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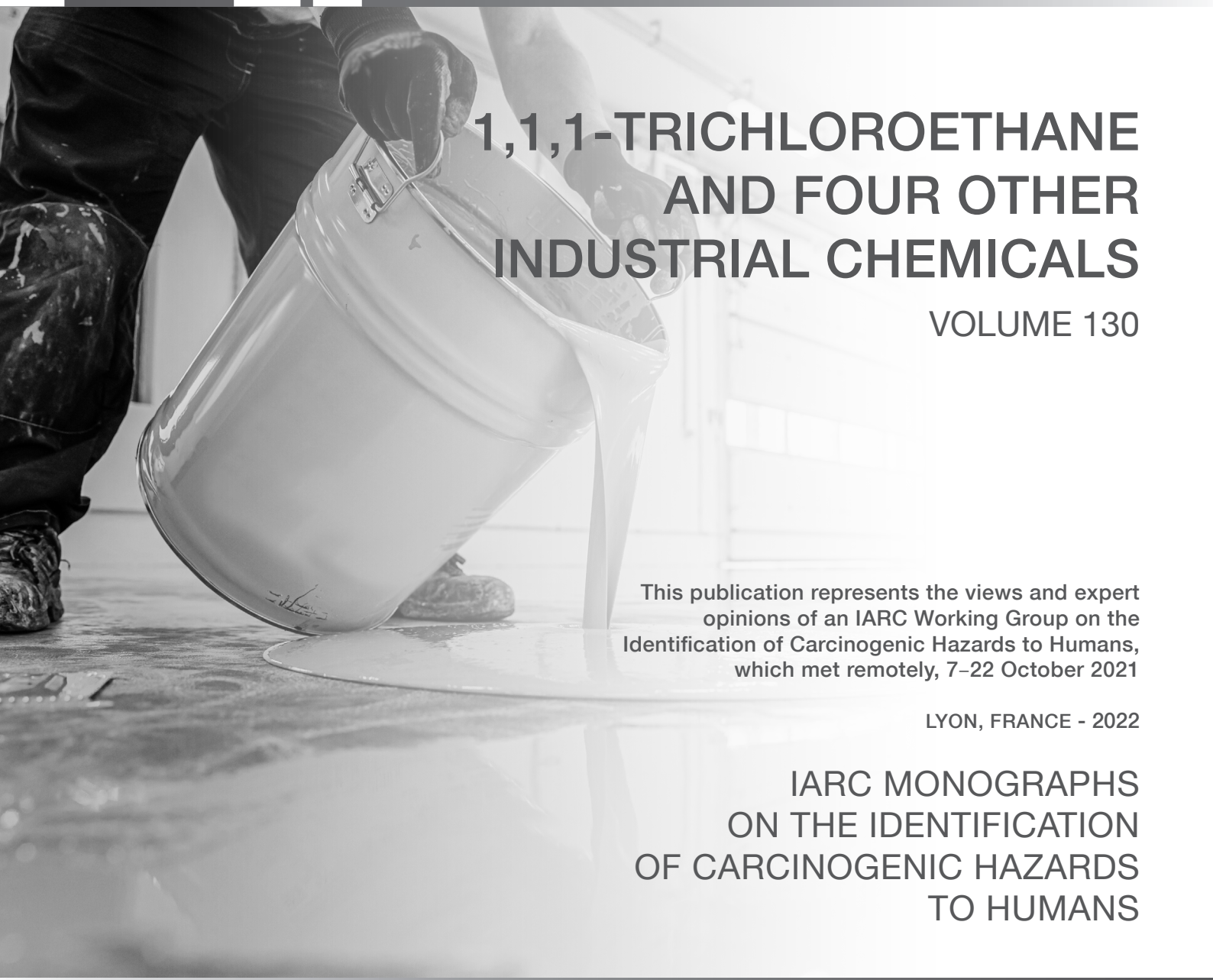

1,1,1-TRICHLOROETHANE AND FOUR OTHER INDUSTRIAL CHEMICALS

VOLUME 130

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International Agency for Research on Cancer

World Health Organization

ISOPHORONE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

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

EC/List No.: 201-126-0

Chem. Abstr. Serv. name: isophorone

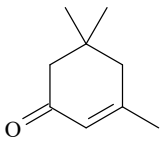
IUPAC systematic name: 3,5,5-trimethyl-cyclohex-2-en-1-one

Synonyms: 3,5,5-trimethylcyclohex-2-enone; 3,5,5-trimethyl-2-cyclohexene-1-one; 1,1,3-trimethyl-3-cyclohexene-5-none; 3,5,5-trimethyl-2-cyclohexen-1-one; isoacetophorone; isooctopherone; α-isophorone; 3,5,5-trimethyl-2-cyclohexenone; and other depositor-supplied synonyms and acronyms (NCBI, 2021).

1.1.2 Structural and molecular information

Relative molecular mass: 138.21 (NCBI, 2021)

Chemical structure:



Molecular formula: $C_9H_{14}O$

1.1.3 Chemical and physical properties

Description: colourless liquid with a peppermint-like odour (IFA, 2021a) or camphor-like odour (NCBI, 2021)

Odour threshold: odour may be noticeable at concentrations of 2–5 ppm (NCBI, 2021)

Boiling point: 215 °C (NCBI, 2021)

Melting point: –8.1 °C (NCBI, 2021)

Density: 0.92 g/cm^3 at 20 °C (IFA, 2021a)

Relative vapour density: 4.77 (air = 1) (IFA, 2021a)

Vapour pressure: 0.59 hPa at 25 °C (IFA, 2021a)

Auto-ignition temperature: 460–470 °C at 1013 hPa (ECHA, 2021a)

Lower explosion limit: 0.87 vol.% (50 g/m^3) (IFA, 2021a)

