HAZARD ASSESSMENT REPORT

POLY(OXYETHYLENE) NONYLPHENYL ETHER

CAS No. 9016-45-9

Chemicals Evaluation and Research Institute (CERI), Japan

This report was prepared by CERI in collaboration with National Institute of Technology and Evaluation (NITE) under the sponsorship of New Energy and Industrial Technology Development Organization (NEDO).

Preface to the English Version of the Hazard Assessment Reports

For six years from April 2001 to March 2007, Chemicals Evaluation and Research Institute (CERI/Japan) was engaged in a project named “Chemical Risk Assessment and Development of Risk Assessment Methods” under "Comprehensive Chemical Substance Assessment and Management Program" funded by New Energy and Industrial Technology Development Organization (NEDO/Japan). Under this project, about 150 chemical substances were selected among those designated as Class-I Chemicals in the Law for Pollutant Release and Transfer Register and Promotion of Chemical Management (hereafter PRTR Law)[1]. The selection criteria of these chemicals were their priorities for risk assessment based on their production levels and environmental/human health concerns.

CERI developed the hazard assessment reports of these selected chemical substances based on the review and evaluation of the environmental and human health hazard data obtained from the existing evaluation documents released by the regulatory agencies and international organizations as well as those from the published scientific literatures. The data review and compilation of the reports were conducted according to the guidelines[2] and the guidance manual[2] developed for this project. The proposed hazard assessment reports by CERI were reviewed by the experts in the relevant scientific fields from both inside and outside this project for accuracy, relevance and completeness. The final reports were published in Japanese after going through the deliberation by the “Council on Chemical Substances” under the Ministry of Economy, Trade and Industry (METI/Japan), which is responsible for regulation of chemical substances in Japan.

This project was the first attempt in Japan to develop comprehensive hazard assessments of chemical substances for application in risk assessment. In order to share the outcomes of the project globally, CERI independently selected the following seven chemical substances and developed the English version of the hazard assessment reports:

(1) Acetaldehyde
(2) Chlorobenzene
(3) Hydrazine
(4) *N, N*-Dimethylformamide
(5) Poly(oxyethylene) nonylphenyl ether
(6) 3,3’-Dichloro-4,4’-diaminodiphenylmethane
(7) Dimethyl 2,2-dichlorovinyl phosphate (Dichlorvos)

We hope that the hazard assessment reports from our project contribute to the risk assessment and management of chemical substances globally, and appreciate your feedback. .

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[1] Details of the PRTR Law, the list of designated chemical substances, and release data in Japan are available on Internet at: http://www.prtr.nite.go.jp/index-e.html.

[2] Guidelines and the guidance manual in Japanese are available on Internet at: http://www.safe.nite.go.jp/risk/riskhykdl01.html. Also, the initial risk assessment reports in Japanese developed in this project which include calculations of margin of exposure based on the result of hazard assessment and exposure assessment, are available on Internet at: http://www.safe.nite.go.jp/risk/riskhykdl01.html.

Date: May, 2007

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Summary

Poly(oxyethylene) nonylphenyl ether is composed of branched 4-nonylphenol and ethylene oxide. In this document, it is abbreviated to NPE, and the number of the addition moles, n, of ethylene oxide (EO) group in NPE is expressed into NPEn. Commercial products have the number of the addition moles of EO around 10. The appearance changes from a liquid to a solid, and the water solubility is increased with increasing the number of addition moles of EO. For an example, NPE with an average number of EO of 9.5, $NPE_{9.5}$, is a colorless liquid and soluble in water. NPE is used extensively as a surfactant in various industries. Specifically, NPE is used as a compounding ingredient for industrial detergents, dispersants, cutting fluids, emulsifiers and spreading agents. Most NPE is used for products for industrial use, whereas some are used for pharmaceutical and cosmetic products.

The production, import and export of NPE in Japan were 22,318, 224 and 8,088 tons, respectively, and the domestic supply was 14,454 tons in 2001. Considering from the information that NPE is added to products mainly as a surfactant and based on the 2001 PRTR Data, the main release route is considered through emissions in the use process of products containing NPE from business institutions and households, not in the NPE-manufacturing process. As the scenario of NPE releases in Japan, it has been estimated that 38 tons of NPE is released annually into air, 1,622 tons into water, and 406 tons into land.

NPE is considered to be transformed to mono(oxyethylene) nonylphenyl ether (NPE_1) by a sequential break in the EO chain in the aquatic environment including river water, and in anaerobic conditions including sediment, and by a further break in the EO chain to nonylphenol. NPE released to the aquatic environment dissolves and transits in water and is eliminated by biodegradation in aerobic conditions. NPE is, however, hardly eliminated by volatilization. With an increase in the moles of EO, NPE lipophilicity is decreased and water solubility is increased, which probably leads to reduced bioaccumulation of NPE. NPE_{10} is bioaccumulative but $NPE_{30\text{-}50}$ is hardly bioaccumulated.

In the environmental conditions, NPE with a shorter EO chain has stronger toxicity to aquatic organisms including aquatic bacteria, alga, crustacea and fish. The toxicity of NPE to algae was studied for the freshwater alga. The lowest EC_{50} of various NPEs is 12 mg/L as the 96-hr EC_{50} of NPE_9 for growth inhibition of the alga, and the lowest NOEC is 8 mg/L as the 96-hr NOEC of NPE_9. The acute toxicity of various NPEs to invertebrates is reported for the freshwater water fleas and the marine mysid. The 48-hr LC_{50} of NPE with EO 9 to 10 ranged from 1.23 to 14.0 mg/L and that of NPE with EO 1 to 7 ranged from 0.11 to 10 mg/L. The lowest value of these toxicity data is 0.11 mg/L as the 48-hr LC_{50} of $NPE_{1.5}$ in the mysid. The acute toxicity of NPE to

fish has been studied for the freshwater fish, fathead minnow, Japanese killifish, bluegill, goldfish, brown trout, golden orphe and rainbow trout, and the marine fish, Atlantic cod. The 48 to 96-hr LC_{50} of NPE with EO 10 and less ranged from 1.0 to 18 mg/L. The lowest LC_{50} of NPE is 1.0 mg/L as the 96-hr LC_{50} of $NPE_{9\text{-}10}$ for the brown trout. The 7-day NOEC of NPE_9 for growth of the fathead minnow was 1.0 mg/L. The lowest value of these toxicity data for aquatic organisms is 0.11 mg/L as the 48-hr LC_{50} of $NPE_{1.5}$ for the crustacean, mysid.

The effects of NPE on the endocrine and reproductive systems of aquatic organisms were studied by using *in vitro* and *in vivo* assays. In *in vitro* assays, NPE_2 and NPE_9 induced the expression of vitellogenin, a yolk protein precursor, in rainbow trout hepatocytes. In *in vivo* assays, NPE (EO chain length unknown) decreased the number of offspring and changed the excretion of testosterone metabolites in freshwater water flea. NPE_2 enhanced plasma vitellogenin concentrations and decreased the relative testis weight in male rainbow trout. These results suggest the possibility that NPE has effects on the endocrine and reproductive systems in crustacea and fish.

In experimental animals, NPE orally administered is absorbed through the gastrointestinal tract. After absorption, NPE is metabolized to nonylphenol and polyethylene glycol with a shortend EO chain and carboxylated terminal. The excretion ratio varies according to the EO chain length. However, most NPE administered is excreted in the feces and urine within 7 days regardless of EO chain length.

Studies in volunteers suggested that NPE produces primary skin irritation and skin sensitization in humans. However, it has been reported that NPE produces no photosensitization.

In acute toxicity studies with experimental animals, the oral LD_{50} values of NPE with EO 2 to 15 for rats ranged from 1,300 to 7,400 mg/kg and that of NPE with EO 20 exceeded 15,900 mg/kg. The LD_{50} of NPE with EO 9 was 4,290 mg/kg in mice and ranged from 620 to 4,400 mg/kg in rabbits. The lowest LD_{50} values of NPE are 620 and 1,800 mg/kg in rabbits with oral and dermal administration, respectively. No data of acute inhalation exposure have been obtained. Common acute symptoms in animals with oral and dermal administration are tremor, lethargy and liver congestion.

NPE-induced irritation varies according to EO chain length, ranging from no irritation to strong skin and eye irritation. Skin irritation is moderate to severe by EO 2 to 9, and none to slight by EO 10 and more. Eye irritation is moderate to severe by EO 2 to 15, and none to slight by EO 30 and more. NPE_6 produced no sensitization.

In repeated dose toxicity, oral administration of NPE to rats and dogs causes a decrease in food consumption, suppression of body weight gain and an increase in the relative liver weight although the toxicity varies according to the EO chain length. NPE_{20} only caused focal necrosis in

the myocardium in dogs but not in rats.

The toxicity varies according to the EO chain length, but main target organ of NPE is considered to be the liver. In the case of repeated dose oral toxicity studies for 2 years on NPE_9, the actual NOAEL is not determined, but it is considered that NOAEL is the highest dose of 0.27% (corresponding to 135 mg/kg /day) in rats, and highest dose of 0.27% (corresponding to 88 mg/kg /day) in dogs.

In reproductive and developmental toxicity, oral and intravaginal administration of NPE_9 have reproductive and developmental toxicity, whereas oral administration of NPE_{30} has not. Oral administration to pregnant rats induces a significant decrease in litter size, an increase in fetal extra rib and a significant increase in dilated pelvic cavity, and intravaginal administration induces a decrease in the number of normal implantation sites and an increase in the number of resorption sites. The NOAEL of oral NPE for reproductive and developmental toxicity is 50 mg/kg/day as the NOAEL of NPE_9.

In genotoxicity, except for the positive result of a DNA strand break assay of NPE_4, four NPEs of NPE_4, NPE_9, $NPE_{9.5}$ and NPE_{12} showed negative results in all assays of *in vitro* reverse and forward mutation, unscheduled DNA synthesis and cell transformation assays, and *in vivo* micronucleus and dominant lethal tests. Although genotoxicity studies have been conducted only with NPE_4, NPE_9, $NPE_{9.5}$ and NPE_{12},, it can be considered that NPE is not genotoxic.

In carcinogenicity studies, NPE_4 and NPE_9 did not induce carcinogenic responses in rats and dogs. Based on the available data with only two NPEs, however, it is not possible to evaluate carcinogenic potential of NPE in general. It has been reported that NPE (EO chain length unknown) promoted gastrointestinal tumorigenesis induced by a known carcinogen. No evaluation of carcinogenicity with NPE has been conduced by international and national organizations.

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1. Identification of the substance

Poly(oxyethylene) nonylphenyl ether (NPE) can be synthesized from nonylphenol and ethylene oxide. Nonylphenol is produced by reaction of phenol and propylene trimer. 4-Nonylphenol is mainly produced. Nonylphenols may vary in two ways: the substitution position of nonyl group on the phenol molecule; and the degree of branching of the nonyl group. Nonylphenol can have 170 isomeric compounds in speculation. Poly(oxyethylene) nonylphenyl ether is generally synthesized from isomeric mixture consisted mainly of 4-nonylphenol. In this document, poly(oxyethylene) nonylphenyl ether is used as isomeric mixture of branched nonylphenol unless otherwise noted. However, the number of the addition moles of ethylene oxide of poly(oxyethylene) nonylphenyl ether is not prescribed in Law for PRTR and Promotion of Chemical Management.

In this document, NPEn is used with "NPE" as the abbreviation of poly(oxyethylene) nonylphenyl ether and "n" indicating the number of the addition moles of ethylene oxide (EO) group.

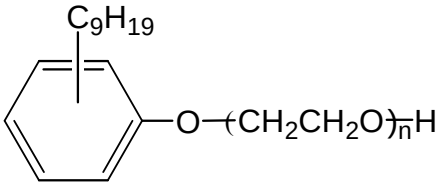
1.1 Chemical name : Poly(oxyethylene) nonylphenyl ether

1.2 Class reference number in Chemical Substance Control Law[1)] : 7-172

1.3 PRTR[2)] **Number (Law for PRTR and Promotion of Chemical Management)** : 1-309

1.4 CAS registry number : 9016-45-9

1.5 Structural formula



Note: In commercial products, the number of the addition moles of ethylene oxide is around 10.
(CERI/Japan, 2003a)

1.6 Molecular formula : $C_{15+2n}H_{24+4n}O_{1+n}$
$C_{35}H_{64}O_{11}$ (NPE_{10})

1.7 Molecular weight : 661.2 (NPE_{10})

2. General information

2.1 Synonyms

Nonylphenol ethoxylate, NPE, Polyethylene glycol monononylphenyl ether

2.2 Purity

>99% (Commercial products) (CERI/Japan, 2003a)

[1)] The Low Concerning the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc., Japan. Provisional translation is available on Internet at: http://www.safe.nite.go.jp/english/kasinn/kaiseikasinhou.html

[2)] Pollutant Release and Transfer Register

2.3 Impurities

Polyethylene glycol (Commercial products) (CERI/Japan, 2003a)

2.4 Additives/Stabilizers

No additives/Stabilizers (Commercial products) (CERI/Japan, 2003a)

2.5 Current regulations in Japan[1)]

Law for PRTR and Promotion of Chemical Management	Class I-designated chemical substance
Waterworks Law	Water quality standard 0.02 mg/L[2)]
Law Relating to the Prevention of Marine Pollution and Maritime Disasters	Noxious liquid substance category B[3)]
Agricultural Chemicals Regulation Law	Registered pesticide (Spreader agent)
Law of Maintenance of Sanitation in Buildings	Water quality standard 0.02 mg/L[2)]

3. Physico-chemical properties

Appearance:	Colorless liquid ($NPE_{9.5}$)	(CERI/Japan, 2003a)
	The appearance changes to a solid from liquid with an increase in the number of the addition moles of ethylene oxide	(CERI/Japan, 2003a)
Melting point:	-20°C ($NPE_{9.5}$, freezing point)	(CERI/Japan, 2003a)
Boiling point:	No data	
Flash point:	282°C ($NPE_{9.5}$)	(CERI/Japan, 2003a)
Ignition point :	No data	
Explosion limit :	No data	
Specific gravity:	1.06 ($NPE_{9.5}$, 20°C)	(CERI/Japan, 2003a)
Vapor density:	16.7 (NPE_6, air = 1)	
Vapor pressure:	3.2×10^{-8} Pa (NPE_6, 25°C, estimated)	(U.S. NLM: HSDB, 2003)
Partition coefficient:	No data	

[1)] As this document covers basic information on Japanese regulations (unofficial translations), you should confirm the details using it.

[2)] By the absorbance measurement of 510 nm, the concentration of nonionic surfactant in water is calculated for the concentration of heptaoxyethylene dodecyl ether.

[3)] NPEs with the addition moles of ethylene oxide of more than 4, and their mixtures.

Dissociation constant :	No functional groups capable of dissociation.	
Mass spectrum:	Main mass fragments : No data	
Soil adsorption coefficient:	Koc = 6.1 (NPE_6, measured)	(U.S. NLM: HSDB, 2003)
Solubility:	water: soluble ($NPE_{9.5}$) Water solubility is increased with an increase in the number of addition moles of EO. NPEs with longer chains (n≥7) are soluble in water.	(CERI/Japan, 2003a) (Talmage, 1994)
Henry's constant:	4.2×10^{-7} Pa·m^3/mol (4.1×10^{-12} atm·m^3/mol) (NPE_6, estimated)	(U.S. NLM: HSDB, 2003)
Conversion factor:	(air, 20°C) 1 ppm = 20.2 mg/m^3, 1 mg/m^3 = 0.050 ppm (NPE_6)	
Others:	Critical micelle concentration (CMC): 5.4 mM (NPE_9, equilibration method)	(Heinze et al., 1999)

4. Sources of release to the environment

4.1 Production, import, export and domestic supply

The total amount of production, import, export and domestic supply of poly(oxyethylene) nonylphenyl ether (NPE) in Fiscal Year 2001 ranged from 10,000 to 100,000 tons in Japan (METI/Japan, 2003).

In another investigation, NPE production was 22,318 tons, import and export were 224 and 8,088 tons, respectively, and domestic supply was 14,454 tons in Japan (NITE/Japan, 2004).

4.2 Uses

NPE is used extensively as a surfactant in various industries. Specifically, NPE is used as a compounding ingredient for industrial detergents, dispersants, cutting fluids, emulsifiers and spreading agents. NPE is mostly used for industrial products, and partly used for pharmaceutical and cosmetic products (NITE/Japan, 2004).

