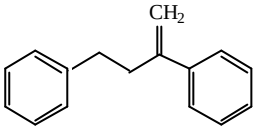
Hazard assessment of Styrene dimer, Styrene trimer

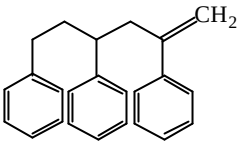
[Styrene dimer, CAS No. 25247-68-1; Styrene trimer, , 28213-80-1]

Chemical name	:	Styrene dimer	Styrene trimer
Synonyms	:	Omitted as there are too many isomers (Attachment-1.2)	
Molecular formula	:	$C_{16}H_{16}$	$C_{24}H_{24}$
Molecular weight	:	208.2	312.3
Structural formula	:	Among structural isomers, 4 of the dimeric isomers, 7 of the trimeric isomers and 2 of the trimer mixture (cf. Attachments 1 and 2) are included in the present hazard assessment.	

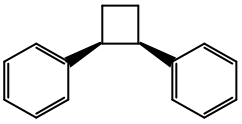
The structural formula of some of them are shown below.



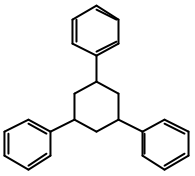
2,4-diphenyl-1-butene



2,4,6-triphenyl-1-hexene



1,2-diphenylcyclobutane



1,3,5-triphenylcyclohexane

Appearance	:	No Report[1)]	No Report[1)]
Melting point	:	No Report[1)]	No Report[1)]
Boiling point	:	No Report[1)]	No Report[1)]
Specific gravity	:	No Report[1)]	No Report[1)]
Vapor pressure	:	No Report[1)]	No Report[1)]
Partition coefficient	:	No Report[1)]	No Report[1)]
Degradability		Hydrolyzability: No Report[1)]	No Report[1)]
		Biodegradability: No Report[1)]	No Report[1)]
Solubility	:	No Report[1)]	No Report[1)]
		No Report[1)]	

Amount of Production/: Import	:	Not manufactured because there is no specific usage for these compounds. There is a report that 760 - 21,430 μg/g (mean 9,590 μg/g) of combined styrene dimers and trimers were detected in various polystyrene containers[2)].

Usage	:	Styrene dimer and trimer are byproducts generated when polystyrene resin is manufactured and there is no specific usage for these substances[3, 4].
Applied laws and regulations	:	None.

[1] HSDB, 2001; [2] Kawamura et al., 1998; 3)Mayo, 1968; 4)Kurze et al., 1970.

1. Toxicity Data

1) Information on adverse effects on human health

There is no report on the effects on human health due to exposure to styrene dimer and trimer.

2) Information on endocrine system and reproductive system

(1) *in vitro* test results related to receptor binding (Attachment-3)

A receptor binding assay was conducted using synthesized styrene dimers and trimers (Attachment-1), all of which were confirmed to be eluted from cup noodle containers, but none of these compounds demonstrated affinity to estrogen receptor (ER), androgen receptor (AR) and thyroid hormone receptor (TR) derived from rat (Nobuhara et al., 1999; Azuma et al., 2000; Hirano et al., 2001; Ohno et al., 2001; Date et al., 2002). Furthermore, these compounds did not accelerate the growth of human breast cancer cell (MCF-7) (Nobuhara et al., 1999; Ohno et al., 2001).

With an estrogen receptor/reporter gene assay of MCF-7 cells, a mixture of styrene dimer and trimer extracted from polystyrene (weight ratio of 0.143: 0.955) did not influence trascription activity (Fail et al., 1998). On the other hand, growth of MCF-7 cell was enhanced by 2 out of 4 styrene dimer isomers and 4 out of 7 trimer isomers. Whereas the EC_{50} of 17β-estradiol (E2), a positive control (concentration corresponding to 50% of the cell growth count at 10^{-10} M E2) was 1.4×10^{-11} M, the EC_{50} demonstrated by dimers and trimers were 10^{-6} - 10^{-5} M, that is, corresponding to 1/68,000 or less of that of E2. In a human estrogen receptor α binding test, binding of 4 types of dimers and 5 types of trimers was observed. Their affinity was 1/43,000 or less of that of E2 (Ohyama et al., 2001).