Upper explosion limit: 3.8 vol.% (220 g/m^3) (IFA, 2021a)

Solubility: sparingly soluble (12 g/L at 20 °C) in water (IFA, 2021a); soluble in ether, acetone, and alcohol; high solvent power for vinyl resins, and cellulose esters (NCBI, 2021)

Flash point: 84 °C (NCBI, 2021)

Stability and reactivity: the combustible substance can react dangerously with air, and the formation of peroxides is possible (IFA, 2021a); exposure to sunlight in aqueous

solutions can result in the formation of photodimers by 2+2 photocycloaddition (Gonçalves et al., 1998)

Octanol/water partition coefficient (P): log K_{ow} = 1.70 (NCBI, 2021)

Conversion factor: 1 ppm is equivalent to 5.74 mg/m^3 at 1013 mbar [101.3 kPa] and 20 °C (IFA, 2021a)

Dynamic viscosity: 2.62 cP at 20 °C [2.62 × 10^{-5} hPa.s] (NCBI, 2021).

1.1.4 Impurities

Impurities include up to 4% isomeric β-isophorone (3,5,5-trimethyl-3-cyclohexen-1-one) and traces (< 1%) of 1,3,5-trimethylbenzene, mesityl oxide (2-methyl-2 pentene-4-one), phorone (2,6-dimethyl-2, 5-heptadiene-4-one), and isoxylitones (NCBI, 2021).

1.2 Production and use

1.2.1 Production process

Isophorone is produced by the aldol condensation of acetone at high temperature (200 °C) and pressure (3.6 MPa) in the presence of aqueous potassium hydroxide. Alternatively, isophorone can be manufactured using calcium oxide, hydroxide, or carbide, or mixtures thereof, at atmospheric pressure and high temperature (350 °C) (NCBI, 2021).

1.2.2 Production volume

Isophorone is listed as a High Production Volume chemical by the Organisation for Economic Co-operation and Development (OECD) (OECD, 2004, 2009). Worldwide production capacity has been estimated at 50 000 tons [45 400 tonnes] in 1990 (NCBI, 2021). In 2016, the United States Environmental Protection Agency (US EPA) estimated an aggregated production volume of 10 000 000–50 000 000 pounds [~4536–22 680 tonnes] in the USA (US EPA, 2016). Isophorone is registered under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation, and more than 100 tonnes per year are manufactured in and/or imported to the European Economic Area (ECHA, 2021a).

1.2.3 Uses

Isophorone is a widely used solvent and chemical intermediate used at industrial sites and in the manufacture of lacquers and vinyl/acetate-based polymers, inks and paints, nitrocellulose finishes, and washing and cleaning products (US EPA, 2000; ECHA, 2021a; NCBI, 2021). Isophorone is used in the manufacture of agrochemicals and is a constituent of certain pesticides. For example, in the USA, isophorone is exempt from the requirement of a tolerance when used as an inert ingredient in pesticide formulations applied to beet, ginseng, rice, spinach, sugar beet, and Swiss chard (Federal Register, 2006). Isophorone is also used as an intermediate for the manufacture of other chemicals, such as 3,5-xylenol, 3,3,5-trimethylcyclohexanol, and trimethylcyclohexanone (US EPA, 2000).

1.3 Detection and quantification

The methodology used to measure isophorone in environmental samples is broadly synonymous with the approach used for other organic solvents, i.e. typically involving solid- or liquid-phase extraction followed by chromatographic analysis. The volatility of isophorone permits determination by headspace analysis, whereby sorbents can be used to extract volatilized isophorone from the headspace above samples, or the headspace can be sampled and analysed inline. Selected representative methods for the analysis of isophorone in different sample matrices are summarized in Table 1.1.

Table 1.1 Representative methods for the detection and quantification of isophorone in various matrices

Sample matrix (method number)	Sample preparation	Analytical technique	LOD (unless otherwise specified)	Reference
Air				
Indoor/workplace air (NIOSH Method 2556)	Solid-phase extraction (XAD-4 resin collection matrix)	GC-FID	1 µg per sample; working range, 0.24–33.2 µg/m^3 for a typical 25 L sample	NIOSH (2003), based on Levin & Carleborg (1987)
Headspace over electrical equipment	Sample components heated in a headspace chamber for inline analysis	GC-MS	NR	Paz et al. (2012)
Water				
Drinking-water (US EPA Method 525.3)	Solid-phase extraction (styrene divinylbenzene or divinylbenzene *N*-vinylpyrrolidone copolymer sorbents)	GC-MS	0.004–0.014 µg/L	US EPA (2012)
Industrial wastewater (US EPA Method 609)	Solvent extraction (methylene chloride)	GC-FID or GC-ECD	5.7 µg/L	US EPA (1984)
Lake water	Solid-phase microextraction (polydimethylsiloxane-coated fibre)	GC-FID	15 µg/L	Horng & Huang (1994)
Seawater	HSPME (activated carbon fibre)	GC	1.4–3.2 ng/L [0.0014–0.0032 µg/L]	Ma et al. (2009)
Soil or sediment				
Lake sediment	Solvent extraction (diethyl ether), centrifugation, and fractionation by gel permeation column chromatography	GC-MS	NR	McFall et al. (1985)
Lake sediment	Freeze-drying, homogenization, Soxhlet extraction with acetone and *n*-hexane, cleaning with alumina/silica adsorption chromatography	GC-MS	NR	Wang et al. (2002)
Soil, experimentally treated	HSPME (divinylbenzene/carboxen/polydimethylsiloxane fibre)	GC-Q-TOF-MS	NR	Brown et al. (2021)
Soil, spiked samples	Shaken and centrifuged with water, methanol or dichloromethane and extracted by florisil column	GC-MS	NR	Singh et al. (1998)
Food				
Oysters and clams, environmental samples	Solvent extraction (diethyl ether), homogenization, centrifugation, and fractionation by gel permeation column chromatography	GC-MS	NR	McFall et al. (1985)
32 supermarket-bought food samples, including soft and alcoholic drinks, condiments, grains, vegetables, spices, seafood, dairy and meat products	Solid and semi-solid samples were homogenized, suspended in distilled water, and saturated with sodium chloride, magnetically stirred at 60 °C and underwent HSPME (polydimethylsiloxane/divinylbenzene fibre)	GC-MS	0.5 pg/mL [0.0005 µg/L]	Kataoka et al. (2007)