4.3 Releases

4.3.1 Releases under PRTR system

According to “Total Release and Transfers for Fiscal Year 2001 (hereafter 2001 PRTR Data)” under the PRTR system (METI/Japan and MOE/Japan, 2003a), 11 tons of NPE was released into air, 295 tons into public water, 283 tons into sewers, and 606 tons was transferred as wastes in Fiscal Year 2001. There were no reports on release of NPE into land.

In addition, it was estimated that 729 tons of NPE was released from the business institutions in the

industries that were designated under the PRTR system but exempted from notification, 947 tons from the industries outside the scope of the PRTR system, and 84 tons from households. No estimation was made for the amount of releases from mobile sources.

a. Release and transfer from the industries within the scope of PRTR system

Table 4-1 shows the amounts of releases into the environmental media (air, water and land) and transfer by the designated industries summarized from the 2001 PRTR Data of NPE. METI/Japan and MOE/Japan (2003a) do not provide the amounts of release by environmental media for the estimations of releases from the business institutions exempted from notification. The ratio for each environmental medium of the releases estimated for the business institutions exempted from notification was calculated based on the assumption that the ratios of releases by environmental media were the same as those obtained by notification (NITE/Japan, 2004).

Based on this estimation, respective releases from textile, laundry and pulp and paper industries were approximately 20% of the total release amount.

Table 4-1 Releases and transfer of poly(oxyethylene) nonylphenyl ether to environmental media by industries (tons/year)

Industries	By Notification					Notification Exempted			Total amount of releases by notification and by estimation	
	Release			Transfer		Release (estimated)[1)]				
	Air	Water	Land	Sewer	Wastes	Air	Water	Land	Total release	Ratio (%)
Textile products	＜0.5	62	0	235	38	7	189	＜0.5	258	25
Laundries	0	3	0	27	92	9	220	＜0.5	232	22
Pulp, paper and paper products	1	202	0	＜0.5	2	＜0.5	4	＜0.5	207	20
Plastic products	＜0.5	＜0.5	0	0	14	3	80	＜0.5	83	8
Apparel and other textile goods	0	＜0.5	0	1	＜0.5	2	51	＜0.5	54	5
Petroleum and coal products	0	＜0.5	0	0	12	1	19	＜0.5	20	2
Electrical machinery, equipment and supplies	＜0.5	12	0	0	10	＜0.5	3	＜0.5	15	1
Fabricated metal products	0	＜0.5	0	0	37	1	14	＜0.5	15	1
Transportation equipment	＜0.5	1	0	＜0.5	7	＜0.5	12	＜0.5	13	1
Others [2)]	10	15	＜0.5	20	394	4	110	＜0.5	140	15
Total	11	295	0	283	606	27	702	＜0.5	1,037	100

（NITE/Japan, 2004）

1) Based on the assumption that ratios of releases into the air, water and land were the same as those of the releases

obtained by notification, the amounts of releases from the business institutions exempted from notification were estimated.
2) "Others" indicates the industries other than those described above.
The release and transfer of less than 0.5 tons was mentioned as "<0.5" without exception.

Based on the production level and the emission factor at manufacturing sites of NPE in 2001 (Japan Chemical Industry Association, 2002), the release of NPE is not considered to occur during the manufacturing process (NITE/Japan, 2004). Therefore, based on the 2001 PRTR Data, the releases of NPE from the industries within the scope of the PRTR system are considered to occur not during the manufacturing process but in use as a compounding ingredient or during the use of products containing NPE.

b. Releases from the non-designated industries, households and mobile sources

Based on the 2001 PRTR Data, the amounts of release from the non-designateded industries and households are summarized in Table 4-2. METI/Japan and MOE/Japan (2003a) do not provide the amounts of release by environmental media for the estimations of releases. The ratio for each environmental medium of the releases is estimated based on the purpose of use.

Consequently, from the industries outside the scope of the PRTR system, 552 tons of NPE was released into water in use for industrial detergents/cleaners and 395 tons into land in use for agricultural chemicals. From households, 73 tons was released into water in use for cosmetic products and 11 tons into land in use for agricultural chemicals (NITE/Japan, 2004). NPE is not a subject for estimation of releases from mobile sources (METI/Japan and MOE/Japan, 2003b).

Table 4-2 Releases of poly(oxyethylene) nonylphenyl ether from the non-designated industries and households into environmental media (tons/year)

	Air	Water	Land
Non-designated industries[1)]	0	552	395
Households[1)]	0	73	11
Total	0	625	406

（NITE/Japan, 2004）
1) The distribution to air, water and land was considered from the use and the physico-chemical property.

4.3.2 Releases from other PRTR

Other information on NPE release sources than estimations based on the 2001 PRTR Data was not obtained in this investigation.

4.4 Estimated routes of releases

Considering from the information that NPE is mainly used as a surfactant in various products and the 2001 PRTR Data, the main release route is through emissions in the use process of products containing

NPE from business institutions and households, not during the NPE-manufacturing process.

As the scenario of NPE releases in Japan, it is estimated that 38 tons of NPE is released annually into the air, 1,622 tons into the water, and 406 tons into the land. Releases into the environment after processing of wastes at waste disposal facilities are not considered for estimation of the amount transferred into sewers and that transferred as wastes.

5. Environmental fate

5.1 Stability in the atmosphere

No reports on the stability of poly(oxyethylene) nonylphenyl ether (NPE) in air were obtained in this investigation.

5.2 Stability in water

5.2.1 Abiotic degradation

No reports on the abiotic degradation of NPE were obtained in this investigation.

5.2.2 Biodegradation

NPE_{30-50} is ranked as a persistent substance based on the result of the aerobic biodegradation study required under the Chemical Substance Control Law. The study result indicated that the degradation rate of NPE was 1% in biological oxygen demand (BOD) determination under the conditions of 30 mg/L of test substance concentration, 30 mg/L of activated sludge concentration and 3 weeks of test period. The degradation rates were 2%, 2% and 0% in the total organic carbon (TOC) determination, by measurement with high-performance liquid chromatography (HPLC) and gas chromatography (GC), respectively (MITI/Japan, 1982).

In a biodegradation study of NPE (the moles of ethylene oxide (EO) were unknown), the degradation rate ranged from 14% to 65% by measurement of carbon dioxide production (Hughes et al., 1989; Kravetze et al., 1991). In another biodegradation study of NPE with 9 moles of EO (NPE_9) and with 1.5 moles of EO ($NPE_{1.5}$) and nonylphenol, the degradation rates by measurement of carbon dioxide production were 70%, 59% and 48%, respectively (Staples et al., 2001). These results suggest that not only EO chains in NPE but also nonylphenol are biodegraded.

In biodegradation studies of intermediates in NPE biodegradation, nonylphenoxyethoxy acetic acid ($NPEC_2$) and nonylphenoxy acetic acid ($NPEC_1$), the degradation rates by measurement of carbon oxide production were 65% and 59-66%, respectively (Ahel et al., 1994; Staples et al., 1999). A metabolite analysis of biodegradation has shown that the alkyl group is biodegraded (Jonkers et al., 2001).

These results suggest that NPE biodegradation varies according to the test conditions (concentrations of test materials and microorganisms or microbiota etc.) and that some parts of EO chains, alkyl groups and benzene rings are degraded in appropriate conditions. However, many studies on biodegradation of NPE have shown that EO chains are most easily degraded and that benzene rings are degraded less than EO chains.

In addition to the formation of nonylphenol polyethoxy acetic acid by chain shortening associated with oxidation and breaking of EO chains, it has been reported that poly(oxyethylene) glycol and mono- and dicarboxylated poly(oxyethylene) are produced by oxidation and breaking of the ether bond (Franska et al., 2003). In aerobic conditions, NPE changes into mono(oxyethylene) nonylphenyl ether (NPE_1) by the sequential breaking of EO chains, and in anaerobic conditions, into nonylphenol by the further breaking of EO chains (Kojima and Watanabe, 1998).

NPE is used as a spreading agent for agricultural chemicals and is assumed to be transferred into agricultural lands. It has been reported that NPE was biodegraded in soil and not detected within 30 days after application (Mihaich et al., 2001).

5.2.3 Removal in sewage treatment

NPE is eliminated by the activated sludge sewage system. According to the Report of the Ministry of Land, Infrastructure and Transport (MLIT) in 1998, 1999 and 2000, 99% of NPE and above was eliminated in urban sewage plants. This report estimated the elimination rates of related compounds including NPE homologs with a short EO chain. The median elimination rates of all compounds were around 97% (MLIT, 2001).

The elimination rates estimated by foreign and domestic organizations (Naylor, 1995; Tokyo Metropolitan Government, 1999) are similar to the above rate, and the rate estimated by a monitoring research project (CERI/Japan, 2003b) is not very different.

The results of research by MLIT (Table 5-1) indicated that the ratio of NPE_{1-4} (moles of the EO chain: 1 to 4) to $NPE_{\geq 5}$ (moles of the EO chain: 5 or more) was higher in treated sewage than inflowing sewage (MLIT, 2001), suggesting that sewage treatment shortens the EO chain.

Table 5-1 Density (median elimination rates) comparison of poly(oxyethylene) nonylphenyl ether between inflow sewage and treated sewage in a sewage facility

NPE	Inflowing sewage (μg/L)		Treated sewage (μg/L)	
	1998	1999	1998	1999
NPE_{1-4}	25	29	1.3	0.7
$NPE_{\geq 5}$	150	81	1.6	tr (0.3)

tr: below the limit of quantitation and above the limit of detection
(MLIT, 2001)

The concentrations of nonylphenol and NPE_{1-19} in the wastewater of a paper factory for bleached kraft and the primary and secondary treated water of a sewage facility in Canada were determined. It has been reported that sewage treatment increased NPE with short EO chains including NPE_1 and NPE_2 (Afonso et al., 2002).

Furthermore, it has been reported that NPE, which was absorbed and remained in the sludge, was degraded in the composting process of sludge when surplus sludge in a sewage facility was composted and reused (Moeller and Reeh, 2003).

5.3 Behavior in the aquatic environment

It was reported in a biodegradation study using river water that NPE produced long-chain ethoxycarboxylic acid, which is NPE with the EO terminal oxidized, and that the EO chain was shortened further (Jonkers et al., 2001). NPE is considered to be transformed into mono(oxyethylene) nonylphenyl ether (NPE_1) by a sequential break in the EO chain in the aquatic environment including river water, and in anaerobic conditions including sediment, and by a further break in the EO chain, into nonylphenol (see 5.2.2). Considering a soil adsorption coefficient, Koc, of 6.1 (NPE_6, see Chapter 3), it is assumed that NPE is hardly adsorbed into suspended solids in water and sediment. NPE is water soluble ($NPE_{9.5}$) and its vapor pressure is extremely low (3.2×10^{-8} Pa: NPE_6 at 25°C), and Henry's constant is also extremely low (4.2×10^{-7} Pa・m^3/mol: NPE_6) (see Chapter 3). Therefore, NPE is assumed to be hardly released from the aquatic environment into the air.

Based on the information summarized here and in Section 5.2, it is assumed that NPE released into the aquatic environment dissolves and transits in water and is eliminated by biodegradation in specific aerobic conditions such as acclimation. It is assumed that NPE is hardly eliminated by volatilization.

5.4 Bioaccumulation

NPE_{30-50} is ranked as a “non- or low bioaccumulative substance” based on the result of a 6-week bioaccumulation study in the common carp required under the Chemical Substances Control Law, Japan. The study result indicated that the bioaccumulation factor (BCF) of NPE was less than 0.2 and less than 1.4 at 0.2 and 2 mg/L of NPE concentration in water, respectively (MITI/Japan, 1982).

Cod (*Gadus morhua*) were exposed to 5 mg/L of NPE_{10} (EO chain labeled with ^{14}C) and bioaccumulation and excretion were examined. The state of equilibrium was reached in 8 hours and the concentration was approximately 100 mg/L in the gills and blood, 500 mg/L in the liver and kidney and 4,000 mg/L in the gall bladder. In that study, except for the gall bladder, 60% of the radioactivity administered was excreted within 24 hours (Granmo and Kollberg, 1976). The apparent BCF was approximately 20 in the gills and blood, 100 in the liver and kidney, and 800 in the gall bladder.

It was reported that nonylphenol, NPE_1 and NPE_2 are more lipophilic than NPE with large moles of ethylene oxide and easily concentrated in aquatic organisms (Kvestak and Ahel, 1994). The BCFs of NPE_1 and NPE_2 are unknown, but the BCF of nonylphenol was estimated to be 540 from log Kow of 5.76 for nonylphenol (SRC: BcfWin, 2003).

As the moles of ethylene oxide increase, NPE lipophilicity is decreased and water solubility is increased (Talmage, 1994, see Chapter 3). Therefore, bioaccumulation is considered to be reduced. From the study results that substances with molecular weight of more than 1,000 are difficult to bioaccumulate (Wakabayashi, 2003), it is considered that NPE_{10} (molecular weight: 661) is bioaccumulative but that NPE_{30-50} (molecular weight: 1,542 to 2,423) is hardly bioaccumulated.

6. Effects on organisms in the environment

6.1 Effects on aquatic organisms

6.1.1 Microorganisms

Toxicity data of poly(oxyethylene) nonylphenyl ether (NPE) for microorganisms are shown in Table 6-1.

In a growth inhibition study of NPE for *Pseudomonas* sp., NPE inhibited bacterial growth, and the toxicity threshold increased from 50 to 1,000 mg/L as the average moles of ethylene oxide (EO) (hereafter EO chain length) increased from 4 to 30, which suggests that toxicity was weakened (Janicke et al., 1969). The 16-hr EC_{50} of NPE_{12} (abbreviation of NPE with EO chain length of 12 units) for growth inhibition of *Pseudomonas fluorescens* exceeded 2,000 mg/L (Torslov, 1993).

The 5-min EC_{50} of NPE_9 (EO chain length: 9 units) for luminescence inhibition in marine luminescent bacteria (*Photobacterium phosphoreum*) was 60.6 mg/L (Dorn et al., 1993).

Table 6-1 Toxicity of poly(oxyethylene) nonylphenyl ether for microorganisms

Species	NPE composition	Tem-perature (°C)	Endpoint		Concent-ration (mg/L)	Refrence
Bacteria *Pseudomonas* sp. (*Pseudomonas*)	NPE_4 NPE_6 NPE_7 NPE_{10} NPE_{20} NPE_{30}	ND	Toxicity threshold	Growth inhibition	50 500 63-500 1,000 1,000 1,000	Janicke et al., 1969
Pseudomonas fluorescens (*Pseudomonas*)	NPE_{12}	20	16-hr EC_{50}	Growth inhibition	>2,000	Torslov, 1993
Photobacterium phosphoreum (marine luminescent bacterium)	NPE_9	ND	5-min EC_{50}	Lumines-cence inhibition	60.6 (n)	Dorn et al., 1993

ND: No data available
(n): Nominal concentration

6.1.2 Algae

Toxicity data of poly(oxyethylene) nonylphenyl ether for algae are shown in Table 6-2.

The toxicity of NPE for the freshwater alga *Selenastrum capricornutum* was studied. The 48-hr EC_{50} of NPE_8 for growth inhibition was 20 mg/L and that of NPE_9 was 50 mg/L (Yamane et al., 1984). The 96-hr EC_{50} of NPE_9 for growth inhibition was 12 mg/L and NOEC was 8 mg/L (Dorn et al., 1993). NPE_{30} enhanced growth in the range of 100 to 500 mg/L (Nyberg, 1988).

These results suggest that NPE with a longer EO chain is less hazardous to growth of the freshwater alga *Selenastrum capricornutum*. Of the toxicity values of NPE in algae, the lowest EC_{50} and NOEC values were 12 mg/L (96-hr EC_{50} of NPE_9) and 8 mg/L (96-hr NOEC of NPE_9) for *Selenastrum capricornutum*, respectively.

