

FOREWORD

INTRODUCTION

5-Ethylidene-2-norbornene

CAS N°: 16219-75-3

SIDS Initial Assessment Report

For

SIAM 14

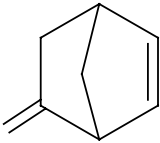
Paris, 26-28th March 2002

1. **Chemical Name:** 5-Ethylidene-2-norbornene
2. **CAS Number:** 16219-75-3
3. **Sponsor Country:** Japan
National SIDS Contact Point in Sponsor Country:
Mr. Yasuhisa Kawamura
Ministry of Foreign Affairs, Economic Affairs Bureau,
Second International Organizations Division
4. **Shared Partnership with:**
5. **Roles/Responsibilities of the Partners:**
 - Name of industry sponsor /consortium Mr. Masahito Goto
Nippon Petrochemicals Co., LTD
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 - Process used
6. **Sponsorship History**
 - How was the chemical or category brought into the OECD HPV Chemicals Programme ? This substance is sponsored by Japan under the ICCA Initiative and is submitted for first discussion at SIAM 14.
7. **Review Process Prior to the SIAM:** The industry consortium collected new data and prepared the updated IUCLID, and draft versions of the SIAR and SIAP. Japanese government peer-reviewed the documents and audited selected studies.
8. **Quality check process:**
9. **Date of Submission:** February 2002
10. **Date of last Update:** November 2003
11. **Comments:** No testing (X) Testing ()

SIDS Initial Assessment Documents were prepared by ExxonMobil Biomedical Sciences, Inc.

ENB Consortium Members include ExxonMobil Chemical Company, INEOS, nv, and Nippon Petrochemicals Co., LTD. The Dow Chemical Company also sponsored this effort, but is not a member of the ENB Consortium.

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	16219-75-3
Chemical Name	5-Ethylidene-2-norbornene
Structural Formula	 <chem>H3C=CH2</chem>
RECOMMENDATIONS	
The chemical is currently of low priority for further work.	
SUMMARY CONCLUSIONS OF THE SIAR	
Human Health	
<p>Ethylidene norbornene (ENB) has a relatively low degree of acute toxicity in several species via oral (LD₅₀: 2276-5071 mg/kg), dermal (LD₅₀: >7168 mg/kg), and inhalation (LC₅₀: 13.3-14.8 mg/L or 2717-3015 ppm) routes of administration. The substance is a mild irritant to skin and is a slight eye irritant to rabbits. There are no data available on skin sensitization. Repeated dose toxicity data include one 28-d oral (gavage) study and 3 subchronic inhalation studies up to 14-wk in duration. In the 28-d repeated oral dose study [TG 407], relative kidney weights were increased in rats of both sexes given 100 mg/kg/d. Histopathological examination revealed increased hyaline droplets in proximal tubular epithelium of the kidney, and hypertrophy of follicular epithelium, as well as a decrease in colloid or irregularly shaped follicles in the thyroids of males given 4 mg/kg/d or more ENB. Hypertrophy of thyroid follicular epithelium and a decrease in colloid were also observed in females given 100 mg/kg/d. The LOAEL of ENB in the 28-d repeated dose study was reported as 4 mg/kg/d for males, and the NOAEL was 20 mg/kg/d for females. Because the male rat kidney effects are consistent with alpha-2u-globulin nephropathy they are not relevant to humans. The mechanism producing thyroid effects in rats has little or no relevance to humans. Therefore, the oral NOAEL for systemic effects other than thyroid and kidney is 20 mg/kg/d, based on reduced body weight of females in the 100 mg/kg group. In inhalation exposure studies in rats, the major toxicity also appeared in the thyroid. For the most recent rat study, the NOAEL was reported to be 5 ppm based on thyroid effects. Other than the thyroid, no exposure related lesions were observed at concentrations up to 149 ppm. Because the increased relative liver weights were seen in both sexes at 149 ppm, the inhalation NOAEL based on effects other than thyroid is considered to be 25 ppm. ENB was not mutagenic with and without an exogenous metabolic activation system in bacteria and mammalian cells <i>in vitro</i> [OECD TG 471, 472, 473]. The chemical induced neither chromosomal aberrations nor sister chromatid exchanges in mammalian cells in culture. It also did not induce dominant lethal mutation in rats. There are two key studies that evaluated reproductive and developmental toxicity. One is an oral reproductive / developmental toxicity screening test [OECD TG 421], and the other is an inhalation development toxicity (teratogenicity) study. In the OECD TG 421 study conducted in rats administered 0, 4, 20, and 100 mg/kg/day of ENB, a prolongation of the gestation period was noted in the 100 mg/kg/d group compared to controls but was within the normal historical range for the laboratory. The implantation and delivery indices were significantly lower in the 100 mg/kg/d group compared to controls. No other changes attributable to the compound were observed in any parameters including the mating index, the fertility index, the gestation index, number of corpora lutea, parturition state and lactation behavior. The total number of births and number of live offspring on day 4 of lactation were decreased in the 100 mg/kg/d group. Among the pups, no other changes attributable to the compound were observed in parameters including the sex ratio, the live birth index, and the viability index on day 4, necropsy findings or external examination. Based on these findings, the oral NOAEL for reproductive/ developmental toxicity was 20 mg/kg/d. A teratogenicity study was conducted in rats exposed by inhalation to 0, 25, 100 and 354 ppm ENB (0, 123, 492, 1740 mg/m³) during days 6-15 of pregnancy. There was no maternal mortality. Maternal body weights, body weight gain, and food consumption were reduced over the exposure period at 100 and 354 ppm, with partial or complete recovery post exposure. Increased relative liver weights were measured for the 100 and 354 ppm groups. There were no increases in the incidence of malformations</p>	

or external and visceral variations. Three skeletal variants (bilobed 12th thoracic centrum, split 12th thoracic centrum, and poorly ossified second sternabra) were increased at 354 ppm, and one (bilobed 12th thoracic centrum) was increased at 100 ppm. Thus, fetotoxicity (skeletal variants) was seen in the 100 and 354 ppm group litters in the presence of maternal toxicity. For both maternal and developmental toxicity, 25 ppm (123 mg/m³) was a NOAEL.

Environment

ENB has been tested for aquatic toxicity in three trophic levels including fish, daphnia and algae. For acute toxicity, a 72hEC₅₀ of 2.61 mg/L and a 96hEC₅₀ of 3.68 mg/L for algae (OECD TG 201, *Selenastrum capricornutum* biomass), 48hEC₅₀ values of 3.34 and 7.3 mg/L for daphnid (OECD TG 202, *Daphnia magna*, immobilization), and for fish a 96hLC₅₀ of 7.0 mg/L (OECD TG 203, *Oryzias latipes*) and of 7.6 mg/L (*Brachydanio rerio*) were available. In chronic studies, a 72-h NOEC of 0.852 mg/L in *Selenastrum* (OECD TG 201, biomass) and a 21-d NOEC of 1.51 mg/L in *Daphnia magna* (OECD TG 211, reproduction) were reported, respectively. The EC₅₀ of multiple studies in different species of fish and in the daphnia and algae were consistent, however algae was the most sensitive among three trophic levels.

Exposure

The production volume of ENB is estimated to be ca. 20,000 tonnes/year in Japan, and ca. 54,000 tonnes/year worldwide; major producers are located in Japan, EU and the United States. ENB is a bicyclic diene compound used as a co-polymer in the production of ethylene-propylene diene monomer (EPDM) elastomers. ENB is produced in a closed system by a limited number of companies. At one company in Japan, ENB was not detected in the wastewater, rain sewer or in the air at the borderline of the Japanese manufacturing plant site. Data from one US manufacturer indicates 979 pounds (445 kg) per year are released as fugitive emissions to the atmosphere during production and storage of ENB. There are no discharges to soil or water (data reported to USEPA Toxic Release Inventory in 2000). The product use pattern can be described as "closed systems; non-dispersive use in the chemical industry as an intermediate." The major use of ENB is in EPDM rubber production, which occurs under controlled conditions. Data from a US and European EPDM plant have been obtained, and McKay Level III fugacity calculations indicate "nanogram" quantities of ENB will be present in water that enters the waste water treatment plant where most will be released to atmosphere prior to discharge. Based on physical/chemical properties [log Pow (3.82), water solubility (80 mg/L), vapor pressure (5.6 hPa), and Henry's Law constant (>5 atm·m³·mol⁻¹)] ENB released in the environment is readily volatile and will rapidly partition to the air (Fugacity level I calculations). ENB is not readily biodegradable (OECD 301C) and is expected to be slightly to moderately mobile in soil based on calculated soil adsorption coefficients (log K_{oc}) ranging from 2.96 to 3.01. Measured BCF of 61-160 in Carp confirm low potential for bioaccumulation (OECD 305C). If released into water, ENB is expected to volatilize to the atmosphere. The atmospheric half-life of ENB is estimated to be 52 minutes. Vapor phase ENB will be degraded in the atmosphere by reaction with photochemically produced hydroxy radicals and ozone molecules.

Occupational exposure to ENB may occur through inhalation and dermal contact with this substance at workplaces where ENB is produced and used. ACGIH and US/OSHA set a ceiling limit at 5 ppm (25 mg/m³) for ENB to protect against eye and skin irritation. Since ENB is produced in a closed system the potential for exposure is primarily during maintenance operations and/or upset conditions. Workplace air monitoring in the EPDM production area has found full shift personnel exposures normally below 0.5 ppm with a range of <0.01 to 1.39 ppm. In the rubber production areas, potential for worker exposures exist in and around the distribution conveyors to the baling pits. Short-term area samples from open points in the system vary from 1 to 5 ppm. The exposure to the general population via the environment is theoretically possible through consumption of fish, which may accumulate this chemical to a limited degree. However, due to the anticipated short residence time of ENB in aquatic ecosystems, chronic exposure of aquatic organisms is not expected. Another possible exposure route may be via migration of the chemical from food packaging polymers. However, estimation of worst case exposures revealed very low exposure levels which were considered insignificant.

NATURE OF FURTHER WORK RECOMMENDED

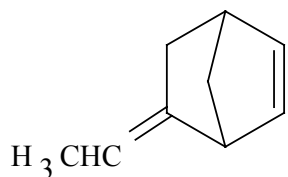
The chemical is currently of low priority for further work. This conclusion is based on negligible human exposure and very low environmental releases.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 16219-75-3
 IUPAC Name: 5-Ethylidene-2-norbornene
 Molecular Formula: C₉H₁₂
 Structural Formula:



Synonyms: 5-Ethylidenebicyclo(2.2.1)hept-2-ene
 ENB
 Ethylidene norbornene
 5-Ethylidene-norbornene

1.2 Purity/Impurities/Additives

Purity: > 98.5% weight/weight.
 Impurities: (a) 2-Vinylbicyclo[2,2,1]hept-5-ene < 0.7 wt %
 (b) Unknown including (a) < 2.0wt%
 Additives: 2,6-Di-tert-butyl-4-methylphenol 100-150 ppm

1.3 Physico-Chemical properties

Table 1. Summary of physico-chemical properties (IUCLID):

Items	Protocol	Results
Melting Point	Unknown	< -80 °C
Boiling Point	Unknown	147.6 °C (at 1,013 hPa)
Vapor Pressure	Unknown	5.6 hPa (at 20 °C)
Partition Coefficient (Log Pow)	Flask shaking method OECD TG 107	3.82
(Log Koc)	Calculated	2.96-3.01
Water Solubility	OECD TG 105	80 mg/L
Relative Density	Unknown	0.9 (at 20 °C)
pH/pKa		No data
Oxidation/Reduction potential		No data

2 GENERAL INFORMATION ON EXPOSURE

The production volume of 5-Ethylidene-2-norbornene (ENB) is estimated ca. 20,000 tonnes/year in Japan, and ca. 54,000 tonnes/year worldwide; major producers are located in Japan, EU and the United States. ENB is produced in a closed system in Japan. ENB is a bicyclic diene compound used as a co-polymer in the production of ethylene-propylene diene monomer (EPDM) elastomers that are used in the production of synthetic rubber (EPDM Rubber).

In the EPDM reaction, the norbornene double bond co-polymerizes with ethylene and propylene to produce an ethylene-propylene terpolymer that contains the ethylidene double bond as residual unsaturation. The ethylidene bond is subsequently used to provide crosslinking during the curing process. ENB is cleared by the US-FDA for certain applications as listed in 21 CFR under part 177-indirect food additives: Polymers (closures with sealing gaskets for food containers, olefin polymers and rubber articles intended for repeated use).

ENB is manufactured and used in closed systems and has not been detected in the air or wastewater from a sewage treatment plant at its Japanese manufacturing plant site.

2.1 Environmental Exposure and Fate

ENB's production and use as a monomer may result in its release to the environment through various waste streams.

ENB is volatile (vapor pressure 5.6 hPa) and hydrophobic (log Pow 3.82). Water solubility of ENB is 80 mg/L. The substance is not biodegradable, according to the OECD 301C: 0% after 28 days based on BOD and 1% based on GC analysis (MITI, 1992). Measured BCFs of 61-160 in carp (*Cyprinus carpio*) confirm the low potential for ENB to bioaccumulate (MITI, 1992). Using the EPIWIN program, the atmospheric half-life of ENB is estimated to be 52 min and the half-life of reaction with ozone radicals is estimated to be 26 min (EPIWIN, 1999). ENB was not detected in the drainage from the manufacturing plant, the rain sewer or air at the borderline of the manufacturing plant site.

ENB is expected to be slightly to moderately mobile in soil based on calculated soil adsorption coefficients (log Koc) ranging from 2.96 to 3.01. However, if spilled on soil, Fugacity level I calculations suggest that the majority of ENB released to the environment would partition to air. Vapor phase ENB will be degraded in the atmosphere by reaction with photochemically produced hydroxy radicals and ozone molecules. If released into water, ENB is expected to volatilize to the atmosphere (EPIWIN, 1999).

ENB is produced in a closed system by a limited number of companies. Data from one US manufacturer indicates 979 pounds (445 kg) per year are released as fugitive emissions to the atmosphere during production and storage of ENB. There are no discharges to soil or water (data reported to USEPA Toxic Release Inventory in 2000). The product use pattern can be described as "closed systems; non-dispersive use in the chemical industry as an intermediate." The major use of ENB is in EPDM rubber production, which occurs under controlled conditions. Data from a European EPDM plant have been obtained, will be used in a McKay Level III fugacity calculation, and will be presented at the SIAM. Preliminary results indicate "nanogram" quantities of ENB will be present in water that enters the waste water treatment plant where most will be released to atmosphere prior to discharge. ENB will be degraded before it reaches the stratosphere, thus it will have no effect on the protective ozone layer. The fate of ENB in the troposphere during daylight hours will be largely dictated by a degradation process initiated by hydroxyl radicals (OH⁻). ENB is expected to degrade by OH⁻ attack at a relatively rapid rate with a calculated half-life of approximately one hour. ENB can potentially participate in atmospheric photochemical reactions

that could contribute to higher levels of ozone (O₃). In the air, ENB can form peroxy radicals that react with nitrous oxide (NO). This reaction results in a temporary removal of NO that could otherwise participate in a series of chemical reactions, which include a reaction with O₃ to form NO₂ and O₂, thereby reducing O₃ levels. Although several variables influence O₃ levels, a net O₃ increase in the presence of ENB would be dependent on atmospheric ENB levels, which if transient would allow NO used in OH- attack reactions to return to reactions with O₃.

2.2 Human Exposure

2.2.1 Occupational Exposure

Occupational exposure to ENB may occur through inhalation and dermal contact with this substance at workplaces where ENB is produced and used. However, prolonged exposure is unlikely due to the extreme odor of ENB, which has an odor threshold of approximately 0.01 ppm (Kinkead et al., 1971). Dermal contact is minimized through the use of protective equipment. There is minimal potential for contact with residual unreacted ENB in finished products.

There is currently no internationally accepted workplace standard for ENB. The American Conference of Governmental Industrial Hygienists (ACGIH) and US Occupational Safety and Health Administration (OSHA) set a ceiling limit at 5 ppm (25 mg/m³) for ENB to protect against eye and skin irritation (ACGIH, 2001). The 5 ppm ceiling limit was accepted in Canada. Denmark and France adopted a 5 ppm short-term exposure limit (STEL) while Australia, the Netherlands and Switzerland adopted 5 ppm as TWA. Finland uses a 5 ppm TWA and a 10 ppm STEL.

Potential worker exposures to ENB could occur during ENB production and the rubber manufacturing process. ENB is produced in a closed system and therefore the potential for exposure is primarily during maintenance operations and/or upset conditions. Workplace air monitoring in the production area has found airborne concentrations to be less than 0.5 ppm. In the rubber production areas, potential for worker exposures exist in and around the distribution conveyors to the baling pits. Full shift personnel exposure concentrations are normally below 0.5 ppm. Short-term area samples from open points in the system vary from 1 to 5 ppm (unpublished data as reported by ExxonMobil Chemical Company, INEOS, nv, and Nippon Petrochemical Company, 1999).

The estimated human exposure (EHE) in the occupational setting can be calculated using the full-shift sampling data (C_{air} of 0.5 ppm or 2.5 mg/m³), an 8-hr exposure time (t), and standard values for volume of breath (V, m³/hr) and body weight (bw, kg).

$$\begin{aligned} \text{EHE} &= (C_{\text{air}} \times V \times t) / \text{bw} \\ &= (2.5 \text{ mg/m}^3 \times 1.25 \text{ m}^3/\text{hr} \times 8 \text{ hr}) / 70 \text{ kg} \\ &= 0.36 \text{ mg/kg} \end{aligned}$$

2.2.2 Consumer Exposure

Since ENB is used only as a chemical intermediate for synthetic rubber production, consumer exposure is not expected. To support use of EPDM in food contact applications in Europe, the maximum possible migration of ENB into food was calculated with the worst case assumptions. Residual ENB was set at 5 mg/kg (food contact limit for monomers) and 100% migration of ENB was assumed, representing the worst case scenario; maximum migration was calculated to be 0.066 mg ENB/kg food. The specific migration of ENB was also calculated for EPDM seal caps and hoses containing a maximum 12% ENB. Calculations on the migration of ENB were based on an

inlay of a cap for a 1 L bottle (area = 7 cm²) and a tube or hose which holds 1 kg foodstuff (area = 80 cm²). For the bottle cap, the migration was calculated to be 0.00077 mg ENB/kg food for the tube or hose, the migration was calculated to be 0.0088 mg ENB/kg food. Analytical results for actual EDPM test materials showed that residual ENB concentration was less than 5 mg ENB/kg test material. The detection limit by HPLC is 5.0 ug (INEOS, 1999).

2.2.3 Indirect exposure via the environment

The exposure to the general population via the environment through drinking water processed from surface water and through fish is low since ENB is manufactured in closed systems. Although not readily biodegradable, ENB has a low potential for bioconcentration. If released to the environment, ENB will rapidly volatilize to the atmosphere where it undergoes rapid photodegradation (EPIWIN, 1999).

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

There is no available information on toxicokinetics and metabolism of this substance. However, ENB has a log Pow of 3.82, a water solubility of 80 mg/L, a vapor pressure of 5.6 hPa and a molecular weight of 120. Both the log Pow and water solubility favor absorption, and the toxicity seen in acute studies indicates that the compound is absorbed via the oral, dermal and inhalation routes of exposure. The small molecular weight and a log Pow >0 suggest that ENB is likely to be widely distributed, and in studies ENB appears to be widely distributed within the body, as systemic effects are seen in a variety of organs after repeated administration via oral or inhalation dosing, including the kidney, thyroid, and liver. In two repeated dose studies, carried out with unknown purity of ENB, adaptive and reversible changes in the liver indicated metabolism. However in modern studies carried out on ENB of known purity these liver effects were not always seen, so it is uncertain if metabolism occurs in the liver or not. No information regarding excretion of ENB can be extracted from the available data.'

3.1.2 Acute Toxicity

There are nine acute toxicity studies referenced in the dossier, six of which were by inhalation, two were by the oral route and one was dermally applied. The data described in Ballantyne et al (1997a) were assigned a Klimisch code of 1 (reliable without restriction) and selected as the key studies since the analytical purity of the ENB was specified and because they are well reported studies. These data are summarized in Table 2. The acute oral LD₅₀ value was 2276 mg/kg for male rats and 5071 mg/kg for female rats. The acute inhalation LC₅₀ values were 13.3 mg/L (2717 ppm) for male rats and 14.8 mg/L (3015 ppm) for female rats. The dermal LD₅₀ value was greater than 7168 mg/kg for male and female rabbits.