Table 1.1 (continued)

Sample matrix (method number)	Sample preparation	Analytical technique	LOD (unless otherwise specified)	Reference
Fish, multiple species, environmental samples	Homogenized samples underwent Soxhlet extraction with acetone and hexane, concentration, and cleaning with gel permeation chromatography	GC-MS	0.02 mg/kg	Camanzo et al. (1987)
Honey	Storage at –18 °C; sample vials were maintained at 60 °C in a water bath during HSPME (divinylbenzene/carboxen/polydimethylsiloxane fibre)	GC-MS	NR	Alissandrakis et al. (2007)
Honey	Magnetically stirred and maintained at 70 °C during HSPME (divinylbenzene/carboxen/polydimethylsiloxane fibre)	GC-MS	NR	El-Sayed et al. (2018)
Honey	Magnetically stirred and maintained at 60 °C during HSPME (polyacrylate fibre)	GC-MS	NR	Anand et al. (2019)
Saffron	Steam distillation to extract essential oil, received in ethyl acetate	GC-MS	NR	Liu et al. (2018)
Saffron	Vials heated to 50 °C in water bath and headspace directly sampled into PTR-TOF-MS instrument	PTR-TOF-MS	NR	Masi et al. (2016)
Jiashi muskmelon juice	Heated to 40 °C and magnetically stirred for HSPME (polydimethylsiloxane/divinylbenzene/carboxen fibre)	GC-MS	NR	Pang et al. (2012)
Other consumer products				
Clofibrate preparations	Dissolution of 0.5 mL of capsule content in 0.5 mL of chloroform and injection into GC-MS	GC-MS	NR	Johansson & Ryhage (1976)
Inflatable aquatic toys and swimming learning devices	Solvent extraction from plastic matrix with dichloromethane, filtration, and volatile fraction isolation with SAFE	GC-MS	NR	Wiedmer et al. (2017), Wiedmer & Buettner (2018)
Human biospecimens				
Urine	HSPME (divinylbenzene/carboxen/polydimethylsiloxane fibre)	GC-TOF-MS	NR	Hanai et al. (2012)
Plasma (unspecified origin), spiked	Centrifugation with acetonitrile and extraction by florisil column	GC-MS	NR	Singh et al. (1998)

FID, flame ionization detector; GC-ECD, gas chromatography with electron capture detection; GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography with mass spectrometry; GC-TOF, gas chromatography time-of-flight; GC-Q-TOF-MS, gas chromatography quadrupole time-of-flight mass spectrometry; HSPME, headspace solid-phase microextraction; LOD, limit of detection; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; PTR-TOF-MS, proton-transfer-reaction time-of-flight mass spectrometry; SAFE, solvent-assisted flavour evaporation; US EPA, United States Environmental Protection Agency.

1.3.1 Air

Established methods exist for the measurement of isophorone in indoor workplace air, with protocols dated as early as 1955 (Kacy & Cope, 1955). Modern methods commonly involve the use of sampling tubes containing a solid adsorbent matrix through which a volume of air is passed and can be used in conjunction with personal air samplers. Early approaches to this method employed adsorption on a charcoal matrix (White et al., 1970), e.g. National Institute for Occupational Safety and Health (NIOSH) Method 2508 (NIOSH, 2003). Owing to the decomposition of isophorone desorbed on charcoal, polymer-based collection matrices are now favoured (Brown & Purnell, 1979; Levin & Carleborg, 1987). These developments formed the basis of (superseding) NIOSH Method 2556, which uses a XAD-4 resin mesh as the adsorptive collection matrix with desorption using diethyl ether and analysis by gas chromatography (GC) with flame ionization detection (FID). This method has a limit of detection (LOD) of 1 µg per sample and has achieved average recoveries of isophorone of 94.1%, in the range of 55–831 µg (Levin & Carleborg, 1987; NIOSH, 2003). One study made inline measurements of emissions of isophorone and other volatile organic compounds (VOCs) from headspace chambers in which electronic components were heated to 75–200 °C. Analysis was performed using gas chromatography-mass spectrometry (GC-MS) (Paz et al., 2012). [The Working Group noted that this study was conducted in the context of detecting the onset of equipment overheating and electrical fires in on-board instruments found in aircraft, submarines, and other vessels. While determining human exposure was not the primary rationale for this study, such electrical emissions may still be a relevant potential source of isophorone.]

Methods for the determination of isophorone in workplace air have also been developed in China. These methods are based on the earlier principle of adsorption to a charcoal matrix but report good recoveries (> 90%) if the analysis is carried out within 10 days of sampling (Kang et al., 2006; Chen & He, 2009).