Table 6-2 Toxicity of poly(oxyethylene) nonylphenyl ether for algae

Species	NPE composition	Method/ Condition	Temperature (°C)	Endpoint		Concentration (mg/L)	Reference
Freshwater species							
Selenastrum capricornutum [1] (green alga)	NPE_8 NPE_9	Static, closed	24±2	48-hr EC_{50}	Growth inhibition Growth rate	20 50	Yamane et al., 1984
	NPE_6 NPE_9 NPE_{30}	Static (synthetic medium)	25	3-week LOEC 3-week EC_{50} 3-week EC_{100}	Growth inhibition Growth inhibition Growth stimulation Number of cells	100 100-500 100-500	Nyberg, 1988
	NPE_9	U.S. EPA, TG Static	25	96-hr EC_{50} 96-hr LOEC 96-hr NOEC	Growth inhibition Number of cells	12 16 8 (m)	Dorn et al., 1993

(m): Measured concentration
Closed system: a test container and water bath is closed with a cover such as a lid, and a headspace is kept.
1) Current scientific name: *Pseudokirchneriella subcapitata*

6.1.3 Invertebrates

Toxicity data of poly(oxyethylene) nonylphenyl ether for invertebrates are shown in Table 6-3.

The acute toxicity of NPE for freshwater crustacea, water fleas (*Daphnia magna*, *Daphnia pulex* and one of *Ceriodaphnia dubia*), has been reported. The 48-hr LC_{50} values of NPE with EO 9 and less ranged from 0.148 to 14.0 mg/L (Ankley et al., 1990; Dorn et al., 1993; Janicke et al., 1969; Maki et al., 1998; Moore et al., 1987; Salanitro et al., 1988). The 24-hr LC_{50} of NPE_{11} for insect, *Aedes aegypti*, was 500 mg/L (Van Emden et al., 1974).

The acute toxicity for marine crustacean, mysid, ranged from 0.11 to 2.2 mg/L as the 48-hr LC_{50} of NPE with EO 9 and less (Hall et al., 1989; Patoczka and Pulliam, 1990). The 48-hr LC_{50} of NPE_{10} for crustacean, acartia, was 2.8 mg/L and the 5-day EC_{50} for developmental inhibition from egg to nauplius larva was 0.15 mg/L (Andersen et al., 2001). The 96-hr LC_{50} of NPE_{10} for shellfish *Cardium edule* was 5 mg/L (Swedmark et al., 1971).

The long-term toxicity of NPE to growth of *Daphnia magna* was 10 mg/L as the 7-day NOEC of NPE_9 (Dorn et al., 1993).

The relationship between the EO chain length of NPE and toxicity level was investigated for one of the freshwater water flea (*Daphnia* sp.) and the marine crustacean, mysid. For *Daphnia* sp., the toxicity threshold was increased from 5 to 10,000 mg/L with the increase in EO chain length from 4 to 30 (Janicke et al., 1969). For the mysid, when the EO chain length was 1.5, 9, 15 and 40, the 48-hr LC_{50} was 0.11, 0.7-2.2, 2.57 and more than 100 mg/L (Hall et al., 1989). These results suggest that the increase in the EO chain length of NPE decreases acute toxicity of NPE.

Based on the above data, the lowest LC_{50} of NPE for invertebrates is 0.11 mg/L as the 48-hr LC_{50} of

$NPE_{1.5}$ for the crustacean, mysid.

Table 6-3 Toxicity of poly(oxyethylene) nonylphenyl ether for invertebrates

Species	NPE composition	Growth Stage	Method/ Condition	Temperature (°C)	Hardness (mg $CaCO_3$ /L)	pH	Endpoint	Concentration (mg/L)	Refrence
Acute toxicity: freshwater species									
Daphnia magna (crustacea, water flea)	NPE_2	<12 hours	ISO6341-1982	20	ND	ND	48-hr LC_{50}	0.148	Maki et al., 1998
	NPE_9	<12 hours	Semi-static	25	150	ND	48-hr LC_{50}	14.0 (m)	Dorn et al., 1993
	NPE EO chain length unknown	<24 hours	Semi-static	22 ±1	ND	8.0	48-hr LC_{50}	8.6	Baldwin et al., 1998
Daphnia pulex (crustacea, water flea)	NPE_{10}	ND	U.S. EPA TG	20 -21	25-40	7-8	48-hr LC_{50}	12.5	Moore et al., 1987
	NPE_9	ND	ND	ND	ND	ND	48-hr EC_{50} Immobilization	2.87	Salanitro et al., 1988
Daphnia sp. (crustacea, one species of water flea)	NPE_4 NPE_6 NPE_7 NPE_{10} NPE_{20} NPE_{30}	ND	ND	ND	ND	ND	Toxicity threshold	5 5 10 10 1,000 10,000	Janicke et al., 1969
Ceriodaphnia dubia (crustacea, water flea)	NPE_{1-2}	ND	U.S. EPA TG	25	ND	ND	48-hr LC_{50}	1.04	Ankley et al., 1990
Aedes aegypti (insect, mosquito)	NPE_{11}	ND	ND	25	ND	ND	24-hr LC_{50}	500	Van Emden et al., 1974
Acute toxicity: marine species									
Americamysis bahia (crustacea, mysid)	NPE_9	ND	Semi-static	ND	ND	ND	48-hr LC_{50}	1.23	Patoczka & Pulliam, 1990
	n-$NPE_{1.5}$ [1)] n-NPE_9 n-NPE_{50} $NPE_{1.5}$ NPE_9 NPE_{15} NPE_{40}	3 to 8 days	U.S. EPA TG Semi-static	25 ±1	Salinity: 24-29‰	7.7 - 8.0	48-hr LC_{50}	1.66 1.59 4,148 0.11 0.7-2.2 2.57 >100	Hall et al., 1989
Acartia tonsa (crustacea, acartia, copepod)	NPE_{10}	10 to 12 days	Static, closed	20	Salinity: 18‰	ND	48-hr LC_{50}	2.8 (n)	Andersen et al., 2001
		Egg	Semi-static	20	Salinity: 18‰	ND	5-day EC_{50} Larva (nauplius) growth inhibition	0.15 (n)	
Leander adspersus (crustacea, prawn)	NPE_{10}	ND	Flow-through	6-8 or 15 -17	Salinity: 32-34‰	ND	96-hr LC_{50} (6-8°C) (15-17 °C)	>100 10-50	Swedmark et al., 1971
Mytilus edulis (shell, common blue mussel)	NPE_{10}	ND	Flow-through	6-8	Salinity: 32-34‰	ND	96-hr LC_{50}	12	Swedmark et al., 1971

Species	NPE composition	Growth Stage	Method/ Condition	Temperature (°C)	Hardness (mg $CaCO_3$ /L)	pH	Endpoint	Concentration (mg/L)	Refrence
Cardium edule (shell, cockle)	NPE_{10}	ND	Flow-through	6-8	Salinity: 32-34‰	ND	96-hr LC_{50}	5	Swedmark et al., 1971
Long-term toxicity: freshwater species									
Daphnia magna (crustacea, water flea)	NPE_9	<24 hours	Semi-static	25	150	ND	7-day NOEC Growth	10 (m)	Dorn et al., 1993

ND: No data available, (n): Nominal concentration, (m): Measured concentration
Closed system: A test container and water bath is closed with a cover such as a lid, and a headspace is kept.
1) n-: normal; normal chain

6.1.4 Fish

Toxicity data of poly(oxyethylene) nonylphenyl ether for fish are shown in Table 6-4.

The acute toxicity of NPE to freshwater fish including fathead minnow, Japanese killifish, bluegill, goldfish, brown trout, golden orphe and rainbow trout has been studied. The 48- to 96-hr LC_{50} values of NPE with EO chain length of 10 and less ranged from 1.0 to 18 mg/L (Calamari and Marchetti, 1973; Dorn et al., 1993; Macek and Krzeminski, 1975; Marchetti, 1965; Reiff et al., 1979; Salanitro et al., 1988; Yoshimura, 1986; Kurata et al., 1977; Toyama, 1974). The 96-hr LC_{50} of NPE_{10} for the Atlantic cod was 6.0 mg/L at a water temperature of 6 to 8°C and 2.5 mg/L at 15 to 17°C. The acute toxicity of NPE_{10} to the cod as cold-water fish had a tendency to be stronger at high water temperatures (Swedmark et al., 1971). The 7-day NOEC of NPE_9 for growth of the fathead minnow was 1.0 mg/L (Dorn et al., 1993).

In studies to investigate the effects of EO chain length on NPE toxicity in the Japanese killifish and bluegill, the 48- to 96-hr LC_{50} of NPE with EO chain length of 10 and less ranged from 1.3 to 18 mg/L, and that of NPE with EO chain length of 10 to 30 ranged from 48 to 1,000 mg/L and more (Macek and Krzeminski, 1975; Yoshimura, 1986). These results suggest that the increase in the EO chain length of NPE decreases acute toxicity of NPE.

Based on the above data, the lowest LC_{50} of NPE for fish is 1.0 mg/L as the 96-hr LC_{50} of $NPE_{9\text{-}10}$ for the brown trout, and the lowest NOEC for growth inhibition was 1.0 mg/L as the 7-day NOEC of NPE_9 for the fathead minnow.

Table 6-4 Toxicity of poly(oxyethylene) nonylphenyl ether for fish

Species	NPE composition	Growth stage	Method/ Condition	Temperature (°C)	Hardness (mg $CaCO_3$ /L)	pH	Endpoint	Concentration (mg /L)	Refrence.
Acute toxicity: freshwater species									
Pimephales promelas	NPE_9	ND	ND	ND	ND	ND	96-hr LC_{50}	1.6	Salanitro et al., 1988

Species	NPE composition	Growth stage	Method/ Condition	Temperature (°C)	Hardness (mg $CaCO_3$ /L)	pH	Endpoint	Concentration (mg /L)	Refrence.
(fathead minnow)	NPE_9	7 to 27 days	U.S. EPA, 600/4 -85-014 Semi-static	25	150	ND	96-hr LC_{50}	4.6 (m)	Dorn et al., 1993
	NPE_9	Fry 1 days	U.S. EPA, 600/4 -85-014 Semi-static	25	150	ND	7-day LC_{50} 7-day NOEC Death Growth	2.9 1.8 1.0 (m)	
Oryzias latipes (Japanese killifish)	NPE_9	Mean body weight 0.2 g	JIS K0102-1971	25	ND	ND	24-hr LC_{50} 48-hr LC_{50}	18 18	Toyama, 1974
	NPE_1 $NPE_{3.3}$ NPE_5 $NPE_{6.4}$ $NPE_{8.4}$ $NPE_{8.9}$ NPE_{13} $NPE_{16.6}$	Mean body size 2.0 cm Mean body weight 0.2 g	JIS K0102 ND	ND	50	7.2	48-hr LC_{50}	3.0 2.5 3.6 5.4 11.6 11.2 48 110	Yoshimura, 1986
Lepomis macrochirus (bluegill)	NPE_4 NPE_5 NPE_9 NPE_{30}	Mean body weight 1.0 g 1 month breeding	Static	18 ±0.5	35	7.1	96-hr LC_{50}	1.3 2.4-2.8 7.6-7.9 >1,000 (m)	Macek & Krzeminsk, 1975
	NPE_9		Flow-through	21 ±1	38	7.1	8-day LC_{50}	6.3 (m)	
Carassius auratus (goldfish)	NPE_{9-10}	Body size 5.0-6.0 cm	Static	20	100	ND	6-hr LC_{50}	6.9	Reiff et al., 1979
	NPE_{10}	Body size 5.0 ± 0.5 cm	JIS K0102 Static	ND	ND	ND	48-hr LC_{50}	5.4	Kurata et al., 1977
Rasbora heteromorpha (harequin fish, cyprinidae)	NPE_{9-10}	Body size 1.3-3.0 cm	Semi-static	20	20	ND	96-hr LC_{50}	8.6	Reiff et al., 1979
Salmo trutta (brown trout)	NPE_{9-10}	2.8, 5.8 cm	Semi-static	15	26-30	ND	96-hr LC_{50}	1.0	Reiff et al., 1979
Leuciscus idus (golden orphe, cyprinidae)	NPE_{9-10}	Body size 5.0-7.0 cm	Static Flow-through	20	268	ND	96-hr LC_{50}	11.2 7.0	Reiff et al., 1979
Oncorhynchus mykiss (rainbow trout)	NPE_8	Body size 12-16 cm	Flow-through	15.0 -15.6	290-310	7.3 -7.4	96-hr LC_{50} 14-day LC_{50}	4.7 4.25	Calamari & Marchetti, 1973
	NPE_{10}	<u>Early larval fish</u> Immediately after hatch 6 days 12 days <u>Late larval fish</u> 23 days 40 days <u>Fry</u> 210 days	Static	10-14	240-260	7.3 -7.4	6-hr LC_{50}	 42 22 5.2 2.1 5.2 5.2	Marchetti, 1965

Species	NPE composi-tion	Growth stage	Method/ Condi-tion	Tem-peratur e (°C)	Hardness (mg $CaCO_3$ /L)	pH	Endpoint	Conc-ent-ration (mg /L)	Refrence.
Acute toxicity: marine species									
Gadus morhua (Atlantic trout)	NPE_{10}	30 cm	Flow-through	6-8 15-17	Salinity: 32-34‰	ND	96-hr LC_{50}	6.0 2.5	Swedmark et al., 1971

ND: No data available; (m): Measured concentration

6.1.5 Other aquatic organisms

Toxicity data of poly(oxyethylene) nonylphenyl ether for other aquatic organisms are shown in Table 6-5.

The acute toxicity of NPE_8 to amphibians has been studied in the larvae (tadpoles) of African clawed frog, *Xenopus laevis* and cane toad, *Bufo marinus*. Acute toxicity was studied with two endpoints of mild narcosis (when stopping swimming, the tadpole resumes swimming within one second after contact stimulus to the tail) and full narcosis (even after receiving contact stimulus, the tadpole remains at rest). The 48-hr EC_{50} of NPE_8 for mild and full narcosis was 1.1 and 2.8 mg/L for *X. laevis*, and 2.8 and 5.1 mg/L for *B. Marinus*, respectively. These results indicated that African clawed frogs were more sensitive to NPE_8 toxicity than cane toads (Mann and Bidwell, 2001).

Table 6-5 Toxicity of poly(oxyethylene) nonylphenyl ether for other aquatic organisms

Species	NPE composi-tion	Growth/ stage	Method/ Condi-tion	Tem-pera-ture (°C)	Hardness (mg $CaCO_3$ /L)	pH	Endpoint	Conc-entra-tion (mg/L)	Refrence
Amphibian *Xenopus laevis* (african clawed frog)	NPE_8	Larva (tadpole)	ASTM TG (1993) Semi-static	18.9 -21.4	40-48	7.0 -7.9	48-hr EC_{50} Mild Full narcosis	 1.1 2.8	Mann & Bidwell, 2001
Bufo marinus (cane toad)		Larva (tadpole)					48-hr EC_{50} Mild Full narcosis	 2.8 5.1	

6.2 Effects on terrestrial organisms

6.2.1 Microorganisms

The toxicity data of poly(oxyethylene) nonylphenyl ether for microorganisms are shown in Table 6-6.

The toxic effects of NPE on growth of the soil bacteria *Bacillus subtilis* and *Bacillus megaterium* and the nitrogen-fixing bacterium *Azotobacter chroococcum* were studied. In an inoculated agar medium, a hole (diameter: 9 mm) was opened and 40 μL of 20 to 800 mg/L NPE with EO chain length of 4 to 30 was added to the hole. After incubation at 30°C for 48 hours, growth of bacteria was observed. NPE with EO chain length of 4 to 9 inhibited the growth of *B. subtilis* and *B. megaterium*, but NPE with EO

chain length of 10 and above did not up to 800 mg/L. The growth inhibitory effect of NPE was reduced as the EO chain length increased. The growth of *A. chroococcum* was not inhibited by NPE even at 800 mg/L (Cserhati et al., 1991).

Table 6-6 Toxicity of poly(oxyethylene) nonylphenyl ether for microorganisms

Species	AE composition	Tem-perature (°C)	Endpoint		Concent-ration (mg/L)	Refrence
Bacteria		30	48-hr	Growth		Cserhati et al., 1991
Bacillus subtilis	NPE_4		LOEC	inhibition	20	
	NPE_6		LOEC		20	
	NPE_9		NOEC		200	
	NPE_{10}		NOEC		>800	
	NPE_{15}		NOEC		>800	
	NPE_{30}		NOEC		>800	
Bacillus megaterium	NPE_{10}	ND	48-hr LOEC	Growth	20	
	NPE_{11}		NOEC	inhibition	50	
Azotobacter chroococcum	NPE_{10}	ND	48-hr NOEC	Growth	>800	
	NPE_{11}		NOEC	inhibition	>800	

ND: No data available

6.2.2 Plants

The toxicity data of poly(oxyethylene) nonylphenyl ether for plants are shown in Table 6-7.