(2) *in vivo* test results in mammals (Attachment-4)

When single intraperitoneal administration of a condensate mixture of byproducts obtained in the manufacturing process of polystyrene (styrene dimer: 13.35%, trimer: 69.5%, ethylbenzene: 3.17%, styrene monomer: 4.52%, cumene: 1.65%, mineral oil: about 6%) was given to female SD rats (22 days old) at the concentrations of 0, 100, 300 and 1,000 mg/kg, premature vaginal opening was observed in the 1,000 mg/kg group. This concentration corresponded to 1 mg/kg of estron (Rao et al., 1978).

When styrene oligomer with styrene tetramer as the major ingredient (styrene dimer: 12.4%, trimer: 15.2%, tetramer: 54.3%) at 0, 10, 20, 40, 80 and 160 ppm (equivalent to 0, 1.6, 2.9, 6.9, 13.3 and 27.2 mg/kg/day, respectively) was mixed in diet and given to female Wistar rats (19 days old) for 4 days, increase of uterus weight was observed in the 160 ppm group. This activity was assessed as 1/20,000 of that of diethylstilbestrol used as a positive control (Prinsen, 1996). However, since this result was obtained from the chemicals with styrene tetramer as a major ingredient, the result is not acceptable to be demonstrated by styrene dimers and trimers.

No effects on endocrine system were demonstrated in the following reports.

A mixture of high purity styrene dimers and trimers (weight ratio of 0.143: 0.955) extracted from polystyrene administered to female SD rats (21 days old) by oral gavage for 3 days did not induce significant change in weight of uterus (although practical dose administered was not stated in the report, the amounts were estimated to be 8 and 52 mg/kg/day of styrene dimer and trimer respectively, from the procedure of extraction reported) (Fail et al., 1998).

When the eluate of 23 types of polystyrene classified into 3 categories [9 types of general purpose polystyrene (GPPS), 8 types of high impact polystyren (HIPS) and 6 types of expandable polystyrene (EPS)] (though chemical structure of styrene oligomer, styrene dimers and trimers are unknown, the concentrations of styrene dimers and trimers in the mixture that contained the largest amount of these substances were 0.33 mg/*l* and 0.75 mg/ *l* respectively) was administered to female Wistar rats (22±1 day old) by oral gavage for 3 days in uterotrophic assay, no significant change was observed in uterus weight by any of the extracts. With consideration to the human exposure, the extracts from GPPS, HIPS and EPS in the amounts corresponding to the contents of food

consumed by adults were combined and administered at 2 doses each at the concentration ratio of 10:1 in the test (though the doses are not stated in the literature, the amounts corresponding to 6.6 mg/kg/day of styrene dimer and 15.0 mg/kg/day of trimer among the 23 extracts are assumed to have been administered as the maximum doses in view of the procedure of extraction) (Bachmann et al., 1998).

Styrene monomer, and synthesized styrene dimers (3types) and trimers (3types) (Attachment-1) eluted from cup noodle containers were administered separately to female SD rats (21 days old) by oral gavage in uterotrophic assay. No significant changes were noted by any of the test substances in weight of uterus and prolactin concentration in serum (Nobuhara, et al., 1999; Date et al., 2002).

In a Hershberger assay, styrene monomer and synthesized styrene dimers (3 types) and trimers (3 types) were each given at 0, 20 and 200 mg/kg/day to castrated male SD rats (4 weeks old) to detect anti-androgenic action (in accordance with the OECD guideline) by oral gavage for 7 days together with subcutaneous administration of testostreone propionate at 50 μg/head/day. None of the test substances induced any significant changes in weight of prostatic ventral lobe, seminal vesicle, lavator ani muscle + bulbospongiosus muscle (Azuma, et al., 2000; Date et al., 2002).