Ethylidene norbornene (ENB) has a relatively low degree of acute toxicity in several species via oral, dermal, and inhalation routes of administration.

Table 2. Acute toxicity of 5-ethylidene-2-norbornene

Route	Species	Values	Type	References
Oral	Rat	2276 mg/kg (male) 5071 mg/kg (female)	LD ₅₀	Ballantyne, B. et al. (1997a)
Inhalation	Rat	13.3 mg/L (male) (2717 ppm) 14.8 mg/L (female) (3015 ppm)	LC ₅₀	Ballantyne, B. et al. (1997a)
Dermal	Rabbit	> 7168 mg/kg (male & female)	LD ₅₀	Ballantyne, B. et al. (1997a)

There are two skin irritation and two eye irritation studies referenced in the dossier. The most recent studies in rabbits were selected as the key studies since the analytical purity of ENB was specified (Ballantyne et al., 1997a). In the skin irritation study, a volume of 0.5 ml undiluted ENB was applied to the shaved dorsal trunk skin of 6 rabbits. Material was maintained in contact with the skin for 4-hr under an occlusive dressing and then the test site was wiped moist gauze to remove excess ENB. Sites of application were inspected for signs of inflammation and injury at 1 hr and 1,2,3,7, and 10 days. Erythema and edema were scored and recorded on a 5 point scale (0= no reaction, 4= severe reaction). There was mild to moderate erythema and edema, most marked at 1-2 days and thereafter slowly resolving, completely resolved by 14 days. Desquamation was observed in all animals from day 7. Necrosis was not seen. In the eye irritation study, a volume of 0.1 or 0.01 ml undiluted ENB was applied to the conjunctival sac of one eye of each rabbit. Eyes were examined for signs of ocular and periocular inflammation and injury at 1, 4, and 24hr and 2,3,7 days postinstillation. Iritis and conjunctival irritation were scored according to Draize. Corneal injury was not observed. At 0.1 mL, there was slight conjunctival hyperemia, which began to resolve after 24 hr, was minimal by 2 days and all eyes normal by 7 days. Slight chemosis was at a maximum at 1-4 hr and resolved by 2 days. At 0.01 mL the only findings were mild conjunctival hyperemia and discharge of less than 4 hr duration.

Conclusion

Ethylidene norbornene (ENB) has a relatively low degree of acute toxicity in several species via oral (LD₅₀: 2276-5071 mg/kg), dermal (LD₅₀: >7168 mg/kg), and inhalation (LC₅₀: 13.3-14.8 mg/L or 2717-3015 ppm) routes of administration. The substance is a mild irritant to skin and is a slight eye irritant to rabbits. There are no data available on skin sensitization.

3.1.3 Repeated Dose Toxicity

Studies in Animals

There are five repeated dose toxicity studies referenced in the dossier and four studies are summarized in Table 3. One study was of less than 2 wk duration and served as a range finding study for a subchronic study. The other studies include one 28-d oral (gavage) study and 3 subchronic inhalation studies up to 14-wk in duration. The early studies, with limited monitoring, showed that exposure to high concentrations (e.g., 237 ppm) of ENB vapor produced liver, kidney, and testicular injury in the rat accompanied by a high incidence of mortality, and liver and testicular injury in the dog (Kinkead, E. et al., 1971). These were assigned a Klimisch code of 2 (reliable with restrictions). In the more recent rat studies where the purity of the ENB was measured and exposure concentrations maintained at or below 149 ppm, there is no biochemical or histologic

evidence for testicular effects, minor adaptive and reversible changes in the liver, and male-rat specific kidney effects (Ballantyne, B. et al., US, 1997b). Thyroid effects were evaluated in the rat oral 28-d study (MHLW, Japan, 2001) and the rat inhalation 14-wk study (Ballantyne, B. et al, US, 1997b). These two were selected as key studies and considered to be the most reliable because they were conducted under well-designed protocols, reported analytical purity of the test material, and provide detailed information. They were assigned a Klimisch code of 1 (reliable without restriction) and are described below. The most important observation common to these two studies is the effect of ENB on the thyroid.

A rat 28-day repeated oral dose toxicity test was conducted under the guidelines of the Japanese government (MHLW, Japan, 2001). Sprague-Dawley rats (7 animals/sex/dose with recovery groups of 7 animals/sex for the control and high dose groups held an additional 14-d) received doses of 0, 4, 20 and 100 mg/kg/day for 28-d by oral gavage.

In the 100 mg/kg group females the mean body weight was significantly reduced at dosage termination but not in the recovery group. Food consumption was not affected. These changes were not found at the end of recovery period. Urinalysis revealed an increase in the number of animals with protein-positive urine and a decrease in water consumption in males given 100 mg/kg. These changes were also not found at the end of recovery period. In the hematological examination, no effect was observed after administration of ENB. In the blood biochemical examination, 20 and 100 mg/kg group males showed a decrease in alpha-1 globulin level. However, these changes were not found at the end of the recovery period. In high dose males there was increased brain/body wt ratio and increased kidney/body wt ratio; both effects were absent in recovery males. At autopsy, pale discoloration of the kidneys was observed in males given 100 mg/kg, and histopathology showed increased hyaline droplets in renal tubule epithelium of all male rats in the 20 and 100 mg/kg groups. Histopathological examination of the thyroid indicated hypertrophy of follicular epithelium, as well as decrease in colloid or irregular shape of follicles in males given 4 mg/kg or more. Hypertrophy of follicular epithelium and decrease in colloid were also observed in females given 100 mg/kg. There were no effects on the testes. The NOAEL of ENB for repeated dose toxicity was reported to be less than 4 mg/kg/day for males (based on thyroid effects at 4 mg/kg and kidney effects at 20 mg/kg) and 20 mg/kg/day for females (based on thyroid effects at 100 mg/kg). However, the male rat kidney effects are consistent with alpha-2u-globulin nephropathy and are not relevant to humans (IARC, 1998). The thyroid effects are also likely to be a species specific effect not relevant to humans (IARC, 1998). For these reasons, the oral NOAEL based on systemic effects other than thyroid and kidney is 20 mg/kg/d, based on reduced body weight of females in the 100 mg/kg/d group.

The 9-d inhalation study was conducted as a range-finder for an inhalation subchronic study (Ballantyne et al., 1997b). Groups of CDF rats (10/sex/dose) were exposed to analytically measured ENB vapor concentrations of 0, 52, 148, or 359 ppm (0, 255, 732, or 1763 mg/m³, respectively) for 6 hr/day for 5 days, a 2 day rest period, then an additional 4 exposure days. The 14-wk inhalation study of ENB was conducted in CDF rats (15/sex/dose) exposed to analytically measured ENB vapor concentrations of 0, 4.9, 24.8, or 149 ppm (0, 24, 122, 732 mg/m³, respectively) for 6 hr/day, 5 days/week for 14 weeks (a total of 65 exposures) (Ballantyne, B. et al., 1997b).

No deaths occurred during the 9-d range-finding study. Thyroid gland weight was increased for males of the 148 and 359 ppm exposure groups. Vacuolar depletion of thyroid colloid was observed in all male and female exposure groups. Thyroid morphometry showed a statistically significant dose-related decrease in thyroid colloid. Liver weights increased in a dose-related fashion for all exposure groups, and histopathology examination indicated enlargement of hepatocytes in all exposed male groups. Kidney weights were slightly increased for males of all groups, and the 359 ppm exposed females.

Table 3. Repeated dose toxicity of 5-ethylidene-2-norbornene

Route	Species	Period	Doses	Reported NOAEL mg/kg/d or mg/m ³	Toxic effects seen at higher doses	Reference
Oral	Rat	28-d	0,4, 20, 100 mg/kg	<4 (M) 20 (F)	kidney thyroid	MHLW, Japan (2001)
Inhalation	Rat	88-d	0, 300, 442, 1165 mg/m ³ (0, 61, 90, 237 ppm)	300(F) <300(M)	liver kidney testis	Kinkead (1971)
	Dog	89-d	0,108, 300, 457 mg/m ³ (0, 22, 61, 93 ppm)	108	liver testis	Kinkead (1971)
	Rat	14-wk	0,24, 122, 732 mg/m ³ (0, 4.9, 24.8, 149 ppm)	24 (thyroid) 122 (other)	liver kidney thyroid	Ballantyne (1997b)

No deaths occurred during the 14-wk study. Overall, toxicity was greater in males than in females. Clinical chemistries were unaffected with the exception of reduced tri-iodothyronine (T3) uptake in males of the 24.8 and 149 ppm groups at 14 wk by not after the 4-wk recovery. T3 uptake was slightly reduced (not statistically significant) in male rats of the 4.9 ppm exposed group only at 14 wk. Urine osmolality and creatinine were decreased in males of the 149 ppm group at wk 14, but not in the recovery animals. Urine protein was increased in the 149 ppm exposed females at 14 wk and after the 4-wk recovery. Relative kidney weights increased in all groups of males and the females exposed to 149 ppm ENB. Relative kidney weights were increased (not statistically significant) in 149 ppm exposed males at the end of the 4-wk recovery period. Relative liver weight was increased in 149 ppm exposed males, and also at the end of the recovery period. Relative liver weight was increased in 149 ppm exposed females only after the recovery period. The following organ weights were unaffected: adrenal glands, brain, heart, pituitary gland, spleen, thymus, thyroid gland, and lungs. Principal target organ effects were to the thyroid gland, which showed an exposure concentration-related, but not exposure time-related, depletion of follicular colloid that resolved during the recovery period in all exposed groups except the 4.9 ppm exposed females. There was light microscopic evidence for a hypertrophic and hyperplastic response in the follicular epithelium that resolved more slowly. The thyroid colloid depletion was a graded effect without a clear no-effect concentration, but was not accompanied by any clinical or clear biochemical evidence for thyroid dysfunction. The depletion was generally not seen in the recovery animals. A no-effect concentration of 4.9 ppm (24 mg/ m³) was established for the follicular cytological effects. Other than the thyroid gland, no exposure related lesions were observed following histopathology, therefore, if the thyroid effects are not considered, the NOAEL is 24.8 ppm (122 mg/ m³) based on changes in organ weights and urinalysis findings observed at 149 ppm (732 mg/m³).

Studies in Humans

There is no available information on human toxicity.

Conclusion

The NOAEL for repeated oral dose toxicity in rats is considered to be 20 mg/kg/day for male and female rats, based on the 28-d oral toxicity study. Based on the 14-wk inhalation study, the NOAEL

for repeated inhalation toxicity in the rat is 24.8 ppm (122 mg/m³). These values are based on systemic effects other than thyroid and kidney, which are not relevant to humans (IARC, 1998).

3.1.4 Mutagenicity

In vitro Studies

ENB has been investigated in *in vitro* tests, and did not induce gene mutation in bacterial systems or chromosomal aberration in mammalian cultured cells, with or without an exogenous metabolic activation system. Several well conducted and reported studies were identified.

Reverse gene mutation assay was conducted by OECD TG 471 and TG 472. The substance was not mutagenic in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 *uvrA* at concentration of ranging from 0.00781 to 0.25 mg /plate, with and without an exogenous metabolic activation system (MHW, Japan, 1998). ENB was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537 and TA1538 at concentrations of 0.001 to 0.1 mg/plate (Ballantyne, 1998).

Chromosomal aberration test by OECD TG 473 was conducted in cultured Chinese hamster lung (CHL/IU) cells. Structural chromosomal aberrations and polyploidy were not induced up to a maximum concentration of 0.050 mg/mL on continuous treatment, and 0.1 mg/ml on short-term treatment, with and without an exogenous metabolic activation systems, respectively (MHW, Japan, 1998). ENB at concentrations ranging from 0.006 to 0.06 mg/mL did not produce increases in chromosome aberrations in Chinese hamster ovary (CHO) cells with or without metabolic activation (Ballantyne, 1998). ENB was not mutagenic in the CHO/HGPRT forward gene mutation assay at concentrations of 0.02 to 0.08 mg/mL (without activation) and 0.02 to 0.10 mg/mL (with activation), and was not mutagenic in the sister chromatid exchange test at concentration of 0.01 to 0.06 mg/mL with and without metabolic activation (Ballantyne, 1998).

In vivo Studies

The rat dominant lethal test showed a no-observed effect concentration for dominant lethality greater than 254 ppm ENB (Neeper-Bradley and Ballantyne, 1996). There are no other available data on genotoxicity *in vivo*.

Conclusion

This substance is not genotoxic with and without an exogenous metabolic activation system in bacteria and mammalian cells.

3.1.5 Carcinogenicity

There are no available data on carcinogenicity.

3.1.6 Toxicity for Reproduction

Studies in Animals

There are two studies referenced in the dossier that evaluated reproductive and developmental toxicity. One is a Preliminary Reproduction Toxicity Screening Test [OECD TG 421], and the other is a development toxicity (teratogenicity) study. Both were conducted under well-designed protocols and give detailed information. These key studies are described below. Also, the dominant lethal study described above produced no effect on the testes of rats exposed to ENB concentrations up to 254 ppm for 5 days (Neeper-Bradley and Ballantyne, 1996)

An OECD reproductive/developmental toxicity screening test was conducted in Sprague-Dawley rats at doses of 0, 4, 20, and 100 mg/kg/day (MHW, Japan 1999). ENB was administered for 46 days in males and from 14 days before mating to day 4 of lactation in female rats. Two male rats in the 100 mg/kg group died. Suppressed body weight gain and decreased food consumption was observed in both sexes in this group. The relative liver weights were increased in the 100 mg/kg male group, and histopathological examination revealed hypertrophy and vacuolation of hepatocytes in these animals. Among dams, prolongation of the gestation period, and a trend for a decrease in the number of implantation and delivery indices were observed in the 100 mg/kg group. No other changes attributable to the compound were observed in any parameters including the mating index, the fertility index, the gestation index, number of corpora lutea, parturition state and lactation behavior. Among offspring, total number of births and number of live offspring on day 4 of lactation were decreased in the 100 mg/kg group. No other changes attributable to the compound were observed in parameters including the sex ratio, the live birth index, the viability index on day 4, necropsy findings or external examination. The NOAEL for reproductive and developmental toxicity is considered to be 20 mg/kg/day for the parental animals and offspring.

A teratogenicity test was conducted in CD (Sprague-Dawley) rats by inhalation of 0, 25, 100 and 354 ppm ENB (0, 123, 492, 1740 mg/m³) during days 6-15 of pregnancy (Neeper-Bradley, et al. 1995). There was no maternal mortality. Maternal body weights, body weight gain, and food consumption were reduced over the exposure period at 100 and 354 ppm, with partial or complete recovery post exposure. Increased relative liver weights were measured for the 100 and 354 ppm groups. Vacuolar depletion of thyroid follicular colloid was present in all groups of pregnant animals, including air-only controls, but the incidence and extent was greater in ENB vapor-exposed rats. There were no effects on tri-iodothyronine (T3), tetra-iodothyronine (T4) or T3 uptake. There were no increases in the incidence of malformations or external and visceral variations. Three skeletal variants (bilobed 12th thoracic centrum, split 12th thoracic centrum, and poorly ossified second sternabra) were increased at 354 ppm, and one (bilobed 12th thoracic centrum) was increased at 100 ppm. Thus, minimal fetotoxicity in the form of skeletal variants was observed in the 100 and 354 ppm group litters in the presence of maternal toxicity. For both maternal and developmental toxicity, 25 ppm (123 mg/m³) was a NOAEL.

Studies in Humans

There is no available information on humans.

Conclusion

The NOAEL for reproductive and developmental toxicity for ENB administered orally is 20 mg/kg/day for the parental animals and offspring. A NOAEL of 25 ppm (123 mg/m³) was established in an inhalation developmental toxicity study for maternal animals and offspring.

3.1.7 Other human health related information

Data on the structurally related substance, vinyl norbornene

ENB and 5-vinyl-2-norbornene (VNB)(CAS No. 3048-64-4) are structural isomers. ENB is used extensively as the third monomer in terpolymer elastomers and VNB as an intermediate in manufacture of ENB and ethylene-propylene-diene monomer (EPDM) rubber. Comparison of the norbornenes shows no significant differences in acute toxicity by the inhalation route and that ENB was slightly more lethally toxic than VNB by the oral route. For both substances there is a statistically significant sex difference with males more susceptible (Ballantyne, B., et al., 1997a). VNB did not produce chromosomal aberrations in an in vivo genotoxicity study (rat bone marrow micronucleus) (Vergnes and Ballantyne, 1998).

Conclusions:

A structurally related compound, VNB, did not produce *in vivo* genotoxicity.

3.2 Initial Assessment for Human Health

Ethylidene norbornene (ENB) has a relatively low degree of acute toxicity in several species via oral (LD₅₀: 2276-5071 mg/kg), dermal (LD₅₀: >7168 mg/kg), and inhalation (LC₅₀: 13.3-14.8 mg/L or 2717-3015 ppm) routes of administration. The substance is a mild irritant to skin and is a slight eye irritant to rabbits. There are no data available on skin sensitization. Repeated dose toxicity data include one 28-d oral (gavage) study and 3 subchronic inhalation studies up to 14-wk in duration. In the 28-d repeated oral dose study [TG 407], relative kidney weights were increased in rats of both sexes given 100 mg/kg/d. Histopathological examination revealed increased hyaline droplets in proximal tubular epithelium of the kidney, and hypertrophy of follicular epithelium, as well as a decrease in colloid or irregularly shaped follicles in the thyroids of males given 4 mg/kg/d or more ENB. Hypertrophy of thyroid follicular epithelium and a decrease in colloid were also observed in females given 100 mg/kg/d. The LOAEL of ENB in the 28-d repeated dose study was reported as 4 mg/kg/d for males, and the NOAEL was 20 mg/kg/d for females. Because the male rat kidney effects are consistent with alpha-2u-globulin nephropathy they are not relevant to humans. The mechanism producing thyroid effects in rats has little or no relevance to humans. Therefore, the oral NOAEL for systemic effects other than thyroid and kidney is 20 mg/kg/d, based on reduced body weight of females in the 100 mg/kg group. In inhalation exposure studies in rats, the major toxicity also appeared in the thyroid. For the most recent rat study, the NOAEL was reported to be 5 ppm based on thyroid effects. Other than the thyroid, no exposure related lesions were observed at concentrations up to 149 ppm. Because the increased relative liver weights were seen in both sexes at 149 ppm, the inhalation NOAEL based on effects other than thyroid is considered to be 25 ppm. ENB was not mutagenic with and without an exogenous metabolic activation system in bacteria and mammalian cells *in vitro* [OECD TG 471, 472, 473]. The chemical induced neither chromosomal aberrations nor sister chromatid exchanges in mammalian cells in culture. It also did not induce dominant lethal mutation in rats. There are two key studies that evaluated reproductive and developmental toxicity. One is an oral reproductive / developmental toxicity screening test [OECD TG 421], and the other is an inhalation development toxicity (teratogenicity) study. In the OECD TG 421 study conducted in rats administered 0, 4, 20, and 100 mg/kg/day of ENB, a prolongation of the gestation period was noted in the 100 mg/kg/d group compared to controls but was within the normal historical range for the laboratory. The implantation and delivery indices were significantly lower in the 100 mg/kg/d group compared to controls. No other changes attributable to the compound were observed in any parameters including the mating index, the fertility index, the gestation index, number of corpora lutea, parturition state and lactation behavior. The total number of births and number of live offspring on day 4 of lactation were decreased in the 100 mg/kg/d group. Among the pups, no other changes attributable to the compound were observed in parameters including the sex ratio, the live birth index, and the viability index on day 4, necropsy findings or external examination. Based on these findings, the oral NOAEL for reproductive/developmental toxicity was 20 mg/kg/d. A teratogenicity study was conducted in rats exposed by inhalation to 0, 25, 100 and 354 ppm ENB (0, 123, 492, 1740 mg/m³) during days 6-15 of pregnancy. There was no maternal mortality. Maternal body weights, body weight gain, and food consumption were reduced over the exposure period at 100 and 354 ppm, with partial or complete recovery post exposure. Increased relative liver weights were measured for the 100 and 354 ppm groups. There were no increases in the incidence of malformations or external and visceral variations. Three skeletal variants (bilobed 12th thoracic centrum, split 12th thoracic centrum, and poorly ossified second sternabra) were increased at 354 ppm, and one (bilobed 12th thoracic centrum) was increased at 100 ppm. Thus, fetotoxicity (skeletal variants) was seen in the 100 and

354 ppm group litters in the presence of maternal toxicity. For both maternal and developmental toxicity, 25 ppm (123 mg/m³) was a NOAEL.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute and chronic toxicity data of ENB to test organisms are summarized in Table 4. All of these studies are reliable without restriction (Klimisch code 1), except the acute and chronic daphnid studies conducted by the Environment Agency of Japan which have a code of 2, reliable with restrictions.