NPE is used as a spreading agent for agricultural chemicals that are directly sprayed onto plants. Thus, the effects of NPE itself on the germination of fruit trees were studied. Branches cut before germination from 5 kinds of apple (*Malus domestica*), 2 kinds of grape (*Vitis* sp.), peach (*Prunus persica*) and pear (*Pyrus* sp.) trees were hydrocultured and 0, 1, 3 and 5% NPE_5 solution was directly sprayed once. Then, the effects on the germination rate and period were studied for 3 weeks (spray volume unknown). In the apple branches, excluding Golden Delicious that was sprayed at a concentration of 3% and higher, germination was delayed by 2 to 5 days and the germination rate was decreased by 30% to 50%. In the aurore grape branches that were sprayed at a concentration of 1% and higher, the germination was not delayed; however, the germination rate was decreased by 10% to 30%, and in the concord grape branches that were sprayed at a concentration of 3% and higher, germination was inhibited by 100%. The germination rate in the peach (*Prunus persica*) branches was inhibited by 75% to 84%; however, no effect on the germination rate in the pear (*Pyrus* sp.) branches was found (Spotts and Ferree, 1979).

Aqueous solutions of NPE_7, NPE_{10}, NPE_{14}, NPE_{20} and NPE_{30} at 0.2% (w/v) were dropped onto cabbage leaves and damage to the leaves was observed. NPE_{10} induced the severest damage of color change and necrosis in 50% of the leaf surface surrounding the solution drops (Knoche et al., 1992).

Table 6-7 Toxicity of poly(oxyethylene) nonylphenyl ether for plants

Species	NPE composi-tion	Growth/ stage	Method/ Condition	Tem-perature (°C)	Endpoint		Concent-ration (%w/v)	Refrence
Apple: Cortland Delicious Golden Delicious McIntosh Rome Beauty Grape: Aurore Concord Peach Pear	NPE_5	Branch before germina-tion	Direct spray once	23 ±2	3-week NOEC	Inhibited germina-tion Delayed germina-tion	<1 3 5 1 1 <1 <1 <1 5	Spotts & Ferree, 1979
Brassica oleracea (cabbage)	NPE_7 NPE_{10} NPE_{14} NPE_{20} NPE_{30}	2 to 4 months	0.2%(w/v) aqueous solution was dropped to the leaf surface.	24 ±2	24-hr	Damaged leaf	NPE_{10} had the severest effect.	Knoche et al., 1992

6.2.3 Animals

No reports on the toxicity of NPE in animals were obtained in this investigation.

6.3 Other effects

6.3.1 Effects on the endocrine and reproductive systems

In vitro and *in vivo* test results about influence to the endocrine and reproductive systems of NPE are shown in Table 6-8.

a. *in vitro* assays

The estrogen-like activity of NPE was studied in the hepatocytes of the rainbow trout. As it was known that 17ß-estradiol (E2), a natural estrogen, induces gene expression and secretion of vitellogenin, a yolk protein precursor in the hepatocytes, NPE was administered to the hepatocytes and the amount of vitellogenin that was secreted for 4 days was determined by radioimmunoassay. The EC_{50} for the vitellogenin production (the NPE concentration that increases the vitellogenin concentration by 50%: it is estimated from the dose-response curve of NPE and vitellogenin concentrations) of E2 was 1.8 nM and those of NP, NPE_2, NPE_9 were 16, 17 and 82 μM, respectively. NPE_{40} did not induce vitellogenin. The estrogen-like activity of NPE_2 was 1/10,000 of E2 activity and similar to that of NP. The estrogen-like activity of NPE_9 was 1/44,000 of E2 activity (Jobling and Sumpter, 1993).

These results suggest that NPE_2 and NPE_9 have weak estrogen-like activity on the hepatocytes of the rainbow trout.

[Relevant information]

Human estrogen receptor-binding activity was studied using reporter gene assay with yeast. The LOECs (the lowest concentration showing binding activity) for the receptor-binding activity were 3 ng/L in E2 and 1,500 μg/L in NPE_2. The relative binding activity of NPE_2 to E2 was 1/500,000. NPE_{12} showed no binding activity (Routledge and Sumpter, 1996). The REC_{10} for the rat estrogen receptor-binding activity (concentration corresponding to 10% of agonist activity of E2 100 nM) of E2 was 0.3 nM and that of NPE_2 was 1 mM. The relative binding activity of NPE_2 to E2 was 1/3,300,000 (Nishihara et al., 2000).

b. *in vivo* assays

The effects of NPE on fertility and excretion of testosterone metabolites were studied in the crustacea *Daphnia magna*. *D. magna* within 24 hours after birth were exposed to NPE (EO chain length unknown) at concentrations of 0, 0.31, 0.62, 1.2, 2.5 and 5.0 mg/L for 3 weeks under a flow-through condition. No significant differences in the survival rate and the number of offspring in females were found at any of the concentrations except 5.0 mg/L. At 5.0 mg/L, the survival rate and the number of offspring decreased by 30% and 35%, respectively, and on the last day of the 3-week exposure period, no offspring were born. In another experiment, testosterone metabolites excreted were quantitatively determined at 0, 2.5 and 5.0 mg/L. The excretion rates of glucose and sulfate conjugates were significantly decreased but those of reduced derivatives and hydroxy derivatives were not changed at 5.0 mg/L. The inhibitory effects of NPE on the excretion of testosterone metabolites and fertility on the last day of the 3-week exposure were similar to the effect of 4-nonylphenol. Therefore, it is considered that these inhibitions are induced by *in vivo* metabolites of NPE (Baldwin et al., 1998).

To investigate the effect of NPE on the gonads of the freshwater fish fathead minnow, the adult male and female fish were exposed to NPE_9 at 0, 0.15, 0.43, 1.45 and 5.5 μg/L for 42 days under a flow-through condition. The effects of NPE on the growth rate of male Sertoli cells, the damage rate of seminiferous tubules, testis size, and the development of female follicular cells were studied. No significant exposure-induced changes were found (Miles-Richardson et al., 1999).

To investigate the effect of NPE on the fertility of fathead minnow, the adult male and female fish were exposed to $NPE_{9.5}$ at 0, 0.21, 0.65, 2.1 and 7.9μg/L for 42 days under flow-through condition. Plasma vitellogenin, 17β-estradiol and testosterone concentrations were determined by competitive enzyme-linked immunosorbent assays as well as the survival and egg-laying rates. The dose dependency of the female egg-laying rate, male vitellogenin and testosterone concentrations to NPE were investigated and these values were shown to be highest at concentrations of 0.21, 0.21 and 0.65 μg/L of NPE, respectively. However, these changes were not significantly different from those of the control group. From these results, it is concluded that the NOEC of $NPE_{9.5}$ for fertility is 7.9 μg/L (Nichols et al., 2001).

The effect of NPE_2 on spermatogenesis in the rainbow trout was investigated. Adult male fish aged 13 months were exposed to NPE_2 at concentrations of 0, 0.45 and 1.80 μg/L for 3 weeks under flow-through condition, and 4.5 weeks after exposure, the testes were observed. The relative testis

weight to body weight was reduced by 0%, 18% and 40%, respectively. The ratios of germ cells in the developmental stage in the spermatoblasts and sperm were 60.5%, 48% and 31.8%, respectively, showing a dose-dependent delay in spermatogenesis. The plasma vitellogenin concentrations after 21-day exposure were 23, 27 and 4,750 ng/mL, which high-level exposure induced a high concentration (Le Gac et al., 2001).

Table 6-8 Effect on the endocrine and reproductive systems of poly(oxyethylene) nonylphenyl ether

a. *in vitro* assays

Species	Method/Period	Results	Reference
Fish *Oncorhynchus mykiss* (rainbow trout)	Primary-cultured hepatocytes from male 4-day culture with NPE Determination of vitellogenin production by radioimmuno assay	EC_{50} for vilellogenin production E2 1.8 nM NP 16 μM NPE_2 17 μM NPE_9 82 μM NPE_{40} —	Jobling & Sumpter, 1993
[Reference data]	Reporter gene assay with estrogen receptor (ER)	Human ER: LOEC for gene transcriptional activity / relative gene transcriptional activity E2 [1)] 3 ng/L 1 NPE_2 1,500μg/L 1/500,000 NPE_{12} —	Routledge & Sumpter, 1996
		Rat ER: REC_{10} for gene transcriptional activity / relative gene transcriptional activity E2 0.3 nM 1 NPE_2 1 mM 1/3,300,000	Nishihara et al., 2000

b. *in vivo* assays

Species	Method/Period	Results	Reference
Crustacea *Daphnia magna* (water flea)	<24 hours, female NPE (EO chain length unknown) 0, 0.31, 0.62, 1.2, 2.5, 5.0 mg/L 3-week exposure under flow-through condition	5.0 mg/L: Decreases in female survival rate and the number of offspring by 30% and 35% No offspring on the last day of 3-week exposure period	Baldwin et al., 1998
	0, 2.5, 5.0 mg/L 3-week exposure under flow-through condition	5.0 mg/L: Excretion rate of testosterone metabolites Glucose conjugate: significantly low Sulfoconjugate: significantly low Oxidated/reduced derivative: no change Hydroxy derivative: no change	
Fish *Pimephales promelas* (fathead minnow)	Adult, male and female NPE_9 0, 0.15, 0.43, 1.45, 5.5 μg/L 42-day exposure under flow-through condition	Male: no significant changes in growth rate of male Sertoli cells, damage rate of the seminiferous tubules, testis size Female: no significant changes in development of female follicular cells	Miles-Richardson et al., 1999

Species	Method/Period	Results	Reference
	Adult, male and female $NPE_{9.5}$: 0, 0.21, 0.65, 2.1, 7.9 μg/L 42-day exposure under flow-through condition Determination of survival and egg-laying rates Determination of plasma vitellogenin, 17β-estradiol (E2) and testosterone concentrations	0.21 μg/L and above: No significant differences in survival and egg-laying rates No significant differences in plasma vitellogenin, 17β-estradiol (E2) and testosterone concentrations	Nichols et al., 2001
Oncorhynchus mykiss (rainbow trout)	Adult male fish aged 13 months NPE_2 0, 0.45, 1.80 μM 3-week exposure under flow-through condition Observation of the testis 4.5 weeks after exposure Determinaiton of plasma vitellogenin concentration	NPE_2 (μM) 0 0.45 1.80 Decrease in relative testis weight (%) 0 18 40 Ratios of germ cells in the spermatoblasts and sperms (%) 60.5 48 31.8 Plasma vitellogenin concentration (ng/mL) 23 27 4,750	Le Gac et al., 2001

1) E2, 17β-estradiol

6.4 Summary of effects on organisms in the environment

It is postulated that NPE is converted to mono(oxyethylene) nonylphenyl ether (NPE_1) by sequential breaks in the EO chain in the aquatic environment including river water, and under anaerobic conditions including sediment, further breaks in the EO chain occur to generate nonylphenol (see 5.3). In such environmental conditions, NPE with a shorter EO chain has stronger toxicity to aquatic organisms including aquatic bacteria, algae, crustacea (water fleas and mysid) and fish (Japanese killifish and bluegill).

NPE inhibited the growth of the bacteria *Pseudomonas*, and the toxicity threshold increased from 50 to 1,000 mg/L as the EO chain length increased from 4 to 30. The 16-hr EC_{50} of NPE_{12} for growth inhibition exceeded 2,000 mg/L. NPE_9 inhibited luminescence of marine luminescent bacteria, and the 5-min EC_{50} for luminescence inhibition was 60.6 mg/L

For the freshwater alga *Selenastrum capricornutum*, the lowest EC_{50} of various NPEs is 12 mg/L as the 96-hr EC_{50} of NPE_9 for growth inhibition. The lowest NOEC is 8 mg/L as the 96-hr NOEC of NPE_9.

The acute toxicity of various NPEs to invertebrates is reported for the freshwater crustacea, water fleas (*Daphnia magna*, *Daphnia pulex* and one of *Ceriodaphnia dubia*) and the marine crustacean, mysid. The 48-hr LC_{50} of NPE with EO chain length of 9 to 10 ranged from 1.23 to 14.0 mg/L and that of NPE with EO chain length of 1 to 7 ranged from 0.11 to 10 mg/L. The lowest value of these toxicity data is 0.11 mg/L as the 48-hr LC_{50} of $NPE_{1.5}$ in the mysid. The 24-hr LC_{50} of NPE_{11} for the insect *Aedes aegypti* was 500 mg/L and the 96-hr LC_{50} of NPE_{10} for the shellfish *Cardium edule* was 5 mg/L.

The acute toxicity of NPE to fish has been studied for the freshwater fish, fathead minnow, Japanese killifish, bluegill, goldfish, brown trout, golden orphe and rainbow trout, and the marine fish, Atlantic cod. The 48- to 96-hr LC_{50} of NPE with EO 10 and less ranged from 1.0 to 18 mg/L. The lowest LC_{50} of

NPE is 1.0 mg/L as the 96-hr LC_{50} of NPE_{9-10} for the brown trout. The 7-day NOEC of NPE_9 for growth of the fathead minnow was 1.0 mg/L.

In other aquatic organisms, the acute toxicity of NPE has been studied for amphibians. NPE_8 induced narcosis to the larvae (tadpoles) of African clawed frogs and cane toads. The 48-hr EC_{50} ranged from 1.1 to 5.1 mg/L.

As for terrestrial organisms, the toxicity of NPE has been studied in bacteria and plants. No report on the toxicity of NPE in animals was obtained. NPE with EO 4 to 30 inhibited the growth of the soil bacterium *Bacillus* at concentrations of 20 to 800 mg/L but not the growth of nitrogen-fixing bacteria *Azotobacter*.

NPE is used as a spreading agent of agricultural chemicals for the plants. The effects of NPE itself on the germination of fruit trees were studied. Solution of 5% NPE_5 inhibited germination of dormant apple, grape and peach but not pear trees. Of the 0.2% (w/v) aqueous solutions of NPE_7, NPE_{10}, NPE_{14}, NPE_{20} and NPE_{30}, NPE_{10} induced the severest damage, suggesting that toxicity varies among NPE types and concentrations.

The effects of NPE on the endocrine and reproductive systems were studied by using *in vitro* assays. NPE_2 activity inducing the expression of vitellogenin, a yolk protein precursor, in rainbow trout hepatocytes, was 1/10,000 of the activity of 17ß-estradiol (E2), a natural estrogen, and NPE_9 activity was 1/44,000 of E2 activity. These results suggest that NPE_2 and NPE_9 have weak estrogen-like activity in rainbow trout hepatocytes. The results of *in vivo* studies showed that NPE at concentrations around LC_{50} decreased the number of offspring and changed the excretion of testosterone metabolites. NPE_2 enhanced plasma vitellogenin concentrations and decreased the relative testis weight in male rainbow trout. These results suggest the possibility that NPE has effects on the endocrine and reproductive systems in aquatic organisms including crustacea and fish.

Although formal classification criteria is not used in this investigation, it can be considered that the acute toxicity values of NPE to aquatic organisms crustacea and fish are corresponding to the GHS acute toxicity hazard category I (very toxic). The lowest value of these toxicity data for aquatic organisms is 0.11 mg/L as the 48-hr LC_{50} of $NPE_{1.5}$ for the crustacean, mysid.

7. Effects on human health

7.1 Kinetics and metabolism

No reports were obtained on the kinetics and metabolism in humans of poly(oxyethylene) nonylphenyl ether (NPE). Studies on the kinetics and metabolism of NPE to experimenal animals are summarized in Table 7-1.

a. Absorption/Distribution

The absorption of NPE was studied using NPE with ^{14}C-labeled phenyl group or oxyethylene (EO) chain.

[Phenyl-^{14}C]-NPE_9 or [ethylene-^{14}C]-NPE_9 was orally administered (gavage) to male Carworth-Elias rats at a dose of 10 mg/animal, and absorption and excretion were investigated. Within 7 days after NP[^{14}C]E_9 administration, approximately 52% of the radioactivity administered was excreted in the feces, 40% in the urine and 1.2% through exhalation as CO_2. After ^{14}C-NPE_9 administration, 78% of the radioactivity administered was excreted in the feces and 20% in the urine but not through exhalation as CO_2. In both NPEs, 90-95 % of the radioactivity administered was excreted in the feces and urine within 7 days, and NPE did not remain in the body (Knaak et al., 1966). Judging from the radioactivity excreted in the urine, not less than 40% of the radioactivity administered was absorbed in the body.