1.3.2 Water

Several methods have been used to measure isophorone in various aqueous samples, including drinking-water (US EPA, 2012), natural waters (Sheldon & Hites, 1978; Horng & Huang, 1994; Ma et al., 2009), industrial effluents (US EPA, 1984), and landfill leachate (Ghassemi et al., 1984). Solid-phase or solvent extraction techniques are capable of separating isophorone from an aqueous matrix before chromatographic analysis. The US EPA has developed several approved methods for the determination of isophorone in water. US EPA Method 525.3 (US EPA, 2012) is the most recent issue of a series of methods used to detect isophorone and more than 100 other organic chemicals in drinking-water. In brief, 1 L of sample is passed through a solid-phase extraction (SPE) device. Analytes are eluted from the SPE device with organic solvents. The eluent is then dried by passing through an anhydrous sodium sulfate column, concentrated by evaporation with nitrogen gas, and made up to a volume of 1 mL with ethyl acetate and internal standard solutions. Samples are then analysed by GC-MS, with reported LODs of 0.004–0.014 µg/L. US EPA Method 609 (US EPA, 1984) covers the determination of isophorone and selected nitroaromatics in industrial wastewater using solvent extraction with methylene chloride [dichloromethane]. Extracts are dried, concentrated to a volume of 10 mL in hexane and can be analysed by GC with FID, or GC with electron capture detection (ECD), although the former is recommended since the LOD is lower (LOD of 5.7 µg/L with GC-FID compared with 15.7 µg/L with GC-ECD). The abovementioned analytes were also analysed using an SPE approach in

lake water (Horng & Huang, 1994). Low concentrations of isophorone (LODs, 1.4–3.2 ng/L) have also been measured in sea water by a method using GC coupled with headspace solid-phase microextraction (HSPME) with activated carbon fibre (Ma et al., 2009).

1.3.3 Soil and sediment

No methods were identified for the measurement of isophorone in natural soils.

Isophorone has been measured in lake sediments using both solvent (diethyl ether) (McFall et al., 1985) and Soxhlet (Wang et al., 2002) extraction methods followed by chromatographic clean-up of extracts and analysis by GC-MS. Two studies have reported isophorone measurements made in soils subjected to various aerobic and anaerobic conditions in vitro (Brown et al., 2021) and spiked with known quantities of isophorone (Singh et al., 1998). These studies used HSPME followed by gas chromatography quadrupole time-of-flight mass spectrometry (GC-Q-TOF-MS), and florisil column extraction followed by GC-MS, respectively. [The Working Group noted that although the soils analysed in these two studies were subjected to experimental conditions, the sample preparation, extraction, and analysis steps are probably applicable to environmentally sampled or natural soils.]

1.3.4 Food and consumer products

Methods have been described for the determination of isophorone in various edible samples, including fish and shellfish sampled in their natural habitat (McFall et al., 1985; Camanzo et al., 1987) and shop-bought food items (Kataoka et al., 2007). Isophorone was measured in samples of oyster and clam using solvent extraction with diethyl ether, homogenization, and centrifugation, followed by fractionation by gel-permeation chromatography and analysis by GC-MS (McFall et al., 1985). A similar approach, using Soxhlet extraction with GC-MS, was used to analyse isophorone in several fish species (Camanzo et al., 1987). Kataoka et al. (2007) collected samples of 32 food items and determined isophorone content using HSPME and GC-MS, with a reported LOD of 0.5 pg/mL [0.0005 µg/L] and recoveries of > 84% [the Working Group noted the versatility of this method across the wide variety of food items analysed].

Additionally, several reports have described methods for the chemical profiling of a variety of natural products cultivated for human consumption (see Section 1.4.1). These methods have assessed the VOC composition of different samples of which isophorone is a major constituent. The rationales for undertaking such analyses include the VOC profiling of honeys for identification and for description of antifungal, medicinal and aromatic properties (Alissandrakis et al., 2007; El-Sayed et al., 2018; Anand et al., 2019); the VOC profiling of plant extracts (e.g. *Clerodendrum infortunatum* L.; Gera et al., 2020) to determine taxonomy and aromatherapeutic and pharmaceutical applications; the analysis of saffron for the purposes of geographical and commercial discrimination (Masi et al., 2016; Liu et al., 2018); the analysis of the intermediate distillate of the Chinese traditional medicine, Xingnaojing injection, to optimize its extraction process (Fang et al., 2017); and the analysis of Jiashi muskmelon juice for odour profiling – a major determinant of consumer acceptance (Pang et al., 2012). [The Working Group noted that, while the rationales for the abovementioned analyses were not related to human exposure, the methods used would be applicable to determinations of isophorone made in this context.] Selected methods for measure of isophorone in these items are summarized in Table 1.1 and mostly involved HSPME techniques or direct headspace analysis by GC-MS.

Other consumer products for which methods of isophorone determination have been reported

in the context of human exposure include clofibrate (a pharmaceutical used to control high blood levels of cholesterol and triglyceride), which was analysed directly by GC-MS after dissolution in chloroform (Johansson & Ryhage, 1976). Children's inflatable aquatic toys and learning devices (armbands, beach balls and bathing rings) were also analysed by GC-MS after solvent extraction with dichloromethane and filtration (Wiedmer et al., 2017; Wiedmer & Buettner, 2018). [The Working Group noted that the two studies quantifying isophorone in aquatic toys used a combination of sensory analyses (i.e. a panel of trained assessors) and instrumental analyses to identify the principal chemical signatures of odours, a technique termed "gas chromatography-olfactometry". The instrumental aspects of this approach are relevant to exposure assessment more broadly.]