The 96-hr LC₅₀ for Medaka (*Oryzias latipes*) exposed to ENB is 7.00 mg/L with 95% confidence limits of 6.17 and 8.51 mg/L. The 96-hr LC₀ (highest concentration causing 0% mortality) was 4.41 mg/L, and the 96-hr LC₁₀₀ (lowest concentration causing 100% mortality) was 8.51 mg/L (EA Japan, 1999a). Results for Zebra fish (*Brachydanio rerio*) were similar; the 96-hr LC₅₀ was 7.6 mg/L and the 96-hr NOEC was 5.02 mg/L (LISEC, 1999c).

Two studies using an alga (*Selenastrum capricornutum*) showed that growth curves were logistic with logarithmic growth for 48 hours and showed a concentration related response (EA Japan, 1999c; LISEC, 1999a). The 0.852 mg/L (measured concentration) treated group showed similar growth to that of control (98-fold increase after 72 hr) while the 6.81 mg/L group showed obvious inhibition of growth after 24 hr. The intermediate groups (4.14 mg/L, 2.46 mg/L and 1.45 mg/L) showed inhibition and less growth than that of control (EA Japan, 1999c). The 72-hr EC₅₀ for biomass was 2.61 mg/L, which compares favorably to the 96-hr EC₅₀ for algae growth (biomass) was 3.68 mg/L (LISEC, 1999a).

Two acute toxicity studies in the water flea (daphnid) showed comparable results. The EC₅₀ at 24-hr was 3.34 mg/L (95% confidence limits: 2.78-5.00 mg/L), based on immobility. The 48-hr EC₅₀ was 3.34 mg/L (95% confidence limits: 2.78-5.00 mg/L) in one study (EA Japan, 1999b), while the 48-hr EC₅₀ was 7.3 mg/L (98% C.I.: 6-11.2 mg/L) in the other study (LISEC 1999b). The highest concentration which did not immobilize daphnids within 48 hours was 2.8 mg/L.

Table 4. Aquatic toxicity of 5-ethylidene-2-norbornene

Organism	Test duration	Result (mg/L)	Reference
<i>Microorganisms</i> Green algae (<i>Selenastrum capricornutum</i>)	72 hr (cl,s) 96 hr (cl)	EC ₅₀ (bms) =2.61(mc) NOEC (bms) =0.852(mc) EC ₅₀ (bms) =3.68(mc) NOEC (bms) <1.20(mc) EC ₅₀ (gr) =8.93(mc) NOEC (gr) <1.20(mc)	EA Japan (1999c) LISEC (1999a)
<i>Invertebrates</i> Water flea (<i>Daphnia magna</i>)	48 hr (cl, s) 48 hr (cl, s) 21 d (cl, ss)	EC ₅₀ (imm) =7.3(mc) EC ₅₀ (imm) =3.34(mc) LC ₅₀ >2.57 (mc) EC ₅₀ (rep) =2.41 (mc) NOEC (rep) =1.51 (mc)	LISEC (1999b) EA Japan (1999b) EA Japan (1999d)
<i>Fish</i> Medaka (<i>Oryzias latipes</i>) Zebra fish (<i>Brachydanio rerio</i>)	96 hr (cl, ss) 96 hr (cl, ss)	LC ₅₀ =7.00(mc) LC ₅₀ =7.6 (mc)	EA Japan (1999a) LISEC (1999c)

cl ; closed system, op ; open system

s ; static, ss ; semi-static

nc; nominal concentration (actual concentration not measured), mc; measured concentration, nc*; nominal concentration (actual concentration measured and greater than 80% of the nominal)

bms ; biomass, gr ; growth rate, imm ; immobility, rep ; reproduction

One chronic daphnid study was conducted. The cumulative mortality at end of exposure of parent daphnids was 5% in the control group and 20% in the vehicle control groups. In the 2.57 mg/L group, the cumulative mortality at the completion of exposure was 5%, while no death occurred in the 0.307 to 1.51 mg/L groups. The mean time to the first brood production was 8.0 days in the control, the vehicle control and 0.307 to 1.51 mg/L groups. However, it was 9.0 days in the 2.57 mg/L group. The mean cumulative number of offspring produced per parent was 113 in the control group and 85 in the vehicle control group. There was no significant difference from controls with 115, 125, 120, and 100 live offspring per parent in the 1.51, 0.888, 0.516, and 0.307 mg/L groups, respectively. In the 2.57 mg/L group the mean cumulative number of offspring per parent was 47, which was significantly lower than in the control groups. Growth inhibition and hypoactivity was observed in almost all daphnids in the 2.57 mg/L group compared to controls from the second day of exposure. The LC₅₀ of > 2.57 mg/L (14 and 21 days) for parent daphnids was calculated based on the measured concentration. The calculated 21-d EC₅₀ (reproduction) was 2.41 mg/L during 21-d exposure. The NOEC and LOEC for the reproductive performance of parent daphnids during 21-d exposure were considered to be 1.51 and 2.57 mg/L, respectively. Although mortality in the vehicle control group is reported to be within the guideline limits of 20%; this treatment showed the second greatest effect on reproduction; and the total number of live offspring from the vehicle control was 30% less than the total number of offspring produced from the control treatment. Reduction in reproduction is expected to be even lower in the vehicle control group compared to the control

group if calculated based on the total number of offspring of those adults still alive at the end of the test (EA Japan, 1999d).

There is no available information on the toxicity to sediment dwelling organisms.

4.2 Terrestrial Effects

There is no available information.

4.3 Other Environmental Effects

There is no available information.

4.4 Initial Assessment for the Environment

Based on physical/chemical properties [log Pow (3.82), water solubility (80 mg/L), vapor pressure (5.6 hPa), and Henry's Law constant ($>5 \text{ atm}\cdot\text{m}^3\cdot\text{mol}^{-1}$)] ENB released in the environment is readily volatile and will rapidly partition to the air (Fugacity level I calculations). ENB is not readily biodegradable (OECD 301C) and is expected to be slightly to moderately mobile in soil based on calculated soil adsorption coefficients (log Koc) ranging from 2.96 to 3.01. Measured BCF of 61-160 in Carp confirm low potential for bioaccumulation (OECD 305C). If released into water, ENB is expected to volatilize to the atmosphere. The atmospheric half-life of ENB is estimated to be 52 minutes. Vapor phase ENB will be degraded in the atmosphere by reaction with photochemically produced hydroxy radicals and ozone molecules.

ENB has been tested for aquatic toxicity in three trophic levels including fish, daphnia and algae. For acute toxicity, a 72hEC50 of 2.61 mg/L and a 96hEC50 of 3.68 mg/L for algae (OECD TG 201, *Selenastrum capricornutum* biomass), 48hEC50 values of 3.34 and 7.3 mg/L for daphnid (OECD TG 202, *Daphnia magna*, immobilization), and for fish a 96hLC50 of 7.0 mg/L (OECD TG 203, *Oryzias latipes*) and of 7.6 mg/L (*Brachydanio rerio*) were available. In chronic studies, a 72-h NOEC of 0.852 mg/L in *Selenastrum* (OECD TG 201, biomass) and a 21-d NOEC of 1.51 mg/L in *Daphnia magna* (OECD TG 211, reproduction) were reported, respectively. The EC50 of multiple studies in different species of fish and in the daphnia and algae were consistent, however alga was the most sensitive among three trophic levels.

5 RECOMMENDATIONS

The chemical is currently of low priority for further work.

This conclusion is based on negligible human exposure and very low environmental releases.

6 REFERENCES

- ACGIH (2001), Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices, 7th Edition, Cincinnati, OH.
- Ballantyne, B., Myers, R.C., and Klonne, D.R. (1997a). Comparative acute toxicity and primary irritancy of the ethylidene and vinyl isomers of norbornene. *J. Appl. Toxicol.*, 17:211-221.
- Ballantyne, B. Norris, J.C., Dodd, D.E. et al. (1997b). Short-term and subchronic repeated exposure studies with 5-ethylidene-2-norbornene vapor in the rat. *J. of Applied Toxicology* 17: 197-210.
- Ballantyne, B. (1998). In vitro genetic toxicology investigations with 5-ethylidene-2-norbornene. *Toxic Substance Mechanisms* 17(4), 133-151.
- Ballantyne, B. and Cawley, T. (1999). Toxicology Update: 5-ethylidene-2-norbornene. *J. Applied Toxicology*: 19: 291-294.
- Capen, C.C. (1997). Mechanistic Data and Risk Assessment of Selected Toxic End Points of the Thyroid Gland. *Toxicologic Pathology*. 25(1): 39-48.
- Chemical Week (1999), April 7, p. 19.
- Environmental Agency of Japan (1999a), Ecotoxicity testing report (draft), Test No.92068 (acute Medaka)
- Environmental Agency of Japan (1999b), Ecotoxicity testing report (draft), Test No.92066 (acute Daphnia)
- Environmental Agency of Japan (1999c), Ecotoxicity testing report (draft), Test No.92065 (algae inhibition)
- Environmental Agency of Japan (1999d), Ecotoxicity testing report (draft), Test No.92067 (chronic Daphnia)
- EPIWIN (1999). Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.
- Hill, R.N., Erdreich, L.S., Paynter, O.E., Roberts, P.A., Rosenthal, S.L., and Wilkinson, C.F. (1989). Thyroid Follicular Cell Carcinogenesis. *Fund. Appl. Toxicol.* 12:629-697.
- IARC (1998). Consensus report. In: Species Differences In Thyroid, Kidney, And Urinary Bladder Carcinogenesis. IARC Scientific Publications No. 147. International Agency for Research on Cancer, Lyon.
- INEOS (1999). Technical dossier submitted to European Commission Directorate General Industry, dated 11 June, 1999. Request for the re-evaluation of a monomer, PM//REF.N :17110, Belgium.
- IUCLID Data Sheet (1995). 5-ethylidene-8,9,10-trinorborn-2-ene. European Chemicals Bureau - Existing Chemicals.
- Kinkead, E.R., Pozzani, U.C., Geary, D.L., and Carpenter, C.P. (1971). The mammalian toxicity of ethylidenenorbornene [5-Ethylidenebicyclo(2,2,1)hept-2-ene], *Toxicol. Appl. Pharmacol.*, 20: 250-259.

LISEC (1999a) unpublished report, Alga growth inhibition test effect of 5-ethylidene-2-norbornene, report WE-06-236. Genk, Belgium.

LISEC (1999b) unpublished report, acute toxicity of 5-ethylidene-2-norbornene for *Daphnia magna*, report WE-01-214. Genk, Belgium.

LISEC (1999c) unpublished report, acute toxicity of 5-ethylidene-2-norbornene for fish, *Brachydanio rerio*, report WE-03-192. Genk, Belgium.

LISEC (1999d) unpublished report, biodegradation study of 5-ethylidene-2-norbornene, Headspace CO₂ evolution test, report WB-13-013. Genk, Belgium.

MHW, Japan (1998) Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, 6, 579-591. (The reverse mutation and in vitro chromosomal aberration tests were performed by the Hatano Research Institute, Food and Drug Safety Center).

MHW, Japan (1999) Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, 7, 667-676. (The reproductive / developmental toxicity screening test was performed by the Mitsubishi Chemical Safety Institute Ltd.).

MHLW, Japan (2001) Ministry of Health, Labour and Welfare, Toxicity Testing Reports of Environmental Chemicals, 8, 1087-1100. (The 28 days repeated dose toxicity test was performed by the Safety Research Institute for Chemical Compounds Co., Ltd).

MITI Japan 1992. Green Card of "biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan"

Neeper-Bradley, T.L., Ballantyne, B., Losco, P.E., Pritts, I.M., and Neptun, D.A. (1995). Investigation of the developmental toxicity potential of 5-ethylidene-2-norbornene vapor in the CD rat. *International Journal of Occupational Medicine and Toxicology*. 4: 371-381.

Neeper-Bradley, T.L. and Ballantyne, B. (1996). Absence of dominant lethal effects in male CD rats exposed to 5-ethylidene-2-norbornene vapor. *Toxic Substance Mechanisms* 15:389-404

USEPA: U.S. Environmental Protection Agency (1998). Assessment of Thyroid Follicular Cell Tumors. Report EPA/630/R-97/002. Risk Assessment Forum, US Environmental Protection Agency, Washington, DC.

Vergnes, J.S. and Ballantyne, B. (1998) Investigations on the in vitro and in vivo genotoxicity potential of 5-vinyl-2-norbornene. *Journal of Applied Toxicology* 18: 129-142.

ANNEX

The following databases were searched: Medline, Toxline, NIOSH: National Institute for Occupational Safety and Health, HSDB: Hazardous Substance Data Bank, RTECS: Registry of Toxic Effects of Chemical Substances, Reptext and CHEMINFO: Canadian Centre for Occupational Health and Safety. Additional proprietary data were provided by The Dow Chemical Company (former Union Carbide data), ExxonMobil Biomedical Sciences, Inc. Published & Proprietary Collection, the Japanese government, Nippon Petrochemical Company, and INEOS, na.

I U C L I D

Data Set

Existing Chemical	:	ID: 16219-75-3
CAS No.	:	16219-75-3
EINECS Name	:	5-ethylidene-8,9,10-trinorborn-2-ene
EC No.	:	240-347-7
TSCA Name	:	Bicyclo[2.2.1]hept-2-ene, 5-ethylidene-
Molecular Formula	:	C ₉ H ₁₂
Producer related part		
Company	:	ExxonMobil Biomedical Sciences Inc.
Creation date	:	16.12.2003
Substance related part		
Company	:	ExxonMobil Biomedical Sciences Inc.
Creation date	:	16.12.2003
Status	:	
Memo	:	
Printing date	:	16.04.2004
Revision date	:	
Date of last update	:	19.12.2003
Number of pages	:	1
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION**1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR****1.0.3 IDENTITY OF RECIPIENTS****1.0.4 DETAILS ON CATEGORY/TEMPLATE****1.1.0 SUBSTANCE IDENTIFICATION****1.1.1 GENERAL SUBSTANCE INFORMATION**

Purity type :
Substance type : organic
Physical status : liquid
Purity :
Colour :
Odour :

Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
11.02.2000

1.1.2 SPECTRA**1.2 SYNONYMS AND TRADENAMES****5-ethylidene bicyclo(2.2.1)hept-2-ene**

Source : BP Chemicals Limited LONDON
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
31.05.1995

ENB

Source : BP Chemicals Limited LONDON
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
31.05.1995

Ethylidene norbornene

Source : BP Chemicals Limited LONDON
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
31.05.1995

1.3 IMPURITIES

1.4 ADDITIVES**1.5 TOTAL QUANTITY****1.6.1 LABELLING**

18.12.2003

1.6.2 CLASSIFICATION**1.6.3 PACKAGING****1.7 USE PATTERN****1.7.1 DETAILED USE PATTERN****1.7.2 METHODS OF MANUFACTURE****1.8 REGULATORY MEASURES****1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES**

Type of limit : TLV (US)
Limit value : 5 ml/m³
Short term exposure limit value
Limit value : 25 ml/m³
Time schedule :
Frequency : times

Remark : US ACGIH 8 hour time weighted average and 15 minute STEL values. Both are ceiling values.

Source : BP Chemicals Limited LONDON
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

31.05.1995

1.8.2 ACCEPTABLE RESIDUES LEVELS**1.8.3 WATER POLLUTION**

1.8.4 MAJOR ACCIDENT HAZARDS**1.8.5 AIR POLLUTION****1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES****1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS****1.9.2 COMPONENTS****1.10 SOURCE OF EXPOSURE**

- Remark** : ENB is manufactured and used in closed systems. It has a strong distinctive odour with a low olfactory threshold (circa 0.01ppm) and so any break of containment leading to exposure should be readily detected. ENB is used as a speciality monomer in the production of synthetic rubbers (EPDM rubber).
- Source** : BP Chemicals Limited LONDON
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
31.05.1995

1.11 ADDITIONAL REMARKS

- Remark** : This substance was produced at BP Chemicals Antwerp plant. This plant has been transferred to the ownership of International Speciality Chemicals (Inspec) with effect from April 1995.
- Source** : BP Chemicals Limited LONDON
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
16.12.2003

1.12 LAST LITERATURE SEARCH**1.13 REVIEWS**

2.1 MELTING POINT

Value	:	< -80 °C	
Decomposition	:	no, at °C	
Sublimation	:	no	
Method	:		
Year	:		
GLP	:	no data	
Test substance	:		
Source	:	BP Chemicals Limited LONDON EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
31.05.1995			(28)
Value	:	< -80 °C	
Sublimation	:		
Method	:	other: not specified	
Year	:		
GLP	:	no data	
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)	
Test substance	:	5-Ethylidene-2-norbornene (CAS No. 16219-75-3)	
Conclusion	:	Melting point is < -80 Degree C.	
Reliability	:	(2) valid with restrictions	
Flag	:	Critical study for SIDS endpoint	
16.12.2003			(26)

2.2 BOILING POINT

Value	:	= 147.6 °C at 1013 hPa	
Decomposition	:	no	
Method	:		
Year	:		
GLP	:		
Test substance	:		
Remark	:	Decomposition in air begins at around 300 degrees C.	
Source	:	BP Chemicals Limited LONDON EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)	
18.12.2003			(6)
Value	:	= 147.6 °C at 1013 hPa	
Decomposition	:	yes	
Method	:	other: not specified	
Year	:		
GLP	:	no data	
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)	
Test substance	:	5-Ethylidene-2-norbornene (CAS No. 16219-75-3)	
Conclusion	:	Boiling point is 147.6 degree C at 1013 hPa.	
Reliability	:	(2) valid with restrictions	
Flag	:	Critical study for SIDS endpoint	
16.12.2003			(5)

2.3 DENSITY

Type : relative density
Value : = .896 at 20 °C

Remark : (Specific gravity water = 1.00)
Source : BP Chemicals Limited LONDON
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
 16.12.2003 (28)

Type : density
Value : = 893.3 kg/m³ at 20 °C

Source : BP Chemicals Limited LONDON
 EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
 16.12.2003 (6)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : = 5.6 hPa at 20 °C
Decomposition :
Method : other (measured): not specified
Year :
GLP : no data
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Test substance : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Conclusion : Vapor pressure is 5.6 hPa at 20 degree C.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
 16.12.2003 (27)

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : = 3.82 at 25 °C
pH value :
Method : OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year : 1992
GLP : yes
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Remark : Partition coefficient calculated to be 3.67 by EPIWIN model (ExxonMobil Biomedical Sciences, 2000).
Test substance : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
 Source: Tokyo Kasei Kogyo Co. Ltd., TCI grade, Purity: 99.8%.
Conclusion : Log Pow is 3.82.
Reliability : (1) valid without restriction
 Well conducted study, carried out by Chemical Evaluation and Research Institute (CERI), Japan.
Flag : Critical study for SIDS endpoint
 16.12.2003 (23)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in Value	:	Water
	:	= 80 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	of low solubility
Stable	:	
Deg. product	:	
Method	:	OECD Guide-line 105
Year	:	1992
GLP	:	yes
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Remark	:	Solubility calculated to be 21 mg/L at 25 oC by EPIWIN model for surrogate structure in program database (ExxonMobil Biomedical Sciences, 2000). Water accommodated fraction loadings of 50 and 100 mg/L attained in LISEC aquatic toxicity tests.
Test substance	:	5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Source: Tokyo Kasei Kogyo Vo. Ltd., TCI-grade, purity = 99.8%
Conclusion	:	The chemical solubility is 80 mg/L in water at 25 degree C.
Reliability	:	(1) valid without restriction Well conducted study, carried out by Chemical Evaluation and Research Institute (CERI), Japan.
Flag	:	Critical study for SIDS endpoint
16.12.2003		(23)

2.6.2 SURFACE TENSION**2.7 FLASH POINT**

Value	:	= 29 °C
Type	:	closed cup
Source	:	BP Chemicals Limited LONDON EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
31.05.1995		(6)
Value	:	= 38 °C
Type	:	open cup
Source	:	BP Chemicals Limited LONDON EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
31.05.1995		(28)

