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ISOPHORONE

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European Chemical Industry
Ecology & Toxicology Centre

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JACC Report No. 10

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THE ECETOC SCHEME FOR THE

"JOINT ASSESSMENT OF COMMODITY CHEMICALS" (JACC)

This report has been produced as part of a programme for reviewing critically the toxicity and environmental hazards of selected industrial chemicals. A number of organisations world-wide produce such reviews so that, based on up-to-date knowledge, existing chemicals can continue to be produced and used safely. ECETOC is contributing to this with its JACC reviews.

In general, commodity chemicals, that is those produced in large tonnage by several companies and having widespread and multiple uses, are reviewed. Every effort is made to discover whether an adequate review exists already, but when this is not so a review is produced jointly by experts from a number of companies with interests in the chemical. Whenever good scientific reviews on certain toxicological or ecotoxicological aspects exist, their conclusions are summarised and only the subsequent literature is assessed. Only the uses of the chemical as such are considered; its occurrence as an impurity in other products is not normally taken into account.

In this document a critical assessment of the toxicology and ecotoxicology of isophorone is presented.

CONTENTS

1. SUMMARY AND CONCLUSIONS

Isophorone is a solvent which enters into the environment from numerous industries, waste disposal and its use as a pesticide carrier. Following release to water or soil, environmental concentrations will decrease as a result of volatilising and biodegradation. The atmospheric half-life was estimated to be about 30 minutes. The results of biodegradation studies are variable, limited and possibly of questionable value. Water solubility, soil adsorption coefficients and polarity indicate that significant adsorption by suspended solids and sediments is unlikely to occur.

Isophorone has been identified in surface waters, groundwater, finished drinking water, urban runoff and in coal fly-ash. Acute LC_{50} values indicate low toxicity to a range of aquatic species. Although isophorone has been found in fish tissues the data and the physico-chemical properties suggest that significant bioaccumulation is unlikely. After application of isophorone as a pesticide carrier to bean plants, rice and sugar beet, no residues were found in the edible parts at the time of harvest.

The odour of isophorone is detectable at a concentration of 0.2 ppm. Eye, nose and throat irritation has been reported at concentrations as low as 5 ppm; above 200 ppm nausea, headache, dizziness, faintness and inebriation have been reported.

Animal studies indicate that the predominant acute systemic effect is central nervous system depression. Death following exposure to high atmospheric concentrations has been ascribed to respiratory paralysis. Percutaneous LD_{50} values indicate that isophorone is rapidly absorbed through the skin. Effects have been reported on occluded application to rabbit skin ranging from mild reversible erythema to more persistent scaring, depending on dose. Conjunctivitis and corneal damage have been reported on direct application to the eye or exposure to high concentrations of vapour.

Distribution studies in rat using ^{14}C-isophorone have shown that 93% of orally administered radioactivity appears in urine, faeces and expired air within 24 hours. The tissues retaining the highest concentration after this period were the liver, kidney and preputial glands.

The metabolic transformations identified in the rabbit were oxidation of the 3-methyl group, reduction of the ketone group and hydrogenation of the cyclohexene ring.

Studies in animals have not indicated any selective toxicity to the embryo or foetus or any teratogenic potential.

Isophorone does not induce gene mutation in bacteria, chromosomal aberration in vitro, bone-marrow micronuclei in mice, DNA repair in primary cultures of rat hepatocytes and no DNA binding was observed in a DNA binding study using 1,3,5-^{14}C-isophorone. In the absence of metabolic activation weak mutagenic responses were obtained in the L5178Y TK +/- mouse lymphoma mutagenesis assay and an increase of sister chromatid exchange (SCE) was observed in CHO cells. The weight of the evidence of all mutagenicity data supports the contention that isophorone has no mutagenic activity in vivo.

Animal studies on the effect of repeated exposure to isophorone vapour indicate the lung, liver and kidney to be target organs. After oral administration, kidney lesions have been found only in male rats; the lesions were similar to the hyaline droplet degeneration produced by some hydrocarbon solvents. The role of alpha-2μ-globulin accumulation in the aetiology of these lesions is widely accepted; isophorone and some of its metabolites, like the hydrocarbons, have been shown to bind to this protein.

The carcinogenicity of isophorone has been investigated in mice and rats. Isophorone, administered by gavage at doses of 250 and 500 mg/kg on 5 days/week for 2 years, produced degenerative changes in the kidneys of male rats and livers of male mice. An increased incidence of liver, mesenchymal and lymphatic tumours in male mice and of renal and preputial gland tumours

in male rats was reported. These data were interpreted by the NTP as showing "some evidence of carcinogenicity" in male rats and as showing "equivocal evidence of carcinogenicity" in male mice. "No evidence of carcinogenicity" was found in the females of either species.

The variable spontaneous incidence of lymphomas, liver, mesenchymal and preputial gland tumours in previous studies suggest that the slightly elevated incidences observed in the isophorone treated animals are likely to be chance occurrences. Although there was evidence of a treatment related increase in renal tumours in male rats, the presence of hyperplasia and of alpha-2u-globulin in their renal tubular cells, and the absence of renal tumours in female rats and male and female mice together with the lack of mutagenic activity, suggest that male rat kidney tumours develop by a non-genotoxic mechanism and that their occurrence does not indicate a carcinogenic risk to man.

Overall, the results of this study do not indicate that isophorone represents a significant carcinogenic hazard at exposure levels at or below the current occupational exposure guidelines.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1 Identity

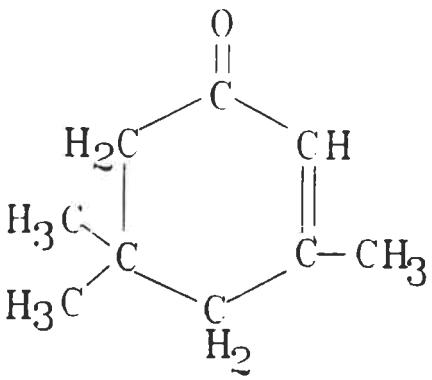
Chem. Abstr. Serv. Reg. No.: 78-59-1

EEC No.: 606-012-00-8

EINECS No.: 2011260

Synonyms: 2-Cyclohexen-1-one, 3,5,5-trimethyl; 3,5,5-Trimethyl-2-cyclohexene-1-one; 1,1,3-Trimethyl-3-cyclohexene-5-one; alpha-Isophorone; Isoacetophorone; Isoforone; Izoforon.

Formula: $C_9H_{14}O$



Molecular weight: 138.2

2.2 Physical and Chemical Properties

Isophorone is a colourless liquid. Its odour has been described as similar to peppermint and camphor. It is moderately soluble in water and is miscible in all proportions with aliphatic and aromatic hydrocarbons, alcohols, ethers, esters, ketones and chlorinated hydrocarbons. Its physical and chemical data are summarised in Table 1.

A typical commercial sample of Isophorone may contain 1-3% of the isomer beta-Isophorone (3,5,5-trimethyl-3-cyclohexene-1-one, CAS-No. 471-01-2, EINECS No. 2074341) with the sum of alpha- and beta-isomers exceeding 99% (Hüls, 1981; Atochem, 1986).

2.3 Conversion Factors

The following conversion factors have been calculated for 22° C and 1013 hPa:

1 ppm = 5.71 mg/m^3
1 mg/m^3 = 0.175 ppm

2.4 Analytical Methods

Isophorone may be assayed for purity by gas chromatography using a flame ionisation detector (FID). Typical conditions are given in Table 2. Its presence in environmental media and animal tissue has been determined by GC-mass spectrometry following suitable extraction procedures (Jungclaus et al, 1976; Sheldon and Hites, 1979; Camanzo et al, 1987).

