



Executive Summary Styrene-Acrylonitrile Trimer (SAN Trimer)

Background Information on Styrene-Acrylonitrile Trimer (SAN Trimer)

June 15, 1999

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Working definition: SAN trimer is a mixture of isomers with a molecular weight of 210 formed by the condensation of two moles of acrylonitrile and one mole of styrene.

Chemical names: 4-cyano-1,2,3,4-tetrahydro- α -methyl-1-naphthaleneacetonitrile (THNA), found in 4 forms in waste stream:

- Cis-R-THNA
- Trans-R-THNA
- Cis-S-THNA
- Trans-S-THNA

4-cyano-1,2,3,4-tetrahydro-1-naphthalene-propionitrile (THNP), found in 2 forms in waste stream:

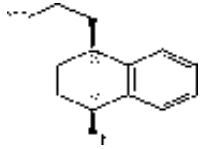
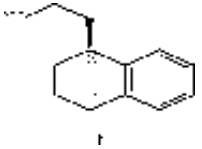
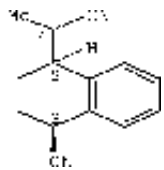
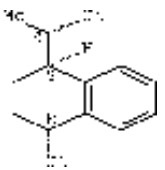
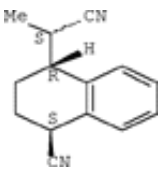
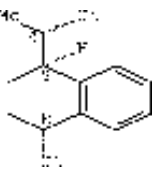
- Cis-THNP
- Trans-THNP

CAS Numbers:

- THNA: 57964-39-3;
- Cis-R-THNA: 142759-38-4;
- Trans-R-THNA: 142759-37-3;
- Cis-S-THNA: 142759-39-5;
- Trans-S-THNA: 142759-40-8;
- THNP: 57964-40-6; Cis-THNP: 142681-91-2;
- Trans-THNP: 142681-92-3

Molecular weight: 210

Chemical structures:

	-		-
Cis-THNP	-	Trans-THNP	-
8% of bulk*	-	6% of bulk*	-
			
Cis-S-THNA	Trans-S-THNA	Cis-R-THNA	Trans-R-THNA
17% of bulk*	14% of bulk*	30% of bulk*	25% of bulk*

Rationale for nomination

A member of Congress brought SAN trimer to our attention in June 1998 in response to citizen concerns in the Toms River area of New Jersey regarding a childhood cancer cluster in this area. We were asked whether or not it would be appropriate to get involved in this case by performing a study on pregnant rats exposed to the trimer and their offspring. In September 1998, both William McCabe, Deputy Director of the Emergency Remedial and Response Division, EPA Region 2, and Timothy Fields, Acting Assistant Administrator of the Office of Solid Waste and Emergency Response, EPA, requested to appoint a scientist of NIEHS to be a member of the Interagency Workgroup for the Toxicity Testing of SAN trimer. This Interagency Workgroup consists of members of the US Environmental Protection Agency, the New Jersey Department of Environmental Protection, the New Jersey Department of Health and Senior Services, the US Agency for Toxic Substances and Disease Registry, Union Carbide, and NIEHS. The workgroup considers only those

issues that are relevant to existing toxicological data on the SAN trimer, the determination of additional toxicity testing needs, and the development of study protocols to address those testing needs.

SAN trimer was identified in groundwater and was determined to have leached into the groundwater from the Reich Farm Superfund site, where spent process streams from the manufacture of polymers of styrene and acrylonitrile by Union Carbide Corporation (UCC) were dumped by a contractor for UCC. The SAN trimer was a component of the process streams.

Although more chemicals were identified in the well water, the SAN trimer appeared to occur consistently in the water in the ppb range and was determined to be a site contaminant.

The New Jersey Department of Health and Senior Services (NJDHSS) analyzed cancer incidence data on children living in the Toms River section of Dover Township, Ocean County diagnosed with cancer between birth and 19 years of age from 1979 through 1991. They found an elevated rate of brain and central nervous system cancer in children under the age of five. In Ocean County they found the same cancers elevated in children under the age of five and under the age of 20 (NJDHSS, 1996).