A mixture of styrene dimers and trimers was orally given to female SD rats (9 weeks old) at 0.04 mg/kg/day (dimer: 0.781 ppm, trimer: 8.73 ppm), 0.2 mg/kg/day (2.64 ppm and 32.8 ppm respectively) and 1.0 mg/kg/day (16.9 ppm and 166 ppm respectively) from day 6 of gestation to day 21 after delivery. No changes were observed in the body weight, food consumption and gestation period of dams at any of the doses. Nor any changes were noted in survival rate, growth, reproductive organ formation and learning behavior of offspring. The concentration of thyroid stimulating hormone in serum was significantly higher in male offsprings of the 0.2 and 1.0 mg/kg/ groups than that in the control group. The concentrations of thyroid hormone T3 and T4 in the serum were off similar to the control values. No changes were noted in thyroid tissue of male spring in any of the dose groups (Nagao et al., 2000).

In an *in vitro* study, leydig cells from 7 weeks old male SD rats were given the above mentioned styrene monomer and synthesized 3 types of styrene dimer and 3 types of trimer, and human chorionic gonad stimulatimg hormone (hCG) was added one hour

thereafter. Then, after 3 hours no significant suppression of testosterone synthesis was not observed up to the level of 10^{-5}M hCG (Nobuhara et al., 1999).

3) Information on general toxicity

(1) Acute toxicity

Currently, there is no report on acute toxicity.

(2) Repeated-dose toxicity

Currently, there is no report on repeated-dose toxicity.

4) Information on mutagenicity/genotoxicity and carcinogenicity

(1) Mutagenicity/genotoxicity (Table 1)

Reverse mutation assay of some of styrene dimers and trimers using *Salmonella typhimurium* (TA98 + S9 mix) gave negative results (Grifoll et al., 1990).

Table 1 Results of mutagenicity/genotoxicity tests

Test method		Cell and animal species used	Result*	References
in vitro	Reverse mutation test	*Salmonella typhimurium* TA98 S9(+) 0.001-1 mg/plate	−	Grifoll et al., 1990

* -: Negative

(2) Carcinogenicity (Table 2)

No report on carcinogenicity is now available.

Table 2 Carcinogenicity assessment by national and international organizations

Organizations	Classification	Significance	References
EPA	−	No evaluation	IRIS, 2002
EU	−	No evaluation	ECB, 2000
NTP	−	No evaluation	NTP, 2000
IARC	−	No evaluation	IARC, 2001
ACGIH	−	No evaluation	ACGIH, 2001
Japan Society for Occupational Health	−	No evaluation	Japan Society for Occupational Health, 2001

5) Information on immune system

No report has been available on the effects on immune system.

6) Fate and Metabolism

There are no detailed reports regarding metabolism of styrene dimers and trimers.

2. Hazard Assessment at present

There is no report regarding the effects of styrene dimer and trimer on human health. One styrene oligomer mixtures mainly composed of tetramers showed estrogenic activity. But it is not clear whether this was caused by styrene dimmers/trimers contained in the mixture.

Since 1998, experiments focused on human exposure to styrene dimer and trimer that were eluted especially from polystyrene containers, and those using chemically synthesized styrene dimers and trimers eluted from polystyrene containers have been conducted and no estrogenic or anti-androgenic activity was observed in any of them. No effects on dams and offsprings was noted in oral adminsitration test conducted from day 6 of gestation to day 21 of delivery in rats. No increase in serum prolactin level was observed in the rats given the synthesized styrene dimers and trimers.

Binding assays for estrogen receptor, androgen receptor and thyroid hormone receptor, estrogenic assay on human breast cancer cell MCF-7 and the experiment on biosynthesis of testosterone using testicular cells were all negative. However, the growth of human breast cancer cells was noted and the binding to human estrogen α receptor was reported in an MCF-7 cell growth test using synthesized styrene dimers and trimers at 1 - 10 μM, but the estrogenic activity observed in these tests was 1/68,000 or less of that of E2, and extremely weak.