3. PRODUCTION, STORAGE, TRANSPORT AND USE

Isophorone is produced commercially by catalytic condensation of acetone at elevated temperature and pressure and is purified by distillation. Worldwide annual production capacity was estimated to be 92,000 tonnes in 1988 (Hüls, 1989).

Isophorone is stable and may be stored in steel or aluminium containers. Prolonged periods of storage may lead to slight yellowing.

Isophorone is a solvent for a number of natural and synthetic resins and polymers such as polyvinyl chlorides and -acetates, cellulose derivatives, epoxy- and alkyd resins and polyacrylates. It is therefore used as a high boiling solvent in industrial air drying and stoving paints, nitro emulsion leather finishes and the manufacture of vinyl resin based printing inks for plastic surfaces.

It is also used as a solvent for plant protection products, especially for emulsifiable concentrates of anilides and carbamates.

Isophorone is also used as a chemical intermediate for the synthesis of a variety of organic chemicals (Hüls, 1981).

4. ENVIRONMENTAL DISTRIBUTION, BIOTRANSFORMATION AND ENVIRONMENTAL FATE

4.1 Environmental Distribution

There are no known naturally occurring sources of isophorone (Abrams et al, 1975). In view of its ubiquity as a solvent for polymers, resins, waxes, oils and pesticides, there is a wide potential for release into the environment. Isophorone has been detected in river, surface, ground and finished drinking water (cf Section 5.1.2.), waste water (40 µg/l) from a tyre manufacturing plant (Jungclaus et al, 1976) and in effluents from latex and chemical plants (Shackelford and Keith 1976). The detection of isophorone in coal fly ash suggests that this may also be a source of environmental release (Harrison et al, 1985).

4.2 Biotransformation and Environmental Fate

4.2.1. Atmospheric fate

By virtue of its vapour pressure of 0.4 hPa at 20°C atmospheric isophorone will exist mainly in the vapour state (ECETOC, 1988). Mathematical modelling predicts that the half-life for reaction with both ambient ozone and photo-chemically generated hydroxyl radicals will be 32 minutes. This estimate assumed a concentration of 8×10^5 molecules per cm^3 and a reaction rate constant of 8.14×10^{-11} cm^3 $molecule^{-1}$ sec^{-1} at 25°C for hydroxyl radicals and 1.0×10^{12} molecules per cm^3 with a reaction rate constant of 5×10^{16} cm^3 $molecule^{-1}$ sec^{-1} at 25°C for ozone (US-EPA 1986).

4.2.2. Aquatic Fate

Following release to water, isophorone may either volatilise or biodegrade. Its high solubility in water and its partition coefficient suggest that adsorption onto sediment is unlikely to influence significantly its fate in aquatic systems (Callahan et al, 1979).

From an estimated Henry's Law Constant of 5.8×10^{-6} atm m^3 $mole^{-1}$, based upon a water solubility of 12 g/l at 20°C and a vapour pressure of 0.5 hPa at 20°C, the volatilisation half-life in a model river flowing at 1 m/sec was calculated to be 7.5 days (Lyman et al, 1982). Based on a water solubility of 17.5 g/l (Table 1) the volatilisation half-life would be 11 days.

The oxidation of isophorone by alkylperoxy radicals or singlet oxygen in water is unlikely to be significant in the environment (Mabey et al, 1981). Although dimerisation has been reported in water irradiated at wavelengths > 200nm and in organic solvents at >300nm, such products are also considered unlikely at the dilutions existing in the environment (Callahan et al, 1979).

Evidence that isophorone is photo-oxidised is provided by Borup and Middlebrooks (1986). Treatment with hydrogen peroxide (250 mg/l) followed by UV radiation reduced an isophorone concentration of 62 mg/l to <0.05 mg/l in 60 mins.

4.2.3. Terrestrial fate

Loss from soil, as from surface water, will be by volatilisation and biodegradation. When considering both the vapour pressure and Henry's Law constant, volatilisation from both wet and dry soil surfaces would be slow.

Based on a log $k_{o/w}$ (see Table 1) of 2.22 and assuming water solubility as being 12 g/l at 25° C, a soil adsorption coefficient (k_{oc}) of 25 has been estimated (US EPA 1987). These values suggested that isophorone would be mobile in soil and that adsorption onto suspended solids and sediment in water would be insignificant (Swann et al, 1983).

4.2.4. Biodegradation

Tabak et al (1981a,b) reported concentrations of 5 and 10 mg/l isophorone to be rapidly degraded over 7 days by adapted micro-organisms based on an aerobic-static culture procedure

incorporating settled domestic waste water as the microbial inoculum. Care should be taken when interpreting data from this paper as no precautions were taken to prevent volatilisation losses.

Aerobic incubation of 100 mg/l with 30 ppm activated sludge for 2 weeks resulted in < 30% degradation (Kawasaki, 1980; Sasaki, 1980). Price et al (1974) reported the removal of 9 and 42% isophorone from salt or fresh water respectively following incubation for 20 days with a settled non-adapted domestic wastewater inoculum.

The losses of isophorone from waste water treated using a trickling filter, activated sludge, aerated lagoon and facultative lagoon were 19, 98, 24 and 30% respectively (Hannah et al, 1986). Intermediate degradation products of isophorone identified by Mikami et al (1981) after incubation with Aspergillus niger were:

3,5,5-trimethyl-2-cyclohexene-1,4-dione,
3,5,5-trimethylcyclohexane-1,4-dione,
(S)-4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one and
3-hydroxymethyl-5,5-dimethyl-2-cyclohexen-1-one

4.2.5. Bioaccumulation

Veith et al (1978) reported a bioconcentration factor of 7 for the bluegill sunfish exposed for 14 days to a mean water concentration of 92.4 ± 10.5 µg/l. The half-life in the tissues of this species was 1 day.

Isophorone was one of the three contaminants on the US EPA priority pollutant list to be detected in fish taken from Lake Michigan and 13 of its tributaries. The tissue concentrations ranged from 'not detected' to 3.61 mg/kg in Boardman River small mouth bass; the approximate limit of detection was 0.02 mg/kg using GC/MS. Elevated levels were also found in samples of common carp, largemouth bass, bowfin, northen pike, rock bass and lake trout (Camanzo et al, 1987).

5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 Environmental levels

5.1.1 Air

Harrison et al (1985) identified isophorone at a level of 490 ppb by GC/MS in electrostatically precipitated coal fly ash suggesting that coal-fired power stations may be a source of emission to the atmosphere.

5.1.2 Water

Isophorone was detected in 1% of 795 surface water samples with a median concentration of < 10 µg/l (Hauser and Bromberg, 1982). Concentrations up to 3 µg/l were reported in the Delaware river (Sheldon and Hites, 1979) and a concentration of 10 µg/l in urban runoff from Washington D.C. (Cole et al, 1984).

The US Environmental Protection Agency has identified isophorone in finished drinking water at concentrations ranging from 0.02 - 9.5 µg/litre (US EPA, 1980). A maximum concentration of 1 µg/l has also been reported in ground water in the Netherlands (Zoeteman, 1981). Specific sources of this contamination were not identified.

5.1.3. Soil

Isophorone was identified in sediments and soil taken from Love Canal (Hauser and Bromberg, 1982) and in sediments taken from Lake Pontchartrain (McFall et al, 1985). The concentrations in the latter were 0.98-12 ng/g dry weight.