1.3.5 Biological specimens

Although isophorone is mostly excreted via the urine in animal systems, data on excretion in humans are sparse (see Section 4.1 of the present monograph). There are no standardized protocols for the measurement of biomarkers of isophorone exposure in humans, and only one study reporting the determination of isophorone in human urine was available. In this study with a case–control design (Hanai et al., 2012), isophorone and other urinary VOCs were investigated as potential non-invasive diagnostic markers of lung cancer. Urine samples were frozen at −80 °C until use and, after thawing, underwent centrifugation and filtration. In an approach similar to that used by Ma et al. (2009) for sea water samples, isophorone and other VOCs were extracted from urine samples by HSPME using divinylbenzene/carboxen/polydimethylsiloxane fibres. Fibres with adsorbed compounds were analysed by GC-TOF-MS [limits of detection were not reported]. [The Working Group noted that although there was a lack of studies reporting methods for isophorone determination in human urine, methods used for other aqueous samples and in animals (e.g. hexane extraction of rabbit urine followed by GC analysis; Dutertre Catella et al., 1978) may be used for the analysis of human urine.]

Finally, an experimental study reported isophorone measurements on spiked plasma samples that underwent florisil column extraction and analysis by GC-MS (Singh et al., 1998). [The Working Group noted that the authors did not specify the origin (i.e. human or animal) of the plasma used in this experiment, and that samples were spiked with isophorone. Nevertheless, the study provided an example of isophorone determination in a plasma matrix, which may be relevant to human biomonitoring.]

1.4 Occurrence and exposure

1.4.1 Occurrence in the environment, food, and consumer products

The wide use of isophorone as a chemical intermediate and solvent for lacquers, inks, vinyl resins, herbicides, copolymers, coatings, and other products in a variety of industrial settings permits its entry into the environment from urban centres and industrial sites via atmospheric emissions due to volatilization; and via water and soil contamination due to waste disposal, industrial effluents and runoff. While isophorone is rapidly removed from the air by photochemical breakdown, and to a lesser extent washout, it may persist in natural waters and soil for longer periods. In water, volatilization and sorption to sediments and particulates are not expected to be significant removal mechanisms of isophorone and, in soils, microbial degradation is expected to occur (ATSDR, 2018). Isophorone is also present in food items, and in products whose manufacture involves its application, including food packaging (Sasaki et al., 2005; Skjevrak et al., 2005) and children's aquatic

toys (Wiedmer et al., 2017; Wiedmer & Buettner, 2018). While the dominant sources of isophorone in the environment appear to be anthropogenic in nature, it has been found to occur naturally, including in several botanical specimens, such as cranberries (NCBI, 2021) and saffron, and in honey (see Section 1.3.4), and in the defensive froth or secretions of grasshoppers (Eisner et al., 1971). [The Working Group noted that the precise origin of isophorone in these natural specimens is a subject of inquiry.] Concentrations of isophorone reported in different environmental media, including food and other consumer products, are summarized in Table 1.2 and described throughout the following sections. [The Working Group noted that many of the measurements reviewed throughout Section 1.4.1 were made for method development and validation purposes and do not necessarily reflect the actual distribution of isophorone in the environment.]

(a) Environmental occurrence

There is a notable scarcity of ambient air measurements of isophorone in the literature, despite its volatility and known sources of atmospheric emissions. The US EPA publishes national estimates of isophorone emissions via its National Emissions Inventory (NEI) on the basis of data provided by state, local, and tribal air agencies, and supplemented by data collected by the US EPA. Estimated isophorone emissions by sector in the USA in 2017 are presented in Table 1.3. This suggests that the five sectors with the highest emissions of isophorone are coal-powered electricity generation, waste disposal, industrial surface coating and solvent use, industrial processes not elsewhere classified, and chemical manufacturing, which contribute 38%, 29%, 19%, 6.5%, and 3.4%, respectively, of total emissions (US EPA, 2017). Atmospheric emissions of isophorone may be produced by coal combustion; isophorone was measured at a concentration of 490 ppb [0.49 mg/kg] in coal fly ash from a power station in the USA (Harrison et al., 1985). Overheating electrical components have also been shown to be a source of atmospheric emission of isophorone. Under experimental conditions, a resistor heated at a constant temperature of 200 °C for 5 hours emitted isophorone at 128 ng/g of component per hour (Paz et al., 2012). [The Working Group noted that these data indicated that isophorone exposure may occur as a result of electrical and other fires involving the combustion of isophorone-containing materials. The former is of relevance to those involved in the burning of electronic waste, an activity that is particularly prevalent in low- and middle-income countries such as those in West Africa and from where occurrence and exposure data for isophorone appear to be absent.]

Data on the occurrence of isophorone in surface waters (excluding effluents from industrial sites) are sparse, with the few measurements available from the USA not detecting the compound or detecting trace amounts (< 2 µg/L) (Sheldon & Hites, 1978; US EPA, 1982; Hall Jr et al., 1987). Lake sediments in both the USA and China have been found to contain isophorone. Mean concentrations at three sites at Lake Pontchartrain, Louisiana, USA, between May and June 1980, were between 0.9 and 12 ng/g [0.0009–0.012 mg/kg] (McFall et al., 1985). Much higher concentrations (1.01–17.21 mg/kg) were measured in five sediment samples collected from Donghu Lake, Wuhan, China, in November 2000 (Wang et al., 2002). A small number of studies (US EPA, 1974, 1975; Keith et al., 1976; Feng et al., 2020) have measured isophorone in drinking-water. A range of 1.5–2.9 µg/L was reported for an unknown number of samples collected in New Orleans, USA in 1974 (US EPA, 1974). Isophorone was detected in 3 out of 11 samples collected in Philadelphia, USA, between 1975 and 1977 (Suffet et al., 1980), but the concentrations were not reported. A study conducted in China comparing methods for the removal of isophorone from reservoir-sourced drinking-water

Table 1.2 Occurrence of isophorone in environmental samples, food, consumer products, and biological specimens

