TMSN does migrate at 1 ng/mL in *n*-heptane. Although it is hard to compare the migration of TMSN from different resins or plastics, the release of TMSN from the plastics examined in the present paper may be less than 1 ng/mL in food-simulating solvents under the conditions of 60 °C for 30 min because the highest concentration of TMSN was 134 μg/g in MABS, while others were 20 μg/g or less.

Confirmation of TMSN by GC–MS. TMSN in PAN, PMMA, and MABS was confirmed by GC–MS. Although no contaminants in the test solution were observed on gas chromatograms as shown in Figure 1 because of the use of an NPD, many coexisting substances were found in the test solutions obtained from these samples by the use of an FID. Cleanup of the test solutions was then carried out by HPLC using a mixture of methanol and water (1:1, v/v) as a mobile phase. TMSN in each fraction was monitored by GC after extraction of the eluate with dichloromethane, since it shows no ultraviolet absorption. GC-FID showed that TMSN was eluted in the fraction between 8 and 9 min

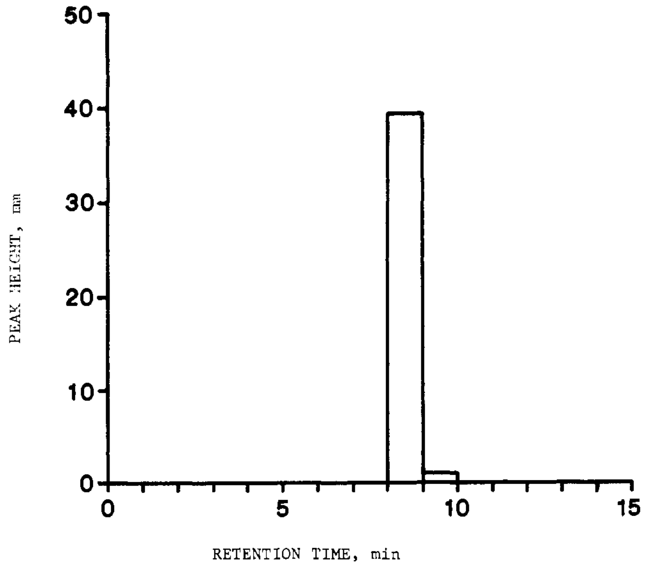

Figure 2. Elution pattern of TMSN by HPLC of the extract from PMMA. TMSN in fractions was determined by GC–NPD.

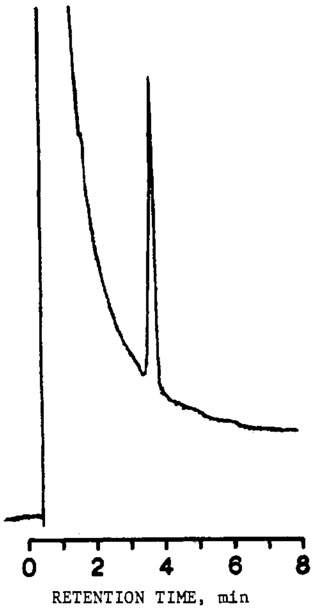

Figure 3. Gas chromatogram of the HPLC fraction between 8 and 9 min using FID.

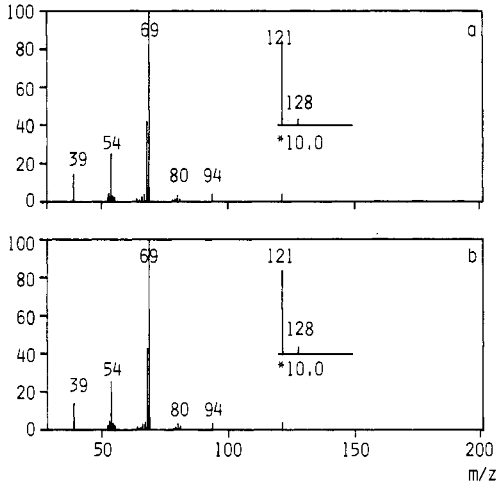

Figure 4. Mass spectra of authentic TMSN (a) and the extract of MABS (b). The test solution for GC was applied to HPLC, and the eluate between 8 and 9 min was applied to GC–MS.

(Figure 2) without any peaks except that of TMSN (Figure 3), showing the same retention times of authentic TMSN in both HPLC and GC, respectively.

Retention times and mass spectra of the dichloromethane extract from the HPLC fraction between 8 and 9 min obtained from PAN, PMMA, and MABS coincided with those of authentic TMSN, though the M^+ (m/e 136) could not be detected in either the authentic standard or samples. The mass spectra of authentic TMSN and that obtained from a sample are shown in Figure 4,ab.

The results presented here clarified the carring over of TMSN, a decomposition product of 2,2′-azobisisobutyronitrile, into final plastic products. TMSN was detected in 10 samples (six kinds of resin) from 76 plastic products (18 kinds of resin), and the highest concentration, 134.4 μg/g of TMSN, was observed in MABS. Experiments of migration of TMSN from these plastic materials into contents are undergoing.

Registry No. PE, 9002-88-4; PSF, 9003-53-6; PAN, 25014-41-9; PMMA, 9011-14-7; MABS, 9010-94-0; ABS, 9003-56-9; PVC, 9002-86-2; TMSN, 3333-52-6; 2,2′-azobis(isobutyronitrile), 78-67-1.

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