5.1.4 Plants

To estimate the decline of isophorone in plants treated with pesticides containing isophorone as a carrier, ^{14}C-isophorone was sprayed on bean plants and rice at a rate equivalent to 7.5 kg/ha. Samples of the plants were taken periodically and assayed for total

radioactivity. No attempt was made to characterise metabolites or degradation products. In bean plants total residues declined rapidly from 16 mg/kg one hour after application to below 0.1 mg/kg on day 42. Beans harvested on day 56 did not contain detectable radioactivity. Residues in rice plants declined from 7.3 mg/kg one hour after application to 3.1 mg/kg on day 35 and 0.12 mg/kg on day 128. Immature rice heads did not contain radioactivity on days 110 and 128. The relatively slow decay in rice plants was considered by the authors to be due to unfavorable growing conditions in this particular study (Rohm & Haas, 1972).

In a similar study, sugar beet was sprayed at the 2-leaf stage with a herbicide containing ^{14}C-isophorone. Total radioactivity found on day 30 was reported to be 10% of the initial value. On day 90, residues in the plants were below 0.01 mg/kg except in dry leaves where 0.07 mg/kg were found. The results suggested some uptake of radiolabel from the soil from day 60 onwards; this was considered likely to be due to the uptake of small carbon fragments or $^{14}CO_2$ resulting from degradation of isophorone in the soil (Schering, 1974).

5.2 Hygiene standards - Occupational Exposure Levels

In the USA, the ACGIH have adopted a ceiling limit of 5 ppm (29 mg/m^3) and NIOSH a 4 ppm 10h-TWA based primarily on unpublished reports, supplied to the TLV committee, of fatigue and malaise in workers exposed to concentrations of 5-8 ppm for 1 month. On lowering the concentration to between 1 and 4 ppm no further complaints were received (ACGIH 1986). Similar values have been adopted by other countries (e.g. Federal Republic of Germany, Netherlands, Sweden, and UK). Exposure to isophorone in a screen printing plant, where "several environmental and working conditions favoured evaporation of this solvent and/or increase of risk of employee exposure" has been determined. Highest exposures were reported to be 23 $\pm$ 5.4 ppm (8h TWA) in the breathing zone of printing press workers (Samimi, 1982).

6. EFFECTS ON ORGANISMS IN THE ENVIRONMENT

6.1 Microorganisms

Yoshioka et al (1985) studied the acute toxicity of isophorone in Tetrahymena pyriformis. The EC_{50} after 24 hours exposure was 420 mg/l. The same authors (Yoshioka et al, 1986) found that the EC_{50} in the "Activated Sludge Respiration Inhibition Test" was 100 mg/l.

6.2 Aquatic Organisms

The acute toxicity of isophorone to fish, crustaceae, Daphnia and algae is summarised in Table 3. With the exception of Mysidopsis bahia (US EPA, 1978) all acute LC_{50}-values were above 100 mg/l indicating a relatively low aquatic toxicity. This was consistent with the results of a subacute (14 d) study with the marine red algae Champia parvula. The toxicity of isophorone was determined by means of various biological endpoints, namely vegetative growth, formation of tetrasporangia (asexual reproduction) and production of cystocarps (sexual reproduction). Depending on the toxicological endpoint, the lowest concentration resulting in a significant difference from controls ranged between 50 and 138 mg/l (Thursby et al, 1985).

In addition to the results of acute toxicity tests shown in Table 3, an early life stage toxicity test was conducted with the fresh water fathead minnow Pimephales promelas (Cairns and Nebeker, 1982) using concentrations of 11, 19, 30, 56 and 112 mg isophorone/l. Survival was effected at a concentration of 112 mg/l but not at 56 mg/l and lower; fork length was affected at 30 mg/l but not at 19 mg/l or lower; body weight gain was decreased at 19 mg/l and above but not at 11 mg/l. The authors calculated a no-effect concentration of 14 mg/l.

6.3 Terrestrial Organisms

Except for results of tests on laboratory animals no data are available.

7. KINETICS AND METABOLISM

7.1 Human

No data are available on toxicokinetics and metabolism of isophorone in man.

7.2 Experimental

Isophorone is absorbed when vapour or aerosol are inhaled, and (to a lesser extent) when in contact with the skin.

Following a single oral administration of isophorone to rats (4 g/kg) or one rabbit (1 g/kg), the substance was distributed rapidly in the body and detected in stomach, pancreas, adrenals, spleen and liver. Following inhalation (400 ppm for 4 h) isophorone was detected in the kidney, adrenals, liver, pancreas, and brain of rats (Dutertre-Catella, 1976).

Strasser et al (1988) studied the disposition of isophorone in male rats following administration by gavage of a single dose (3.6 mmol/kg) of ^{14}C-isophorone (in corn oil) containing 177 uCi/kg. Radiolabel distribution of ^{14}C-isophorone 24 hours after dosing showed that 93 % of the label had been excreted in the urine, faeces and expired air. The relative amounts were not stated. The remainder of radiolabel was highest in the liver, kidney and preputial glands which contained 3.7%, 1.1% and 0.7%, respectively of the original dose. The high concentration of isophorone in the preputial gland may be due to the high concentrations of alpha-2μ-globulin to which it could bind.

Following oral administration of 1 g/kg isophorone to rabbits the substance was partly eliminated unchanged in expired air and urine; the remainder was metabolised (see Fig. 1) to:-

a) 5,5-dimethylcyclohex-1-en-3-one-1-carboxylic acid, (i) derived from isophorone by methyloxidation,

b) isophorol (3,5,5-trimethyl-cyclohex-2-en-1-ol) (ii) formed by the reduction of the ketonic group into a secondary alcohol and eliminated as a glucuronide, and

c) dihydroisophorone (3,5,5-trimethyl-cyclohexanone) (iii) proceeding from the hydrogenation of the cyclohexene ring, and small quantities of *cis*- and *trans*-3,5,5-trimethyl-cyclohexanol-1 (iv), likely to have been formed from dihydroisophorone by dismutation.

The amounts of the identified metabolites as a proportion of the administered dose was not stated by the authors (Truhaut et al, 1970; Dutertre-Catella et al, 1978).

8. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

8.1 Acute Toxicity

The acute LD_{50}/LC_{50}-values for various routes of exposure are presented in Table 4.

8.1.1 Oral

Median lethal doses for isophorone in laboratory mammals ranging from 1,000 to 3,200 mg/kg body weight have been reported. The signs of toxicity were similar to those of solvents and narcotics, prostration being followed rapidly by coma. Deaths occurred within 24 hours, otherwise recovery was complete. Degenerative changes in the liver were reported in animals that died (Dutertre-Catella, 1976).

8.1.1.1 beta-Isophorone

This compound is present in an amount of 1-3% in technical isophorone (see Section 2.2). An oral LD_{50} of 2,950 mg/kg in rats has been reported. The predominant systemic effect was non-specific CNS depression shortly after dosing. Cirrhosis-like changes on the surface of the liver and severe irritation of the stomach were described in the animals that died following dosing (Hüls, 1988d).

8.1.2 Dermal

Dermal LD_{50} values indicate that isophorone is rapidly absorbed through the skin under occlusion. During the first 6 hours of occluded application an increase in respiratory rate followed by prostration and narcosis was reported in rabbits (Dutertre- Catella, 1976). Skin contact for 24 hours resulted in erythema followed after several days by scaring. Skin damage was still evident after 14 days.