3. Risk assessment and other necessary future measures

Various *in vitro* screening tests to assess endocrine disrupting activity of styrene dimers and trimers have already been conducted. And for highly pure synthesized, dimers and trimers most of researches to detect estrogenic activity was negative. Although there are some reports showing estrogenic activity, the activity was weak.

Furthermore, no estrogenic activity nor anti-androgenic activity was noted in an *in vivo* experiment using rats. Thus, no specific actions are judged to be necessary for the time being, since no evidence indicating the endocrine disrupting activity of styrene dimers and trimers has been found from the test results obtained so far.

Reference

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Date, K., Ohno, K., Azuma, Y., Hirano, S., Kobayashi, K., Sakurai, T., Nobuhara, Y. and Yamada, T. (2002) Endocrine-disrupting effects of styrene oligomers that migrated from polystrene containers into food. Food Chem. Toxocol., 40, 65-75.

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Nagao, T., Wada, K., Kuwagata, M. and Ono, H. (2000) Effects of prenatal exposure to styrene dimers and trimers on reproductive function in rats. Reprod. Toxicol., 14, 403-415.

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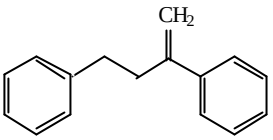
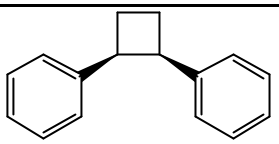
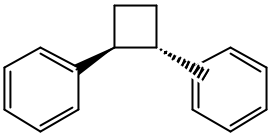
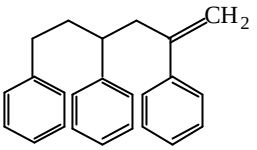
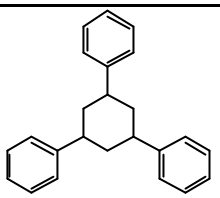
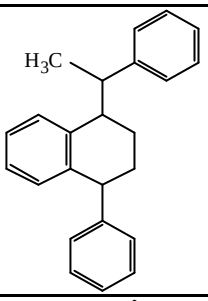
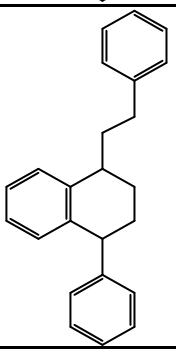
Attachment-1 Synthesized styren dimers and trimers used in the tests (manufactured by Nisshin Food Laboratories) (1)

	Abbreviations	Names of compounds	Structural formula	References
Styrene dimer	NSD-01	2,4-Diphenyl-1-butene		Nobuhara, etc., 1999; Azuma, etc., 2000
	NSD-08	*cis*-1,2-Diphenylcyclobutane		
	NSD-09	*trans*-1,2-Diphenylcyclobutane		
Styrene trimer	NST-01	2,4,6-Tripheyl-1-hexene		
	NST-02 *)	1,3,5-Triphenylcyclohexane （mixture of 1e,3e,5a-Triphenylcyclohexane, 1e,3e,5e-Triphenylcyclohexane）		
	NST-03	1e-Phenyl-4e-(1´-phenylethyl)tetralin (mixture of 1S*-Phenyl-4S*-(1R*-phenylethyl)tetralin, 1S*-Phenyl-4R*-(1S*-phenylethyl)tetralin, 1S*-Phenyl-4S*-(1S*-phenylethyl)tetralin, 1S*-Phenyl-4R*-(1R*-phenylethyl)tetralin）		
	NST-12 **)	1e-Phenyl-4a-(2-phenylethyl)tetralin		

*) Test only according to the report by Nobuhara et al. (1999)