In an oral study of NPE_7, NPE_{10}, NPE_{12} and NPE_{15} whose EO chains were labeled with ^{14}C, the effect of EO chain length on the excretion route was investigated. As the EO chain length increased, the radioactivity excreted in the urine and expiration was decreased and that in the feces was increased. For all NPEs, 90-95% of the radioactivity administered was excreted in the feces and urine within 7 days. From these results, it is considered that NPE with a long EO chain was less absorbed in the intestine and was more excreted in the feces (Knaak et al., 1966).

NPE_9 has been used as a spermicidal contraceptive (see 7.2). Absorption of NPE_9 through the vaginal wall was studied. An aqueous solution of NPE_9 with ^{14}C-labeled nonylphenyl group was administered to the vagina of female Wistar rats on gestation day 15, and blood radioactivity was determined. Radioactivity was detected in the blood at 10 minutes after administration. The blood radioactivity concentration reached a peak at one hour after administration, gradually decreased until 3 hours after administration and thereafter remained constant. Approximately 56% of NPE was not absorbed and remained in the vagina at 6 hours after administration (Buttar, 1982).

In the above study, radioactivity distribution of NPE_9 with ^{14}C-labeled nonylphenyl group was examined at 6 hours after administration. The radioactivity concentration/g tissue was the highest in the liver, followed by the kidney, adrenal gland, thyroid, heart and spleen. Of the tissues determined, the lowest concentration was in the brain. The radioactivity concentration in the urinary bladder was 12 times as high as that in the plasma. Assuming that the specific gravity of the uterus and placenta is 1, the radioactivity concentrations in the uterus and placenta were similar to the maternal plasma concentration. The radioactivity concentrations in the amniotic fluid and fetuses were one third of the maternal plasma concentration (Buttar, 1982).

In a kinetic study of plasma and urinary NPE_9 in female Japanese albino rabbits following injection of 5 mL of NPE_9 into the auricular vein at a dose of 2 mg/L, the plasma concentration immediately after administration was 1.48 μg/mL and was decreased to 0.30 μg/mL by one hour after administration (Minami et al., 2000).

NPE film (EO chain length unknown) was inserted into the vaginas of female Japanese albino rabbits once, and the kinetics of blood NPE concentrations was studied and the plasma nonylphenol (NP) was quantitatively determined. Following injection of 100 mg of NPE into the vagina, the plasma NPE concentration reached a peak (1.28 μg/mL) at 2.25 hours after administration and was decreased below the quantitation limit (0.2 μg/mL) by 6 hours after administration. The plasma half-life was 1.50 hours. The plasma NP concentrations within 24 hours were below the quantitation limit (10 ng/mL) (Minami

et al., 2000).

b. Metabolism

[Phenyl-^{14}C]-NPE_9 or [ethylene-^{14}C]-NPE_9 was orally administered to male rats, and the metabolites excreted in the urine within one day were identified. The major metabolite was neutral, followed by acid metabolites. The former was glucuronoconjugate of nonylphenol (NP) and the latter were mono- and di-carboxylic acid conjugates of polyethylene glycol. Approximately 1.2% of ^{14}C-NPE_9 was metabolized to CO_2 (Knaak et al., 1966). These results suggest that the ether linkage of NPE is broken and NP and polyethylene glycol are produced. Most polyethylene glycol terminals are carboxylated and furthermore, some of them are metabolized to CO_2. The NP produced was conjugated with glucuronic acid.

NPE film (100 mg/animal; EO chain length unknown) was once inserted into the vagina of female Japanese albino rabbits. Within 24 hours after administration, 0.02% and 0.20% of radioactivity administered were detected as NP and NP glucuronoconjugate, respectively, in the urine (Minami et al., 2000). These results suggest that NPE is absorbed through the vaginal mucosa and metabolized to NP and its glucuronoconjugate after blood circulation.

c. Excretion

[Phenyl-^{14}C]-NPE_9 or [ethylene-^{14}C]-NPE_9 was orally administered to male rats, and excretion of the radioactivity was determined. Within 7 days after ^{14}C-NPE_9 administration, approximately 78% of the radioactivity administered was excreted in the feces and 20% in the urine. Radioactivity was not detected in the exhalation. Within 7 days after ^{14}C-NPE_9 administration, approximately 52% of the radioactivity administered was excreted in the feces, 39% in the urine and 1.2% through exhalation. In both NPEs, 90-95 % of the radioactivity administered was excreted in the feces and urine within 7 days. In the urine only within one day after administration, unchanged NPE (1%) was excreted (Knaak et al., 1966).

In summary, NPE orally administered is absorbed through the gastrointestinal tract. After absorption, NPE is metabolized to nonylphenol and polyethylene glycol with a shortend EO chain and carboxylated terminal. The excretion ratio varies according to the EO chain length. However, most NPE administered was excreted in the feces and urine within 7 days regardless of EO chain length.

Table 7-1 Kinetics and metabolism of poly(oxyethylene) nonylphenyl ether

Species	NPE composition	Route	Exposure condition	Result	Reference
Rat Carworth-Elias Male Approximately 150 g	[Phenyl-^{14}C]-NPE_9 or [ethylene-^{14}C]-NPE_9	Oral	Single dose 10 mg/animal	<u>Absorption</u> Within 7 days, 90-95 % of the radioactivity administered was excreted in the feces and urine. It is suggested that NPE is absorbed in the gastrointestinal tract. <u>Metabolism</u> Major metabolites in the urine: glucuronoconjugate of nonylphenol, mono- and dicarboxylic acid conjugates of polyethylene glycol small amount of CO_2 <u>Excretion</u> Within 7 days, 90-95 % of the radioactivity administered was excreted in the feces and urine. <u>Excretion rate (%)</u> Feces Urine Expiration N[^{14}C]PE_9 78 20 0 NP[^{14}C]E_9 52 40 1.2	Knaak et al., 1966
	^{14}C-NPE_7 ^{14}C-NPE_{10} ^{14}C-NPE_{12} ^{14}C-NPE_{15}	Oral	Single dose Each 10 mg/animal	<u>Absorption/ Excretion</u> With an increase in oxyethylene (EO) chain length, the radioactivity excreted in the urine and exspiration was decreased and that in the feces was increased. It is suggested that NPE with long EO chain was less absorbed in the gastrointestinal tract.	
Rat Wistar pregnant Female 5 animals	[nonylphenyl-^{14}C]-NPE_9	Intrava-ginal	Single dose Gestation day 15 25 mg/kg	<u>Absorption</u> Kinetics of blood radioactivity concentration after administration: 10 mins after: radioactivity was detected in the blood. 1 hour after: The peak gradually decreased until 3 hours after constant until 6 hours after approximately 56% of radioactivity administered remained in the vagina 6 hours after. <u>Distribution</u> <u>Radoactivity distribution in tissues 6 hours after administration:</u> (μg/g) Liver 6.17 Kidney 3.71 Adrenal gland 2.55 Thyroid 2.07 Salivary gland 1.52 Heart 1.39 Spleen 1.37 Lung 1.21 Brain 0.26 (μg/mL) Plasma 1.25	Buttar, 1982

Species	NPE composition	Route	Exposure condition	Result	Reference
				Bladder 15.09 The radioactivity concentration in the uterus and placenta (assuming that the specific gravity of the tissues was 1) was similar to the maternal plasma concentration. The radioactivity concentration in the amniotic fluid and fetus was 1/3 of the maternal plasma concentration.	
Rabbit Japanese albino Female 4 to 6 months 3 to 4 animals /group	NPE_9	Intrave-nous	Single dose 10 mg/animal	Plasma concentration Immediately after administration: 1.25 μg/mL 1 hour after: 0.30 μg/mL Unchanged NPE in the urine Within 24 hours, 0.02% of the administered amount	Minami et al. 2000
	NPE film (NPE 50 mg/sheet: EO chain length unknown	Intravag-inal	100 mg/animal	Plasma concentration 2.25 hours after administration: peak Peak concentration 1.25 μg/mL 6 hours after: under determination limit The plasma half-life: 1.50 hours Nonylphenol (NP) concentration (within 24 hours) Plasma: Under detection limit Urine: (of radioactivity administered) NP 0.02% NP glucuronoconjugate 0.20%	

7.2 Epidemiological studies and case reports

The epidemiological studies and case reports of poly(oxyethylene) nonylphenyl ether are summarized in Table 7-2.

In addition to the epidemiological studies and case reports, there are some studies in volunteers.

Some epidemiological studies showed a high incidence of abortion and many anomalies in newborns after the use of spermicides containing NPE_9 before pregnancy (Jick et al., 1981; Smith et al., 1977; Warburton et al., 1980). The results of epidemiological studies with control groups without birth control and with other contraceptives showed that the incidence of congenital anomaly in the NPE group was not higher than that in the control groups without birth control and with other contraceptives (Huggins et al., 1982; Linn et al., 1983; Louik et al., 1987; Warburton et al., 1987). Based on these results, NPE is considered not to induce congenital anomaly.

NPE_9 is still used as a spermicide in the United States, while the manufaturing of contraceptives containing NPE has been terminated in Japan (Ortho-McNeil Pharmaceutical, 2001; Taiho Pharmaceutical 2001).

A skin irritation and sensitization study of NPE_2 was conducted with 102 male and female volunteers. NPE_2 of 5% was applied to the posterior part of the neck at a dose of 0.2 mL, 3 times/week for 3 weeks

and the application site was occluded to induce sensitization. After a 14-day period without application, NPE was applied and covered for 48 hours to challenge sensitization response, and then, 48 and 96 hours after the challenge period, skin responses were examined. Slight to moderate erythema was observed in 3 subjects at 48 hours after the induction period for sensitization. Skin responses were observed in 3 subjects after the challenge. However, these responses were not considered to be allergic contact dermatitis (Jordan, 1994).

A similar study with 10% NPE_2 was conducted with 103 male and female volunteers. At the end of induction period for sensitization, slight to moderate erythema was found in 15 subjects and severe erythema in one subject. After challenge, skin responses were found in 23 subjects, and 9 of them developed allergic contact dermatitis. In a retest with 30-min application for 7 subjects, 2 of them showed allergic reaction (Jordan, 1995).

A skin irritation and sensitization study of NPE_4 was conducted with 107 male and female volunteers in which 10%-NPE_4 was applied to the posterior part of the neck at a dose of 0.2 mL, 3 times/week for 3 weeks and the application site was occluded. After a 14-day period without application, NPE was applied and covered for 48 hours to induce irritation, and then, 48 and 96 hours after, skin responses were examined. At the end of the sensitization period, slight to moderate erythema was found in 36 subjects. After induction, skin responses were observed in 31 subjects, and 3 of them developed allergic contact dermatitis. In a retest with 30 mins induction in 3 subjects, one of them showed a weak allergic reaction (Jordan, 1995).

In a skin irritation study of cosmetics containing NPE_4, NPE_9 and NPE_{12} (1.75% to 20%) in volunteers (20 subjects/group), minimal to slight irritation was observed at 24 hours after application to the inner forearm and occlusion of the application site (CIR Expert Panel, 1983).

Undiluted NPE_4 was applied to the backs of male and female volunteers (25 each) and the application site was occluded for 48 hours, and sensitization was repeated 14 times every 24 hours. In a skin sensitization study after a 2-week period without application, no sensitization response was observed. In a similar sensitization study of NPE_9 with 50 male and 50 female volunteers, no response due to skin sensitization was found and it was suggested that NPE_4 and NPE_9 produced no skin sensitization (CIR Expert Panel, 1983).

A patch test of NPE was conducted in 12 patients who developed allergic contact dermatitis due to disinfectant containing NPE. Ten of them used a disinfectant containing NPE_9 and two used one containing $NPE_{8.3}$ and NPE_{10}. In a patch test, respective 2% solutions of the disinfectant ingredient, $NPE_{8.3}$, NPE_9 and NPE_{10}, were applied, and the application sites were occluded for 48 hours, and then, after 48, 72 and 96 hours, skin responses were examined. All 12 patients except one showed negative to the disinfectant ingredient, but all developed severe edema or vesicular eruption responses to the NPE that they used. From these results, it is concluded that NPE induces allergic contact dermatitis (Dooms-Goossens et al., 1989).

Skin sensitization and photosensitization by NPE_{15} and NPE_{50} were investigated. The solution (50%) of each NPE_{15} and NPE_{50} was applied to the dorsal skin of 53 male and 115 female volunteers and the application sites were occluded for 48 hours, 3 times/week for 3 weeks, and at the induction after a

3-week interval, no skin sensitization was observed. In a photosensitization study, 50% solution of each NPE_{15} and NPE_{50} was applied to the dorsal skin of 28 volunteers and the application sites were occluded for 48 hours, and after rising, UV-A (320-400 nm) or UV-B (280-320 nm) was irradiated. This procedure was repeated 3 times/week for 3 weeks, and at photoinduction after a 3-week interval, no photosensitization was found (CIR Expert Panel, 1983).

As NPE had been used as a spermicidal contraceptive, an irritation study of spermicides containing 10% NPE_4 in 30 females was conducted. Slight local irritation was observed in the vaginas of 2 subjects and itching in one subject (Lichtman et al., 1973).

It has been reported that leukoderma was developed in two female workers who were engaged in washing metal parts with 2 alkaline detergents containing NPE or poly(oxyethylene) octylphenyl ether (OPE) with EO chain length of 3 to 16. In the washing process, a worker routinely used a detergent solution containing 10% NPE_3 at 80°C, and another worker used detergents containing 10% of NPE_{10} or OPE_{10}. Both workers developed tumid erythema associated with pruritus on both hands, depigmentation of the skin and leukoderma on the forearms. These detergents were analyzed to contain nonylphenol (NP) and octylphenol (OP). From these results and a report that OP and alkylphenol induced leukoderma in humans and experimental animals (Hara and Nakajima, 1969), it is concluded that leukoderma was induced not by NPE and OPE but by NP and OP, which were contained as unreacted materials or degradation products (Ikeda et al., 1970).

Based on these results, it is suggested that NPE produces primary skin irritation in humans, and that the severity depends on the EO chain length. Sensitization was induced by 10% NPE_2 but not by 5% NPE_2. Sensitization was induced by NPE_4 and NPE_9 in a few cases. In addition, patients with allergic contact dermatitis who used disinfectants containing $NPE_{8.3}$, NPE_9 and NPE_{10} showed sensitization reactions to the respective NPEs. However, NPE with EO chain length of 15 and 50 produces neither skin sensitization nor photosensitization. Therefore, it is highly possible that NPE with EO chain length of 10 and less causes skin sensitization. Routine use of detergents containing $NPE_{3\text{-}16}$ in alkaline conditions at 80°C possibly induces leukoderma.