8.1.3 Inhalation

It was reported that rats and guinea pigs were exposed to atmospheres containing isophorone concentrations of 300 or 750 ppm for 24 hours, of 880 ppm for 12 hours or of 1,370 to 4,600 ppm for 8 hours (Smyth and Seaton, 1940). From details of the method of atmosphere generation provided by the authors it follows that it was not possible to produce concentrations higher than saturated vapour concentration (about 500 ppm at 23°C) in their experiments. It would appear that at least some of the atmospheric concentrations quoted are an order of magnitude higher than could practically be achieved. It is therefore not possible to draw any conclusions from these experiments with respect to the relationship between the observed effects and the stated doses. Nevertheless certain toxicological features were noted and these are reported below.

In guinea pigs the signs of toxicity seen in the order of development were: irritation of the nose and eyes, lachrymation, swelling of the nose, ataxia, dyspnoea, diarrhoea, light narcosis and death. Fluorescein staining revealed corneal damage in guinea pigs but not rats exposed for periods of 4 hours or more. Rats developed the same symptoms as guinea pigs within a shorter period of exposure.

Post-mortem examination of rats dying following exposure showed haemorrhage in the lung, congestion of the stomach and liver, a peritoneal effusion and discoloration of the kidneys and spleen; death was ascribed to respiratory paralysis. It was stated that at room temperature it was not possible to generate a vapour concentration of isophorone sufficient to kill guinea pigs in 8 hours or rats in less than 4 hours.

8.2 Skin and Eye Irritation, Sensitisation

8.2.1 Skin irritation

The skin irritancy of isophorone was studied by Truhaut et al (1972). A single application of 0.5 ml isophorone under an occlusive patch for a period of 24 hours on the shaved or scarified skin of 6 rabbits produced a light erythema which disappeared rapidly after exposure. Microscopical examination did not show any histopathological changes.

In the rabbit, occlusive and semi-occlusive contact with 0.5 ml neat isophorone for 1 or 4 hours was non-irritating (Potokar et al, 1985).

8.2.1.1 beta-Isophorone

A single application of 0.5 ml of beta-isophorone under an semi-occlusive patch over a period of 4 hours on the shaved skin of 3 rabbits produced moderate erythema and swelling (Hüls, 1988e).

8.2.2 Respiratory irritation

De Ceaurriz et al (1981) estimated the concentration of various chemicals causing a 50% decrease in respiratory rate in mice (RD_{50}). The RD_{50} for isophorone was 27.8 ppm. For comparison the RD_{50} values for toluene di-isocyanate and acetone were 0.24 and 23,480 ppm respectively.

8.2.3 Eye irritation

A single instillation of 0.1 ml of isophorone in the eyes of 6 rabbits caused opacity in 4 animals, which in some instances covered the entire area of the cornea, inflammation of the conjunctivae and purulent discharge. A subsequent study indicated that considerable recovery from these effects occurred over 7 days (Dutertre-Catella, 1976).

Grant (1974) reported that application of one drop of isophorone to rabbit cornea caused mild transient injury, graded 4 on a scale of 1

to 10 after 24 hours. Pronounced irritation of eyes and nose in rats and guinea pigs exposed to atmospheres containing isophorone occurred at apparently higher concentrations (see Section 8.1.3) (Smyth and Seaton, 1940; Smyth et al, 1942).

8.2.3.1 beta-Isophorone

A single instillation of 0.1 ml in the eyes of 3 rabbits produced moderate conjunctival and corneal opacities. Iridial changes were also reported in 2 animals. Although the corneal and iridial changes had resolved by 7 days, minor conjunctival irritation was still in evidence (Hüls, 1988f).

8.2.4 Sensitisation

Isophorone, administered at a concentration of 10% intradermally (in maize germ oil) and 100% topically to female guinea pigs in the Magnusson-Kligman test, showed no sensitising potential (Hüls, 1988a).

8.3 Subchronic Toxicity

8.3.1 Inhalation

The effects of repeated exposure to isophorone vapour were reported by Smyth et al (1942). Groups of ten male Wistar rats and ten guinea pigs of mixed sex were exposed to concentrations of 25, 50, 100, 200 and 500 ppm isophorone 8 hours/day, 5 days/week, for 6 weeks. In the presentation of these results no distinction was made between the two species and no control data were presented. Growth retardation was noted in all animals exposed to concentrations at 100 ppm and above. There was a dose-related increase in mortality, the number of deaths increasing from 12% at 50 ppm to 45% at 500 ppm; the time of death was not reported. Post-mortem examination of animals dying subsequent to exposure revealed severely injured kidneys and lungs. The kidneys of surviving animals were congested

with dilation of Bowman's capsule and cloudy swelling of tubular cells. The lungs and liver were also reported to be congested with desquamation of the bronchial epithelium in the lungs and cloudy swelling in the liver cells. There was no apparent relationship between the incidence of these findings and the exposure concentrations. Chronic conjunctivitis and nasal irritation were reported only in animals exposed to 500 ppm isophorone. No effects were reported in animals exposed to 25 ppm isophorone.

Dutertre-Catella (1976) exposed groups of 10 male and 10 female rats to atmospheres containing 500 ppm 8 hours/day, 5 days/week for 6 and 4 months respectively. One female and two males exposed to isophorone died; there were no deaths in the control animals. The only reported effects were irritation of the eyes and nose.

8.3.2 Oral administration

Groups of 10 male and 10 female albino rats were fed diets containing 750, 1,500 or 3,000 ppm isophorone for 90 days. Haematology, serum chemistry and urine analyses were carried out on 5 animals of each sex from each group at week 4 and at termination. Comprehensive histopathology examination was confined to 5 animals of each sex from the control and high dose groups, and to liver and kidney only from the same number of animals of the intermediate dose levels. Under the conditions of this study, no effects on the general appearance of the test animals, on their behaviour, on body weight gains or on food consumption were observed at a dietary level of 1,500 ppm isophorone or less. Isophorone did not alter the composition of the formed elements of the blood, nor did it interfere with the general metabolism as well as liver and kidney function. No detectable gross or microscopic pathology was noted in any of the animals examined after 28 or 90 days of feeding. Organ/body weight ratios for vital organs were not changed. The only untoward finding reported was a reduction in body weight gain in the male rats receiving diet containing 3,000 ppm isophorone. The no observable effect level was considered to be 1,500 ppm in the diet, equivalent to approximately 100 or 150 mg/kg/day in males and

females respectively (Affiliated Medical Enterprises, 1972a).As part of a preliminary investigation prior to an NTP carcinogenicity study, rats and mice were given 12 oral doses per day up to 2,000 mg isophorone/kg body weight administered in corn oil over a 16 day period (NTP, 1986). Lethargy was reported in all rats following dosing while in mice uncertain locomotion was reported among animals receiving 1,000 mg/kg. All mice and 50% of the rats receiving 2,000 mg/kg died. The weight gain of animals surviving 2,000 mg/kg and all animals receiving 1,000 mg/kg was reduced. As part of the same programme, rats and mice were given daily doses of 0, 62.5, 125, 250, 500 and 1,000 mg/kg isophorone/kg body weight for 90 days (NTP 1986). At the highest dose 1 female rat and 3 female mice died. The rats were drowsy and lethargic following administration. No macroscopic or microscopic changes were observed in the organs examined from either study. A subsequent histopathological review of the kidney, which included re-sectioning and additional staining, also failed to reveal any treatment-related effects.

Groups of 4 male and 4 female beagle dogs were given 90 daily oral doses of isophorone in gelatin capsules at dosage rates of 35, 75 or 150 mg/kg/day. Comprehensive haematology, clinical chemistry and urine analyses were carried out initially and at 1, 2 and 3 months. Apart from "a mild intermittent incidence of soft stools" in animals of the high dose group there was no treatment-related effect as demonstrated by the data on general appearance and conditions as well as on haematology or biochemistry. At autopsy, no changes in the organ/body weight ratios and in the histology were observed. It was concluded that the no observable effect level for isophorone in the dog was 150 mg/kg/day (Affiliated Medical Enterprises, 1972b).