2.8 AUTO FLAMMABILITY

Value	:	= 272 °C at
Source	:	BP Chemicals Limited LONDON EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
31.05.1995		(6)

2.9 FLAMMABILITY

Result : flammable

Remark : BASED ON APPLICATION OF EU DANGEROUS SUBSTANCES
DIRECTIVE
DEFINITION TO THE CLOSED CUP FLASH POINT

Source : BP Chemicals Limited LONDON
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

31.05.1995

2.10 EXPLOSIVE PROPERTIES

Result : other

Remark : FLAMMABLE LIMITS IN AIR :
Lower limit 0.9%v/v
Upper limit 6.4%v/v

Source : BP Chemicals Limited LONDON
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

31.05.1995

2.11 OXIDIZING PROPERTIES**2.12 DISSOCIATION CONSTANT****2.13 VISCOSITY****2.14 ADDITIONAL REMARKS**

Source : Enichem SpA San Donato Milanese
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

06.11.1998

3.1.1 PHOTODEGRADATION

Type	:	
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	at 25 °C
INDIRECT PHOTOLYSIS		
Sensitizer	:	other: OH radical
Conc. of sensitizer	:	1500000 molecule/cm ³
Rate constant	:	.000000000148 cm ³ /(molecule*sec)
Degradation	:	% after
Deg. product	:	
Method	:	other (calculated): Calculated by AOPWIN ver.1.89
Year	:	2000
GLP	:	no
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Method	:	Method based on the work of R. Atkinson. Calculated SAR result for surrogate structure contained in program database.
Remark	:	For the O ₃ radical the rate constant is 6.3E-16 cm ³ /molecule-sec and the half-life is 26 minutes.
Test substance	:	5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Reliability	:	(2) valid with restrictions EPIWIN is used and advocated by the USEPA for chemical property estimation.
Flag	:	Critical study for SIDS endpoint
19.12.2003		(19)

3.1.2 STABILITY IN WATER

Deg. product	:	
Method	:	OECD Guide-line 111 "Hydrolysis as a Function of pH"
Year	:	1992
GLP	:	no data
Test substance	:	other TS: 5-Ethylidene-2-norbornene(CAS No. 16219-75-3)
Result	:	This chemical is not degraded at pH 4, 7, 9 (at 50 degree C).
Test substance	:	5-Ethylidene-2-norbornene(CAS No. 16219-75-3) Source: Tokyo Kasei Kogyo Co. Ltd. TCI-grade), Purity: >99%
Conclusion	:	This chemical is stable at pH 4,7,9. Hydrolysis is unlikely.
Reliability	:	(1) valid without restriction Well conducted study, carried out by Chemical Evaluation and Research Institute, Japan.
Flag	:	Critical study for SIDS endpoint
16.12.2003		(12) (23)

3.1.3 STABILITY IN SOIL**3.2.1 MONITORING DATA****3.2.2 FIELD STUDIES**

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	
Media	:	other: soil - air - water - suspended sediment - sediment
Air	:	96 % (Fugacity Model Level I)
Water	:	.6 % (Fugacity Model Level I)
Soil	:	3.3 % (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Method	:	other: Calculation
Year	:	2000
Method	:	Mackay level I. This model is based on chemical fugacity. Physical properties input include molecular weight (120.2), melting point (-80 oC, measured value), vapor pressure (5.6 hPa at 20 oC, measured value), partition coefficient (log Pow of 3.82 at 25 oC, measured value), water solubility (80 mg/L 25 oC, measured value). Partitioning to the environment is calculated by the EPIWIN Estimation 3.04 program based on the input parameters.
Result	:	Estimated Distribution and Media Concentration (level I)
		Compartment
		Air 96.0%
		Soil 3.3%
		Water 0.6%
		Sediment <0.1%
		Suspended sediment <0.1%
		Chemical will partition rapidly to air, where it will be rapidly oxidized by OH radicals.
Test substance	:	5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Conclusion	:	This chemical is expected to partition primarily to air.
Reliability	:	(2) valid with restrictions EPIWIN is used and advocated by the US EPA for chemical property estimation.
Flag	:	Critical study for SIDS endpoint
19.12.2003		(18)

3.3.2 DISTRIBUTION**3.4 MODE OF DEGRADATION IN ACTUAL USE****3.5 BIODEGRADATION**

Type	:	aerobic
Inoculum	:	other: activated sludge, fresh
Contact time	:	28 day(s)
Degradation	:	(±) % after
Result	:	other: not readily biodegradable
Deg. product	:	
Method	:	OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year	:	1992
GLP	:	yes
Test substance	:	other TS: 5-Ethylidene-2-Norbornene (CASNo.16219-75-3)

Method	:	Fresh activated sludge. Concentration of test chemical = 100 mg/L. Concentration of activated sludge = 30 mg/L [as the concentration of suspended solid]. Volume of solution = 300 mL. Temperature of incubation = 25°C.	
Result	:	0% by BOD after 28 days, 1% by GC after 28 days Under test condition no biodegradation observed.	
Test substance	:	5-Ethylidene-2-Norbornene (CASNo.16219-75-3) Source: Tokyo Kasei Kogyo Co. Ltd. TCI-grade), Purity: >99%	
Conclusion	:	This chemical is not readily biodegradable.	
Reliability	:	(1) valid without restriction Well conducted study, carried out by Chemical Evaluation and Research Institute, Japan.	
Flag 16.12.2003	:	Critical study for SIDS endpoint	(4)
Type	:		
Inoculum	:	other: activated sludge, fresh, nonacclimated	
Contact time	:	28 day(s)	
Degradation	:	(±) % after	
Result	:		
Deg. product	:		
Method	:	other: ISO/TC 147/SC 5/WG 4 N 182 (1995)	
Year	:	1999	
GLP	:	yes	
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CAS# 16219-75-3)	
Method	:	Water Quality - Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds - methods by analysis of released inorganic carbon in sealed vessels. Fresh activated sludge, nonacclimated. Obtained from Zonhaven waste water treatment plant which receives & treats predominantly domestic sewage. Washed 2x, added to test nutrient medium. Final test medium concentration = 4mg/L solids; 15E3 CFU/mL.	
Result	:	Sodium benzoate-95% degraded by day 14, ENB - no biodegradation by day 28. Degradation based on CO2 produced as a percentage of total carbon present as test material.	
Test condition	:	One hundred mL of inoculated test medium added to 150 mL OBUS flasks. Treatments-blank; sodium benzoate (positive control); ENB; abiotic blank & control. ENB added directly (1.3 microliters) to vessels, sodium benzoate added as aliquot of stock solution. All vessels sealed w/ screw caps, incubated in dark at 100 rpm on shaker. IC analysis performed on days 0, 3, 7, 10, 14, 17, 21, 25 & 28. Samples acidified w/ conc H3PO4, agitated for one hour, then 1 mL aliquots of headspace gas removed and analyzed for CO2 using O.I. carbon analyzer. The mean measured concentration of ENB in the test vessels was 10.99 mg/L. The nominal concentration was 11.57 mg/L. Sodium benzoate concentration was 18.6 mg/L.	
Test substance	:	5-Ethylidene-2-norbornene (CAS# 16219-75-3)	
Conclusion	:	Not degradable under test conditions - <1% @ day 28.	
Reliability	:	(1) valid without restriction	
Flag 16.12.2003	:	Critical study for SIDS endpoint	(17)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 56 day(s) at 25 °C
Concentration :
Elimination :
Method : OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year : 1992
GLP : no
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Method : Test organisms
 Source, supplier, any treatment and breeding method: test organisms (Oryzias latipes and Cyprinus carpio) were supplied from fish firm. The breeding was conducted according to the guideline.

Average body weight: 21.2 g, average total length: 9.2 cm and average lipid content: 4.3% at start of the test.

Test conditions

Stock solution preparation and stability: the stock solution of 1,000 mg/L was prepared by dispersing the mixture of a necessary weight of the test substance and 10 volumes of a dispersant, polyoxyethylene(20) sorbitan monooleate 40, into distilled water in closed system. The concentration of the stock solution to be maintained in closed system at ambient temperature was checked by GC method.

Exposure concentration: The exposure concentrations were set at 0.1 and 0.01 mg/L taking account of acute toxicity value and detection limit of analytical method used. These value were about one hundredth and one thousandth of the 48 h-LC50 value to Oryzias latipes (9.21 mg/L, below), at which the acute toxicity is expected not to affect the bioconcentration behavior of the test substance.

Test system: the stock solution of 400 times the test concentration was mixed with air-saturated dilution water (well water) and introduced into test tank of 100 liter (70cm x 39cm x 35cm height) in closed system. The stock solution was stored in refrigerator to depress volatilization.

Flow rate: 2 ml/min for stock solution, 800 ml/min for dilution water.

Test temperature: 25 ± 2°C

Test: 20 fish per each exposure level were used at start of the test. Three test fish were removed after 2, 4 and 6 weeks exposure and all remaining fish were removed after 8 weeks exposure. Commercially available food at about one percent of body weight of test fish was fed two times a day except the day before collecting the test fish.

Analyses of the test concentration

Schedule of the analyses: The test substance concentration in test water was determined two times a week and that in test organism was done with each of two fish removed after 2, 4, 6 and 8 weeks of exposure.

Analytical method: Instrument: Gas chromatograph-mass spectrometer

Water quality of the test solution during the test:

DO range: 3.0 - 6.0 mg/L and 3.5 - 6.2 mg/L for high and low level test, respectively.

	Temperature range: 24-27? and 24.6-27? for high and low level test, respectively.															
	Recovery test: The recovery rate for analysis of test water: 92.5% The recovery rate for analysis of test fish : 88.2%															
	Depuration test Depuration test was not conducted.															
Result	: Concentration of test substance in water:															
	<table border="0"> <thead> <tr> <th>Nominal conc.</th> <th>2 wk</th> <th>4 wk</th> <th>6 wk</th> <th>8 wk</th> </tr> </thead> <tbody> <tr> <td>0.1mg/L</td> <td>0.105</td> <td>0.105</td> <td>0.103</td> <td>0.103</td> </tr> <tr> <td>0.01mg/L</td> <td>0.00971</td> <td>0.00972</td> <td>0.00979</td> <td>0.00980</td> </tr> </tbody> </table>	Nominal conc.	2 wk	4 wk	6 wk	8 wk	0.1mg/L	0.105	0.105	0.103	0.103	0.01mg/L	0.00971	0.00972	0.00979	0.00980
Nominal conc.	2 wk	4 wk	6 wk	8 wk												
0.1mg/L	0.105	0.105	0.103	0.103												
0.01mg/L	0.00971	0.00972	0.00979	0.00980												
	Bioconcentration factor:															
	<table border="0"> <thead> <tr> <th>Exposure conc.:</th> <th>2 wk</th> <th>4 wk</th> <th>6 wk</th> <th>8 wk</th> </tr> </thead> <tbody> <tr> <td>0.1mg/L (Level 1)</td> <td>160, 153</td> <td>125, 70</td> <td>86, 104</td> <td>70, 99</td> </tr> <tr> <td>0.01mg/L (Level 2)</td> <td>61, 97</td> <td>121, 119</td> <td>159, 86</td> <td>81, 75</td> </tr> </tbody> </table>	Exposure conc.:	2 wk	4 wk	6 wk	8 wk	0.1mg/L (Level 1)	160, 153	125, 70	86, 104	70, 99	0.01mg/L (Level 2)	61, 97	121, 119	159, 86	81, 75
Exposure conc.:	2 wk	4 wk	6 wk	8 wk												
0.1mg/L (Level 1)	160, 153	125, 70	86, 104	70, 99												
0.01mg/L (Level 2)	61, 97	121, 119	159, 86	81, 75												
Test substance	: The concentration of the test substance in test water was maintained at more than 97% of the nominal concentration. Test fish observation: No abnormality in behavior or appearance was noted. : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Source: Tokyo Kasei Kogyo Co., LTD., Lot No. FBT01 Purity: >99 %, kept at 5 °C until use. Stability during use was checked by IR spectrum.															
Conclusion	: BCF of the test substance to fish was 70-160 and 61-159 at level 1 and level 2, respectively.															
Reliability	: (1) valid without restriction The data was approved by the Japanese government.															
Flag 18.12.2003	: Critical study for SIDS endpoint (7)															
Species	: Cyprinus carpio (Fish, fresh water)															
Exposure period	: at °C															
Concentration	:															
BCF	: <= 61 - 160															
Elimination	: no data															
Method	:															
Year	:															
GLP	:															
Test substance	:															
Source 16.12.2003	: MITI Japan															

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : semistatic
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 5.02
LC50 : = 7.6
Method : other: OECD (1992) Guideline 203; Annex to Dir. 92/69/EEC (1992): Part C.1: Acute Toxicity for Fish; USEPA (1996) 712-C-96-118: OPPTS 850.1075
Year : 1999
GLP : yes
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Method : Moving average, Probit and Binomial (TOXDAT method)
Result : Nominal test concentrations: Control, 2.13, 4.70, 10.33, 22.73, and 50 mg/L.
 Mean measured test concentrations: <DL, 1.07, 2.19, 5.02, 11.50, and 39.15 mg/L.

Test condition : Mean measured values used in calculation of LC50. Analytical samples taken at beginning and end of each renewal period. Measured values remained mostly consistent during the exposure period.
 : Test treatments were prepared for each 24 hour renewal by dilution of a 50 mg/L stock solution with reconstituted water. The stock solution was equilibrated for 24 hours prior to use for each renewal by stirring the solution in a sealed glass vessel. Test chambers were 11L borosilicate glass, closed bottles containing 11L of solution. Two replicates of seven organisms were tested per treatment. Analytical methodology was static headspace-gas chromatography (GC) with mass spectrometry. Test temperature was 20.9 to 21.0 C., The pH range was 7.71 to 8.43. The mean dissolved oxygen ranged from 6.82 to 9.21 mg/L. Fish were obtained from de Siervis, Leuven.

Test substance : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
 > 99% purity

Conclusion : 96 hr LC50: = 7.6 mg/L
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint

16.12.2003

(15)

Type : semistatic
Species : Oryzias latipes (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 7 measured/nominal
Limit test :
Analytical monitoring : yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1999
GLP : yes
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Method : Binomial method
 Test fish (Age/length/weight, loading, pretreatment). Obtained from commercial domestic hatcheries

Test conditions, e.g.

Details of test : Semi-static, test media renewed twice per day
 Dilution water source : Tap water after dechlorination by passing through activated carbon.
 Dilution water chemistry : Hardness 52.0 mg/L as CaCO₃ ;pH of 7.5
 Concentrations of 0, 5.21, 7.29, 10.2, 14.3, and 20.0 mg/L
 solubilizer (100 mg/L of hardened castor oil and laboratory water control.
 Stability of test chemical solutions.
 Exposure vessel type : 3L glass beaker without aeration under room light.
 Number of replicates, fish per replicate : 2 replicates, 5 fish per replicate
 Water chemistry in test (O₂, pH) in the control and one concentration where effects were observed.
 Dissolved oxygen reading and pH values were taken daily during 96hr exposure period.
 Dissolved oxygen concentration: 7.0 to 8.0 mg/L
 pH values : 7.1 to 7.4

Test temperature range: Water temperature during expose period: 23.2 to 23.8°C
 Method of calculation mean measured concentrations: Time-weighted mean.

Result

: Nominal concentrations:
 0, 5.21, 7.29, 10.2, 14.3, 20.0 (mg/L)

Measured concentrations:
 n.d, 4.55, 6.35, 9.07, 12.2, 16.8 (mg/L) (0hr)
 n.d, 4.44, 6.16, 7.95, 10.9, 15.7 (mg/L) (16hr)
 n.d, 3.99, 5.79, 8.12, ---, --- (mg/L) (24hr)

Statistical results:
 95% confidence limits = 6.17 to 8.51 mg/L

Mortality of orange killfish (*Oryzias latipes*) exposed to 5-ethylidene-2-norbornene (% mortality)

Measured Concentration (mg/L)	24 hour	48 hour	72 hour	96 hour
Control	0(0)	0(0)	0(0)	0(0)
Solvent Control	0(0)	0(0)	0(0)	0(0)
4.41	0(0)	0(0)	0(0)	0(0)
6.17	0(0)	0(0)	0(0)	1(10)
8.51	2(20)	7(70)	10(100)	10(100)
11.5	10(100)	10(100)	10(100)	10(100)
16.2	10(100)	10(100)	10(100)	10(100)

Lowest test substance concentration causing 100% mortality: 8.51 mg/L

Mortality of controls: No mortality observed during test period.

Abnormal responses: Abnormal swimming is observed behavior at 4.41 to 16.2 mg/L and impossibility of swimming is observed at 8.5 mg/L and higher concentrations.

Reference substances (if used) - results Copper (?) sulfate pentahydrate. LC50 at 96h was 0.56 mg/L.

Any observations, such as precipitation that might cause a difference between measured and nominal values.: No precipitation and color

Test substance	: formation by the test chemical. : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Source: Aldrich Chemical Co., Lot No.07809TQ Purity: 99.2% Vapor Pressure: 5.6 hPa (20°C) Stability during use was checked by IR absorption spectrum; Kept in refrigerator until use.
Conclusion	: The 96 hours LC50 for Medaka (<i>Oryzias latipes</i>) exposed to ENB is 7.00 mg/l from the concentration-response curve, with 95% confidence limits of 6.17 and 8.51 mg/l. The highest 96hLC0 was 4.41 mg/l, and the lowest 96hLC100 was 8.51 mg/l.
Reliability	: (1) valid without restriction Dispersant (HCO40) was used, however its concentration was less than 100 mg/L which TG 203 allowed without causing any toxic symptoms in vehicle control and the exposure concentration and toxicity value of this chemical was lower than the water solubility.
Flag 18.12.2003	: Critical study for SIDS endpoint

(8)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	: static
Species	: <i>Daphnia magna</i> (Crustacea)
Exposure period	: 48 hour(s)
Unit	: mg/l
Analytical monitoring	: yes
Method	: OECD Guide-line 202
Year	: 1999
GLP	: yes
Test substance	: other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Method : Binomial method

Test organisms:

Source, supplier, any pre-treatment, breeding method: Supplied by NIES (Japan); test organisms were cultured in the laboratory.

Age at study initiation: within 24 hr of birth

Control group: Yes.

Test conditions

Stock solutions preparation and stability: A stock solution was prepared by combining an unspecified weight of ENB with 5 volumes (apparently 5 x the mass of ENB) of a dispersant, hardened castor oil (HCO-40) and kneading this mixture thoroughly. The mixture was then diluted with chlorine-free tap water to achieve 1000 mg/L and stirred. A 5000 mg/L HCO-40 dispersant stock solution was also prepared using "diluent water". Test treatments were prepared by mixing a necessary amount of the ENB/HCO-40 stock solution and the HCO-40 stock solution with diluent water, which were then dispensed into the test chambers. Four replicates of five organisms were tested per treatment. Daphnids were not fed during the test. Chemical analysis was performed by HPLC/UV detection method. Test temperature range: 20±1 °C (measured: 19.9-20.3 °C)
Exposure vessel type: 324 mL glass vessels, containing 250 mL of solution closed with a glass lid.

Dilution water source: Dechlorinated tap water.

Dilution water chemistry: Hardness was 52 mg/L as CaCO₃, pH = 7.5

Lighting: room light, 16h:8h light-darkness cycle

Water chemistry in test: The mean dissolved oxygen ranged from 7.9 to 8.8 mg/L; pH= 7.4 to 7.8.