Table 7-2 Epidemiological studies and case reports of poly(oxyethylene) nonylphenyl ether

Population sex/number	NPE composition	Exposure condition / Dose	Results	Reference
Volunteer 102 males and females	NPE_2	Skin irritation and sensitization test Induction: Application of 5% NPE_2 to the posterior part of neck at 0.2 mL, 3 times/week, for 3 weeks Occlusion of the application site	Subject After induction: 3 persons, slight to moderate erythema After challenge: 3 persons, skin responses without allergic contact dermatitis Conclusion: 5% NPE_2 has skin irritation but not	Jordan, 1994

Population sex/number	NPE composition	Exposure condition / Dose	Results	Reference
		Challenge at 2 weeks after: 48 hours application and occlusion of the application site Skin observation at 48 and 96 hours after induciton	sensitization.	
Volunteer 103 males and females	NPE_2	<u>Skin irritation and sensitization test</u> Sensitization: Application of 10% NPE_2 to the posterior part of neck at 0.2 mL, 3 times/week, for 3 weeks Occlusion of the application site induciton at 2 weeks after: 48 hours application and occlusion of the application site Skin observation at 48 and 96 hours after induciton	<u>Subject</u> After sensitization: 15 persons slight to moderate erythema 1 person severe erythema After induciton 23 persons skin response 9 persons allergic contact dermatitis After re-induciton (30 mins application) in 7 subjects 2 persons slight allergic reaction Conclusion: 10% NPE_2 has skin irritation and sensitization.	Jordan, 1995
Volunteer 107 males and females	NPE_4	<u>Skin irritation and sensitization test</u> Sensitization: Application of 10% NPE_4 to the posterior part of neck at 0.2 mL, 3 times/week for 3 weeks Occlusion of the application site induciton at 2 weeks after: 48 hours application and occlusion of the appliction site Skin obsrvation at 48 and 96 hours after induciton	<u>Subject</u> After sensitization: 36 persons slight to moderate erythema After induciton: 31 persons skin response 3 persons allergic contact dermatitis After re induciton (30 mins application) in 3 subjects 1 persons slight allergic reaction Conclusion: 10% NPE_4 has skin irritation and sensitization.	Jordan, 1995
Volunteer (gender and number unknown)	NPE_4 NPE_9 NPE_{12}	<u>Skin irritation test</u>	Minimal to slight skin irritation	CIR Expert Panel, 1983
Volunteer 25 males and 25 females 50 males and 50 females	NPE_4 NPE_9	<u>Skin sensitization test</u> Sensitization: Application of undiluted solution to the back and 48 hours occlusion of the application site, followed by 14 times application in every 24 hours induciton at 2 weeks after: Skin observation	NPE_4 and NPE_9: negative in skin response Conclusion: NPE_4 and NPE_9 have no sensitization.	CIR Expert Panel, 1983

Population sex/number	NPE composition	Exposure condition / Dose	Results	Reference
Patients with allergic contact dermatitis 12 persons (Use of disinfectant containing NPE_9: 10 persons, use of disinfectant containing $NPE_{8.3}$/NPE_{10}: 2 persons)	$NPE_{8.3}$ NPE_9 NPE_{10}	Patch test Application of $NPE_{8.3}$, NPE_9, NPE_{10} 2% solutions and 48 hours occlusion of the application site skin observation at 48, 72 and 96 hours after application	Response to $NPE_{8.3}$ and NPE_{10}: 2 persons Response to NPE_9: 10 persons All developed severe edema or vesicular response. Conclusion: NPE was suggested to be allergen.	Dooms-Goossens et al., 1989
Volunteer 53 males 115 females	NPE_{15} NPE_{50}	Skin sensitization test Sensitization: Application of 50% solution to the back and 48 hours occlusion of the application site 3 times/week for 3 weeks induciton at 3 weeks after: observation	NPE_{15} and NPE_{50}: negative in skin response Conclusion: No sensitization	CIR Expert Panel, 1983
Volunteer 28 persons	NPE_{15} NPE_{50}	Photosensitization test Sensitization: Application of 50% solution to the back and 48 hours occlusion of the application site and UV irradiation, 3 times/week for 3 weeks Photo induciton at 3 weeks after: Observation	Negative skin responses in all of 28 subjects Conclusion: No photosensitization	CIR Expert Panel, 1983
Volunteer 30 females	NPE_4	Vagina irritation test Intravaginal injection of spermicides containing 10% NPE_4	Normal:27 persons Slight local irritation: 2 persons Itching symptom: 1 person	Lichtman et al., 1973
Metal washing worker 2 females	$NPE_{3\text{-}16}$	2 kinds of alkaline detergents: A. containing 10% NPE_3 B. containing 10% NPE_{10} or OPE_{10} Used in washing metal parts at 80°C	Worker using detergent A: Tumid erythema associated with pruritus in both hands and the outer side of both forearms, With routine use, scaliness and depigmentation of skin, leukoderma Worker using detergent B: After working, pruritic erythemad in both hands, leukoderma in both sides of finger nails, the left forearm and shoulder	Ikeda et al., 1970

7.3 Studies in experimental animals and *in vitro* studies

7.3.1 Acute toxicity

Summary of the acute toxicity of poly(oxyethylene) nonylphenyl ether to experimenal animals is shown in Table 7-3 (Talmage, 1994).

In oral studies of NPE in rats, the LD_{50} values of NPE with EO 2 to 15 ranged from 1,300 to 7,400 mg/kg (CIR Expert Panel, 1983) and that of NPE with EO 20 exceeded 15,900 mg/kg (Schick, 1967). The LD_{50} of NPE with EO 9 was 4,290 mg/kg in mice and ranged from 620 to 4,400 mg/kg in rabbits (CIR Expert Panel, 1983). The LD_{50} of NPE with EO 4 was 2,000 mg/kg and that of NPE with EO 9 ranged 840 to 5,000 mg/kg in guinea pigs (CIR Expert Panel, 1983; Schick, 1967).

No LC_{50} data were obtained from inhalation studies. Rats were exposed to NPE_4 aerosol (213 mg/m^3), NPE_7 aerosol (250 mg/m^3) and concentrated NPE_9 vapor (concentration unknown) for 8 hours and observed for 14 days, and the body weight gain was normal and no deaths were observed (CIR Expert Panel, 1983).

In dermal studies of NPE in rabbits, the LD_{50} of NPE with EO 4 to 40 ranged from 1,800 to over 10,000 mg/kg (CIR Expert Panel, 1983; Monsanto Chemical, 1975).

The intraperitoneal, intravenous and intracutaneous LD_{50} values of NPE_9 with EO 9 in female mice were 210, 44 and 1,000 mg/kg, respectively (CIR Expert Panel, 1983).

Acute symptoms after oral administration were tremor, ataxia, lethargy and coma in rats. At autopsy, liver congestion and discoloration, lung congestion and hemorrhage, kidney discoloration and inflammation of the gastric mucosa were found. In rabbits, salivation, tremor, diarrhea and lethargy were observed, and liver congestion was found at autopsy (Larson et al., 1963; Smyth and Calandra, 1969).

In a dermal study in rabbits, erythema and necrosis were found at the skin application site and diarrhea was also observed. At autopsy, lung congestion and hemorrhage, liver congestion and kidney discoloration were found (CIR Expert Panel, 1983).

Table 7-3 Acute toxicity of poly(oxyethylene) nonylphenyl ether

	Mouse	Rat	Rabbit	Guinea pig
Oral LD_{50} (mg/kg)	4,290 (NPE_9)	1,300->15,900 (NPE_{10}) (NPE_{20})	620-4,400 (NPE_9)	840-5,000 (NPE_9)
Inhalation LC_{50} (mg/m^3)	ND	ND	ND	ND
Dermal LD_{50} (mg/kg)	ND	ND	1,800->10,000 (NPE_7) (NPE_{40})	ND
Intraperitoneal LD_{50} (mg/kg)	210 (NPE_9)	ND	ND	ND
Intravenous LD_{50} (mg/kg)	44 (NPE_9)	ND	ND	ND
Subcutaneous LD_{50} (mg/kg)	1,000 (NPE_9)	ND	ND	ND

ND: No data available

7.3.2 Irritation and corrosion

In a primary skin irritation study of undiluted solution of NPE with EO 2 to 9 in New Zealand White rabbits, NPE solution was applied to the shaved skin on the back and the application site was occluded. NPE induced moderate to severe irritation. However, NPE with EO 10 and more induced no or slight irritation (CIR Expert Panel, 1983; Talmage, 1994).

In an eye irritation study of undiluted NPE solution at a dose of 0.1 mL, NPE solution was applied to the conjunctival sacs of New Zealand White rabbits and symptoms were observed. NPE with EO chain length of 2 to 15 induced moderate to severe irritation. NPE with EO chain length of 30 and more induced no irritation (CIR Expert Panel, 1983; Talmage, 1994). Washing eys immediately after applying NPE solution relieved irritation (Gershbein and McDonald, 1977; Olson et al., 1962).

Based on these results, it is suggested that NPE-induced irritation varies by EO chain length, ranging from no irritation to strong skin and eye irritation. Skin irritation was moderate to severe by NPE with EO chain length of 2 to 9, none to slight by EO chain length of 10 and more. Eye irritation was moderate to severe by EO chain length of 2 to 15, none to slight by EO chain length of 30 and more.

7.3.3 Sensitization

A maximization study of NPE_6 sensitization was conducted in Hartley-Dalkin guinea pigs. A solution of 0, 1.7, 3, 9 and 27% (w/w) NPE_6 in propylene glycol and complete Freund's adjuvant (1:1) was injected into the shoulder at a dose of 0.1 mL. After 7 days, undiluted NPE_6 was applied and the application site was occluded for 48 hours for sensitization. After a 21-day interval without application, 2.7% NPE_6, which induces no skin irritation, was applied and the application site was occluded for 24 hours to induce irritation, and 48 hours after, skin responses were observed. The positive response rates were 5/40, 2/5, 0/5, 1/4 and 2/5, respectively, and no significant differences were found among NPE_6 concentrations. From these results, it is concluded that NPE_6 causes no sensitization in guinea pigs (Nethercott and Lawrence, 1984).

7.3.4 Repeated dose toxicity

Studies on the repeated dose toxicity of poly(oxyethylene) nonylphenyl ether to experimental animals are summarized in Table 7-4.

In repeated dose toxicity study, various strains of rats or dogs were orally administered NPE via diet for 90 days or 2 years.

Male and female SD rats (10 animals/group) were fed diet containig NPE_4, NPE_6, NPE_{15} (0, 40, 200 and 1,000 mg/kg/day), NPE_{20} or NPE_{30} (0, 200, 1,000 and 5,000 mg/kg/day) for 90 days. At 40 mg/kg/day and above, the relative liver weight was significantly increased in the NPE_6-treated group, and at 200 mg/kg/day and above, the relative liver weight was significantly increased in the NPE_4-treated group, the absolute liver weight was significantly increased in the NPE_6-treated group, and body weight gain was suppressed in the NPE_{15}-treated group. At 1,000 mg/kg/day, body weight gain was suppressed in the NPE_4- and NPE_6-treated groups and the absolute liver weight was

significantly increased in the NPE_4-treated group. At 5,000 mg/kg/day, body weight gain was suppressed in the NPE_{20}-treated group but not in the NPE_{30}-treated group. Based on the result that no histopathological changes in the liver of the NPE_4- and NPE_6-treated groups were observed, Smyth and Calandra considered that the increase in liver weight was due to the hypertrophy of hepatocytes caused by the increase in metabolic enzyme activity (Smyth and Calandra, 1969). In a histopathologic examination of 33 tissues, no abnormal findings were observed in the heart of the NPE_{20}-treated group (focal necrosis in the myocardium was observed in dogs: described later).

Male and female Wistar rats (30 animals/control group, 15 animals/treated group) were given diet containing NPE_9 at 0, 0.01, 0.04, 0.16, 0.64, 2.5 and 5.0% (corresponding to 0, 5, 20, 80, 320, 1,250 and 2,500 mg/kg/day: values converted by Talmage (1994); hereafter Talmage's conversion) for 90 days. At 0.64% and above, a decreasing tendency of food consumption, significant suppression of body weight gain, and an increasing tendency of relative liver, kidney and brain weight were found in the males and females, and an increasing tendency of relative testis weight was observed in the males. At 5%, body weight loss and an increase in mortality (death: 11/15) were found in the males and females (56-day administration). In a histopathologic examination of 19 tissues in males and females, no toxicity-induced histopathological changes were observed in any of the dose groups, and red and white blood cell counts and hemoglobin were normal in hematology. From these results, it is concluded that significant suppression of body weight gain was induced by a decrease in feed palatability (Smyth and Calandra, 1969). However, the study data showed that the mean decrease in food consumption and suppression of body weight gain in the 2.5% group were 78% and 25% of the control group in the males and 92% and 22% in the females, respectively. Since in the males and females, body weight gain was suppressed more than the decrease in food consumption, it is difficult to consider that the suppression of body weight gain was caused only by a decrease in food consumption. Therefore, it is considered in this assessment that NPE_9 has toxicity resulting in the suppression of body weight gain.

Following oral administration of NPE_9 to male and female CFE rats (10 animals/group) at 0, 10, 50, 250 and 1,250 mg/kg/day for 90 days, food consumption was reduced in the females at 10 mg/kg/day and above. Body weight gain was suppressed in the males and females and the relative liver weight was increased in the females at 250 mg/kg/day and above. In the histopathologic examination, hepatic fatty changes were observed in the males and females. At 1,250 mg/kg/day, food consumption was reduced and the relative liver weight was increased in the males, but the relative kidney weight was not changed in the males and females. Focal necrosis in hepatocytes and renal tubular necrosis were observed in the males and females (Smyth and Calandra, 1969).

NPE_9 was administered to male and female rats (strain unknown, 10 animals/group) at 0, 0.1, 0.3 and 1.0% (corresponding to 0, 50, 150 and 500 mg/kg/day: Talmage's conversion). At 0.1% and above, the survival rate and mean food consumption for the first 30 days were not changed in the males and females. At 0.3% and above, the relative liver weight was significantly increased in the males and females. At 1.0%, body weight gain was significantly suppressed and the relative kidney weight was increased in the males, and the relative spleen weight was increased in the females. In the histopathologic examination of 8 organs, slight cloudy swelling was found in the renal tubular

epithelium in the males and slight granular degeneration and necrosis were observed in the liver centrilobules of the males and females. However, these findings were also observed in the animals of the control group. Therefore, it is considered that those are reversible adaptive responses, not significant changes (Smyth and Calandra, 1969).

Male and female rats (strain unknown, 10 animals/group) were fed diet containig NPE_{40} at 0, 0.03, 0.1, 0.3, 1.0 and 3.0% (corresponding to 0, 15, 50, 150, 500 and 1,500 mg/kg/day: Talmage's conversion) for 90 days. No significant changes were found in the survival rate, food consumption, body weight, relative weight of the heart, liver, kidney, spleen of the males and females and testis (males). In a histopathologic examination, slight granular degeneration and necrosis were observed in the liver centrilobules of the males at 1% and above (Smyth and Calandra, 1969).

A 2-year oral feeding study of NPE_4 at 0, 40, 200 and 1,000 mg/kg/day in male and female SD rats (35 animals/group) was conducted. At 200 mg/kg/day and above, food consumption was reduced in the females, and body weight gain was suppressed after 12 months but recovered and no difference compared to the body weight of the control group was observed after 24 months. At 1,000 mg/kg/day, and food consumption was reduced and body weight gain was suppressed and then recovered in the males. This suppression of body weight gain was observed in a dietary restriction study, therefore, it was concluded that the suppression of body weight gain was induced by a decrease in food consumption due to reduced feed palatability. Of results of hematology, measurements of the liver, kidney and testis weights and histopathology of 28 tissues, only the increasing tendency of the relative liver weight of males and females was observed at 1,000 mg/kg/day. However, no abnormality was found in a histopathological examination of the liver. From these results, it is considered that the increase in the relative liver weight is not resulted from the toxicity of NPE_4 (Smyth and Calandra, 1969).

Male and female Carworth-Elias rats (36 animals/group) were orally administered NPE_9 at doses of 0, 0.03, 0.09 and 0.27% (corresponding to 0, 15, 45 and 135 mg/kg/day: Talmage's conversion) for 2 years. The liver and kidney weights were measured and a histopathological examination was conducted with 11 tissues. No significant differences were found in food consumption, mortality, relative liver and kidney weights, body weight gain and red blood cell count among any of the NPE-treated groups and the control group in the males and females (Smyth and Calandra, 1969). These results show that the actual NOAEL is not determined up to the highest dose, but it is considered that NOAEL is the highest dose of 0.27% (corresponding to 135 mg/kg /day) in this assessment.

Male and female purebred Beagle dogs (2 animals/group) were orally administered NPE with EO chain length of 4, 6, 9, 15, 20 and 30 at 0, 40, 200 and 1,000 mg/kg/day for 90 days, and general appearance was observed. Body weight and parameters in hematology and serum biochemistry were measured during the administration period. At the end of the administration period, organ weights of the liver, kidney, spleen, heart, brain and testis were measured, and a histopathological examination of 38 tissues was conducted. At 40 mg/kg/day and above, vomiting and salivation (occurring every day for 1 to 3 weeks after administration) were found in the NPE_9-treated groups and focal necrosis in the myocardium was microscopically observed in the NPE_{20}-treated groups. At 200 mg/kg/day and above, vomiting and salivation (occurring every day for 1 to 3 weeks after administration) and an increase in

the relative liver weight were found in the NPE_4-treated groups, vomiting and salivation (often during the administration period) in the NPE_6-treated groups, vomiting and salivation (often for 1 to 3 weeks after administration) in the NPE_{15}-treated groups, and vomiting and salivation (every day for 1 to 3 weeks after administration and very frequently during the administration period) and an increase in the relative liver weight in the NPE_{20}-treated groups. At 1,000 mg/kg/day, reduction of food consumption and suppression of body weight gain (from the beginning of the administration to 4 weeks after) were found in the NPE_4-treated groups, an increase in the relative liver weight in the NPE_6-treated group, vomiting and salivation (during the administration period) in the NPE_{15}-treated groups, and severe vomiting and salivation, reduction of food consumption and suppression of body weight gain (during the administration period) and focal necrosis in the myocardium after the completion of administration was microscopically observed in the NPE_{20}-treated groups. In the NPE_{30}-treated groups, no significant differences from the control group were found at any of the doses (Smyth and Calandra, 1969). These results show that NPE-induced focal necrosis in the myocardium was observed only in NPE_{20}-treated groups.