8.3.3 Dermal

The daily occluded dermal application of 0.1 or 0.2 ml of isophorone to rats for 8 weeks produced erythema and scabs at the site of application (Dutertre-Catella, 1976). The only apparent systemic effect reported was an 8% reduction in mean weight gain in the females compared with controls; the dose levels at which this occurred were not given.

8.4 Mutagenicity

The results of mutagenicity tests are summarised in Table 5.

8.4.1 Gene-mutation in bacteria: Ames Salmonella test

The mutagenic potential of isophorone was examined using Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100, following a preincubation protocol. Test substance concentrations used were between 10 and 5,000 µg per plate. There was no increase in the number of revertants at any of the concentrations tested with and without rat liver S9 fraction (Hüls 1988b).

Two further studies were conducted on Salmonella typhimurium TA 1535, TA 1537 and TA 1538 strains (1 to 1,000 µg/plate) (Atochem 1978a) and TA 98, TA 100, TA 1535 and TA 1537 strains (100 to 10,000 µg/plate) (NTP, 1986). No evidence of mutagenic potential was provided in the presence or absence of rat or hamster liver S9 fractions.

8.4.1.1 Dihydroisophorone

The mutagenic potential of dihydroisophorone, a metabolite of isophorone, was examined using Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 at concentrations of 25 to 2,500 µg per plate. There was no increase in the number of revertants at the concentrations tested with and without rat liver S9 fraction (BP, 1988a).

8.4.1.2 Isophorone oxide

Isophorone oxide is a putative metabolite of isophorone although it has not been identified in animal tissues. Its mutagenic potential (purity ≥ 99.5 %) was studied in a standard Ames Salmonella test using the strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100, following a preincubation protocol. The concentrations of isophorone oxide ranged from 10 to 5,000 µg per plate. No increase in the number of revertants was observed in the presence or in the absence of rat liver S9 fraction (Hüls, 1988g).

8.4.2 Gene mutation in mammalian cells : L5178Y TK +/- mouse lymphoma mutagenesis assay

Isophorone was tested in the L5178Y TK +/- mouse lymphoma mutagenesis assay in the presence and absence of rat liver S9 fraction. The experiment was performed once only and all doses were tested in duplicate. In the absence of S9, concentrations of 0.18 to 1.3 µl isophorone/ml produced total growth of 111% to 12% compared to the control. In the presence of S9, isophorone concentrations of 0.067 to 0.89 µl/ml produced total growth of 86% to 9% compared to the control. None of these cultures exhibited mutant frequencies which were significantly greater than the mean mutant frequency of the solvent control (CMA, 1984a).

Another L5178Y TK +/- mouse lymphoma study employing concentrations of 400 to 1,200 µg/ml was carried out in the absence of rat liver S9 fraction. Duplicate experiments produced gradations of total growth relative to control values of 112 or 118% for the low doses to 7 or 14% for the high doses. Dose-related increases in mutation frequencies compared with control were observed, but even at the highest dose level the increase was only 4-fold (NTP, 1986).

8.4.3 Chromosome aberrations in CHO cells in vitro

Isophorone was tested in an in vitro cytogenetic assay using CHO cell cultures. Treatments were performed both in the absence and

presence of rat liver S9 fraction. Under the conditions of this test, isophorone did not induce chromosomal aberrations at concentrations up to 1,600 or 1,500 µg per ml respectively (NTP, 1986).

8.4.4 Micronucleus test in mice

Doses of 450, 900 and 1800 mg/kg bodyweight were administered by gavage to CFLP mice in 2 equal doses separated by an interval of 24 hours. Six hours after the last treatment the mean micronucleated cell counts and the bone marrow cytotoxicity were similar in all test groups and controls (Atochem, 1978b).

Male and female CD-1 mice were treated by i.p. injection with 0.54 ml/kg bodyweight of isophorone. There was no evidence of micronuclei or cytotoxicity in bone marrow samples collected 12, 24 and 48 hours after administration (CMA, 1984b).

8.4.5 Sister-Chromatid Exchange (SCE)

The ability of isophorone to induce SCE was studied in CHO cells, in the absence and presence of rat liver S 9 fraction at concentrations up to 1,000 µg/ml isophorone. Without S9, isophorone induced a small but dose-related increase in the frequencies of SCE. No effect was observed in the presence of S9 (NTP, 1986).

8.4.6 Unscheduled DNA Synthesis: primary rat hepatocyte culture

Isophorone was tested at dose levels ranging from 0.005 to 0.40 µl/ml using rat primary cultures of hepatocytes. There was no increase in the mean nuclear grain count compared to the controls or in the incidence of cells undergoing repair at any dose level (CMA, 1984c).

8.4.7 DNA-binding in vivo

A DNA binding study was performed with radioactively labelled 1,3,5-^{14}C-isophorone (Thier et al, 1989). Male and female F344 rats

and male and female B6C3F1 mice received doses of 500 mg per kg body weight containing up to 0.4 mCi labelled isophorone. No binding of the radioactivity to liver or kidney DNA was observed in either species.

8.5 Chronic Toxicity and Carcinogenicity

In 18-month inhalation studies, groups of 10 rats and 2 rabbits per sex were exposed to isophorone (250 ppm for 6 h/d, 5 d/wk). Slight conjunctivitis and irritation of the nasal mucosa with a bloody discharge were observed. Additionally, marked microvacuolation of the liver cells was attributed to exposure to the substance. Histopathological examination failed to detect neoplasias in this limited study (Dutertre-Catella, 1976). In a 2-year bioassay isophorone was administered as a solution in corn oil by gavage to 50 males and 50 females F344 rats and B6C3F1 mice in doses of 0, 250 and 500 mg/kg/day, 5 days a week (NTP, 1986). At the high dose, reduction of body weights of rats (male 5%, female 8%) and of female mice (5%) was observed. No overt signs of toxicity were reported. The survival rate was reduced as a result of treatment in male and female rats and male mice. Autopsy of male rats revealed proliferative kidney changes and mineralisation of renal tubular cells. In addition, liver damage was found in male mice. In male rats kidney and preputial gland tumours occurred at a higher incidence than in controls. In male mice tumours of mesenchymal tissue (integumentary tumours) lymphomas and liver tumours occurred at a higher level than in controls. These data were interpreted by the NTP as providing "some evidence of carcinogenicity" in male rats and "equivocal evidence of carcinogenicity" in male mice.

8.5.1 Rats

The incidence of renal tumours is given in Table 6. The tumours observed are rare (<1%) in untreated rats or in rats treated with corn oil (Hasemann et al, 1985). Examination of control data from three carcinogenicity studies carried out as part of the NTP programme revealed one benign renal tumour occurring among a total of 450 male rats in control and treated groups; this tumour was not considered to be treatment related. The relatively high incidence of these tumours in the current study would therefore suggest that they are caused by isophorone treatment. The presence of hyperplasia and of alpha-2μ-globulin in the renal tubules of isophorone treated male rats and the lack of evidence of genotoxicity point to a non-genotoxic mechanism of action which is species and sex specific (see Section 8.6).