A more extensive review and analysis of cancer registry data from 1979 through 1995 was performed in Dover Township, New Jersey (NJDHSS, 1997). In Ocean County, a significantly elevated standardized incidence ratio (SIR) (2.21; 95% CI = 1.29, 3.54) was observed for sympathetic nervous system cancers that appeared to be due to a large number of neuroblastoma cases, particularly in males under age 5. Astrocytomas were also significantly elevated when all age, race, and sex groups were combined (SIR = 1.46; 95% CI = 1.02, 2.03). In Dover Township, the total childhood cancer incidence was elevated for all age, race, and sex groups combined (SIR = 1.99; 95% CI = 1.06, 3.09). For all females under age 20 (SIR = 1.99; 95% CI = 1.06, 3.40) and for females under age five (SIR = 2.65; 95% CI = 1.06, 5.45), leukemia was significantly elevated, which was due to an elevation in acute lymphocytic leukemia. In the Toms River section of Dover Township, all cancers combined were significantly higher than expected for all age, race, and sex groups combined (SIR = 1.70; 95% CI = 1.09, 2.53), with the highest SIR for all cases in females under age 5 (SIR = 6.17; 95% CI = 2.95, 11.34). Brain and central nervous system cancers were elevated in children under age five for both sexes combined (SIR = 7.04; 95% CI = 1.89, 18.03) and females under age five (SIR = 11.6; 95% CI = 2.33, 33.9). Children under age five had also an elevation in astrocytomas (SIR = 9.47; 95% CI = 1.06, 34.2), leukemia in females (SIR = 7.84; 95% CI = 2.11, 20.7), and acute lymphocytic leukemia in females (SIR = 9.68; 95% CI = 2.60, 24.8).

Physical and chemical properties

Table. 1. Some physical and chemical properties.

Property	Status
Physical state	Thick, viscous, opaque, liquid at room temperature (handout workgroup meeting 11-4-98 and MA Bioservices, 1998a)
Color	Light brown (MA Bioservices, 1998a)
Soluble in	Acetonitrile, methanol, methylene chloride (handout workgroup meeting 11-4-98)

Vapor pressure	2.5 mm Hg at 235°C (workgroup meeting 11-4-98, number is really an estimate, workgroup meeting 3-29-99)
Density	1.101 g/mL at 20.0°C (Wildlife International Ltd., 1998a)
Specific gravity	1.103 at 20.0°C (Wildlife International Ltd., 1998a)
Water solubility	84.9 mg/L (Wildlife International Ltd., 1998b)
n-octanol/water partition coefficient	3.1 (Wildlife International Ltd., 1998c)

Production and use

Production and Producers SAN trimer is a byproduct of the production of acrylonitrile styrene plastics (Union Carbide Fact Sheet, 1998). Upon heating (230°C) 2-amino-3-methyl-1-naphthalenecarbonitrile is formed (Stark et al., 1992).

According to Union Carbide, SAN trimer is only created in specific manufacturing processes for polymers of acrylonitrile and styrene. A few manufacturers still use this process (pers. comm. between Dorothy Canter and Union Carbide).

Production of polymers of acrylonitrile and styrene was estimated to be 41,000 tonnes in 1982 in the US (Huff, 1984), and 90,000 tonnes in 1979 in Japan (Kalliokoski, 1984).

The chemical composition of copolymer fragments resembles that of the original polymers. E.g., polystyrene fragments contain styrene units as dimers, trimers, etc. Fragments of styrene-acrylonitrile contain styrene units and even nitrile units (Pfäffli, 1984). These fragments hold isomeric variations (Blaszo et al., 1980).