After feeding of NPE_9 to male and female Beagle dogs (1 animal/group) at 0, 0.04, 0.64 and 5% (corresponding to 0, 10, 160 and 1,250 mg/kg/day: Talmage's conversion) for 90 days, body weight, food consumption, and parameters in hematology were measured, and at autopsy at the end of the administration period, weight measurement of 8 organs and a histopathological examination of 19 tissues were conducted. Only suppression of body weight gain at 0.64% and above was statistically significant (Smyth and Calandra, 1969).

In a 2-year oral feeding study of NPE_4 at 0, 40, 200 and 1,000 mg/kg/day in male and female Beagle dogs (3 animals/group), general appearance was observed, and body weight and parameters in hematology and biochemistry were measured during the administration period. The organ weights of the liver, kidney, spleen, heart, brain and testis were measured, and a histopathological examination of 28 tissues was conducted at the end of the administration period. At 40 mg/kg/day and above, vomiting was observed during the first week after the initiation of the administration in all dose groups. However, the incidence was gradually decreased. At 200 mg/kg/day and above, increases in the relative liver weight and serum alkaline phosphatase activity were found. At 1,000 mg/kg/day, vomiting continued and food consumption and body weight were decreased during the administration period. Whenever high-protein diet was provided, food consumption and body weight were increased. From these results, author reported that the NOEL is 40 mg/kg/day (Smyth and Calandra, 1969).

Male and female dogs (strain unknown, 3 animals/group) were given diet containing NPE_9 at doses of 0, 0.03, 0.09 and 0.27% (corresponding to 0, 8.5, 28 and 88 mg/kg/day) for 2 years. In this test, general appearance was observed, and body weight and parameters in hematology and serum biochemistry were measured during the administration period. The organ weights of the liver, kidney and heart were measured, and histopathological examination of 21 tissues at the termination of administration was conducted. Only the increase in the relative liver weight was found at 0.27%. No abnormality was found in the histopathological examination of the liver (Smyth and Calandra, 1969). From these results, it is considered in this assessment that the increase in the relative liver weight is an

adaptive response, and that the NOAEL is the highest dose of 0.27% (corresponding to 88 mg/kg/day), although actual NOAEL is not determined.

As described above, oral administration of NPE to rats and dogs causes a decrease in food consumption, suppression of body weight gain and an increase in the relative liver weight although the toxicity varies according to the EO chain length. NPE_{20} only caused focal necrosis in the myocardium in dogs but not in rats.

The toxicity varies according to the EO chain length, but main target organ of NPE is considered to be the liver. In the case of repeated dose oral toxicity studies for 2 years on NPE_9, the actual NOAEL is not determined, but it is considered that NOAEL is the highest dose of 0.27% (corresponding to 135 mg/kg /day) in rats, and highest dose of 0.27% (corresponding to 88 mg/kg /day) in dogs.

Table 7-4 Repeated dose toxicity of poly(oxyethylene) nonylphenyl ether

Species sex/number of animals	NPE composition	Route	Period	Dose	Results	Reference
Rat SD Male and female weanling 10 animals/ group	NPE_4 NPE_6 NPE_{15} NPE_{20} NPE_{30}	Oral (via diet)	90 days	0, 40, 200, 1,000 mg/kg/day 0, 200, 1,000, 5,000 mg/kg/day	40 mg/kg/day and above: NPE_6 increase in relative liver weight 200 mg/kg/day and above: NPE_4 increase in relative liver weight NPE_6 increase in absolute liver weight NPE_{15} suppression of body weight gain 1,000 mg/kg/day: NPE_4 increase in absolute liver weight suppression of body weight gain NPE_6 suppression of body weight gain 5,000 mg/kg/day: NPE_{20} suppression of body weight gain	Smyth & Calandra, 1969
Rat Wistar Male and female 95-210 g 30 animals/ control group 15 animals/ treated group	NPE_9	Oral (via diet)	90 days	0, 0.01, 0.04, 0.16, 0.64, 2.5, 5.0% (corre-sponding to 0, 5, 20, 80, 320, 1,250, 2,500 mg/kg/day: Talmage's conversion [1])	0.64% and above: Male and female: significant suppression of body weight gain, increase in relative liver, kidney and brain weight Male: Increasing tendency of relative testis weight 5%: Male and female: increase in mortality	

Species sex/number of animals	NPE composition	Route	Period	Dose	Results	Reference
Rat CFE Male and female young 10 animals/ group	NPE_9	Oral (via diet)	90 days	0, 10, 50, 250, 1,250 mg/kg/day	10 mg/kg/day and above: Female: reduction of food consumption 250 mg/kg/day and above: Male and female: Suppression of body weight gain, hepatocyte degeneration associated with lipid deposit Female: increase in the relative liver weight 1,250 mg/kg/day: Male and female: focal necrosis in hepatocytes and renal tubular necrosis Male: reduction of food consumption, increase in the relative liver weight	
Rat Strain unknown Male and female 45-50 days 10 animals/ group	NPE_9	Oral (via diet)	90 days	0, 0.1, 0.3, 1.0% (corresponding to 0, 50, 150, 500 mg/kg/day: Talmage's conversion)	0.3% and above: Male and female: significant increase in the relative liver weight, a slight granular degeneration and necrosis in the liver centrilobule 1.0%: Male: significant suppression of body weight gain, increase in the relative liver weight, a slight cloudy swelling in the renal tubular epithelium Female: increase in the relative spleen weight	
	NPE_{40}	Oral (via diet)	90 days	0, 0.03, 0.1, 0.3, 1.0, 3.0% (corresponding to 0, 15, 50, 150, 500, 1,500 mg/kg/day: Talmage's conversion)	0.03% and above: Male and female: no significant differences in survival rate, food consumption, body weight, relative weight of the heart, liver, kidney, spleen and testis (male) 1% and above: Male: a slight granular degeneration and necrosis in the liver centrilobule	
Rat SD Male and female weanling 35 animals/ group	NPE_4	Oral (via diet)	2 years	0, 40, 200, 1,000 mg/kg/day	200 mg/kg/day and above: Female: reduction of food consumption, suppression and recovery of body weight gain 1,000 mg/kg/day: Male and female: increase in the relative liver weight Male: reduction of food consumption, suppression and recovery of body weight gain	

Species sex/number of animals	NPE composition	Route	Period	Dose	Results	Reference
Rat Carworth -Elias Male and female 60 g 36 animals/ group	NPE_9	Oral (via diet)	2 years	0, 0.03, 0.09, 0.27% (corresponding to 0, 15, 45, 135 mg/kg/day: Talmage's conversion)	0.03% and above: no significant changes in the food consumption, mortality, relative liver and kidney weight, body weight gain and red blood cell count compared with the control group NOAEL : 0.27% (corresponding to 135 mg/kg/day) (in this assessment)	
Dog Beagle Male and female 2 animals/ group	NPE_4 NPE_6 NPE_{15} NPE_{20} NPE_{30}	Oral (via diet)	90 days	0, 40, 200, 1,000 mg/kg/day	40 mg/kg/day and above: NPE_9 vomiting and salivation (1 to 3 weeks after administration) NPE_{20} focal necrosis in the myocardium 200 mg/kg/day and above: NPE_4 vomiting and salivation (1 to 3 weeks after administration), increase in the relative liver weight NPE_6 vomiting and salivation (during administration period) NPE_{15} vomiting and salivation (1 to 3 weeks after administration) NPE_{20} vomiting and salivation (1 to 3 weeks after administration), increase in the relative liver weight 1,000 mg/kg/day: NPE_4 reduction of food consumption and suppression of body weight gain (the beginning to 4 weeks after) NPE_6 increase in the relative liver weight NPE_{15} vomiting and salivation (during administration period) NPE_{20} severe vomiting and salivation, reduction of food consumption and suppression of body weight gain (during administration period) and focal necrosis in the myocardium NPE_{30} no significant changes in all doses	Smyth & Calandra, 1969

Species sex/number of animals	NPE composition	Route	Period	Dose	Results	Reference
Dog Beagle Male and female 1 animal/group	NPE_9	Oral (via diet)	90 days	0, 0.04, 0.64, 5% (corresponding to 0, 10, 160, 1,250 mg/kg/day Talmage's conversion)	0.64% and above: suppression of body weight gain NOEL: 0.04% (corresponding to 10 mg/kg/day)	
Dog Beagle Male and female 3 animal/group	NPE_4	Oral (via diet)	2 years	0, 40, 200, 1,000 mg/kg/day	40 mg/kg/day and above: Male and female: vomiting 1 week after administration and then relief 200 mg/kg/day: Male and female: slight increases in the relative liver weight and serum alkaline phosphatase activity 1,000 mg/kg/day: Male and female: continuous vomiting during administration period, reduction of food consumption, suppression of body weight gain, moderate increase in the relative liver weight, increase in serum alkaline phosphatase activity NOEL: 40 mg/kg/day (author)	
Dog Strain unknown Male and female 3 animal/group	NPE_9	Oral (via diet)	2 years	0, 0.03, 0.09, 0.27% (corresponding to 0, 8.5, 28, 88 mg/kg /day)	0.27%: Male and female: increase in the relative liver weight NOAEL: 0.27% (corresponding to 88 mg/kg/day) (in this assessment)	

1) Conversion values incited from Talmage (1994).

7.3.5 Reproductive and developmental toxicity

Studies on the reproductive and developmental toxicity of poly(oxyethylene) nonylphenyl ether to experimental animals are summarized in Table 7-5.

To study the reproductive and developmental toxicity of NPE, NPE_9 was orally administered (gavage) to pregnant Mol:WIST rats (19 to 25 animals/group) at 0, 50, 250 and 500 mg/kg/day from gestation day 6 to 15 and to other rats at a dose of 500 mg/kg/day from gestation day 1 to 20, and Caesarian section was conducted on gestation day 21. Significant suppression of maternal body weight gain and a significant decrease in litter size were found at 250 mg/kg/day and above. Significant increases in fetal extra ribs and rudiments of ribs were found at doses of 250 mg/kg/day and above. A significant increase in dilated pelvic cavity was found in the fetuses from the dams at 500 mg/kg/day

from gestation day 1 to 20. In a similar oral administration (gavage) study of NPE_{30} at doses of 0, 50, 250 and 1,000 mg/kg/day, no maternal or development toxicity was found. From these results, it is concluded that the NOAEL of NPE_9 for teratogenicity is 50 mg/kg/day (Meyer et al., 1988).

NPE_9 is used as a contraceptive; therefore, the reproductive and developmental toxicity of NPE_9 via intrauterine administration was studied. NPE_9 was injected into the uterine horns of female SD rats (6 animals/group) at 0, 0.05, 0.10, 0.25 and 0.50 mg/animal on gestation day 1 and Caesarian section was conducted on gestation day 8 to 12, the pregnancy rate and the mean number of living embryos per pregnant rat were significantly decreased at 0.50 mg (Stolzenberg et al., 1976).

In a reproductive and developmental study, NPE_9 was injected into the vaginas of female Wistar rats (5 animals/group) on gestation day 3 or 7, which is before or after implantation, at doses of 0 and 50 mg/kg and Caesarian section was conducted on gestation day 6 to 15 or 8 to 15, the number of normal implantation sites was 12.5 or 13.8 /uterus in the control group with saline injection on gestation day 3 or 7, and the number of resorption sites was 0.72 or 0.32/uterus. The number of normal implantation sites was less than 1/uterus and the number of resorption sites was 11.5/uterus in the 50 mg/kg group on gestation day 3. The number of normal implantation sites was 9.2/uterus and the number of resorption sites was 4.2/uterus in the 50 mg/kg group on gestation day 7. These results show that NPE_9 caused a significant decrease in the number of normal implantation sites and a significant increase in the number of resorption sites. All maternal animals developed acute vaginitis in all treated groups and some of them developed acute endometritis. It is concluded that NPE_9 injected into the vagina has toxic effects on the endometrium, placenta and embryo, resulting in embryotoxicity, which is severer at administration on gestation day 3 than that on gestation day 7 (Tryphonas and Buttar, 1986).

NPE_9 was injected into the vaginas of pregnant Long-Evans rats (30 animals/group) from gestation day 6 to 15 at doses of 0, 4 and 40 mg/kg/day and Caesarian section was conducted on gestation day 20. No significant differences in gross and microscopic appearance of vagina, embryotoxicity and teratogenicity were found between the control and the treated groups (Abrutyn et al., 1982).

From results described above, it is considered that oral and intravaginal administration of NPE_9 produces reproductive and developmental toxicity, but that oral administration of NPE_{30} does not. The NOAEL of NPE_9 via oral administration for reproductive and developmental toxicity is considered to be 50 mg/kg/day in this assessment, based on the results that administration of NPE_9 to pregnant rats caused a significant decrease in litter size and an increase in extra ribs in the fetus (Meyer et al., 1988).

Table 7-5 Reproductive and developmental toxicity of poly(oxyethylene) nonylphenyl ether

Species sex/number of animals	NPE composition	Route	Period	Dose	Results	Reference
Rat Mol: WIST pregnant female 19-25 animals/ group	NPE_9	Oral gavage	Gestation day 6-15 (Caesarian section on gestation day 21) Gestation day 1-20 (Caesarian section on gestation day 21)	0, 50, 250, 500 mg/kg/day 500 mg/kg/day	250 mg/kg/day and above: Dams: significant suppression of the maternal body weight gain and significant decrease in litter size Fetus: significant increase in extra rib 500 mg/kg/day: Fetus: significant increase in dilated pelvic cavity NOAEL : 50 mg/kg/day	Meyer et al., 1988
	NPE_{30}	Oral gavage	Gestation day 6-15 (Caesarian section on gestation day 21) Gestation day 1-20 (Caesarian section on gestation day 21)	0, 50, 250, 1,000 mg/kg/day 1,000 mg/kg/day	50 mg/kg/day and above: Dams: no change Fetus: no change	
Rat SD pregnant female 6 animals/ Group	NPE_9	Intra-vaginal injec-tion	Gestation day 1 (Caesarian section on gestation day 8-12)	0, 0.05, 0.1, 0.25, 0.5 mg/animal	0.5 mg/animal group: decreases in pregnancy rate and mean number of embryos	Stolzenberg et al., 1976
Rat Wistar pregnant female 5 animals/ group	NPE_9	Vaginal	Gestation day 3 (Caesarian section on gestation day 6-15) or Gestation day 7 (Caesarian section on gestation day 8-15)	0, 50 mg/kg	50 mg/kg: decrease in normal implantation sites/uterus, increase in resorption sites/uterus	Tryphonas & Buttar, 1986
Rat Long-Evans pregnant female 30 animals /group	NPE_9	Vaginal	Gestation day 6-15 (Caesarian section on gestation day 20)	0, 4, 40 mg/kg/day	4 mg/kg/day and above: no significant differences in embryotoxicity and teratogenicity	Abrutyn et al., 1982

7.3.6 Genotoxicity

Studies on the genotoxicity of poly(oxyethylene) nonylphenyl ether are summarized in Table 7-6, and the summary of these data is shown in Table 7-7.

Of various kinds of NPEs, NPE_4, NPE_9, $NPE_{9.5}$ and NPE_{12} showed negative in all assays of *in vitro* reverse mutation assays in *Salmonella typhimurium* (Meyer et al., 1988; Shibuya et al., 1985; Texaco Chemical, 1983a, 1991e), a forward mutation assay in cultured rat hepatocytes (Buttar et al., 1986), unscheduled DNA synthesis assays in rat hepatocytes (Buttar et al., 1986; Texaco Chemical, 1984, 1992h,i), cell transformation assays in cultured mouse and rat cells (Buttar et al., 1986; Sheu et al., 1988), *in vivo* micronucleus tests in mouse bone marrow cells (Texaco Chemical, 1991f, 1992j,k), and a mouse dominant lethal test (Buttar et al., 1986). On the other hand, positive results were obtained in an *in vitro* cell transformation assay of NPE_9 in BALB/3T3 cells and mouse fibroblasts (10T1/2) (Long et al., 1982), and in a DNA strand break assay of NPE_4 in human peripheral lymphocytes (Harreus et al., 2002).