**) Test only according to the report by Azuma et al. (2000)

Attachment-2 Synthesized styren dimers and trimers used in the tests

	Names of compounds	Abbreviations Ohyama	Abbreviations Nobuhara et al., Azuma et al.	References
Styrene dimer	*cis*-1,2-Diphenylcyclobutane	SD-3	NSD-08	Nobuhara et al., 1999. Azuma et al., 2000. Ohyama et al., 2001.
	trans-1,2-Diphenylcyclobutane	SD-4	NSD-09	
	1,3-Diphenylpropane	SD-1		
	2,4-Diphenyl-1-butene	SD-2	NSD-01	
Styrene trimer	2,4,6-Tripheyl-1-hexene	ST-1	NST-01	
	1,3,5-Triphenylcyclohexane	ST-6	NST-02	
	1e-Phenyl-4e-(1´-phenylethyl)tetralin	ST-5	NST-03	
	1a-Phenyl-4e-(1´-phenylethyl)tetralin	ST-3		
	1a-Phenyl-4a-(1´-phenylethyl)tetralin	ST-2		
	1e-Phenyl-4a-(1´-phenylethyl)tetralin	ST-4		
	1e, 3e, 5a-Triphenylcyclohexane	ST-7		

*) The compounds marked with asterisk (*) were provided in 2 categories, namely, the synthesized product and the synthesized reference standard for analysis manufactured by Hayashi Pure Chemical Ltd.
Ohyama et al. (2001) conducted tests using only 11 types of the synthesized reference standard for analysis manufactured by Hayashi Pure Chemical Ltd.

Attachment-3 Results of *in vitro* test on receptor binding

Item	Cell species, etc	Test compound	Result	Remark	References
Hormone receptor binding test	Binding test using rat ER[1)] (ER derived from rat's uterus)	Styrene monomer (1 type), synthesized styrene dimer (3 types), trimer (3 types)[2)]	IC50: > 10^{-5} M (E2: 1.4×10^{-9} M)	No affinity	Nobuhara, et al., 1999
	Binding test using rat AR (AR derived from rat's prostate)		IC50: > 10^{-5} M (DHT3.4×10^{-9} M)	No affinity	Nobuhara, et al., 1999
	Binding test using rat ER (ER derived from rat's uterus)	Styrene monomer (1 type), synthesized styrene dimer (3 types), trimer (3 types)[2)]	IC50: > 10^{-5} M (E2: 2.6×10^{-9} M)	No affinity	Azuma, et al., 2000
	Binding test using rat AR (AR derived from rat's prostate)		IC50: > 10^{-5} M (DHT: 4.2×10^{-9} M)	No affinity	Azuma, et al., 2000
	Binding test using rat TR (AR derived from rat's liver)		IC50: > 10^{-5} M (T3: 1.2×10^{-8} M)	No affinity	Azuma, et al., 2000
	Method: Competitive binding test using fluorescent E2 ligand Receptor: Human estrogen receptor α	Styrene dimer (4 types), trimer (7 types)[3)]	SD-1,2,3,4 IC20: > 2×10^{-5} M ST-1,2,3,4,5 IC20: > 2×10^{-6} M No inhibition by ST-6, 7 (E2: 6**x** 10^{-11} M)	Affinity 1/43,000 or less	Ohyama et al., 2001
Reporter gene assay	Reporter gene assay by incorporating estrogen receptor responding sequence into human breast cancer cell (MCF-7)	Polystyrene extract solution[4)]	No transcription activity was observed.	No gene activity	Fail et al., 1998
Cell Growth	Method using the growth of human breast cancer cell (MCF-7) as an index Culture solution: D-MEM medium added with 5% FBS Cell count: 12-well plate (2.0×10^4 cells well)	Styrene monomer (1 type), synthesized styrene dimer (3 types) and trimers (3 types [2)]	No growth stimulation occurred by any of test substances.	No influence on cell growth activity	Nobuhara, etc., 1999
	Method using the growth of human breast cancer cell (MCF-7) as an index Cell count: 40×10^4 cells/well	Styrene dimer (4 types), trimer (7 types)[3)]	Growth was noted by SD-3, 4, ST-1, 2, 4 and 6 at 1 - 10^6 M.	Cell growth activity 1/68,000 or less	Ohyama et al. 2001