Use SAN trimer has been described as a flow-modifier in the manufacture of the styrene-acrylonitrile polymer in a Japanese patent (ATSDR, 1997). Polymers of acrylonitrile and styrene are used as plastics in housewares, toys, electronic and electrical appliances, recreational articles, inner parts of a refrigerator, handles, bags, and pipes (Martinmaa, 1982).

Human and environmental exposure

Human exposure

Little information is available. It was mentioned in a fact sheet from Union Carbide Corporation on the single and repeat dose toxicity test results on the SAN trimer that 1 ppb was the maximum amount of SAN trimer found in the drinking water in Toms River (Union Carbide Corporation Fact Sheet, 1999). SAN trimer was identified in a concentrated extract of municipal well water (Grange et al., 1998). Six SAN trimer congeners were identified in well water (Richardson et al., 1999). Samples from well #26 in Toms River, NJ, during the time period 1990-1994 contained 6 ppb of SAN trimer (Richardson et al., 1999). This well contained much higher concentrations of SAN trimer compared to dimers (approximately 2 orders of magnitude). In 1997 untreated ground water of well #26 had a concentration of SAN trimer between 4 and 12 ppb (Richardson et

al., 1999). Samples from treated water of well #26 during the same time period contained less than 12 ppt.

Environmental occurrence

SAN trimer was identified in the Parkway Wellfield at Toms River, NJ (ATSDR, 1998). Samples from the ground water within the Reich farm plume had concentrations of SAN trimer ranging from 1 to 20 ppb in 1997 and typically from 1 to 100 ppb between 1990 to 1994 (Richardson et al., 1999).

Absorption, distribution, metabolism, and excretion

Experimental Animals

No information was found in the literature.

Humans

No information was found in the literature.

Toxicity

Note that the tested SAN trimer also contained 1.4% of 2-amino-3-methyl-1-naphthalenecarbonitrile in the acute, 14-day repeat dose, and genotoxicity studies.

Acute toxicity

The oral (gavage) LD50s in male and female Sprague Dawley rats were reported as 441 and 589 mg/kg, respectively (Huntington Life Sciences, 1998a). Signs included stomach changes (red mucosa and depression), body weight loss, decreased food consumption, labored or slow breathing, tremors, hypothermia, lethargy and/or prostration, decreased activity, yellow anogenital staining, red stains on the snout/ventral surface/extremities, lacrimation, excessive salivation and irregular gait.

14-Day repeat dose toxicity

A two-week oral gavage study was performed with SAN trimer at dose levels of 0, 30, 75, 150, and 300 mg/kg/day, 7 days a week, in 7-wk old male and female Sprague Dawley rats, using 6 animals per dose group (Huntington Life Sciences, 1998b). The highest dose tested resulted in deaths of all six males and 5 out of 6 females. Also in the 150 mg/kg/day dose group one female died. Body weights in the 150 and 300 mg/kg/day males and 300 mg/kg/day females groups were decreased (approximately 12%). Food consumption was decreased in 150 and 300 mg/kg/day males and 300 mg/kg/day females to approximately 85, 70, and 85% of controls, respectively. Abnormalities at the highest dose group included ano-genital stains, black or brown stains on the snout and/or extremities, decreased fecal volume, excessive salivation, labored breathing, tremors, and lethargy or prostration. In the 150 mg/kg/day dose group lethargy, labored breathing, irregular gait and lacrimation were observed. The absolute liver weights of the 150 mg/kg/day males and females and the surviving 300 mg/kg/day female were increased 19, 27, and 41% in comparison to controls, respectively. For the relative liver weights, these were 34, 29, and 65% in comparison to controls, respectively. The absolute and relative heart weights of the 150 mg/kg/day females and the 300 mg/kg/day female were increased approximately 15% in comparison to controls. Weights of the brain, kidneys, ovaries, and testes/epididymides were unaffected, whereas weights of the spleen, thymus, and lung were not determined. Postmortem macroscopic examinations of the animals found dead during the study included changes in the liver (enlarged and/or discolored), lungs (discolored), trachea (fluid), spleen (enlarged), and testes (pale). The 150 mg/kg/day dose groups had changes in the lungs (discolored) and trachea (fluid), sometimes cysts in the