Sample type	Location and collection date	No. of samples	Mean (range)	Analytical method	Comments	Reference
Atmospheric emissions						
Coal fly ash	The Four Corners coal-fired power station, New Mexico, USA, December 1979	1	490 ppb [0.49 mg/kg]	GC-MS	Collected from an electrostatic precipitator	Harrison et al. (1985)
Natural waters and sediments						
River water	Delaware River, USA, August 1977 and March 1978	Not well reported, but interpreted as 16	"trace", i.e. < 0.01 ppb [0.01 µg/L]	GC-MS		Sheldon & Hites (1978)
River water	Potomac River, Quantico, Virginia, USA, Spring 1986	1	< 2 µg/L	GC-MS		Hall Jr et al. (1987)
River water	Olentangy River	1	ND, < 5 µg/L	GC-FID		US EPA (1982)
Lake sediment	Lake Pontchartrain, Louisiana, USA, May–June 1980	10 in total, collected from 3 sites	3 sites: 0.9 ng/g (mean of 8 samples); 12 ng/g (1 sample); 10 ng/g (1 sample)	GC-MS	Lake Pontchartrain is a brackish estuary in the Gulf of Mexico	McFall et al. (1985)
Lake sediment	Donghu Lake, Wuhan, China, November 2000	5	7.9 mg/kg (1.01–17.21 mg/kg)	GC-MS		Wang et al. (2002)

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Analytical method	Comments	Reference
Industrial effluents						
Sewer pump sample receiving wastes from phenolic resins, vinyl acetate, and polyvinyl chloride process areas	USA	1	40.5 µg/L	GC-FID		US EPA (1982)
Brine sample from holding tank receiving washings from ships delivering various commodities	USA	1	< 50 µg/L			
Secondary sewage effluent	Columbus, Ohio, USA	1	120 µg/L			
Final effluent from UNOX treatment system receiving wastes from plants producing plasticizers, butyl rubber, and olefins	USA	1	< 5 µg/L			
Final effluent from organic chemical plants producing nitrobenzene, *ortho*-dichlorobenzene, *ortho*-nitrophenol, aniline, and oil additives	USA	1	< 20 µg/L			
Timber products	USA	2 (positive samples)	83 µg/L (55–111 µg/L)	GC-MS		US EPA (1983), cited in ATSDR (2018)
Petroleum refining		1 (positive sample)	1380 µg/L			
Paint and ink		5 (positive samples)	185 µg/L (24–946 µg/L)			
Pulp and paper		1 (positive sample)	753 µg/L			
Auto and other laundries		2 (positive samples)	43 µg/L (43–44 µg/L)			
Pharmaceuticals		1 (positive sample)	237 µg/L			
Foundries		1 (positive sample)	136 µg/L			
Transportation equipment		2 (positive samples)	173 µg/L (28–318 µg/L)			
Publicly owned treatment works		15 (positive samples)	11.5 µg/L (4.2–114 µg/L)			
Drinking-water						
Drinking-water	Philadelphia, USA, 1975–1977	12	NR	GC-MS	Detected in 17% of samples	Keith et al. (1976), cited in ATSDR (2018)

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Analytical method	Comments	Reference
Drinking-water	New Orleans, USA, 1974	NR	(1.5–9.5 µg/L)	GC-MS		US EPA (1974) cited in ATSDR (2018)
Drinking-water	Cincinnati, USA	NR	≤ 0.02 µg/L	NR		US EPA (1975) cited in ATSDR (2018)
Raw drinking-water (pre-traditional treatment)	Yellow River, eastern China, October–November 2018	5	0.338 ng/L (0.132–0.521 ng/L) [0.338×10^{-3} µg/L ($0.132–0.521 \times 10^{-3}$ µg/L)]	GC-MS	Reservoir-sourced water from two plants using alternative treatment processes was compared	Feng et al. (2020)
Finished drinking-water (traditional treatment)		5	0.2 ng/L (0.07–0.49 ng/L) [0.2×10^{-3} µg/L ($0.07–0.49 \times 10^{-3}$ µg/L)]			
Raw drinking-water (pre-advanced oxidation treatment)		5	1.92 ng/L (0.41–5.18 ng/L) [0.129×10^{-2} µg/L ($0.041–0.518 \times 10^{-2}$ µg/L)]			
Finished drinking-water (advanced oxidation treatment)		5	0.33 ng/L (0.17–0.60 ng/L) [0.33×10^{-3} µg/L ($0.17–0.60 \times 10^{-3}$ µg/L)]			
Swimming-pool water						
Chlorinated water from public, private, outdoor and indoor pools, sports pools, hot tubs, water slides, paddling pools, and recreational pools	Poland	50	Quantified in 89% of samples (LOQ, 0.75 µg/L); for those in which quantified, 0.8 µg/L (range, 0.75–1.0 µg/L)	GC-MS		Lempart et al. (2020)
Dietary occurrence						
Oysters	Lake Pontchartrain, Louisiana, USA, May–June 1980	8	38 ng/g	GC-MS	Environmental samples	McFall et al. (1985)
Clams		2	ND			
Fish, including carp, bass, catfish, pumpkinseed, bowfin, and pike	Lake Michigan tributaries, USA, 1983	28 composite samples from a total of 140 fish caught	[0.76 mg/kg] (< LOD to 3.61 mg/kg) [760 ng/g (< LOD to 3610 ng/g)]	GC-MS	Environmental samples; concentrations reported in wet weight; LOD not reported, but 0.02 ng/g used in Working Group calculation of mean for < LOD values reported as "0.*" in the article	Camanzo et al. (1987)