[1)] ER: Estrogen receptor; AR: Androgen receptor; TR: Thyroid hormone receptor; E2: 17β-estradiol; SD: styrene dimer; ST: Styrene trimer; DHT: Dihydrotestosterone.
[2)] Refer to Attachment-1
[3)] Refer to Attachment-2
[4)] Weight ratio of styrene dimer and trimer: 0.143:0.955

Attachment-4 Results of tests on the endocrine system and reproductive system of mammals

Animal species	Administration method	Administration period	Dose	Result	References
Rat (SD, male, 22 days old)	i.p.	Single	Byproduct[1] in the process of polystyrene manufacture, at 0, 100, 300, 1,000 mg/kg	Premature opening of the vagina was observed in the 1,000 mg/kg group.	Rao et al., 1978
Rat (SD, male, 21 days old)	Oral gavage (uterine growth assay)	3 days	Polystyrene extract[2]	No significant change was noted in the weight of uterus.	Fail et al., 1998
Rat (Wistar, female 22±1 days old)	Oral gavage (uterine growth assay)	3 days	23 types of polystyrene extracts[3]	No significant change was noted in the weight of uterus by anyof the extracts.	Bachmann et al., 1998
Rat (SD, female, 21 days old)	s.c. (uterine growth assay) (juvenile rats)	3 days	Styrene monomer (1 type), styrene dimer (3 types), trimer (3 types)[4], at 0, 20, 200 mg/kg/day	No significant change was noted in the weight of uterus by any of the test substances.	Nobuhara, etc., 1999
Rat (SD, male, 4 weeks old)	Oral gavage (Hershberger assay) (castrated rats, castration when they were 3 weeks old)	For 7 days when they were 7 weeks old	Styrene monomer (1 type), styrene dimer (3 types), trimer (3 types)[4] at 0, 20, 200 mg/kg/day + testosterone propionate at 50 μg/animal/day (s.c.)	No significant changes occurred in the prostatic ventral lobe, seminal vesicle, lavator ani muscle + bulbospongiosus muscle weights by any of the test substances (anti-androgen action)	Azuma etc., 2000
Rat (F344, female, 7 weeks old)	s.c. (ovariectomy when they were 6 weeks old)	For 3 days when they were 7 weeks old	Styrene monomer (1 type), styrene dimer (3 types), trimer (3 types)[4] at 0, 20 mg/kg/day	No significant change was noted in the serum prolactin concentration.	Azuma, etc., 2000

Animal species	Administration method	Administration period	Dose	Result	References
Rat (SD, female 9 weeks old)	p.o.	Day 6 of gestation - day 21 of delivery	Mixture of styrene dimer and trimer[5]) at 0, 0.04, 0.2, 1.0 mg/kg/day	No changes occurred in the body weight, food consumption and gestation period of dams at any of the doses. No changes occurred in the survival rate, growth, reproductive organ formation and learning behavior of offspring.	Nagao et al., 2000
Rat (SD, male, 7 weeks old)	Added to testicular interstitial cells	hCG was added at 1h after addtion, followed by 3 hours of further incubation	Styrene monomer (1 type, dimer (3 types) and trimer (3 types)[4]), 10^{-5} M	No significant inhibition on the testosterone biosynthesis was observed by any of the test substances.	Nobuhara, etc., 1999

1) Styrene dimer: 13.35%, trimer: 69.5%
2) The weight ratio of styrene dimer and trimer was 0.143: 0.955
3) 23 types of polystyrene extracts classified into 3 categories [9 types of general purpose polystyrene (GPPS), 8 types of high impact polystyren (HIPS) and 6 types of expandable polystyrene (EPS)] (though the types of styrene oligomer mixutre, styrene dimer and trimer are unknown, the concentrations of styrene dimer and trimer in the mixture that contained a large amount of these substances were 0.33 mg/L and 0.75 mg/L respectively)
4) Refer to Attachment-1
5) Mixture of styrene dimer and trimer at 0.04 mg/kg/day (dimer: 0.781 ppm, trimer: 8.73 ppm), 0.2 mg/kg/day (2.64 ppm and 32.8 ppm respectively) and 1.0 mg/kg/day (16.9 ppm and 166 ppm respectively)