	Feeding: None.																													
	Element (unit) basis: immobility																													
	Test design: Number of replicates=4; individuals per replicate=5;																													
	Concentrations: 0, 0.476, 0.857, 1.54, 2.78, and 5.00 mg/L.																													
	Method of calculating mean measured concentrations: geometric mean.																													
	Exposure period: 48 h.																													
Result	<p>Analytical monitoring: Yes, by HPLC analysis.</p> <p>: Nominal concentrations: 0, 0.476, 0.857, 1.54, 2.78, and 5.00 mg/L Measured concentrations: 0, 0.416, 0.762, 1.36, 2.37, 4.31 mg/L (85.4-88.9% of nominal) Measured concentrations after 48 hours: 0, 0.404, 0.749, 1.32, 2.33, 4.12 mg/L (82.4-87.4 % of nominal) Number (percent) of immobilized Daphnia (including dead daphnids)</p> <table border="0"> <thead> <tr> <th rowspan="2">Nominal Conc. (mg/L)</th> <th colspan="2">Cumulative Number of Immobilized Daphnids.</th> </tr> <tr> <th colspan="2">(percent immobility)</th> </tr> <tr> <td></td> <th>24-hr</th> <th>48-hr</th> </tr> </thead> <tbody> <tr> <td>control</td> <td>0 (0)</td> <td>0 (0)</td> </tr> <tr> <td>solvent control</td> <td>0 (0)</td> <td>0 (0)</td> </tr> <tr> <td>0.476</td> <td>0 (0)</td> <td>0 (0)</td> </tr> <tr> <td>0.857</td> <td>0 (0)</td> <td>0 (0)</td> </tr> <tr> <td>1.54</td> <td>0 (0)</td> <td>0 (0)</td> </tr> <tr> <td>2.78</td> <td>4 (20)</td> <td>4 (20)</td> </tr> <tr> <td>5.00</td> <td>20 (100)</td> <td>20 (100)</td> </tr> </tbody> </table>	Nominal Conc. (mg/L)	Cumulative Number of Immobilized Daphnids.		(percent immobility)			24-hr	48-hr	control	0 (0)	0 (0)	solvent control	0 (0)	0 (0)	0.476	0 (0)	0 (0)	0.857	0 (0)	0 (0)	1.54	0 (0)	0 (0)	2.78	4 (20)	4 (20)	5.00	20 (100)	20 (100)
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Test substance	<p>Results based on nominal concentrations.</p> <p>: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Source: Aldrich Chemical Company, Lot No.07809TQ, Purity: 99.2%, Vapor Pressure: 5.6 hPa (20°C), Stability during use was checked by IR spectra.</p>																													
Conclusion	<p>: EiC50(24 h) = 3.34 mg/L (95% confidence limits:2.78-5.00 mg/L) EiC50(48 h) = 3.34 mg/L (95% confidence limits:2.78-5.00 mg/L) NOECi = 1.54 mg/L Minimum 100% inhibitory concentration: 5.00 mg/L</p>																													
Reliability	<p>: (2) valid with restrictions The experimental design and analytical procedure were well documented. According to the guideline, hardness of diluent water should be a minimum of 140 mg/L as CaCO₃, and the measured hardness of 52 mg/L was low but should have little impact on study reliability. Hardened castor oil (HCO-40) was used at a concentration of 100 mg/L to assist dispersion of the ENB in water.</p>																													
Flag 18.12.2003	<p>: Critical study for SIDS endpoint</p>																													
Type	:																													
Species	: Daphnia magna (Crustacea)																													
Exposure period	: 48 hour(s)																													
Unit	: mg/l																													
EC50	: = 7.3																													
Method	: other: OECD (1984) Guideline 202; Annex to Dir. 92/69/EEC (1992); Part C.2: Acute Toxicity for Daphnia; USEPA (1996) 712-C-96-114: OPPTS 850.1010, ISO 6341 (1989(E))																													
Year	: 1999																													

(9)

GLP	:	yes
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Method	:	Binomial, using TOXDAT software
Result	:	Nominal test concentrations: Control, 3.13, 6.25, 12.5, 25, and 50 mg/L. Mean measured test concentrations: <DL, 1.28, 2.81, 5.97, 11.24, and 26.84 mg/L. Number (percent) of immobilized Daphnia After 24 hr: 2 (10%) immobilized at 5.97 mg/L, All (100%) immobilized at 11.24 and 26.84 mg/L After 48 hr: 4 (20%) immobilized at 5.97 mg/L; All (100%) immobilized at 11.24 and 26.84 mg/L 24 hr EC50 = 7.6 mg/L; 48 hr EC50 = 7.3 mg/L Mean measured values used in calculation of EC50. Analytical samples taken at beginning and end of each renewal period. Measured values remained mostly consistent during the exposure period.
Test condition	:	Test treatments were prepared for each 24 hour renewal by dilution of a 50 mg/L stock solution with reconstituted water. The stock solution was equilibrated for 24 hours prior to use for each renewal by stirring the solution in a sealed glass vessel. Test chambers were 250 mL borosilicate glass, closed bottles containing 250 mL of solution. Two replicates of ten organisms were tested per treatment. Daphnids were not fed during the test. Analytical methodology was static headspace-gas chromatography (GC) with mass spectrometry. Test temperature=20.4 to 20.5o C., The pH range was 8.23 to 8.41. The mean dissolved oxygen ranged from 7.09 to 8.60 mg/L. The test organisms were cultured in the LISEC laboratory.
Test substance	:	5-Ethylidene-2-norbornene (CAS No. 16219-75-3) > 99% purity
Conclusion	:	48 hr EC50 = 7.3 mg/L (98% C.I. - 6-11.2 mg.L) Highest concentration which did not immobilize exposed daphnids within 48 hours was 2.8 mg/L.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint
		18.12.2003

(14)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
Method	:	other: (see freetext)
Year	:	1999
GLP	:	yes
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Method	:	OECD (1984) Guideline 201: Algal Growth Inhibition Test.; Annex to Dir. 92/69/EEC (1992): Part C.3: Method for Determination of Ecotoxicity, Algal Inhibition Test; USEPA (1996) 712-C-96-164: OPPTS 850.5400 - Algal Toxicity, Tiers I and II, ASTM E1218-90. ANOVA, Dunnett's test, (Bruce and Versteeg,1992, Env. Tox. Chem. 11, 1485-1494) (SAS)
Result	:	Nominal test concentrations: 0 (control), 1.25, 2.6, 5.0, 10.2, and 20.2 mg/L. Mean measured test concentrations: <DL, 1.2, 2.26, 4.57, 9.75, and 17.1 mg/L, respectively.

96 hr EbC50: 3.68 mg/L; 95% CI: 2.41-5.61 mg/L

96 hr NOEC (growth): < 1.20 mg/L

96 hr LOEC (growth): 1.20 mg/L

96 hr ErC50: 8.93 mg/L; 95% CI: 8.22-9.70 mg/L

96 hr NOEC (growth): < 1.20 mg/L

96 hr LOEC (growth): 1.20 mg/L

Mean measured values used for calculations.

Analytical samples taken at beginning and end of test. Measured values remained mostly consistent during the exposure period. Algae grew in all diluted 96-hr solutions of maximum inhibition after 9 days of incubation; the results of this type of inhibition determination showed an increase in cell growth, which indicates ENB effect was partially algistatic.

Test condition : Test treatments at each concentration were prepared on an individual basis by adding known amounts of ENB to the respective volume of medium to achieve the nominal test concentrations defined below. All stock solutions were equilibrated for 24 hours prior to use by stirring each solution in individual sealed glass vessels. Closed system test chambers were 23.5 mL borosilicate glass bottles containing 23.5 mL of test solution and tightly closed. Due to test substance volatility, the test included 6 absorption controls and 17 replicates at each test concentration. The absorption control was defined as the algal medium containing the appropriate concentration of the test substance and used to determine the background absorption. At each time period (0, 24, 72 and 96 hours) 3 replicates and one control for each test concentration were used to determine absorption. After 96 hrs, 0.2 mL of test solution with maximum inhibition, i.e., 10 & 20 mg/L ENB, were diluted to 25 ml with fresh nutrient medium to determine whether effects were algistatic or algacidal. These cultures were incubated for nine days, after which absorption was measured. Analytical methodology was static headspace-gas chromatography (GC) with mass spectrometry.

Test temperature=22.5 to 23.3o C., The pH range was 8.91 to 9.89. The algae culture were developed at the LISEC laboratory (ex. CCAP 278/4). Algae used for inoculation of the test cultures were incubated for six days and had a cell density of 4.58 x 10⁶ cells/mL.

Test substance : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
> 99% purity

Conclusion : 96 hr EbC50: 3.68 mg/L; 96 hr ErC50: 8.93 mg/L

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

16.12.2003

(16)

Species : Selenastrum capricornutum (Algae)

Endpoint :

Exposure period : 72 hour(s)

Unit :

Limit test :

Analytical monitoring : yes

Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"

Year : 1999

GLP : yes

Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Method : Test organisms

Laboratory culture
Method of cultivation
Controls

Result	: Nominal concentrations: 0 4.00 6.80 11.6 19.6 33.4 (mg/L) Measured concentrations in mg/L At start of the test (0 hr) n.d 0.852 1.45 2.46 4.14 6.81 At end of the test (72 hr) n.d 0.743 1.22 1.99 3.56 5.76 Unit: Cell density(cell/mL) Biological observations Cell density at each flask at each measuring point Cell density(x10E4 cells/mL) Measured concentration (mg/L)																																			
	<table border="0"> <thead> <tr> <th></th> <th>0 hr</th> <th>24hr</th> <th>48hr</th> <th>72hr</th> </tr> </thead> <tbody> <tr> <td>Control</td> <td>1.0±0.0</td> <td>6.4±0.3</td> <td>43.8±2.7</td> <td>100.6±2.4</td> </tr> <tr> <td>0.852</td> <td>1.0±0.0</td> <td>6.4±0.3</td> <td>42.2±0.5</td> <td>100.0±6.3</td> </tr> <tr> <td>1.45</td> <td>1.0±0.0</td> <td>5.4±0.6</td> <td>35.4±3.6</td> <td>62.3±4.8</td> </tr> <tr> <td>2.46</td> <td>1.0±0.0</td> <td>4.8±0.3</td> <td>24.1±2.7</td> <td>53.5±1.4</td> </tr> <tr> <td>4.14</td> <td>1.0±0.0</td> <td>4.0±0.2</td> <td>14.5±0.7</td> <td>42.1±4.0</td> </tr> <tr> <td>6.81</td> <td>1.0±0.0</td> <td>2.2±0.1</td> <td>2.6±0.2</td> <td>3.4±0.4</td> </tr> </tbody> </table>		0 hr	24hr	48hr	72hr	Control	1.0±0.0	6.4±0.3	43.8±2.7	100.6±2.4	0.852	1.0±0.0	6.4±0.3	42.2±0.5	100.0±6.3	1.45	1.0±0.0	5.4±0.6	35.4±3.6	62.3±4.8	2.46	1.0±0.0	4.8±0.3	24.1±2.7	53.5±1.4	4.14	1.0±0.0	4.0±0.2	14.5±0.7	42.1±4.0	6.81	1.0±0.0	2.2±0.1	2.6±0.2	3.4±0.4
	0 hr	24hr	48hr	72hr																																
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Test condition	: (Each value represents the mean of three sample counts.) Test conditions: Test temperature range : 23±2°C Growth/test medium chemistry : OECD medium Shaking : 100 rpm Dilution water source : OECD medium Exposure vessel type : 100 mL OECD medium in a 500 ml glass flask. Water chemistry in test (pH) in at least one replicate of each concentration: pH = 7.8 at start and 8.6 to 10.4 at end of the test (72 h). Stock solutions preparation: A stock solution was prepared by combining an unspecified weight of ENB with 5 volumes of a dispersant, hardened castor oil (HCO-40) and kneading this mixture thoroughly. The mixture was then diluted with chlorine-free tap water to achieve 1000 mg/L and stirred. A 5000 mg/L HCO-40 dispersant stock solution was also prepared using dilution water. Test treatment were prepared by mixing a necessary amount of the ENB/HCO-40 stock solution with dilution water, which were then dispensed into the test chambers. Light levels and quality during exposure : 4,000 to 5,000 lux, continuous. Test design Number of replicates: triplicate Concentrations: 0, 4.00, 6.80, 11.6, 19.6, 33.4 (mg/L) Initial cell number in cells/mL: 1x10 ⁴																																			
Test substance	: Method of calculating mean measured concentrations: Geometric mean 5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Source: Aldrich Chemical Company, Lot No.07809TQ, Purity: 99.2%, Vapor Pressure: 5.6hPa (20°C), Stability during use was checked by IR absorption spectrum, Kept in refrigerator until use.																																			
Conclusion	: Growth curves were Logarithmic until end of the test (72 h). 4.00 mg/L group showed normal and similar growth to that of control (98-fold increase after 72 hr). 33.4 mg/L group showed obvious inhibition of growth rate after 24 hr. 19.6 mg/L, 11.6 mg/L and 6.80 mg/L groups showed inhibition and less growth rate than that of control.																																			

The 72hr EC50 value of 2.61 mg/L for biomass was calculated based on the measured concentrations.

Reliability : 72hr EC50: 2.61mg/L; NOEC: 0.852mg/L
 : (1) valid without restriction
 The experimental design and analytical procedure were well documented. Hardened castor oil (HCO-40) was used at a concentration of 100 mg/L to assist dispersion of the ENB in water.

Flag : Critical study for SIDS endpoint
 18.12.2003 (10)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species : Daphnia magna (Crustacea)
Endpoint : reproduction rate
Exposure period :
Unit : mg/l
Analytical monitoring : yes
Method : OECD Guide-line 211
Year : 1999
GLP : yes
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No.16219-75-3)

Method : Test organisms
 Source, supplier, any pretreatment, breeding method: Supplied by NIES (Japan); test organisms were cultured in the laboratory.
 Age at study initiation: Juveniles within 24-h of birth.
 Control group: Yes

Test conditions

Stock solutions preparation and stability: A stock solution was prepared by combining an unspecified weight of ENB with 5 volumes (apparently 5 x the mass of ENB) of a dispersant, hardened castor oil (HCO-40) and kneading this mixture thoroughly. The mixture was then diluted with chlorine-free tap water to achieve 1000 mg/L and stirred. A 5000 mg/L HCO-40 dispersant stock solution was also prepared using dilution water. Test treatments were prepared by mixing a necessary amount of the ENB/HCO-40 stock solution and the HCO-40 stock solution with dilution water, which were then dispensed into the test chambers. Four replicates of five organisms were tested per treatment. Daphnids were not fed during the test. Analytical methodology was performed by HPLC/UV detection (details not available in English).

Test temperature range: 19.8 to 20.4 oC

Exposure vessel type: 1.373 L glass beaker

Dilution water source: Dechlorinated tap water

Dilution water chemistry: Hardness 52.0 mg/L as CaCO₃, pH 7.5

Lighting: <1200 lux, 16h:8h light-darkness cycle

Water chemistry in test : DO=8.4 to 8.8 mg/L, pH=7.37.7

Feeding Chlorella vulgaris, 0.1 to 0.2mgC/day/individual

Element (unit) basis: Lethality and reproduction-inhibition concentration

Test design (number of replicates, individuals per replicate, concentrations: Four replicates of five organisms were tested per treatment. Daphnids were fed 0.2mg C (of *Chlorella vulgaris*)/daphnid daily during the test. 1300 mL of test solution was used per test vessel. Test solutions were changed daily. Analytical methodology was performed by HPLC/UV detection (details not available in English).

Method of calculating mean measured concentrations: geometric mean

Exposure period : 21d

Doses were selected based on the 48-h EC_{50} of 3.34 mg/L (acute swimming inhibition) from a previous study (see robust summary 13(a) Report. 92066 Environment Agency of Japan, (1999) and results of a preliminary study prior to this study.

The following three parameters were calculated based on the data obtained in this study:
Calculation of 50% lethal concentration (LC_{50}) for parent *Daphnia* after 14 and 21 days of exposure.
Calculation of 50% reproduction-inhibitory concentration (EC_{50})
Maximum non-toxic concentration ($NOEC$) and minimum toxic concentration ($LOEC$)

Result : Nominal concentrations: 0 (control), 0 (vehicle control), 0.383, 0.651, 1.11, 1.88, 3.20 (mg/L)

Measured concentrations: Time-weighted mean 0, 0, 0.307, 0.516, 0.888, 1.51, 2.57 (mg/L)

Analytical monitoring: By HPLC analysis. 86.7 to 78.2% of nominal concentration at preparation; 82.1 to 71.2% just before the renewal of the test water (after 1 day exposure)

Unit: mg/L

Cumulative number of dead parental *Daphnia* and mortality rate: At completion of exposure, the cumulative mortality rate of parent *Daphnia* was 5% in the control group and 20% in the vehicle control groups. In the 2.57 mg/L group, the cumulative mortality rate at the completion of exposure was 5%, while no death occurred in the 0.307 to 1.51 mg/L groups.

Time to first delivery: The mean time to first delivery was 8.0 days in the 0.307 to 1.51 mg/L groups, showing no difference from control and vehicle control groups. However, it was 9.0 days in the 2.57 mg/L group, which was significantly longer than in the control group.

The mean cumulative young daphnids per parent was 113 in the control group and 85 in the vehicle control group. There was no significant difference from controls with 115, 125, 120, and 100 young daphnids per parent in the 1.51, 0.888, 0.516, and 0.307 mg/L groups, respectively. In the 2.57 mg/L group the mean cumulative number of young fleas per parent was 47, which was lower than in the control groups. Growth inhibition and hypoactivity was observed in almost all daphnids in the 2.57 mg/L group compared to controls from the second day of exposure.

The LC_{50} for parent daphnids was calculated based on the measured concentrations and was > 2.57 mg/L after both 14 and 21 days of exposure.

The EC_{50} was >2.57 mg/L during 14-d exposure and 2.41 mg/L during 21-

	d exposure.
	The NOEC and LOEC for the reproductive performance of parent fleas during 21-d exposure were considered to be 1.51 and 2.57 mg/L, respectively.
Test substance	: 5-Ethylidene-2-norbornene (CAS No.16219-75-3) Source: Aldrich Chemical Company, Lot No.07809TQ, Purity: 99.2%, Stability during use was checked by IR absorption spectrum, kept at refrigerator until use.
Conclusion	: Study results are to be used with restrictions due to the following reasons: 1) According to OECD 211; hardness of diluent water should be a minimum of 140 mg/L as CaCO ₃ . The measured hardness of 52 mg/L was low. 2) Parent animals were not maintained individually, as is recommended in 211 guideline. When parents are maintained individually, the total number of offspring produced per animal alive at the end of the test are used for evaluation of toxicity. This means that juveniles produced by adults that die during the experiment are excluded from the calculations. Since five organisms were maintained per tank, exclusion of the specific juveniles produced by adults that died was not possible, therefore it is not possible to derive reliable NOEC/LOEC values. 3) Although mortality in the vehicle solvent control group is reported to be within the guideline limits of 20%; this treatment showed the second greatest effect on reproduction since the total number of live offspring from the vehicle control was 30% less than the total number of offspring produced from the control treatment. Reduction in reproduction is expected to be even lower in the vehicle control group compared to the control group if calculated based on the total number of offspring of those adults still alive at the end of the test. For these reasons, the validity of the test is borderline. 4) Two separate studies indicate 48 hr acute toxicity EC ₅₀ of 3.34 mg/L and 7.34 mg/L; the results for this chronic study indicate chronic effects at concentrations lower than the acute EC ₅₀ values, which is expected.
Reliability	: (2) valid with restrictions The experimental design and analytical procedure were well documented. According to the guideline, hardness of diluent water should be a minimum of 140 mg/L as CaCO ₃ , and the measured hardness of 52 mg/L was low but should have little impact on study reliability. Hardened castor oil (HCO-40) was used at a concentration of 100 mg/L to assist dispersion of the ENB in water.
Flag 18.12.2003	: Critical study for SIDS endpoint (11)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value :
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10
Vehicle :
Doses : 1.0, 2.0 2.83, 4.0, or 8.0 ml/kg
Method : other: not specified
Year :
GLP : no data
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Result : LD50 (95% confidence limits):
 males, 2276 (1944-2670) mg/kg;
 females, 5071(2876-8915)mg/kg

Clinical signs of toxicity were observed in dose groups of 2.0 mL/kg and greater and include sluggishness, lacrimation, kyphosis, unsteady gait and diarrhea, which appeared within 30-45 min postdosing, The days following dosing, some rats showed piloerection, intermitant tremors and perinasal/periocular encrustaion. Survivors recovered from these effects between 1 and 6 days postdosing. 1.0 mL/kg was a no-effect dose. The only consistent finding at necropsy of rats that died was mottled dark pink or red lungs. No gross pathology was seen in survivors at necropsy. Body weights were unaffected for survivors.

Test condition : No. of animals: 5/sex/dose
 Weight of rats: between 200-300 grams
 Route of administration: Gavage (liquid)
 Doses: 1.0, 2.0 2.83, 4.0, or 8.0 ml/kg
 Post dose observation period: 7 and 14 days

Test substance : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
 Source: Union Carbide Corporation, Institute, West Virginia.
 Purity: >99% as measured flame ionization gas chromatography.

Conclusion : The LD50 values indicate a moderate degree of toxicity. Females had a LD50 value numerically about twice that for males. The marginally significant difference in LD50 values between males and females were due mainly to the greater variability of female to the peroral toxicity of ENB.