kidneys, and changes in the mandibular lymph nodes (enlarged and/or discolored), and the thymic region (discolored). In the 300 mg/kg/day dose groups, histopathologic changes included vacuolation of periacinar hepatocytes (males) and periacinar hypertrophy (females) of the liver and vacuolation of the cortical tubular epithelial cells in the kidney (males). No changes were observed in the 150 mg/kg/day and lower dose groups in the 20+ organs and tissues evaluated. Irwin Screen evaluations were performed before dosing and on day 7 and 14. One male rat at 300 mg/kg on day 7 exhibited ataxia, slight stupor, slightly impaired locomotion, and slow or absent reflexes. This animal was found dead on day 11. Two male rats in the 150 mg/kg/day dose group had abnormal gait. A 20% decrease in activated partial thromboplastin time was observed in males at 150 mg/kg/day and the survival male rat in the 300 mg/kg/day dose group. A macrocytic, hypochromic, responsive anemia was observed in female rats at 150 mg/kg/day (hemoglobin, hematocrit, and erythrocyte count were approximately 10% lower than controls). These endpoints were markedly decreased (20 to 30%) in the surviving female rat at 300 mg/kg/day. In this same animal, a decrease in lactate dehydrogenase and sorbitol dehydrogenase was observed in comparison to controls. The mean 16-hour urine volume in males and females in all test groups was approximately 3 times that of controls.

Reproductive and Developmental Toxicity

Experimental Animals

No reports were found in the literature.

Humans

No reports were found in the literature.

Carcinogenicity

Experimental Animals

No reports were found in the literature.

Humans

No reports were found in the literature.

Genetic Toxicity

SAN trimer was mutagenic in Salmonella strains TA98 and TA1537 without S9, not mutagenic in TA98 and TA1537 with S9, weakly mutagenic in TA100 without S9, not mutagenic in TA100 with S9, and not mutagenic in TA1535 or TA102 (with or without S9) (MA Bioservices, 1998a). SAN trimer was not mutagenic in the Chinese hamster ovary (CHO)/hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus gene mutation test (MA Bioservices, 1998b). SAN trimer did not induce chromosomal aberrations in bone marrow cells of male and female Sprague Dawley rats, neither did SAN trimer induce micronuclei in bone marrow polychromatic erythrocytes in these rats (MA Bioservices, 1998c). In these experiments bone marrow was harvested at 18 and 42 hours after treatment. The recommended sampling time for bone marrow cytogenetics studies is ca. 1.5 times the (24hr) cell cycle. According to Errol Zeiger, the 42-hr sampling time may have been too long and may have allowed cells containing chromosome aberrations to die or to be diluted out (Memo from Errol Zeiger to Bob Chapin, 11 November 1998). In the same study plasma samples of controls and high dose groups were collected at 18 and 42 hours in both male and female rats and returned to UCC for chemical analysis. (As of now, the method development for the total amount of SAN trimer - not individual congeners - is in the validation stage. This method will be send to us by Nancy Broyles, UCC,

when the validation work is complete). SAN trimer induced chromosomal aberrations in CHO cells with and without S9 (MA Bioservices, 1998d).

Additional Information

Carcinogenicity-Acrylonitrile

Acrylonitrile was classified as a Group 2B carcinogen by IARC, i.e., as possibly carcinogenic to humans based on inadequate evidence in humans and sufficient evidence in experimental animals (IARC, 1998).