Carcinomas of the preputial gland were found in five male rats treated with the highest dose of isophorone. The normal incidence of adenomas and carcinomas of this organ is approximately 6%, the range of incidence of adenomas being 0-16% and that of carcinomas 0-10% (Hasemann et al, 1985). In previous NTP carcinogenicity studies cited in the isophorone report (NTP, 1986) the number of preputial gland adenomas and carcinomas in corn oil controls ranged from zero to seven (ie 0 -14%). In a more recent NTP study on nitrofurantoin (NTP, 1989) 6 adenomas and 6 carcinomas occurred in a group of 50 animals serving as controls and treated only with corn oil. Considering the variable incidence of this type of tumour and the fact that up to a 12% incidence of carcinomas have been reported in one study strongly suggests that the apparently raised incidence of preputial gland tumour in the isophorone study is a chance observation. No evidence of increased tumour incidence was found in female rats (Bucher et al, 1986; NTP, 1986).

8.5.2 Mice

In male mice there was a marginal increase in the incidence of hepatocellular adenomas and carcinomas, mesenchymal tumours and malignant lymphomas (Tables 8-10). Although this was interpreted by the NTP as "equivocal evidence of carcinogenicity" the high and variable spontaneous incidence of the tumours in historical control groups suggests that the incidence in the present study is unrelated to treatment (ECETOC, 1982; Haseman et al, 1985;). This view is strenghtened by the fact that no evidence of increased tumour incidence was found in the females (Bucher et al, 1986; NTP, 1986) and that these tumour types were not increased in incidence in rats.

8.6 The Role of Alpha-2μ-globulin in Renal Tumour Induction

A small increase of nephropathy in male rats (tubular mineralisation and epithelial hyperplasia of the renal pelvis) and tubular neoplasia has resulted from lifetime exposure of experimental animals to a number of substances. The compounds tested did not produce any such changes in female rats or in mice of either sex. To explain this species- and sex-specific phenomenom, special investigations were carried out (Olson et al, 1986; Lock et al, 1987; Charbonneau et al, 1988; Goldworthy et al, 1988; Lehmann-McKeeman and Cladhill, 1988) on d-limonene, decalin, trimethylpentane(TMP),p-dichlorobenzene, and chlorinated hydro-carbons. The findings were as follows.

Alpha-2μ-globulin, a protein found mainly in male rats, is synthesised by the liver and subsequently transported to the kidney. It is normally present in the cytoplasm of the proximal convoluted tubules of untreated animals in the form of hyaline droplets, visible by light microscopy. The xenobiotics, or their metabolites, are bound to the alpha-2μ-globulin in the liver of animals; this conjugate is even more difficult to hydrolyse than the alpha-2μ-globulin itself and induces the formation of the hyaline droplets which accumulate in the tubules (Swenberg et al, 1989). Accumulation of the chemical/alpha-2μ-globulin

complex causes lysomal protein overload and necrosis of the cells with subsequent cellular regeneration.

Short et al (1987) showed conclusively in their studies on unleaded gasoline that this cell necrosis is followed by episodes of hyperplasia in the renal tubules which occurred only in the male rats, thus linking them to the presence of an excess of alpha-2μ-globulin in the proximal renal tubular cells.

It is known that recurrent episodes of cell necrosis and reparative hyperplasia in rodent tissues may lead to tumour development. Examples are the sarcoma produced by prolonged exposure to hypertonic or surface-active solutions, the production of bladder tumours by stones or crystalluria, the production of squamous cell carcinoma of the skin by the repeated application of potassium or sodium hydroxides and the development of tumours in hormonally sensitive organs made hyperactive by an excess of the trophic hormone (Schmahl, 1984; Grasso, 1987).

A similar mechanism is known to operate in the kidney. Lead acetate and nitriloacetic acid, both of which are non-genotoxic, produce both hyperplasia and tumours of the renal tubules in rats when administered at a high dose-level for several months (Anderson et al, 1985). Thus cellular proliferation may contribute to the development of renal tumours. In contrast, the amount of alpha-2μ-globulin in females was 120 times lower (Charbonneau and Swenberg, 1988). Therefore the accumulation of alpha-2μ-globulin observed in the tubules of the renal cortex is specific to male rats and is regarded as the primary toxic effect which, following chronic exposure to high doses, resulted in neoplasia (Short and Swenberg, 1988).

Recent investigations (Charbonneau et al, 1988; Strasser et al, 1988) showed that isophorone, isophorol and dihydroisophorone also bind to alpha-2μ-globulin, resulting in an increased accumulation of hyaline droplets in renal tubular cells. These findings suggest the same sequence of events may be responsible for the small increase in the incidence of renal tubular neoplasias seen in male rats. Since

alpha-2μ-globulin was not detected in significant amounts in female rats and appeared to be absent in mice, dogs and human beings of both sexes, Charbonneau (1988) and Strasser et al (1988) concluded that the low increase in the incidence of tubular adenomas and adenocarcinomas produced in male rats by isophorone was attributable to the accumulation of alpha-2μ-globulin and was sex- and species-specific.

Epidemiological studies on workers occupationally exposed to hydrocarbons have provided no evidence of increased nephropathy or renal cancer, giving support to the hypothesis that the renal neoplasia produced in male rats by these compounds is of questionable relevance to man (Pitha et al, 1987).

8.7 Reproduction, Embryotoxicity, Teratogenicity

The teratogenicity of isophorone to rats and mice was studied by Traul et al (1984). Groups of 22 confirmed mated females of each species were exposed 6 hours/day from days 6-15 of gestation to atmospheres containing 0, 25, 50 or 115 ppm isophorone. At the highest atmospheric concentration there was evidence of maternal toxicity which showed reduced food consumption, alopecia and cervical or anogenital staining in the rats and reduced body weights in the mice. Comprehensive uterine and foetal examinations did not show any significant differences between animals exposed to isophorone and their respective controls.

Groups of 10 rats of each sex exposed 6 hours per day 5 days per week to 500 ppm isophorone for 3 months were mated to exposed animals or to controls (Dutertre-Catella, 1976). All females were reported to have delivered 7-10 pups. Anatomo-pathological examination did not show any abnormalities.

8.8 Neurotoxicity

Central nervous system depression is a characteristic feature of isophorone intoxication in experimental animals (Smith and Seaton, 1940; Dutertre-Catella, 1976; De Ceaurriz et al, 1981).

Isophorone and a number of other aliphatic ketones have been studied using the mouse behavioural despair swimming test (De Ceaurriz et al, 1984). This test which was developed for screening anti-depressant drugs is based on the duration of the periods of immobility exhibited by mice when placed in water. Mice were exposed to isophorone at atmospheric concentrations of 89-137 ppm. The ID_{50} (an estimated concentration producing a 50% reduction in the immobility time) for animals thus exposed was 110 ppm. Comparison of this value with the respiratory irritancy as measured by the RD_{50} (the concentration producing a 50% reduction in respiratory rate) led the authors to conclude that the predominant effect of isophorone was sensory irritation of the upper respiratory tract rather than CNS depression. By application of the same techniques acetone was deemed to exert a greater effect on the CNS than on sensory receptors in the respiratory tract.

9. EFFECTS ON MAN

9.1 Acute

Groups of 11 or 12 subjects exposed for a few minutes to measured atmospheric concentrations of 40, 85, 200 and 400 ppm isophorone experienced irritation of the eyes, nose and throat (Smyth and Seaton 1940). A few complaints of nausea, headache, dizziness, faintness, inebriation and a feeling of suffocation occurred at concentrations of 200 and 400 ppm. The symptoms of irritation and narcosis were said to be less intensive following exposure to atmospheres containing 40 to 85 ppm.