Reliability : (1) valid without restriction
 Comparable to guideline study.

Flag : Critical study for SIDS endpoint
 18.12.2003

(2)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Value :
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10

Vehicle	:	
Doses	:	2206, 2813, or 3431 ppm of ENB vapor in air
Exposure time	:	4 hour(s)
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CASNo.16219-75-3)
Result	:	LC50: 4hr LC50 (95% confidence limits): males, 2717 (2318-3184) ppm (13.3 mg/L); females, 3015(2404-3780) ppm (14.8 mg/L) Number of deaths at each dose level: 7 of 10 rats died during exposure to 3431 ppm ENB. All 7 rats that died during the period after exposure to ENB developed hindlimb paresis, and also showed urogenital area wetness, hypothermia, and absent surface righting and toe and tail pinch reflexes. No gross pathology was seen in survivors at necropsy. Body weights were unaffected for all survivors.
Test condition	:	No. of animals: 5/sex/dose Weight of rats: between 194-262 grams (males) and 137-206 grams (females) Route of administration: inhalation Doses: 2206, 2813, or 3431 ppm of ENB vapor in air 4 hours Post dose observation period: 7 and 14 days Exposure duration (for inhalation studies): 4 hours
Test substance	:	5-Ethylidene-2-norbornene (CASNo.16219-75-3) Source: Union Carbide Corporation, Institute, West Virginia. Purity: >99% as measured flame ionization gas chromatography.
Conclusion	:	The 4-hr LC50 value indicates a moderate degree of vapor toxicity. At these high vapor concentration, signs are predominantly those of a central excitatory nature, particularly hyperactivity, tremors, and convulsions.
Reliability	:	(1) valid without restriction Comparable to guideline study.
Flag	:	Critical study for SIDS endpoint
18.12.2003		(2)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 8 ml/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	10
Vehicle	:	
Doses	:	2.0, 4.0, or 8.0 mL/kg
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Result	:	LD50 (95% confidence limits): males and females > 8.0 mL/kg (> 7168 mg/kg)

No rabbits died. Signs of possible systemic effects were few and included almost immediate vocalization on applying the dose, persisting for about 5-15 min. 2 animals showed emaciation, abdominal distension and diarrhea. On removal of the occlusive dressing there was marked erythema and edema in all groups, with the edema persisting for about 1 week. Necrosis

was seen at 7 and 14 days. Desquamation, fissuring, ulceration and alopecia were seen at 7 and 14 days in all groups, with scab formation at 7 days. No gross pathology was observed at necropsy. Male and female rabbits in the 8.0 mL/kg dose group lost weight during the first week with some regain during the second week. The 4.0 mL/kg males lost weight over the whole post-dosing period. All 2.0 mL/kg animals gained weight.

Test condition : No. of animals: 5/sex/dose
Weight of rabbits: between 2-3 kg
Route of administration: Dermal, occlusive covering, 24-hr contact
Doses: 2.0, 4.0, or 8.0 mL/kg
Doses per time period: 24-hr contact
Post-dose observation period: 7 and 14 days

Test substance : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Source: Union Carbide Corporation, Institute, West Virginia.
Purity: >99% as measured flame ionization gas chromatography.

Conclusion : Undiluted ENB is of the slight acute percutaneous systemic toxicity and severe irritancy by single 24-hr contact with skin.

Reliability Flag : (1) valid without restriction
: Critical study for SIDS endpoint

18.12.2003 (2)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : undiluted
Exposure : Occlusive
Exposure time : 4 hour(s)
Number of animals : 6
Vehicle :
PDII :
Result : slightly irritating
Classification :
Method : other: not specified
Year :
GLP : no data
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Result : Average (and range) of scores

Inspection Time	Erythema	Edema
1 hour	1.2 (0-2)	1.5 (1-2)
1 day	2.3 (2-3)	2.2 (1-3)
2 days	2.3 (2-3)	1.7 (1-2)
3 days	1.5 (1-2)	1.0 (0-2)
7 days	1.2 (1-2)	1.0 (1-3)
10 days	0.2 (0-1)	0.0
14 days	0.0	0.0

There was mild to moderate erythema and edema, most marked at 1-2 days and thereafter slowly resolving, completely resolved by 14 days. Desquamation was observed in all animals from day 7. Necrosis was not seen.

Test condition : A volume of 0.5 ml undiluted ENB was applied to the shaved dorsal trunk skin of 6 rabbits. Material was maintained in contact with the skin for 4-hr under an occlusive dressing and then the test site was wiped moist gauze

to remove excess ENB. Sites of application were inspected for signs of inflammation and injury at 1 hr and 1,2,3,7, and 10 days. Erythema and edema were scored and recorded on a 5 point scale (0= no reaction, 4= severe reaction).

Test substance : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Source: Union Carbide Corporation, Institute, West Virginia.
Purity: >99% as measured flame ionization gas chromatography.

Conclusion : ENB is a mild irritant to the skin.

Reliability : (1) valid without restriction
Comparable to guideline study.

Flag : Critical study for SIDS endpoint

18.12.2003 (2)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : undiluted
Dose :
Exposure time :
Comment :
Number of animals : 6
Vehicle :
Result : slightly irritating
Classification :
Method : other: not specified
Year :
GLP : no data
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Result : Mean and range of scores for eye irritation produced by ENB instilled in the rabbit eye

Mean (and range) of scores

Norbornene (volume)	Observation time	Conjunctiva	
		Hyperemia	Chemosis
ENB (0.1 ml)	1 h	1.0 (All I)	0.2 (0-1)
	4 h	1.0 (All 1)	0.7 (0-2)
	24 h	0.3 (0-1)	0.2 (0-1)
	2 days	0.2 (0-1)	0.0
	3 days	0.2 (0-1)	0.0
	7 days	0.0	0.0
ENB (0.01 ml)	1 h	0.7 (0-1)	0.0
	4 h	0.0	0.0
	24 h	0.0	0.0
	2 days	0.0	0.0
	3 days	0.0	0.0
	7 days	0.0	0.0
Norbornene (volume)	Observation time	Conjunctiva	
		Discharge	Iritis
ENB (0.1 ml)	1 h	0.5 (0-1)	0.0
	4 h	0.8 (0-2)	0.0
	24 h	0.2 (0-1)	0.0

	2 days	0.0	0.0
	3 days	0.0	0.0
	7 days	0.0	0.0
ENB (0.01 ml)	1 h	0.3 (0-1)	0.0
	4 h	0.0	0.0
	24 h	0.0	0.0
	2 days	0.0	0.0
	3 days	0.0	0.0
	7 days	0.0	0.0
Test condition	: Iritis or corneal injury were not observed. At 0.1 mL, there was slight conjunctival hyperemia, which began to resolve after 24 hr, was minimal by 2 days and all eyes normal by 7 days. Slight chemosis was at a maximum at 1-4 hr and resolved by 2 days. At 0.01 mL the only findings were mild conjunctival hyperemia and discharge of less than 4 hr duration.		
Test substance	: A volume of 0.1 or 0.01 ml undiluted ENB was applied to the conjunctival sac of one eye of each rabbits. Eyes were examined for signs of ocular and periocular inflammation and injury at 1, 4, and 24hr and 2,3,7 days postinstillation. Iritis and conjunctival irritation were scored according to Draize.		
Conclusion	: Source: Union Carbide Corporation, Institute, West Virginia.		
Reliability	: Purity: >99% as measured flame ionization gas chromatography.		
Flag	: ENB is a slight eye irritant.		
18.12.2003	: (1) valid without restriction		
	: Comparable to guideline study.		
	: Critical study for SIDS endpoint		

(2)

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Type	: Sub-chronic
Species	: rat
Sex	: male/female
Strain	: other: CDF
Route of admin.	: inhalation
Exposure period	: 14 weeks
Frequency of treatm.	: 6 hours/day for 5 days/week
Post exposure period	: 5 male, 5 female per group followed for 4-wk recovery period
Doses	: 0, 4.9, 24.8, 149 ppm (24, 122, 732 mg/m ³)
Control group	: other: yes, filtered air
NOAEL	: = 4.9 ppm
LOAEL	: = 24.8 ppm
Method	: other
Year	:
GLP	: no data
Test substance	: other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Method : Quantitative continuous variables were intercompared between ENB groups and the corresponding air-control groups using Bartlett's homogeneity of variance, analysis of variance, and Duncan's multiple range tests. Non-parametric data were statistically analyzed using the Kruskal-Wallis test followed by the Mann-Whitney U test. A fiducial limit of <0.05(two-tailed)was used as the critical level of significance for all comparisons.

Test Subjects:

Age at study initiation: 7 weeks

No. of animals per sex per dose: 15

Test conditions:

ENB vapor was generated by metering liquid ENB into a heated glass evaporator. A preliminary chamber distribution study was conducted at which ENB concentrations were measured at five positions in a chamber, using 3 or 4 sampling cycles, each of 30 min. During each 6-hr exposure, at least two chamber atmosphere samples were taken each hour and analyzed by calibrated GC.

Vehicle: not applicable

Satellite groups and reasons they were added: Four week recovery group (5 M, 5 F per treatment group) to evaluate reversibility of effects.

Clinical observations performed and frequency: Daily observations for toxic effects. Body weights and food and water consumption measured weekly. Hematology, urinalysis, and serum chemistry including thyroid-related measurements after completion of exposure and recovery periods.

Organs examined at necropsy: Detailed examination for signs of gross pathology. The following organs were weighed: brain, liver, kidneys, lungs, heart, spleen, thymus, pituitary gland, thyroid gland (post fixation), adrenal glands, and testes. Tissues for histological examination were fixed in 10% neutral-buffered formalin, except for eyes and testes which were fixed in Bouin's fluid. Morphometric analysis was conducted on thyroids from 10 males in the control and all exposure groups.

Result

: NOAEL (NOEL) 4.9 ppm (24 mg/m³)
LOAEL (LOEL) 24.8 ppm (122 mg/m³)

No animals died during the study.

Periocular swelling and/or encrustation was seen in females of all groups and males of the 149 ppm group. Corneal dystrophy (linear opacity) was seen with equal frequency in both ENB and air control groups. There were no effects on absolute body weight gain. Body weight gains for the 24.8 and 149 ppm groups of male rats were 13% and 21% lower, respectively, than the controls at the end of the first exposure week, with subsequent recovery.

During the 14-wk exposure, food and water consumption was increased in the 149 ppm group for most periods for males and females and remained increased during the recovery period compared to controls.

Serum chemistry: Other than small changes in certain thyroid hormone concentrations there were no statistically significant effects on serum chemistry. Serum T3 uptake was slightly reduced, with statistical significance, in 24.8 and 149 ppm group males at the 14-wk sacrifice, but not at the recovery sacrifice.

Urinalysis: The only findings were statistically significant decreased urine osmolality (15%) and creatinine (20.8%) at the 14-wk sacrifice, but not the recovery sacrifice, in the male rats exposed to 149 ppm ENB.

Hematology: Males had statistically significantly decreased erythrocyte count, hemoglobin concentration, and hematocrit, with increased MCHC at 149 ppm (732 mg/ m³). Males in the 24.8 ppm (122 mg/ m³) group had decreased erythrocyte count and hematocrit, with increased MCHC. No statistically significant effects were seen in the recovery group.

Necropsy: Relative liver weights were increased only in the 149 ppm males, and no histopathological findings were observed. Relative kidney weights were increased for males of all ENB-treatment groups and females exposed to 149 ppm ENB.

Histology: Other than the thyroid gland, no exposure related lesions were observed. Principal target organ effects were to the thyroid gland, which showed an exposure concentration-related, but not exposure time-related, depletion of follicular colloid that resolved during the recovery period. There was light microscopic evidence for a hypertrophic and hyperplastic response in the follicular epithelium that resolved more slowly. The thyroid colloid depletion was a graded effect without a clear no-effect concentration, but was not accompanied by any clinical or clear biochemical evidence for thyroid dysfunction. The depletion was generally not seen in the recovery animals. A no-effect concentration of 4.9 ppm was established for the follicular cytological effects.

Test substance : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Source: Union Carbide Corporation, South Charleston, West Virginia, Lot No. 1609
Purity: 99%, No compositional changes were measured over the period of the study.

Conclusion : No histopathologic findings, other than in the thyroid gland, were found. A no-effect concentration of 4.9 ppm ENB was established for the follicular cytological effects. The NOAEL for effects other than thyroid is 25 ppm (122 mg/m³).

Reliability : (1) valid without restriction
Well conducted study.

Flag : Critical study for SIDS endpoint
18.12.2003 (3)

Type : Sub-chronic
Species : rat
Sex : male/female
Strain : other: Harlan-Wistar
Route of admin. : inhalation
Exposure period : 88 days
Frequency of treatm. : 7 hours/day for 5 days/week
Post exposure period :
Doses : 0, 61, 90, 237 ppm
Control group : other: yes, filtered air
Method : other
Year :
GLP : no data
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Method : Body weight changes and kidney and liver weight as percentage of body weight of all animal groups were intercompared statistically by use of the following tests: Bartlett's homogeneity of variance, analysis of variance, and Duncan's multiple range tests. The last test was used if F for analysis of variance was significantly high in order to delineate which group differed from the control. If the Bartlett test indicated heterogeneous variances, the t-test was used for each group versus the control. A fiducial limit of <0.05 was used as the critical level of significance for all comparisons.

Test Subjects:
Body Weight at study initiation: 190-265 g (males); 168-210 g (females)

No. of animals per sex per dose: 12

Test conditions:
Chamber concentrations were analyzed by GC an average of 3 times per

	week.
	Clinical observations performed and frequency: Daily observations for toxic effects. Body weights were measured after 4, 23, 43, 62, 80, and 88 exposure days.
	Organs examined at necropsy: Detailed examination for signs of gross pathology. Twenty tissue samples from the thoracic and abdominal cavities were taken from each rat for histopathologic examination.
Result	: NOAEL (NOEL) 61 ppm (females); < 61 ppm (males) LOAEL (LOEL) 90 ppm (females); 61 ppm (males)
	Males in the 61 ppm group showed decreased body weights. The mean body weight gains of males at 237 ppm were statistically significantly lower at every interval of comparison up to 80 days, at which point there were not enough survivors to make a comparison. The mean body weight gains for females at 237 ppm were statistically significantly lower after 43, 62, and 80 days, after which time there were not enough survivors for a comparison. In addition, the 90 ppm dose group showed dose-related effects on the liver and kidney. The mean weights of the livers and kidneys expressed as percentages of body weight of both sexes of rats at the 90 ppm level were statistically significantly higher than the corresponding values for controls after 88 days. Statistical analysis at the 237 ppm level was precluded as there were too few survivors. Male and female rats of the 237 ppm group had bloody exudate around their nostrils after day 45. Eleven of 12 male rats in the 237 ppm dose group died between day 43 and the end of the study. Ten of 12 females died between day 51 and the end of the study. Ten of 12 males at the 237 ppm level had testicular atrophy, but this was not present in animals exposed to 90 or 61 ppm. The 237 ppm level and to a lesser degree the 90 ppm level caused renal lesions, i.e., dilated tubules, pink casts, tubular degeneration followed by regeneration, interstitial nephritis, and marked nephrosis. These kidney lesions were more frequent and of greater severity among the males than among the females. At the 237 ppm level, hydrothorax was evident in 9 of 12 males and 12 of 12 females, and ascites was noted in 6 of the 12 males and 11 of the 12 females. These findings were not seen at either of the 2 lower exposure levels. Liver lesions were seen at the 237 ppm level only and were found in both male and female rats. Grossly, the livers were firm with full rounded edges and adhesions. The most pertinent liver lesion found microscopically was central cord cell degeneration in 5 male and 4 female rats. Effects on the thyroid were not evaluated in this study. No exposure related lesions were observed at any level in the spleen, adrenal, trachea, prostate, uterus, colon, and mesentery. Ectopic cartilage was found in the hearts of both females that survived, and some deviation from normal occurred in the pancreas of all 3 survivors of the 237 ppm exposure group.
Test substance	: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Source: Union Carbide Corporation, South Charleston, West Virginia Purity: unknown
Conclusion	: Histopathologic findings indicate exposure to high concentrations of ENB produced significant lethality and kidney, liver, and testes effects. A NOAEL was not established for male rats.
Reliability	: (2) valid with restrictions Well conducted study, but purity of test material not specified.
18.12.2003	(13)
Type	:
Species	: rat
Sex	: male/female
Strain	: other: CrjCD (SD)
Route of admin.	: gavage

Exposure period : 28 days
Frequency of treatm. : once daily
Post exposure period : 14 days
Doses : 0, 4, 20, 100 (mg/kg)
Control group : other: yes, corn oil
Method : OECD Guide-line 407 "Repeated Dose Oral Toxicity - Rodent: 28-day or 14-d Study"
Year : 1996
GLP : yes
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Method : Bartlett's test and analysis of variance as necessary and Dunnett's test. Kruskal-Wallis and Mann-Whitney U-test for histopathology.

Test Subjects:

Rats were housed individually in suspended wire mesh metallic cages in a room maintained at 22-24°C, 31-79% relative humidity and 12 hour light-dark cycle. Water and rat chow diet was provided ad libitum. Individual rats were identified by ear tattoo and cage cards.

Age at study initiation: 5 weeks

No. of animals per sex per dose: 14 M, 14 F control and high dose, 7 M, 7 F mid and low dose

Study Design:

Doses of test article were administered by gavage in corn oil once/day at 5.0 ml/kg.

Vehicle: corn oil

Satellite groups and reasons they were added: 7 M, 7 F from control and high dose groups retained untreated for additional 14-d period to evaluate recovery.

Clinical observations performed and frequency: Observations for mortality, morbidity and clinical signs were made at least once a day. Body wt and food consumption were determined at initiation and on dosing days 2, 5, 7, 10, 14, 21, and 28 and recovery days 2, 5, 7, 14 and at necropsy. Water consumption was determined and unfasted urines were collected during dosing days 25-26 and recovery days 11-12. Urine was analyzed for pH, protein, glucose, ketones, urobilinogen, bilirubin, occult blood, color, sediment, volume, specific gravity, sodium, potassium, chloride, calcium, and inorganic phosphates. Hematological analysis and clinical chemistries were conducted on day 29 and on day 15 of recovery. Parameters examined were RBC, Hct, Hb, MCV, MCH, MCHC, platelets, reticulocytes, three coagulation factors, WBC and differential counts. Clinical chemistry parameters were GOT, GPT, Al-P, LDH, gamma-GTP, glucose, total cholesterol, triglycerides, bilirubin, BUN, creatinine, sodium, potassium, chloride, calcium, inorganic phosphate, total protein, albumin, and protein fractionation.