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Analytical method	Comments	Reference
"Green tea A"	Japan (supermarket-bought products)	3 per product	129 ± 1 pg/g (standard deviation)	GC-MS	Concentrations in liquid samples are expressed as pg/mL, semi-solid and solid as pg/g	Kataoka et al. (2007)
"Green tea B"			647 ± 5 pg/g			
Liquor			21 ± 1 pg/mL			
Sake			340 ± 19 pg/mL			
Soy sauce (weak)			88 ± 4 pg/mL			
Soy sauce (strong)			3306 ± 107 pg/mL			
Tomato juice			994 ± 43 pg/mL			
Tomato juice			262 ± 14 pg/mL			
Milk			200 ± 9 pg/mL			
Honey			4142 ± 141 pg/mL			
Maple syrup			1252 ± 37 pg/g			
Sugar			1568 ± 22 pg/g			
Soybean flour			13 258 ± 312 pg/g			
Miso			3322 ± 74 pg/g			
Wheat flour			1674 ± 25 pg/g			
Rice			2868 ± 67 pg/g			
Cod roe			260 ± 4 pg/g			
Sea urchin			2868 ± 67 pg/g			
Scallop			238 ± 11 pg/g			
Fresh fish			150 ± 8 pg/g			
Chicken			184 ± 14 pg/g			
Pork			220 ± 10 pg/g			
Worcester sauce, potato, carrot, green pepper, mustard, wasabi, white pepper, dried bonito, egg yolk, beef			ND (LOD, 0.5 pg/mL)			
Thyme honey	Greece	28	46 ng/kg (0–1114 ng/kg) [0.046 ng/g (0–1.1 ng/g)]	GC-MS		Alissandrakis et al. (2007)

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Analytical method	Comments	Reference
Grains and their products	Japan, 2002–2003	17	1.55 ng/g (0.8–2.8 ng/g)	GC-MS		Sasaki et al. (2005)
Beans and their products		30	3.0 ng/g (< 0.1–8.9 ng/g)			
Vegetables and their products		26	0.2 ng/g (< 0.1–1.7 ng/g)			
Fish		10	0.3 ng/g (< 0.1–1.8 ng/g)			
Meat		5	< 0.1 ng/g (< 0.1 to < 0.1 ng/g)			
Milk and butter		2	< 0.1 ng/g (< 0.1 to < 0.1 ng/g)			
Sake		3	0.1 ng/g (< 0.1 to < 0.2 ng/g)			
Food containers		8	12.6 ng/g (4–19 ng/g)		Containers for rice, soy sauce, miso, and beans, made from either polyethylene terephthalate, polyethylene, or polypropylene	
Water exposed to polyolefin bottles at ambient temperature for 72 h	Norway	3	≤ 4 µg/L	GC-MS		Skjevrak et al. (2005)
Consumer product occurrence						
Inflatable pool toys: armbands, bathing rings, and beach balls	Online suppliers located in Germany	20	Detected in 8/20 samples; for those in which detected, [1.95 g/kg] (< 0.16–5.25 g/kg) [1080 mg/kg (< 160–5250 mg/kg)]	GC-MS	0.08 mg/kg was assigned to values reported as < 0.16 mg/kg (LOQ) in the Working Group calculations of mean	Wiedmer & Buettner (2018)
Biological specimens						
Urine	Pennsylvania, USA	20	130 nM (39–1412 nM) [18 µg/L (5.4–195 µg/L)]	GC-TOF-MS	Control participants in a lung cancer case–control study	Hanai et al. (2012)

GC-FID, gas chromatography with flame-ionization detection; GC-MS, gas chromatography with mass spectrometry; GC-TOF-MS, gas chromatography time-of-flight with mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; ND, not detected; NR, not reported; ppb, parts per billion.

Table 1.3 Estimated isophorone emissions in the USA in 2017 by emission sector[a]

Sector	Estimated emissions		Contribution to total estimated emissions (%)[b]
	(pounds)	(tonnes)	
Fuel combustion – electric generation – coal	117 802	[53.4]	38%
Waste disposal	89 208	[40.5]	29%
Solvent use – industrial surface coating and solvent use	60 097	[27.3]	19%
Industrial processes – not elsewhere classified	20 475	[9.3]	6.5%
Industrial processes – chemical manufacturing	10 550	[4.8]	3.4%
Industrial processes – ferrous metals	6256	[2.8]	2%
Fuel combustion – industrial boilers, ices – coal	3307	[1.5]	1%
Solvent use – consumer and commercial solvent use	1659	[0.75]	< 1%
Solvent use – graphic arts	1136	[0.52]	< 1%
Fuel combustion – commercial/institutional – coal	749	[0.34]	< 1%
Other fuel combustion	960	[0.44]	< 1%
Other industrial processes	236	[0.11]	< 1%
Bulk gasoline terminals	166	[0.08]	< 1%
Solvent use – degreasing	0.19	[0.09×10^{-3}]	< 1%

[a] The 10 highest emitting sectors are displayed with the 15 remaining sectors collapsed.
[b] Calculated by the Working Group using National Emissions Inventory Data (US EPA, 2017).

reported concentrations that were all below 5.18 ng/L [0.005 µg/L] (Feng et al., 2020).

The highest environmental concentrations of isophorone have been measured in industrial effluents (Table 1.2): concentrations were 120 µg/L in sewage effluent, 185 µg/L in paint and ink effluent, 237 µg/L in pharmaceutical effluent, 753 µg/L in pulp and paper effluent, and as high as 1380 µg/L in effluent from petroleum refining (US EPA, 1982, 1983). Isophorone has also been found in the turf crumb rubber of synthetic sports pitches, which is made from recycled tyres (Perkins et al., 2019).