9.2 Sub-chronic

Complaints of fatigue and malaise were reported among workers exposed for 1 month to atmospheres containing 5 to 8 ppm isophorone (Ware, 1973). No further complaints were received following a reduction of the concentration to between 1 and 4 ppm. On the basis of these data the ACGIH recommended a ceiling limit of 5 ppm for isophorone.

9.3 Irritation and Sensitisation

9.3.1 Skin irritation

No data are available.

9.3.2 Eye and respiratory irritation

Smyth and Seaton (1940) reported eye, nose and throat irritation in man exposed to measured concentrations at 40, 85, 200 and 400 ppm for a few minutes. At 40 and 85 ppm the initial irritation did not persist throughout the exposure.

Silverman et al (1946) estimated the sensory threshold of a number of ketones including isophorone. An average of 12 subjects of both sexes were used for each solvent exposure. The time of exposure was 15 minutes. Irritation of the eyes, nose and throat was experienced at 25 ppm isophorone; the highest concentration the majority of subjects considered "satisfactory" for an 8 hour exposure was 10 ppm.

Amoore and Hautala (1983) reported an air odour threshold for isophorone of 0.20 ppm. The "odour safety factor", which was defined as the threshold limit value (5 ppm) divided by the odour threshold, was 25. From the magnitude of this value the authors predicted that 50% of distracted persons would perceive sensory warning of the TLV (5 ppm).

Exposures to isophorone at 200 ppm for 1 minute or 40 ppm for 4 minutes were reported as intolerable (NTP, 1986).

9.3.3 Skin sensitisation

Isophorone does not cause allergic sensitisation (NTP, 1986).

9.4 Mutagenicity

No data are available.

9.5 Chronic toxicity and carcinogenicity

No surveys have been carried out on occupationally exposed workers or other potentially exposed populations.

9.6 Reproductive Toxicity

No information found.

9.7 Neurotoxicity

Non-specific symptoms of CNS depression consisting of nausea, headache, dizziness, faintness and inebriation were reported by Smyth and Seaton (1940) among a group of subjects exposed to 200 - 400 ppm isophorone.

10. FIRST AID AND SAFE HANDLING ADVICE

10.1 First Aid and Medical Treatment

Eye Contact: Immediately flush eyes with plenty of water. Assure adequate flushing of the eyes by separating the eyelids with fingers. Medical attention should be obtained.

Skin Contact: Contaminated clothing should be removed and the affected area of the skin thoroughly flushed with water. If skin irritation occurs, medical attention should be obtained.

Inhalation: Provide fresh air. Monitor breathing, and give oxygen if breathing is difficult.

Ingestion: Seek medical advice immediately. Wash out mouth with water.

10.2 Safe Handling

Personal Protection: Atmospheric levels should be kept below the recommended occupational exposure limits by local exhaust ventilation. Wear respirator in areas where these limits are likely to be exceeded. Skin and eye protection should be worn where exposure to liquid is likely to occur. Nitrile rubber gloves are recommended. (BP, 1988b).

Storage: Keep containers tightly closed. Store in a well ventilated area.

10.3 Management of Spillage and Waste

Evacuate the area. Remove sources of ignition. Wear self-contained breathing apparatus, gloves, goggles, face shield and boots. Liquid should be prevented from entering sewers, basements and workpits.

Small-scale spillages should be absorbed on paper towels and the paper burnt away from other combustible material.

For medium-scale spillages the liquid should be absorbed with sand or earth and all material should be removed to a safe place for subsequent incineration. The contaminated area should be washed out with plenty of water.

For large-scale spillages, the spilt liquid should be prevented from spreading by the use of sand or earth. The liquid should be transferred to a salvage tank if possible, otherwise it should be treated as for medium-scale spillages. The local authorities should be informed at once if the spilt liquid enters the surface water drains.

Disposal: This combustible material may be burned in a chemical incinerator. The product should not be buried or dumped in a landfill.

Fire: Suitable extinguishing media are water spray, carbon dioxide, foam or dry powder.

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TABLE 1

Physical and Chemical Data of Isophorone

Criterium	Value	Unit	Reference
Specific gravity (20°C/4°C)	0.922		1
Boiling point at 1013 hPa	215	°C	1
Freezing point	-8.1	°C	2
Refractive index	1.4775	n_D^{20}	1
Viscosity at 20°C	2.6	mPa_1s	3
Coefficient of cubical expension	0.00085	$°C^{-1}$	6
at 20°C	0.00078	$°C^{-1}$	5
Surface tension at 20°C	30	mN/m	6
Vapour pressure at 20°C	0.4	hPa	1
	0.26	hPa	4
Vapour density (air=1)	4.7		2
Concentration in saturated air at 20°C and 1013 hPa	340	ppm	2
Solubility at 20 °C			
- Isophorone in water	17.5	g/l	7
- Water in isophorone	53	g/l	7
log $k_{o/w}$ (estimated)	2.22		8
	1.7		9
Solubility parameters (Hansen)			
delta	19.2	$(J/cm^3)^{1/2}$	3
$delta_D$	16.6	$(J/cm^3)^{1/2}$	3
$delta_P$	8.2	$(J/cm^3)^{1/2}$	3
$delta_H$	7.4	$(J/cm^3)^{1/2}$	3
Hydrogen bonding parameter, gamma	14.9		3
Flashpoint, closed cup	85	°C	2
Explosion limits in air	0.8-3.8	vol-%	1
Ignition temperature	470	°C	1
	455	°C	4
Heat of evaporation at 215 °C	349.2	kJ/kg	1
Heat of combustion (p=const at 20°C)	38,100	kJ/kg	1
Relative permittivity at 20°C	19.9		3
Specific resistivity	$1x10^7$	ohm x cm	5

References: 1 = Bartholomé et al (1977)
2 = Cheminfo (1988)
3 = Hüls (1981)
4 = BIBRA (1987)
5 = Atochem (1986)
6 = BP (1988b)
7 = Hüls (1989)
8 = US EPA (1987)
9 = Callahan et al (1979)

TABLE 2

Gas Chromatographic Conditions for the Analysis of Isophorone

Column	Fused silica capillary	Macrobare
Coating	OV - 1701	CP Wax 52 CB
Dimensions	60 m/0.25 mm	25 m/0.53 mm
Injector Temp.	240° C	250° C
Temp.-Program	6 min 70° to 220° @ 4°/min	10 min 105° to 120° @ 6°/min 2 min 120° to 150° @ 10°/min
Detector Temp.	250° C	250° C
Evaluation	100 % peak area	100 % peak area
Reference	Hüls, 1988c	Atochem, 1988

TABLE 3

Isophorone Aquatoxic

Test species	Parameter	Results	Reference
FISH			
Macrochirus lepomis	LC_{50} (96 h)	220 - 224 mg/l	US EPA, 1978
			Buccafusco et al, 1981
Pimephales promelas	LC_{50} (96 h)	145 - 255 mg/l	Cairns & Nebeker, 1982
Cyprinodon variegatus	LC_{50} (96 h)	140 mg/l	Ward et al, 1981
	LC_{50} (96 h)	>166, <295 mg/l	Price et al, 1974
			US EPA, 1978
SALT WATER CRUSTACEA			
Artemia salina	LC_{50} (96 h)	430 mg/l	Price et al, 1974
Mysidopsis bahia	LC_{50} (96 h)	12,9 mg/l	US EPA, 1978
Daphnia, acute	EC_{50} (24 h)	430 mg/l	Le Blanc, 1980
	EC_{50} (48 h)	120 mg/l	Le Blanc, 1980
	EC_{50} (48 h)	117 mg/l	US EPA, 1978
ALGA			
Selenastrum capricornutum	Cell count EC_{50} (96 h)	122 mg/l	US EPA, 1978
	Chlorophyll EC_{50} (96 h)	126 mg/l	US EPA, 1978
Skeletonema costatum	Cell count EC_{50} (96 h)	105 mg/l	US EPA, 1978
	Chlorophyll a EC_{50} (96 h)	110 mg/l	US EPA, 1978