Organs examined at necropsy: At end of dosing and on day 15 of recovery, rats were sacrificed and 46 tissues/organs were fixed and prepared for histopathologic examination: liver, kidneys, spleen, heart, lung, brain, pituitary, adrenal glands, thyroid, parathyroid, thymus, mesenteric lymph nodes, pancreas, tongue, mandibular lymph nodes, submandibular gland, sublingual gland, parotid gland, zymbal gland, skin, sternum, femur, spine, skeletal muscle, thoracic aorta, pharynx, trachea, bronchi, esophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, bladder, seminal vesicle, prostate, ovary, uterus, vagina, ischiatic nerve, testes,

Result	<p>epididymides, eye and Hardarian gland. Brain, pituitary, thymus, thyroid, parathyroid, lung, heart, liver, kidneys, spleen, adrenal glands, testes and ovaries were weighed before fixation.</p> <p>: NOAEL (NOEL): male < 4 mg/kg/day, female = 20 mg/kg/day LOAEL (LOEL): male = 4 mg/kg/day, female = 100 mg/kg/day</p>
Test substance	<p>There were no deaths attributable to test article administration. At dosage termination, mean body wt was significantly reduced in high dose males but not in the recovery group; there was no effect on food consumption. In high dose males, urine protein was increased and water consumption was decreased, however these changes were not seen after the recovery period. Clinical chemistry showed a significant increase in albumin in both sexes at the highest dose that was not present after recovery. Males showed a decrease in alpha-1-globulin at the 20 and 100 mg/kg dose levels that was not present in recovery animals. In high dose males there was increased brain/body wt ratio and increased kidney/body wt ratio; both effects were absent in recovery males. Histopathology showed increased hyaline droplets in renal tubule epithelium of all male rats in the 20 and 100 mg/kg groups. Thyroid effects were hypertrophy of follicular cells, decreased colloid and irregular follicular shape in 6 males, 1 female in 100 mg/kg group, 1 male each in 20 and 4 mg/kg groups. These thyroid effects were not seen in any recovery animals.</p> <p>: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Source: Nippon Petrochemicals Co., Ltd, Lot No.6J01 Purity: 99.4%</p>
Conclusion	<p>Stability during use confirmed by gas chromatography.</p> <p>: Hyaline droplets in the renal tubular epithelium, hypertrophy and irregular shape of follicular cells and decreased colloid in the thyroid, were observed in male rats at doses of 4 mg/kg/day and higher. The kidney effects are likely due to the male-rat-specific alpha 2u globulin accumulation. Increased albumin, and hypertrophy of follicular cells and decreased colloid in thyroid were found in the female 100 mg/kg/day group. Thyroid effects are reversible in both sexes. Therefore, the oral NOAEL for systemic effects other than thyroid and kidney is 20 mg/kg/day, based on reduced body weight of females in the 100 mg/kg/day group.</p>
Reliability	<p>: (1) valid without restriction Well conducted study.</p>
Flag 18.12.2003	<p>: Critical study for SIDS endpoint</p>
Type	<p>: Sub-chronic</p>
Species	<p>: dog</p>
Sex	<p>: male</p>
Strain	<p>: Beagle</p>
Route of admin.	<p>: inhalation</p>
Exposure period	<p>: 89 days</p>
Frequency of treatm.	<p>: 7 hours/day for 5 days/week</p>
Post exposure period	<p>:</p>
Doses	<p>: 0, 22, 61, 93 ppm</p>
Control group	<p>: other: yes, filtered air</p>
NOAEL	<p>: = 22 ppm</p>
LOAEL	<p>: = 61 ppm</p>
Method	<p>: other</p>
Year	<p>:</p>
GLP	<p>: no data</p>
Test substance	<p>: other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)</p>
Method	<p>: Body weight changes and kidney and liver weight as percentage of body weight of all animal groups were intercompared statistically by use of the following tests: Bartlett's homogeneity of variance, analysis of variance, and Duncan's multiple range tests. The last test was used if F for analysis</p>

(20)

of variance was significantly high in order to delineate which group differed from the control. If the Bartlett test indicated heterogeneous variances, the t-test was used for each group versus the control. A fiducial limit of <0.05 was used as the critical level of significance for all comparisons.

Test Subjects:

Age at study initiation: not specified ("young")

No. of animals per dose: 3

Test conditions:

Chamber concentrations were analyzed by GC an average of 3 times per week.

Vehicle: not applicable

Clinical observations performed and frequency: Daily observations for toxic effects. Body weights were measured after 4, 23, 43, 62, 80, and 89 exposure days. Hematology and blood chemistry were performed 5 days before the start of the study and after the 21st, 45th, 61st, and 83rd days. Urinalysis was performed 6 days prior to starting and after day 22, 46, 62, and 85.

Organs examined at necropsy: Detailed examination for signs of gross pathology. Twenty-eight tissue samples from the cranial, thoracic and abdominal cavities of each dog were taken for microscopic examination. The tissues include portions of: lung, liver, kidney, heart, spleen, adrenal, thyroid, parathyroid, esophagus, diaphragm, lymph node, gall bladder, maxillary gland, tongue, stomach, duodenum, pancreas, ileum, jejunum, colon, urinary bladder, prostate, testis, epididymis, brain, pituitary, skin, and eye.

Result

: NOAEL (NOEL) 22 ppm
LOAEL (LOEL) 61 ppm

There were several changes in biochemical test values. At the 22 ppm level, there was a transient alkaline phosphatase depression at the end of 21 days. There was an increase in serum glutamic-pyruvic transaminase (SGPT) values (a liver enzyme) after 45, 61, and 83 exposures at both the 61 and 93 ppm level. After 83 days, an increase in serum glutamic-oxaloacetic transaminase (SGOT) values (a liver enzyme) and a minimal decrease in the lymphocytes was found in the 93 ppm group. Body weight, kidney and liver weights showed no adverse deviation from control values. None of the urine parameters, when compared to the controls, had any dose-related differences. One dog from the 61 ppm group was sacrificed after 63 days because of labored breathing and excessive fluid in the abdominal cavity. Histopathologic examination of the tissues revealed well-marked bile duct proliferation and portal fibrosis. The hepatic lesions of this dog were similar to, but somewhat more severe than, those of the dogs on the 93 and 61 ppm groups sacrificed at the conclusion of the study. This dog also had marked testicular atrophy. Grossly diagnosed mild congenital hydrocephalus that sporadically occurred in the breeding colony was reported in 1 dog in the 61 ppm group and 2 littermates in the 22 ppm group, but was totally unrelated to dosage of ENB. No behavioral changes have ever been associated with such gross findings and histopathologically there was no evidence of brain injury. Testicular atrophy was seen grossly in 2 of the 3 dogs at the 93 ppm level but was noted in only 1 of the dogs microscopically. However, well-marked cystic degeneration of this organ was found in the second dog. This lesion was not present in dogs from lower exposure levels or controls. Hepatic lesions occurred as bile duct proliferation and portal fibrosis at the 93 ppm and 61 ppm levels.

Test substance	: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Source: Union Carbide Corporation, South Charleston, West Virginia Purity: unknown	
Conclusion	: High ENB exposure concentrations produced liver damage and testicular atrophy in male beagle dogs. A NOAEL of 22 ppm was established. No effects on the thyroid or kidney were observed.	
Reliability	: (2) valid with restrictions Well conducted study, but purity of test material not specified.	
18.12.2003		(13)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	: Bacterial reverse mutation assay
System of testing	:
Test concentration	: -S9: 0, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250 µg/plate; +S9: 0, 7.81, 15.6, 31.3, 62.5, 125, 250, 500 µg/plate
Cycotoxic concentr.	:
Metabolic activation	: with
Result	: negative
Method	: other: Guidance for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Guidelines No. 471 and 472
Year	: 1996
GLP	: yes
Test substance	: other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Method	: Test Design: Standard preincubation assay. Dose setting test conducted within limits of 50.0 to 500 µg/plate; all strains without S9 mix were antibacterial above 150 µg/plate. The stains TA1535 and TA98 with S9 were antibacterial above 150 µg/plate while the other strains were above 500 µg/plate. The maximum dose fixed for the final test was 250 µg/plate without S9 mix and 250 µg/plate for TA1535 and TA98 and 500 µg/plate for other strains with S9 mix. Number of replicates: Two (duplicate) Positive and negative control groups and treatment: AF2, SA, 9AA, 2AA (positive) and DMSO (solvent control) Number of metaphases analyzed: Solvent: DMSO Criteria for evaluating results: A two-fold increase over controls was the basis for a positive finding.
Result	: Ethylidene-2-norbornene (ENB) did not increase the number of revertant colonies beyond the solvent control values in any strain of Salmonella or E. coli WP2 uvrA with or without metabolic activation in both the initial and repeat preincubation assays. The test material did induce significant cell toxicity at doses of 125, 250µg/plate -S9 in TA100, WP2uvrA and TA98, and at 62.5, 125µg/plate in TA1535, TA1537 -S9. Cell toxicity +S9 occurred at doses of 500µg/plate for WP2uvrA, at 500, 250µg/plate for TA1537, at 125, 250µg/plate in TA100, TA1535, and at 250µg/plate only for TA98 in both initial and repeat assays, demonstrating adequate interaction of test material with bacteria. Positive control compounds performed appropriately. ENB did not induce gene mutations under the conditions of this test.
Test substance	: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Nippon Petrochemicals Co., Ltd. Purity: 99.4%, kept at room temperature sealed nitrogen gas until use.

Conclusion	:	ENB did not induce bacterial gene mutation with and without metabolic activation.	
Reliability	:	(1) valid without restriction	
Flag	:	Critical study for SIDS endpoint	
18.12.2003			(21)
Type	:	Ames test	
System of testing	:	Bacterial	
Test concentration	:	0.001-0.1 mg/plate	
Cycotoxic concentr.	:		
Metabolic activation	:	with and without	
Result	:	negative	
Method	:	other: not specified	
Year	:		
GLP	:		
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CAS# 16219-75-3)	
Method	:	The test substance was considered positive if it produced at least a two-fold increase in the number of revertant colonies compared to the negative control, and there is evidence for a dose-related increase in the number of revertant colonies.	
Result	:	A preliminary toxicity/initial mutagenicity assay was conducted with 10 doses over a range of 0.01 to 90 mg/mL using strain TA100. with and without S-9. Toxicity was exhibited at dosages in the range of 1-99 mg/plate. Dosages of 0.1 and 0.3 mg/plate allowed only sparse growth. The dosages used in the definitive study were chosen to span the range 0.001-0.1 mg/plate. None of the five strains, with or without induced rat liver S-9, exhibited reversion frequencies substantially different from spontaneous controls in this assay.	
Test condition	:	ENB was tested in triplicate at each of 5 doses. Either 0.5 mL sodium phosphate buffer or S-9/bacteria mix was added to the tubes containing 2 mL top agar, 100 uL bacterial strain and 100 uL of ENB, control substance, or solvent. The contents of the tubes were poured on agar plates which were allowed to harden. Plates were then incubated for 48-72 hours at 37°C in a darkened incubator. Revertant colonies were counted by automatic colony counter. Solvent control was DMSO; positive control chemicals were: 4-nitro-o-phenylenediamine, sodium azide, 2-aminoanthracene, and 9-aminoacridine (depending on strain).	
Test substance	:	5-Ethylidene-2-norbornene (CAS# 16219-75-3) Source: Union Carbide Corporation, South Charleston, West Virginia, Lot No. 1609 Purity: 99%	
Conclusion	:	The test substance was not mutagenic with or without metabolic activation in this test system.	
Reliability	:	(1) valid without restriction Comparable to guideline study.	
Flag	:	Critical study for SIDS endpoint	
18.12.2003			(1)
Type	:	other: Mammalian cell chromosome aberration assay Chinese hamster lung (CHL/IU) cells	
System of testing	:		
Test concentration	:	Continuous treatment assay -S9: 0, 0.013, 0.025, 0.050, 0.10, 0.20 mg/mL in acetone; Short term (6 hr) treatment ±S9: 0, 0.025, 0.050, 0.10mg/ml, 0.20 mg/mL (+S9 only) in acetone	
Cycotoxic concentr.	:		
Metabolic activation	:	with and without	
Result	:	negative	
Method	:	OECD Guide-line 473	
Year	:	1996	

GLP	:	yes
Test substance	:	other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)
Method	:	Frequency of cells with chromosomal aberrations analyzed by Cochran-Armitage's direct probability method ($p < 0.01$). Dosage dependence analyzed by Cochran-Armitage's trend test. Fisher's exact test was used to compare treated groups to controls.
Result	:	The 50% cell growth inhibition concentrations on continuous (24-h) treatment was 0.07 mg/mL, and short term treatment were 0.1 and 0.06 mg/mL with and without S9 mix, respectively.
		No structural aberration and induction of polyploid cells were observed in any treatment group of CHL/IU for 24-h and 48-h continuous treatment. No structural aberration and induction of polyploid cells were observed in short term (6-h) treatment with or without S9 mix.
		In the continuous treatment assays, dose concentrations of 0.10 and 0.20 mg/ml induced too much cytotoxicity to be analyzed; in the 6 hr treatment assay + S9, 0.20mg/ml induced too much cytotoxicity to be analyzed.
		5-Ethylidene-2-norbornene did not induce any chromosome aberrations or polyploidy in CHL/IU cells at any dose level in continuous 24 or 48 hr exposure groups or in any 6 hr exposure group with or without metabolic activation. Positive control compounds performed appropriately.
Test condition	:	NOAEL(NOEL) (C): 0.2 mg/ml CHL/IU cells (2×10^4) were cultured in 5 ml of Eagle MEM supplemented with 10 vol.% fetal bovine serum in glass flasks (T25 cm ²), 2 flasks/dose, and incubated in 5% CO ₂ /air at 37°C. Test material was dissolved in acetone at maximum concentration and diluted to appropriate doses for immediate use at 0.5 vol.% of culture solution. For the continuous treatment assay, test material was added at the 3rd day of culture for 24 and 48 hrs. For short term treatment, cells were exposed to test material at the 3rd day of culture for 6 hr with and without S9 mix. After removal of test material, cells were incubated for an additional 18 hr in fresh culture medium. Dosage concentrations based on a preliminary cell growth inhibition test, were selected to allow >20% growth rate in both flasks and a mitotic index of >0.5% at the highest dose. Positive control compounds were mitomycin C (0.00005 mg/ml) - S9 and cyclophosphamide (0.005 mg/ml) ±S9, diluted in water for intraperitoneal injection. Two hours before termination of incubation, colcemid (0.1 µg/ml) was added to each flask. Cells were harvested and slides (6/flask) were prepared at each dose concentration and stained with Giemsa. In each assay 200 cells from each of three acceptable dose groups were analyzed for chromosome aberrations. Structural aberrations including chromosome and chromatid breaks, exchanges and polyploidy were recorded; gaps were also counted and number of cells with aberrations with and without gaps were calculated. Mitotic index was calculated from a count of 500 cells at the highest dose analyzed for chromosome aberrations, and 800 cells in each dose group were examined for polyploidy.
Test substance	:	5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Source: Nippon Petrochemicals Co., Ltd, Lot No.6J01 Purity: 99.4%
Conclusion	:	5-Ethylidene-2-norbornene is not a chromosome-damaging agent in cultured mammalian lung cells under conditions of this assay.
Reliability	:	(2) valid with restrictions Amount of S9 employed in the assay was not specified.
		18.12.2003 (21)
Type	:	Cytogenetic assay
System of testing	:	CHO-K1-BH4 (subclone D1)

Test concentration	: 0.01-0.07 mg/mL (without activation); 0.01-0.08 mg/mL (with activation)
Cycotoxic concentr.	:
Metabolic activation	: with and without
Result	: negative
Method	: other: not specified
Year	:
GLP	:
Test substance	: other TS: 5-Ethylidene-2-norbornene (CAS# 16219-75-3)
Result	: There were no statistically significant or dose-related increases in chromosome aberrations, compared with concurrent control values, with or without metabolic activation, at both the 6-hr and 10-hr sampling time. When present, chromatid breaks and chromosome fragment were the predominant aberration. Both positive controls produced highly significant increases in the numbers of aberrant cells compared with the DMSO controls.
Test condition	: A structural chromosome aberration test was conducted in vitro with CHO cells according to the method of Preston et al, 1981. Cytotoxicity was determined in CHO cells that were inoculated into culture flasks and treated with ENB concentrations ranging from 0.006-0.06 mg/mL in the first test, and 0.07-0.10 mg/mL in the second test. Cytotoxicity was determined by comparing the relative numbers of surviving CHO cells in the presence of ENB with the survivors in control preparations. The test for mitotic inhibition was conducted as for the growth inhibition, but cells for harvested for chromosome preparations. Slides were prepared and cells stained with dilute Geimsa, and scored for the proportion of cells in metaphase. Clastogenicity was evaluated after 6-hr and 10-hr incubation. Solvent control was DMSO; positive control chemicals were: cyclophosphamide (with metabolic activation) and triethylenemelamine (without metabolic activation).
Test substance	: 5-Ethylidene-2-norbornene (CAS# 16219-75-3) Source: Union Carbide Corporation, South Charleston, West Virginia, Lot No. 1609 Purity: 99%
Conclusion	: The test substance was not clastogenic with or without metabolic activation in this test system.
Reliability	: (1) valid without restriction Comparable to guideline study.
Flag	: Critical study for SIDS endpoint
18.12.2003	(1)
Type	: other: Forward gene mutation assay (Chinese Hamster Ovary HGPRT locus test)
System of testing	: CHO-K1-BH4 (subclone D1)
Test concentration	: 0.02-0.08 mg/mL (without activation) 0.02-0.10 mg/mL (with activation)
Cycotoxic concentr.	:
Metabolic activation	: with and without
Result	: negative
Method	: other: not specified
Year	:
GLP	:
Test substance	: other TS: 5-Ethylidene-2-norbornene (CAS# 16219-75-3)
Method	: Analysis of mutation frequencies followed the procedure of Irr and Snee (1979), employing Box-Cox transformation (Box and Cox, 1964) before parametric analysis using Student's t-test.
Result	: ENB produced a dose-related cytotoxicity to CHO cells with and without metabolic activation. In the study without metabolic activation, ENB did not produce any statistically significant or dose-related increase in the number of mutants/105 clonable cells. In the test with activation, increases in the incidence of mutants were seen with only one of the duplicate cultures at

- each concentration; however, these increases were not statistically significant. A repeat test at the higher concentrations was conducted to confirm the absence of mutagenic effect, using a dose range of 0.06-0.09 mg/mL with metabolic activation. The 0.08 and 0.09 mg/mL doses were completely cytotoxic to CHO cells, but no mutagenic effects were observed in duplicate cultures with ENB concentrations of 0.06 and 0.07 mg/mL.
- Test condition** : CHO cells were inoculated into culture flasks and incubated at 37 C in a 5-6% CO2 atmosphere for 20-24 hr prior to treatment. Appropriate amounts of ENB or control materials were added and the cultures were exposed for 5 hr. Cytotoxicity was determined 1 day after exposure. The mutant fraction was determined in duplicate cultures for each treatment group after a 9 to 12 day subculturing period, in order to allow expression of any mutant phenotype. At 2 to 3 day intervals posttreatment, cells were subcultured and incubated. After about 7 days, the cells were dissociated, counted, and plated at a concentration of 2x10⁵ cells/plate in five 100 mm culture dishes (ie, 1x10⁶ total cells). The number of mutants per 10⁶ total cells and mutants per 10⁶ viable cells were calculated. Solvent control was DMSO; positive control chemicals were: dimethylnitrosamine (with metabolic activation) and ethylmethanesulfonate (without metabolic activation).
- Test substance** : 5-Ethylidene-2-norbornene (CAS# 16219-75-3)
Source: Union Carbide Corporation, South Charleston, West Virginia, Lot No. 1609
Purity: 99%
- Conclusion** : The test substance was not mutagenic with or without metabolic activation in this test system.
- Reliability** : (1) valid without restriction
Comparable to guideline study.
- Flag** : Critical study for SIDS endpoint
18.12.2003 (1)
- Type** : Sister chromatid exchange assay
System of testing : CHO-K1-BH4 (subclone D1)
Test concentration : 0.01 to 0.06 mg/mL
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: not specified
Year :
GLP :
Test substance : other TS: Ethylidene Norbornene (CAS No. 16219-75-3)
- Method** : Criteria used to evaluate the data included dose-resonse relationship, doubling of SCE incidence, and statistical significance. Statistical analysis was performed after Box-Cox transformation (Box and Cox, 1964).
- Result** : Compared to either the medium or DMSO solvent controls, there were no statistically significant increases in the number of SCE/chromosome with the ENB groups. The positive controls had highly statistically significant increases in SCEs compared to the solvent control. Both with and without metabolic activation, there was a dose-related increase in the incidence of CHO cells at the first mitotic division. The moderate levels of mitotic inhibition at the highest ENB dose, average 27% without and 20% with metabolic activation, demonstrated the ENB doses were in a biologically effective range for the evaluation of potential genotoxicity.
- Test condition** : The method was based on that of Perry and Wolff (1974). Cell lines, cytotoxicity testing, metabolic activation procedures, and positive controls were similar to those used in the CHO forward gene mutation test. Cell cultures were incubated at 37°C in a 5-6% CO2 atmosphere for 20 hr (without activation) or 48 hr (with activation) prior to treatment. After treatment, cells were incubated at 37°C for 24-28 hr to allow 2 cycles of cell

division. Cells were then harvested and chromosomes prepared for SCE staining. The number of chromosomes and number of SCEs in a minimum of 25 cells per duplicate culture were counted for each specific treatment group.

Test substance : Ethylidene Norbornene (CAS No. 16219-75-3)
Source: Union Carbide Corporation, South Charleston, West Virginia, Lot No. 1609
Purity: 99%

Conclusion : The test substance was not mutagenic with or without metabolic activation in this test system.

Reliability : (1) valid without restriction
Comparable to guideline study.

Flag : Critical study for SIDS endpoint
18.12.2003 (1)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Dominant lethal assay

Species : rat

Sex : male

Strain :

Route of admin. : inhalation

Exposure period : 6 hours/day for 5 days

Doses : 0, 5, 52, and 254 ppm

Result :

Method : other

Year :

GLP : yes

Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Method : The number of implantation sites and intrauterine deaths per litter for each week were analyzed by analysis of variance. When appropriate, proportions of resorptions and dead or live fetuses per implant were subjected to arcsin transformation and evaluated by analysis of variance. If significant, Duncan's multiple range test was used for intergroup differences.