Exposure to male and female Sprague Dawley rats by inhalation for 6 hours per day, 5 days per week, for 2 years, resulted in an increased incidence of glial-cell tumors of the central nervous system (males and females), Zymbal gland tumors (males and females), mammary gland adenocarcinomas (females), small intestine tumors (males), and squamous-cell tumors of the tongue (males) (IARC, 1999). In a drinking water study with acrylonitrile up to 300 mg/L and Sprague Dawley rats, resulted in an increased incidence of tumors of the forestomach, tongue, Zymbal gland and brain in males and of the mammary gland, Zymbal gland, tongue, forestomach and brain in females (IARC, 1999). In a drinking water study with acrylonitrile (500 mg/L) 49 out of 215 Fischer rats had brain tumors described as similar to astrocytomas or anaplastic astrocytomas (Bigner et al., 1986). However, the incidence of brain tumors in the control groups was not given. In the same study zymbal gland tumors, stomach and skin papillomas were more frequently seen in acrylonitrile-treated groups than in controls (Bigner et al., 1986). Male and female Sprague Dawley rats were exposed to acrylonitrile in utero and an additional 104 weeks postnatally by inhalation. In female offspring, the incidence of glial cell tumors of the brain, malignant mammary tumors, and extrahepatic angiosarcomas were increased (10/54, 9/54, and 3/54, respectively). In male offspring, the incidence of glial cell tumors of the brain, carcinomas of the Zymbal gland, and benign and malignant hepatocellular tumors were increased (11/67, 10/67, and 5/67, respectively) (IARC, 1999).

In a 90-day drinking water study with acrylonitrile in male Sprague Dawley rats, alterations in indicators of oxidative stress was measured in the brain but not in the liver (Jiang et al., 1998). These markers included an increase in 8-hydroxy-2'-deoxyguanosine, malonaldehyde, and reactive oxygen species. A decrease was observed for glutathione, catalase, and superoxide dismutase.

Carcinogenicity-Styrene

Styrene was classified as a Group 2B carcinogen by IARC, i.e., as possibly carcinogenic to humans (IARC, 1994).

A small increase in the incidence of pulmonary tumors in male mice and hepatocellular adenomas in females was observed in a gavage study. Prenatal exposure followed by postnatal gastric intubation resulted in an increased incidence of pulmonary tumors in male and female mice (IARC, 1994). Exposure to styrene up to 1000 ppm by inhalation resulted in non-neoplastic lesions in the olfactory epithelium of the nasal mucosa in Sprague Dawley rats (Cruzan et al., 1998). No styrene-related neoplastic lesions were observed in rats. CD-1 mice exposed to styrene by inhalation at concentrations ranging from 20 to 160 ppm, had an increase in bronchiolar-alveolar adenomas in 40, 80, and 160 ppm male mice and 20, 40, and 160 ppm female mice (Dorothy Canter, pers. comm.). In addition, female mice exposed to 160 ppm had an increase in bronchiolar-alveolar carcinomas.

Genetic Toxicity-Acrylonitrile

Acrylonitrile was mutagenic, usually but not exclusively in the presence of a metabolic system. In cultured mammalian cells, acrylonitrile also induced DNA strand breakage, gene mutation, sister chromatid

exchanges, and chromosomal aberrations. In rodents treated in vivo, acrylonitrile was negative for unscheduled DNA synthesis, chromosomal aberrations in the bone marrow of mice and rats, micronuclei in the bone marrow of mice, or dominant lethal effects in rats or mice. Acrylonitrile induced sister-chromatid exchanges in mouse bone marrow (IARC, 1999).

Genetic Toxicity-Styrene

Styrene was mutagenic in some studies in the presence of a metabolic system and negative without a metabolic system. Chromosomal aberrations were not observed in most studies in rodents, although some studies indicated a weak induction of sister chromatid exchanges in various tissues in rats and mice (IARC, 1994).

Co-exposure to Acrylonitrile and Styrene

Male Sprague Dawley rats exposed to various dose levels of styrene (i.p.), various dose levels of acrylonitrile (p.o.), or a combination of both at all possible combinations were evaluated after 24 hours (Normandeau et al., 1984). It was suggested that styrene-induced renal toxicity was potentiated by acrylonitrile. This suggestion was based on an increase in serum creatinine and serum glutamic-oxaloacetic transaminase (SGOT) by styrene, which was further increased by acrylonitrile.

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