TABLE 4

Acute LD_{50}/LC_{50} values for Isophorone

Route	Species	LD_{50}/LC_{50}	Reference
ORAL(LD_{50})	Rat	1500 mg/kg	Schering, 1968a
"	"	1870 mg/kg	Union Carbide, 1958
"	"	2000 mg/kg	Rohm & Haas, 1975
"	"	2104 mg/kg	Smyth et al, 1969
"	"	2700 mg/kg	Duterre-Catella, 1976
"	"	2100 mg/kg	Duterre-Catella, 1976
"	"	2370 mg/kg	Bukhulovskii & Shugaev, 1976
"	"	>3200 mg/kg	Eastman Kodak, 1967
"	Mouse	2200 mg/kg	Dutertre-Catella, 1976
"	"	2000 mg/kg	Bukhulovskii & Shugaev, 1976
"	"	>3200 mg/kg	Eastman Kodak, 1967
"	Guinea Pig	1000 mg/kg	Rohm & Haas, 1975
"	Rabbit	2000 mg/kg	Smyth et al, 1969
DERMAL(LD_{50})	Rat	1700 mg/kg	Schering, 1968b
"	Rabbit	1200 mg/kg	Dutertre-Catella, 1976
"	"	1384 mg/kg	Union Carbide, 1958
INHALATION(LC_{50})	Rat	>7000ppm(6h)	Dutertre-Catella, 1976
"	Rabbit	>7000ppm(6h)	Dutertre-Catella, 1976
PARENTERAL(LD_{50}) intra-peritoneal	Rat	400-800mg/kg	Eastman Kodak, 1967
"	Mouse	800 mg/kg	Eastman Kodak, 1967

TABLE 5

Isophorone Mutagenicity Tests

Mutation Type	Test System	Results	Reference
Gene mutation	Ames test (± S9)	-	Hüls 1988b
		-	Atochem 1978a
		-	NTP 1986
	Mouse lymphoma assay (- S9)	+	NTP 1986
	(± S9)	-	CMA 1984a
Chromosome aberrations	CHO cells in vitro (± S9)	-	NTP 1986
	Mouse (in vivo) micronucleus test	-	Atochem 1978b
		-	CMA 1984b
Direct DNA damage	SCE in CHO cells (- S9)	+	NTP 1986
	(+ S9)	-	
	Unscheduled DNA synthesis (primary rat hepatocyte culture)	-	CMA 1984c

± S9 = with and without S9
+ S9 = with S9
- S9 = without S9

+ = positive
- = negative

TABLE 6

Number of Male Rats With Renal Lesions in the Two-year Gavage Study of Isophorone

	Vehicle Control	250 mg/kg	500 mg/kg
Number of male rats examined	50	50	50
Tubular cell hyperplasia	0	1	4
Tubular cell adenoma	0	0	2
Tubular cell adenocarcinoma	0	3	1

TABLE 7

Number of Male Rats With Preputial Gland Tumours in the Two-year Gavage Study of Isophorone

	Vehicle Control	250 mg/kg	500 mg/kg
Number of male rats examined	50	50	50
Carcinomas	0	0	5*

* statistically significant ($P<0.05$)

TABLE 8

Number of Male Mice With Integumentary System Tumours in the Two Year Gavage Study of Isophorone

	Vehicle Control	250 mg/kg	500 mg/kg
Number of male mice examined	50	50	50
Fibroma, Sarcoma, Fibro-sarcoma, or Neurofibrosarcoma	6	8	14*

* statistically significant ($P<0.05$)

TABLE 9

Number of Male Mice With Liver Tumours in the Two-year Gavage Study of Isophorone

	Vehicle Control	250 mg/kg	500 mg/kg
Number of male mice examined	50	50	50
Hepatocellular Adenoma	6	7	13
Hepatocellular Carcinoma	14	13	22
Hepatocellular Adenoma or Carcinoma	18	18	29*

* statistically significant ($P<0.05$)

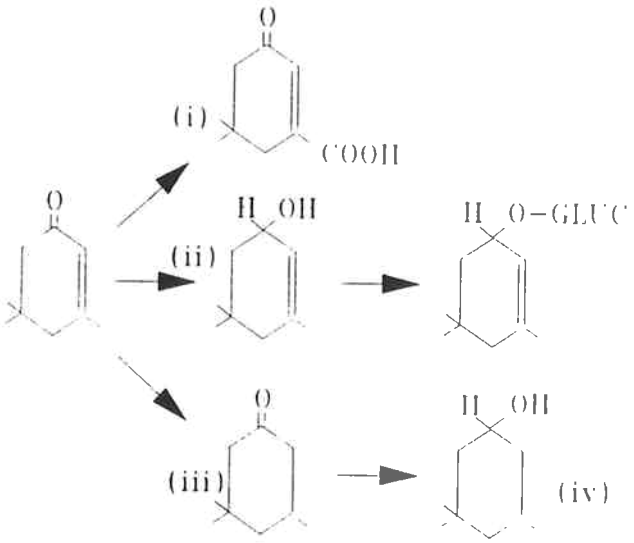
TABLE 10

Number of Male Mice With Hematopoietic System Tumours in the Two Year Gavage Study of Isophorone

	Vehicle Control	250 mg/kg	500 mg/kg
Number of male mice examined	50	50	50
Lymphoma	7	18*	5
Lymphoma or Leukemia	8	18*	5

* statistically significant ($P<0.05$)

Figure 1. Metabolic transformation of isophorone



i = 5,5-dimethylcyclohex-1-en-3-one-1-carboxylic acid
ii = 3,5,5-trimethyl-cyclohex-2-en-1-ol
iii = 3,5,5-trimethyl-cyclohexanone
iv = 3,5,5-trimethyl-cyclohexanol-1 (cis- and trans-)

APPENDIX 1

Classifications and Regulations

EEC-Directive on Dangerous Substances (67/548/EEC et seqq.):

Nature of general risk:	Xi irritant
Nature of special risks:	R36/37/38 irritating to eyes, respiratory system and skin
Safety advices:	S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Regulations on the Transport of Dangerous Goods:

RID/ADR:	class 3, No. 32c
ADNR:	class 3, No. 4, category K 3
GGVSee/IMDG-Code:	not classified
IATA-RAR:	article No. 1939

APPENDIX 2

ALPHA-2μ-GLOBULIN IN RAT PREPUTIAL GLAND

Recent studies show that high levels of alpha-2μ-globulin and its messenger RNA are present in the preputial gland of both male and female rats. The preputial gland in both sexes contained about 3 times more alpha-2μ-globulin m-RNA and about 300 times more alpha-2μ-globulin compared to the male rat liver (Murty et al, 1987). It is claimed, that this high content of alpha-2μ-globulin was primarily due to the cellular and ductal accumulation of the protein in the preputial gland but did not reflect a difference in the rate of transcription of the alpha-2μ-globulin gene. The high amount of ^{14}C-isophorone found in the preputial gland of the male rats following single gavage dosing of 5 ml/kg (Strasser et al, 1988) may therefore be related to its high content of alpha-2μ-globulin. The significance of this information with respect to the aetiology of pathological changes is uncertain at present.

APPENDIX 3

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