Result : ENB did not produce evidence of dominant lethality in rats using a standard protocol (Neeper-Bradley and Ballantyne, 1996). Male rats exposed to analytically measured ENB vapor concentrations of 0, 5, 52, or 254 ppm for 6 hr/day for 5 consecutive days were mated with unexposed females. Reproductive factors, including the number of fertile males and the number of gravid females with viable implants, were unaffected by exposure to ENB vapor. No significant preimplantation loss or dominant lethal effects were observed in females mated to ENB-exposed males during the 10-week breeding period. Males exposed to 254 ppm had slightly reduced body weight gains after the 5-day treatment period, but body weights and body weight gains were comparable to the controls thereafter. Triethylenemelamine (0.5 mg/kg, single dose on day 5 as a positive control) produced clear dominant lethal effects.

Test condition : After five days of exposure, the male rats were mated with unexposed females (two females per week for each male for 10 consecutive weeks). Females were removed from cohabitation after 7 days and sacrificed on gestation day 15. The uterine contents were examined for the total number, position, and status of implantations; the numbers of early and late resorptions; and numbers of live and dead fetuses.

Test substance : 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Conclusion : The ENB-exposed males showed no clinical signs of toxicity. The only objective finding was slight decrease in body weight gain at 254 ppm over the exposure period, but which resolved by the end of the first

postexposure week. No reproductive or gestational effects, including dominant lethality, were noted in the ENB-treated groups during the 10 wk of postexposure mating. This finding confirms the absence of testicular toxicity in the rat from repeated exposure to high concentrations of ENB vapor. The no-observed effect concentration for toxicity (body weight gain) was 52 ppm. The no-observed effect concentration for dominant lethality was greater than 254 ppm ENB.

Reliability : (1) valid without restriction
GLP study.

Flag : Critical study for SIDS endpoint

18.12.2003 (24)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type :
Species : rat
Sex : male/female
Strain : other: CrjCD(SD)IGS
Route of admin. : gavage
Exposure period :
Frequency of treatm. : once daily, in the morning
Premating exposure period
 Male : 14 days
 Female : 14 days
Duration of test : maximum 45 consecutive days
No. of generation studies :
Doses : 0, 4, 20, 100 mg/kg/day; dosing volume 5 ml/kg
Control group : other: yes, corn oil
NOAEL parental : = 20 mg/kg bw
NOAEL F1 offspring : = 20 mg/kg bw
Method : OECD Guide-line 421
Year : 1996
GLP : yes
Test substance : other TS: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3)

Method : Bartlett's test for homogeneity of variance, ANOVA if variance homogeneous or Kruskal Wallis if variance heterogeneous or data non-parametric. Dunnett's or Dunnett's multiple comparison test. Histopathology analyzed by Chi square, then Armitage. Other counted data analyzed by Fisher's exact probability. Maternal animal is specimen unit for neonate data.

Test animals:
Age at study initiation: 9 week old for both sexes
Weight at study initiation: 320-356 g for males , 203-237 g for females
No. of animals per sex per dose: 12 per sex per dose group

Test design:
Animals were quarantined and acclimated for 6 days, then assigned to groups (12/sex/group) by wt stratified randomization. Rats were housed in an animal room maintained at 22±20°C and 55±15% relative humidity with 12 hr light-dark cycle and 12 air changes per hour. Animals were housed in polycarbonate cages spread with Beta chip bedding, individually, in pairs during mating or in litters during lactation period. Rat chow pellets and filtered tap water were available ad libitum. Parameters recorded were

number of days required for successful mating, number of estrus cycles missed until successful mating, mating index and fertility index. Males and females were weighed on the day dosing started, on days 3, 7, 14, and weekly thereafter. Mated females were weighed on days 0, 7, 14, and 20 of gestation, and on days 0 and 4 of lactation. In females, body weight gain during premating, gestation and lactation was calculated from body weight at initiation of dosing, day 0 of gestation, and day 0 of lactation, respectively. Food consumption was measured on the day animals were weighed, except during mating.

Vehicle Corn oil: Test material was prepared in corn oil, stored under refrigeration protected from light and used for dosing within 8 days of preparation. Stability and concentrations of dosing solutions were verified prior to dosing (Analytical method not specified).

Dosing schedules and pre and post dosing observations periods for P, F1 and F2, if appropriate: Male rats were dosed orally by gavage for 45 days, from 14 days prior to mating, during mating and up to the day before necropsy. Females were dosed from 14 days prior to mating, through gestation to day 3 of lactation.

Mating procedures:

After 14 days dosing, animals were mated (1:1) for approximately 5 days. Mating was confirmed by the presence of a vaginal plug or sperm in a vaginal smear =Day 0 of gestation. All successfully mated females delivered naturally; a day was designated Day 0 of lactation if delivery was completed by 9am on that day. Neonates were nursed until 4 days after birth (day 4 of lactation). Animals were observed each day to assess lactation, cannibalism and nesting behavior. Ovaries and uterus of maternal rats were removed at necropsy on day 4 of lactation; and the number of corpora lutea and implantation sites counted. Parameters calculated were gestational period, delivery ($\# \text{females with live births} \div \# \text{females which conceived}$), implantation ($\# \text{implantation sites} \div \text{corpora lutea}$) and gestation ($\text{total } \# \text{ offspring} \div \# \text{implantation sites}$) indices. Neonates were examined at parturition to determine number of offspring born (live-births and stillborn), sex, and external anomalies, and observed daily until day 4 of lactation for general condition and mortality. Live birth index and neonatal viability index on day 4 of lactation were calculated. Surviving offspring were weighed individually on days 0 and 4 of lactation, body wt gain was calculated from day 0 body wt. Offspring were killed on lactation day 4 and necropsied.

Standardization of litters: not applicable

Parameters assessed during study P and F1 as appropriate

Clinical observations performed and frequency: Observations for mortality, external appearance and behavior were made every day before and after dosing.

Estrous cycle length and pattern

Sperm examination: not performed

Parameters assessed during study F1 and F2, as appropriate

Clinical observations performed and frequency (weight gain, growth rate, etc.)

Others, for example anogenital distance, if performed

Organs examined at necropsy (macroscopic and microscopic): After the final day of dosing, all surviving rats of both sexes were killed and necropsied. Livers of males and females, testes and epididymides were weighed and relative organ/body wt at necropsy calculated. Seminal vesicles and prostate, ovaries, uterus and vagina, and mammary glands from maternal animals in which all neonates died, and sites of macroscopic anomalies were also fixed and retained. Slides from livers (both sexes), testes and epididymides from control and high dose groups, sites of any

Result	<p>anomalies, and ovaries from non-pregnant females, were prepared, stained with hematoxylin-eosin and examined microscopically. Livers from males at the 2 lower dose groups and additional lipid (Oil-Red-O) stained sections from selected high dose males were also examined.</p> <p>: NOAEL (NOEL) repeat dose toxicity = 20 mg/kg/day (both sexes) NOAEL (NOEL) reproduction = 20 mg/kg/day (dam and offspring)</p>
Test substance	<p>Repeat dose toxicity: Two males in the high dose group died 25 days and 36 days after start of dosing. Body wt gain was suppressed and food consumption decreased in both sexes in 100 mg/kg group from 7 days after dose initiation to necropsy, but, though a consistent trend, these effects were not always statistically significant. Absolute body wt. in high dose males and females were statistically significantly lower than controls at necropsy ($p < 0.01$). Relative liver wt was higher ($p < 0.01$) and absolute wt of testes and epididymides were significantly lower ($p < 0.01$) in high dose males than controls. Livers from females at all treated doses and males in 4 and 20 mg/kg groups were comparable to controls. Histopathologic examination of livers from high dose males revealed hypertrophy and vacuolization of hepatocytes; liver sections from males at the 2 lower doses were comparable to controls. Examination of the 2 dead high dose male rats revealed pulmonary and hepatic congestion in both animals and milky appearance and petechiae in the thymus of 1 rat, with histopathologic findings of diffuse thymic hemorrhage in that rat, and pulmonary congestion and edema in both rats. (Reviewer's comment - Pulmonary congestion and edema is suggestive of misdosing as a cause of death). No abnormalities were seen in livers of any female or in ovaries of non-pregnant females.</p> <p>Reproduction/Developmental toxicity: All animals mated within 5 days. Mating index, days required for successful mating, and number of estrus cycles missed until mating were comparable for treated animals and controls. Only 10/12 mated females in the 20 mg/kg group and 11/12 females in the 100 mg/kg were pregnant but fertility indices were not statistically different from controls. The gestational period in the 100 mg/kg group (23.1 days) was significantly longer than the control ($p < 0.01$) but was within the normal historical range for this laboratory. Parturition was normal and corpora lutea counts were comparable for all groups. In the 100 mg/kg group, the implantation index (72.8%), total number of offspring born (7.1 pups) and the delivery index (53.1%) were significantly lower than controls ($p < 0.01$), (94.5%, 14.3 pups, and 93.1 %, respectively). The live birth index was 100% and lactation behavior was normal in all groups. All pups from one 20mg/kg dam died on day 2 of lactation. This event did not appear treatment related since no other instances of 100% mortality occurred in other treated groups. The number of surviving offspring on day 4 of lactation in the high dose group (6.8 pups) was significantly lower than controls (14.2 pups) ($p < 0.01$) but the neonatal viability index on day 4 of lactation ($\#$ live pups on day 4 \div $\#$ live pups on day 0) was not statistically significantly different for any treated group compared to controls. There were no changes in sex ratio, offspring body wt, appearance of offspring or necropsy findings attributable to test substance.</p> <p>: 5-Ethylidene-2-norbornene (CAS No. 16219-75-3) Source: SAN-PETROCHEMICALS Co., LTD., Lot No.7K03 Purity: 99.5%</p>
Conclusion	<p>Stability during uses is confirmed by gas chromatography.</p> <p>: 5-Ethylidene-2-norbornene administered to rats by oral gavage for 45 days, induced both parental systemic and reproduction/developmental toxicity at 100 mg/kg/day. Treatment did not appear to affect mating capabilities or fertility index but did affect the embryo and fetus at this high dose that inhibited body wt gain and food consumption in parental animals. However, the lack of effect on the live birth index, day 4 neonatal viability, external appearance and necropsy findings in offspring suggests that the</p>

	test material does not affect postpartum neonatal growth.	
Reliability	: (1) valid without restriction Well conducted study.	
Flag	: Critical study for SIDS endpoint	
18.12.2003		(22)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	: rat
Sex	: female
Strain	: other: CD (Sprague-Dawley)
Route of admin.	: inhalation
Exposure period	: Gestation days (GD) 6-15
Frequency of treatm.	: 6 hours/day
Duration of test	: females sacrificed on GD21
Doses	: 0, 25, 100, or 354 ppm
Control group	: other: yes, air-exposed only
NOAEL maternal tox.	: = 25 ppm
NOAEL teratogen.	: = 25 ppm
Method	: other: Developmental toxicity (Teratogenicity) study
Year	:
GLP	: no data
Test substance	: other TS: 5-Ethylidene-2-norbornene (CAS No.16219-75-3)
Method	: Quantitative continuous variables were intercompared between ENB groups and the corresponding air-control groups using Levene's test for equal variances, analysis of variance, and t-tests. The t-test was used when the F value from the ANOVA was significant. Non-parametric data were statistically analyzed using the Kruskal-Wallis test followed by the Mann-Whitney U test. Incidence data were compared using Fisher's Exact Test. A probability value of <0.05 (two-tailed) was used as the critical level of significance.
Result	: NOAEL maternal toxicity 25 ppm NOAEL developmental toxicity 25 ppm

There were no maternal deaths or abortions. One dam at 100 ppm delivered early. Pregnancy rates ranged from 83.3 to 100% and were equivalent across all groups. Between 20 and 25 litters were available for examination in each group. There were no exposure-related clinical signs of toxicity. However, periorcular and perinasal encrustation was seen at 354 ppm on GD 6-21. Gestational body weights were reduced on GD 9, 12, 15, and 18 at 354 ppm and on GD 15 at 100 ppm. Gestational body weight gains were statistically significantly reduced ($p < 0.01$) 61.5% over GD 6-15 at 354 ppm and 25.2% over GD 9-15 at 100 ppm, both accompanied by decreased food consumption (21.4% and 10.4%, respectively, $p < 0.01$). At 100 ppm, food consumption was less markedly affected, being reduced 16.5% for GD 9-12 and 8.0% for GD 12-15. Food consumption was similar to controls in the post exposure period.

Necropsy: There were no effects on the number of corpora lutea. The corrected body weights and corrected body weight changes were reduced in the 354 ppm group. Liver Weights showed a nonstatistical trend for increase at 100 and 354 ppm. Relative liver weight was increased at 100 and 354 ppm. There were no statistically significant differences among the groups for number of live fetuses per litter, percent resorptions or malformations per litter, placental or fetal body weights, or sex ratio. Three skeletal variants were increased in litters of the 354 ppm group (bilobed 12th thoracic centrum, split 12th thoracic centrum, and poorly ossified second sternabra) and one variant (bilobed 12th thoracic centrum) was

Test condition	<p>increased in the 100 ppm group litters, but these occurred in the presence of maternal toxicity and were not seen in the 25 ppm group. Both absolute and relative thyroid weights were unaffected by Maternal exposure to ENB. There was no difference in thyroid hormones compared to controls. Histology evaluation confirmed vacuolar colloid depletion in all groups, (including controls) that was more marked and numerous in ENB groups. Morphometric evaluation confirmed concentration-related depletion of thyroid colloid in the ENB groups. There were no clinical signs suggestive of thyroid dysfunction.</p> <p>: Analytical chamber concentrations were measured by on-line gas chromatography. The identity of the test substance was independently confirmed by GC-MS and NMR spectrometry. The day a copulation plug was found is designated as Gestation Day 0. Plug-positive females were housed individually for the duration of the study. Maternal food consumption was measured at 3-day intervals. Body weights were recorded on GD 0, 6, 9, 12, 15, 18, and 21. Maternal animals were observed daily for mortality, morbidity, and signs of toxicity and examined for gross tissue abnormalities at necropsy. Necropsy was on GD 21 and included general abdominopelvic gross pathology, gravid uterine weight, liver and kidney weights. Maternal thyroid glands were weighed and prepared for light microscopic examination and morphometric measurements. Ovarian corpora lutea were counted. The uterus was opened to examine the number of implantation sites, resorptions, live and dead fetuses. All fetuses were weighed, sexed, and examined for external variations and malformations. Half of the live fetuses in each litter were examined for thoracic and abdominal abnormalities ; these fetuses were decapitated and the heads fixed in Bouin's solution for examination of craniofacial structures by sectioning. All fetuses were eviscerated and processed for skeletal staining with alizarin red S to examine for skeletal malformations and variations.</p>
Test substance	<p>: 5-Ethylidene-2-norbornene (CAS No.16219-75-3) Union Carbide Corporation, South Charleston, West Virginia, Lot No. 1609 Purity: 99%</p>
Conclusion	<p>: No compositional changes were measured over the period of the study. Exposure of pregnant CD rats to ENB vapor produced maternal toxicity at 100 and 354 ppm. Minimal fetotoxicity (skeletal variants) was present at 354 ppm and 100 ppm. There was no evidence for embryotoxic or teratogenic effects in any of the exposed groups. For both maternal and developmental toxicity, 25 ppm was a no-effect concentration.</p>
Reliability	<p>: (1) valid without restriction Comparable to Guideline study. Well conducted study.</p>
Flag	<p>: Critical study for SIDS endpoint</p>
18.12.2003	(25)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

- (1) Ballantyne B (1998). In vitro genetic toxicology investigations with 5-ethylidene-2-norbornene. *Toxic Substance Mechanisms* 17, 133-151.
- (2) Ballantyne B, Myers R and Klonne D (1997). Comparative acute toxicity and primary irritancy of the ethylidene and vinyl isomers of norbornene. *J. Applied Toxicology* 17, 211-221.
- (3) Ballantyne B, Norris J, Dodd D, et al. (1997). Short-term and subchronic repeated exposure studies with 5-ethylidene-2-norbornene vapor in the rat. *J. of Applied Toxicology* 17, 197-210.
- (4) Biodegradation and Bioaccumulation Data of Existing Chemicals. (1992). Based on the CSCL, Japan. Compiled under the supervision of MITI, Japan.
- (5) BP Chemical Limited London (1995). IUCLID.
- (6) BP Chemicals MSDS
- (7) Chemicals Inspection and Testing Institute Japan (1992). Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan.
- (8) Environment Agency of Japan (1999a). Ecotoxicity Testing Report. Test 92068.
- (9) Environment Agency of Japan (1999b). Acute Toxicity of 5-Ethylidene-2-Norbornene for *Daphnia magna*. Report 92066.
- (10) Environment Agency of Japan (1999c). Ecotoxicity Testing Report. Test 92065.
- (11) Environment Agency of Japan (1999d). Chronic Toxicity of 5-Ethylidene-2-Norbornene for *Daphnia magna*. Report 92067.
- (12) Harris J (1982). Rate of Hydrolysis. In: *Handbook of Chemical Property Estimation Methods*. Chapter 7. Edited by Lyman, Reehl, and Rosenblatt. McGraw-Hill Book Co., New York, NY, USA.
- (13) Kinkead E, et al. (1971). The mammalian toxicity of ethylidenenorbornene [5-Ethylidenebicyclo(2,2,1)hept-2-ene]. *Toxicol. Appl. Pharmacol.* 20, 250-259.
- (14) LISEC (1999). Acute Toxicity of 5-Ethylidene-2-Norbornene for *Daphnia magna*. Report No. WE-01-214. LISEC, Craenevenne, Genk, Belgium.
- (15) LISEC (1999). Acute Toxicity of 5-Ethylidene-2-Norbornene for fish, *Brachydanio rerio*. Report No. WE-03-192. LISEC, Craenevenne, Genk, Belgium.
- (16) LISEC (1999). Alga, growth inhibition test effect of 5-Ethylidene-2-Norbornene on the growth *Selenastrum capricornutum*. Report No. WE-06-236. LISEC, Craenevenne, Genk., Belgium.
- (17) LISEC (1999). Biodegradation Study of 5-Ethylidene-2-Norbornene -Headspace CO₂ Evolution Test. Report No. WB-13-013. LISEC, Craenevenne, Genk, Belgium.
- (18) Mackay D, DiGuardo A, Paterson S and Cowan C (1997). EQC Model, ver. 1.01. Available from the Environmental Modelling Centre, Trent University, Canada. Modelling performed by ExxonMobil Biomedical Sciences, Inc.
- (19) Meylan, M, SRC (1994-1999). AOPWIN is contained in the computer program EPIWIN (Estimate ver.3.04), available from Syracuse Research Corporation, Syracuse, NY, USA. Modelling performed by ExxonMobil Biomedical Sciences, Inc.

-
- (20) MHLW, Japan (2000). Ministry of Health, Labour and Welfare, Toxicity Testing Reports of Environmental Chemicals, 8, 1087-1100. (The 28 days repeated dose toxicity test was performed by the Safety Research Institute for chemical Compounds Co., Ltd.)
- (21) MHW, Japan (1998). Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, 6, 579-591. (The reverse mutation and in vitro chromosomal aberration tests were performed by the Hatano Research Institute, Food and Drug Safety Center.)
- (22) MHW, Japan (1999). Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, 7, 667-676. (The reproductive /developmental toxicity screening test was performed by the Mitsubishi Chemical Safety Institute Ltd.)
- (23) MITI, Japan (1992).
- (24) Neeper-Bradley T and Ballantyne B (1996). Absence of dominant lethal effects in male CD® rats exposed to 5-ethylidene-2-norbornene vapor. Toxic Substance Mechanisms 15, 389-404.
- (25) Neeper-Bradley T, Ballantyne B, Losco P, Pritts I and Neptun D (1995). Investigation of the developmental toxicity potential of 5-ethylidene-2-norbornene vapor in the CD rat. International J. of Occupational Medicine and Toxicology 4, 371-381.
- (26) Organic compound dictionary (KODANSHA) (1978). U.S. Coast Guard, Department of Transportation. CHRIS-Hazardous Chemical Data. Manual Two. Washington, DC.
- (27) The Sigma-Aldrich Library of Regulatory and Safety Data.
- (28) Union Carbide Review