Cell transformation assays similar to that with positive results were conducted later to produce negative results (Buttar et al., 1986; Sheu et al., 1988), and the previous positive results were not reproduced. Harreus et al. concluded the results of their DNA strand break assay to be positive; however, a dose dependency analysis showed no statistical significance, and thus, it is considered in this assessment that the results are not conclusive and further study is necessary to confirm the positive results.

In summary, NPE_4, NPE_9, $NPE_{9.5}$ and NPE_{12} showed negative results in all *in vitro* reverse and forward mutations, unscheduled DNA synthesis and cell transformation assays, and *in vivo* micronucleus and dominant lethal tests, except the positive result of the DNA strand break assay of NPE_4. Although genotoxicity studies have been conducted only with NPE_4, NPE_9, $NPE_{9.5}$ and NPE_{12}, it can be considered that NPE is not genotoxic.

Table 7-6 Genetic toxicity of poly(oxyethylene) nonylphenyl ether

	Test system	NPE Composition	Species (Organisms) /Strain	Experimental condition	Concentration /Dose	Results[1)] -S9	Results[1)] +S9	Reference
in vitro	Reverse mutation	NPE_9	*Salmonella typhimurium* TA98, TA100	Preincubation method	100-10,00 µg/plate	-	-	Shibuya et al., 1985
		NPE_9	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	Plate incorporation method	40-2,000 µg/plate	-	-	Meyer et al., 1988
		NPE_4	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	Plate incorporation method	167-10,000µg /plate	-	-	Texaco Chemical, 1983a, 1991e
		$NPE_{9.5}$	TA1538		100-10,000 µg/plate	-	-	
	Forward mutation	NPE_9	Rat liver T51B cells	Cell culture	0-25 µg/mL	-		Buttar et al., 1986

	Test system	NPE Composition	Species (Organisms) /Strain	Experimental condition	Concentration /Dose	Results[1)] -S9 + S9	Reference
	Unscheduled DNA synthesis	NPE_9	Rat hepatocytes	Primary culture	0-50 μg/mL	-	Buttar et al., 1986
		NPE_4 $NPE_{9.5}$ NPE_{12}	Rat hepatocytes	Primary culture	0.1-5,000 0-10 0.005-1.0 μg/mL	- - -	Texaco Chemical, 1984, 1992h, i
	Cell transformation	NPE_9	BALB/3T3 cells	Cell culture	0.1-10 μg/mL	+	Long et al.,1982
			Mouse fibroblast 10T1/2 cells		0.1 μg/mL	+	
		NPE_9	Rat liver T51B cells	Cell culture	25 μg/mL	-	Buttar et al., 1986
		NPE_9	BALB/3T3 cells	Cell culture	0.01-10 μg/mL	-	Sheu et al., 1988
	DNA strand break	NPE_4	Human peripheral lymphocytes	Cell culture	0.15-150 μg/mL	+	Harreus et al., 2002
in vivo	Micronucleus	NPE_4 $NPE_{9.5}$ NPE_{12}	Mouse/ICR bone marrow cells	Intraperitoneal injection	200 75 40 mg/kg	- - -	Texaco Chemical, 1991f, 1992j, k
	Dominant lethal	NPE_9	Mouse germ cells	Intraperitoneal injection	0-60 mg/kg	-	Buttar et al., 1986

1) +: Positive; -: Negative

Table 7-7 Genetic toxicity of poly(oxyethylene) nonylphenyl ether (Summary)

	Mutation	Chromosal aberration	DNA damage
Bacteria	−	ND	ND
Mold / yeast / plant	ND	ND	ND
Insects	ND	ND	ND
Culture cells	−	ND	−
Mammals (*in vivo*)	ND	−	ND

-: Negative , ND: No data available

7.3.7 Carcinogenicity

Studies on the carcinogenicity of poly(oxyethylene) nonylphenyl ether are summarized in Table 7-8.

In a 2-year oral feeding study, NPE_4 was administered to male and female SD rats and Beagle dogs at 0, 1,000 mg/kg/day and below, and NPE_9 was given male and female Carworth-Elias rats at 0, 140 mg/kg/day and below and male and female Beagle dogs at 0, 88 mg/kg/day and below. No dose-depenent carcinogenic responses were found (Smyth and Calandra, 1969).

A concomitant administration study of NPE with a carcinogen was conducted. *N*-Methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), a known potent mutagen, was used as the carcinogen. Drinking water containing 100 mg/L of MNNG and 2,000 mg/L of NPE (EO chain length unknown) was given to male Wistar rats (13 animals/control group, 15 animals/treated group) for 36 weeks. To animals of the control group, drinking water containing 100 mg/L of MNNG only was given. In the control group with MNNG alone, glandular stomach tumors were developed in 8 of the 13 animals (62%) and in the concomitant group with MNNG and NPE, in 12 of the 15 animals (80%). The

incidence of small intestinal tumor was 1/13 in the control group and 7/15 in the concomitant group. These results suggested that NPE enhanced MNNG-induced glandular stomach and small intestinal tumors. It is considered that NPE with high permeability through the membrane works as a carcinogen carrier and helps the carcinogen to pass through the gastric mucosa and the membrane of the target cells, which are barriers to carcinogens (Takahashi et al., 1975).

As described above, NPE_4 and NPE_9 did not induce carcinogenic responses in rats and dogs. Based on the available data with only two NPEs, however, it is not possible to evaluate carcinogenic potential of NPE in general. It seems that NPE (EO chain length unknown) may promote gastrointestinal tumorigenesis induced by a known carcinogen.

No evaluation of carcinogenicity with NPE has been conduced by international and national organizations (ACGIH, 2003; IARC, 2003; U.S. EPA, 2003; U.S. NTP, 2002; Japan Society for Occupational Health, 2003).

Table 7-8 Carcinogenicity of poly(oxyethylene) nonylphenyl ether.

Species sex/number of animals	NPE composition	Route	Period	Dose	Results	Reference
Rat Male and female SD: 35 animals/group Carworth -Elias: 36 animals/group	NPE_4 NPE_9	Oral (via diet)	2 years	0-1,000 0-140 mg/kg/day	No dose-dependent carcinogenic responses	Smyth & Calandra, 1969
Rat Wistar Male 13 animals/ control group 15 animals /treated group	NPE (EO chain length unknown)	Oral (via drinking water)	36 weeks	MNNG [1]: 100 mg/L ±NPE 2,000 mg/L	Tumor incidence MNNG MNNG/NPE glandular stomach 8/13 12/15 small intestine 1/13 7/15	Takahashi et al., 1975
Dog Male and female Beagle 3 animals/ group 3 animals/ Group	NPE_4 NPE_9	Oral (via diet)	2 years	0-1,000 0-88 mg/kg/day	No dose-dependent carcinogenic responses	Smyth & Calandra, 1969

1) MNNG: N-Methyl-N'-nitro-N-nitrosoguanidine.

7.4 Summary of effects on human health

NPE orally administered is absorbed through the gastrointestinal tract. After absorption, NPE is metabolized to nonylphenol and polyethylene glycol with a shortend EO chain and carboxylated

terminal. The excretion ratio varies according to the EO chain length. However, most NPE administered was excreted in the feces and urine within 7 days regardless of EO chain length.

Studies in volunteers suggested that NPE causes primary skin irritation and skin sensitization in humans. However, NPE produces no photosensitization.

NPE has been used as a spermicidal contraceptive. NPE may induce slight local irritation to the vaginal mucosa when used as a spermicidal contraceptive. There has been a report of possible teratogenicity of NPE in which newborns had congenital anomaly. However, epidemiological studies with control groups of no NPE use and using other kinds of contraceptives showed no possibility of anomaly caused by NPE use. It is, therefore, considered that NPE has no teratogenicity in normal use as a contraceptive.

In acute toxicity studies with experimental animals, the lowest LD_{50} values of NPE are 620 mg/kg with oral administration and 1,800 mg/kg with dermal administration in rabbits. No data of inhalation exposure were obtained. Common acute symptoms in animals with oral and percutaneous administration were tremor, lethargy and liver congestion.

NPE-induced irritation varies according to EO chain length, ranging from no irritation to severe skin and eye irritation. Skin irritation was moderate to severe by NPE with EO chain length of 2 to 9, and none to slight by NPE with EO chain length of 10 and more. Eye irritation was moderate to severe by NPE with EO chain length of EO 2 to 15, and none to slight by NPE with EO chain length of EO 30 and more.

NPE_6 produced no sensitization to experimental animals.

In repeated dose toxicity, oral administration of NPE to rats and dogs causes a decrease in food consumption, suppression of body weight gain and an increase in the relative liver weight although the toxicity varies according to the EO chain length. NPE_{20} only caused focal necrosis in the myocardium in dogs but not in rats.

The toxicity varies according to the EO chain length, but main target organ of NPE is considered to be the liver. In the case of repeated dose oral toxicity studies for 2 years on NPE_9, the actual NOAEL is not determined, but it is considered that NOAEL is the highest dose of 0.27% (corresponding to 135 mg/kg /day) in rats, and highest dose of 0.27% (corresponding to 88 mg/kg /day) in dogs.

In reproductive and developmental toxicity, oral and intravaginal administration of NPE_9 has reproductive and developmental toxicity, but oral administration of NPE_{30} does not. Oral administration to pregnant rats caused a significant decrease in litter size, an increase in fetal extra rib and a significant increase in dilated pelvic cavity, and intravaginal administration caused a decrease in the number of normal implantation sites and an increase in the number of resorption sites. The NOAEL of NPE via oral administration for reproductive and developmental toxicity is 50 mg/kg/day for NPE_9.

From results described above, it is considered that oral and intravaginal administration of NPE_9 produces reproductive and developmental toxicity, but that oral administration of NPE_{30} does not. The NOAEL of NPE_9 via oral administration for reproductive and developmental toxicity is considered to be 50 mg/kg/day in this assessment, based on the results that administration of NPE_9 to pregnant rats caused a significant decrease in litter size and an increase in extra ribs in the fetus

In genotoxicity, NPE_4, NPE_9, $NPE_{9.5}$ and NPE_{12} showed negative results in all assays of *in vitro* reverse and forward mutation, unscheduled DNA synthesis and cell transformation assays, and *in vivo* micronucleus and dominant lethal tests except for the positive result of a DNA strand break assay of NPE_4. Although genotoxicity studies have been conducted only with NPE_4, NPE_9, $NPE_{9.5}$ and NPE_{12}, it can be considered that NPE is not genotoxic.

In carcinogenicity studies, NPE_4 and NPE_9 did not induce carcinogenic responses in rats and dogs. Based on the available data with only two NPEs, however, it is not possible to evaluate carcinogenic potential of NPE in general. It has been reported that NPE (EO chain length unknown) promoted gastrointestinal tumorigenesis induced by a known carcinogen. No evaluation of carcinogenicity with NPE has been conduced by international organizations and national organizations.

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ABBREVIATIONS

ACGIH : American Conference of Governmental Industrial Hygienists
ADH : Alcohol dehydrogenase
ALDH : Aldehyde dehydrogenase
ALP : Alkaline phosphatase
ALT : Alanine aminotransferase
ASAT : Aspartate aminotransferase
AST : Aspartate aminotransferase
ATSDR : Agency for Toxic Substances and Disease Registry
BCF : Bioconcentration Factor
BHK : Syrian hamster kidney culture cells
BOD : Biological Oxygen Demand
BUN : Blood urea nitrogen
CAS : Chemical Abstract Services
CAS Online : Chemical Abstract Services Online
CEPA : Commonwealth Environment Protection Agency
CERHR : Center for the Evaluation of Risks to Human Reproduction
CERI : Chemicals Evaluation and Research Institute, Japan
CHL : Chinese hamster lung cells
CHO : Chinese hamster ovary cells
CICAD : Concise International Chemical Assessment Document
Cmax : Maximum concentration of a compound in the blood, etc.
COD : Chemical Oxygen Demand
CPK : Creatinine phosphokinase
DDT : Dichlorodiphenyltrichloroethane
DOC : Dissolved Organic Carbon
EA : Environment Agency of Japan
EC : European Communities
EC_{10} : 10% Effect Concentration
EC_{50} : 50% Effect Concentration
ECB : European Chemicals Bureau
ECETOC : European Centre for Ecotoxicology and Toxicology of Chemicals
EEC : European Economic Communities
EHC : Environmental Health Criteria
EHI : Estimated Human Intake
EPA : Environmental Protection Agency (USA)
EU : European Union
EUSES : Europian Union System for the Evaluation of Substances
FAD : Flavin adenine dinucleotide
FAO : Food and Agriculture Organisation of the United Nations
GABA : g-Aminobutyric acid
GC : Gas chromatography
GGT : γ-Glutamyl transpeptidase
GLP : Good Laboratory Practice
hr : Hour
HSDB : Hazardous Substances Data Bank
IARC : International Agency for Research on Cancer
IC : Industrial Category
IC_{50} : 50% Immobilisation Concentration or 50% Inhibitory Concentration
ILO : International Labour Organisation
IPCS : International Programme on Chemical Safety
IRIS : Integrated Risk Information System
IUCLID : International Uniform Chemical Information Database (existing substances)
Koc : Soil adsorption coefficient Koc
Kow : Octanol/water partition coefficient
LC_{50} : Median Lethal Concentration

LD_{50} : Median Lethal Dose
LDH : Lactate dehydrogenase
LLNA : Local Lymph Node Assay
LOAEL : Lowest Observed Adverse Effect Level
LOEC : Lowest Observed Effect Concentration
LOEL : Lowest Observed Effect Level
MAO : Monoamineoxydase
MATC : Maximum Acceptable Toxic Concentration
MCH : Mean corpuscular hemoglobin
MCV : Mean corpuscular volume
METI : Ministry of Economy, Trade and Industry, Japan
MHLW : Ministry of Health, Labour and Welfare, Japan
min : Minute
MITI : Ministry of International Trade and Industry, Japan
MNLD : Maximum non lethal dose
MOE : Ministry of the Environment, Japan
MOF : Ministry of Finance, Japan
MOS : Margin of Safety
MTD : Maximum Tolerance Dose
NAT2 : *N*-Acetyltransferase
NCI : National Cancer Institute
NICNAS : Australia's National Industrial Chemicals Notification and Assessment Scheme
NIES : National Institute for Environmental Studies, Japan
NITE : National Institute of Technology and Evaluation, Japan
NMR : Nuclear magnetic resonance
NOAEL : No Observed Adverse Effect Level
NOEC : No Observed Effect Concentration
NOEL : No Observed Effect Level
NTE : Neurotoxic esterase
NTP : National Toxicology Program (USA)
NZW : New Zealand White
OECD : Organisation for Economic Cooperation and Development
OPIDN : Organophosphate-induced delayed neuropathy
OR : Odds ratios
ppm : Parts per million
$polA^-$: DNA polymerase$^-$
$polA^+$: DNA polymerase$^+$
pKa : Negative log of the acid dissociation constant
PRTR : Pollutant Release and Transfer Register
RBC : Radiation Biology Center
RAR : Risk Assessment Report
RC : Risk Characterisation
RfC : Reference Concentration
RfD : Reference Dose
RTECS : Registry of Toxic Effects of Chemical Substances
SCE : Sister chromatid exchange
SDH : Sorbitol dehydrogenase
SER : Smooth endoplasmic reticulum
SG : Syrian golden
SIDS : Screening Information Data Set
SLRL-test : Sex-linked recessive lethal test
SOD : Superoxide dismutase
TDI : Tolerable Daily Intake
TE : Toxic equivalent
TLV : Threshold Limit Value
Tmax : Time until a concentration reaches Cmax.
TOXLINE : Toxicology Literature Online
UV : Ultraviolet

v/v : volume per volume
w/w : weight per weight
WHO : World Health Organization
γ-GTP : γ-Glutamyl transpeptidase
δALS : δ-Aminolevulinic acid synthetase