(b) Dietary exposure

Although represented by only a few studies and individual samples, a substantial variety of food items have been analysed for isophorone content. As mentioned in Section 1.3.4 of the present monograph, some of these studies were conducted for purposes other than human exposure assessment, such as determining the VOC profile of honey, a large fraction of which includes isophorone. Mean concentrations ranged from 0.046 ng/g (Alissandrakis et al., 2007) to 4.12 ng/g (Kataoka et al., 2007). In environmental samples, isophorone was found at a concentration of 38 ng/g in an oyster collected from Lake Pontchartrain, Louisiana, USA, in 1980, but was not detected in two samples from clam (McFall et al., 1985). Two studies conducted in Japan (Sasaki et al., 2005; Kataoka et al., 2007) measured isophorone in a large variety of supermarket-bought food items. These results are summarized in Table 1.2; the majority of samples contained isophorone at less than 1 ng/g, but relatively higher concentrations were found in polished rice (2.8 ng/g), miso (8.9 ng/g), spinach (1.7 ng/g), sole (1.8 ng/g) (Sasaki et al., 2005); and rice and sea urchin (both, 2.9 ng/g), miso (3.3 ng/g), honey (4.1 ng/g), strong soy sauce (3.3 ng/g), soy sauce (5.2 ng/g), fermented soybeans (5.4 ng/g), and soybean flour (13.3 ng/g) (Kataoka et al., 2007). The highest isophorone concentrations in food reported by any study were found in samples of various fish species

collected in 1983 from Lake Michigan tributaries, USA, where there were known influxes of industrial effluent (Camanzo et al., 1987). [A mean concentration of 0.76 mg/kg (range, < LOD to 3.61 mg/kg) [760 ng/g (range, < LOD to 3610 ng/g)] was calculated.] [The Working Group noted that, although these samples were sourced directly from the environment, they pointed to potentially high human exposures from food sourced from polluted areas.]

The origin of isophorone in many food items was not clear; it may be naturally occurring or a result of contamination, including with herbicides and pesticides, some of which include isophorone as a major constituent, e.g. 10–20% in one herbicide (Arysta LifeScience, 2013) and as high as 60% in another (Bayer Crop Science, 2010), both of which are used on beetroot, rice, beans, spinach, and sugar beet (Federal Register, 2006). Isophorone has been detected in food packaging at concentrations many times higher than in the food items themselves. The average isophorone concentration measured in containers made from polyethylene terephthalate, polyethylene, and polypropylene for soy sauce, polished rice, miso, and beans was 12.6 ng/g (range, 4–19 ng/g). The corresponding isophorone concentrations measured in the foods in these containers ranged from 1 to 3.5 ng/g, and estimated migration levels (determined by filling containers with dichloromethane and leaving them at 25 °C for 1 hour) ranged from < 50 to 150 pg/cm^2 [0.05–0.15 ng/cm^2] (Sasaki et al., 2005). In another study (Skjevrak et al., 2005), water exposed to polyolefin bottles at ambient temperature for 72 hours was found to contain an isophorone concentration up to 4 µg/L.

(c) Consumer products

Notably high concentrations of isophorone (160–5250 mg/kg) have been measured in 40% of tested inflatable swimming-pool toys and learning devices, including armbands, bathing rings, and beach balls (Wiedmer & Buettner, 2018). [Direct exposure to these products is most likely to occur among children.] Isophorone has also been shown to migrate from these products and other swimwear (e.g. goggles, earplugs, flip-flops, and swimming caps) into swimming-pool water. In a survey of water from 50 public, private, outdoor and indoor pools, sports pools, hot tubs, water slides, paddling pools, and recreational pools in Poland, isophorone was quantified in 89% of samples, with a mean concentration of 0.8 µg/L (0.75–1.0 µg/L) among those samples in which isophorone was quantified (Lempart et al., 2020). In another study (Danish Ministry of the Environment, 2007), isophorone was detected in a school bag, pencil case, and eraser.

Like other VOCs, isophorone is a component of tobacco smoke (Yang et al., 2006).

1.4.2 Occupational exposure

Few estimates are available of the number of workers exposed to isophorone worldwide. NIOSH estimated in 1978 that about 1.5 million workers were potentially exposed to isophorone in the USA (NIOSH, 1978a). However, the NIOSH National Occupational Exposure Survey estimated that just 47 097 workers (10 353 of whom were women) were exposed to isophorone in 1981–1983, with the highest numbers seen among operators of printing machines, painting and paint spraying machines, textile machines, and miscellaneous machines, as well as hand packers and packagers, assemblers, non-construction labourers, unspecified mechanics and repairers. The industries with the highest representation among exposed workers were rubber and miscellaneous plastics products, printing and publishing, fabricated metal, chemicals and allied products, and miscellaneous manufacturing industries (NIOSH, 1990). [The Working Group noted that it is unclear how representative these estimates are of current exposure prevalence.]

No studies on occupational exposure of workers exposed to isophorone during its manufacture were available to the Working Group. Isophorone has been measured in air in several occupational settings where isophorone is used as an ink in screen-printing and other types of printing and coating, and in plastics manufacture. Although isophorone is a constituent of some herbicides, e.g. Betanal (Bayer Crop Science, 2010) and Satunil (Arysta LifeScience, 2013), no information was available on exposures during manufacture or use of these herbicides. [The Working Group noted that the available data were sparse and were collected in only a few countries.]

In an epidemiological study by Rodrigues et al. (2020), the authors reported isophorone exposures in three facilities, located in East Fishkill in New York, Burlington in Vermont, and San José in California, USA, in which the following operations were carried out: semiconductor manufacture, masking, and module manufacture; and the manufacture of printers, hard disk drives, tape drives, and Winchester disks. [The Working Group noted that, although isophorone was present in at least one of these facilities, no information was provided as to in which operation the isophorone occurred.]

An Institut national de recherche et de sécurité (INRS) database called Solvex (INRS, 2021) is derived from a French occupational exposure database (COLCHIC) of measurements taken by French prevention authorities for risk assessment purposes since 1987 (Mater et al., 2016). Solvex provides summary statistics for personal measurements by industry, occupation, or task. The industry categories that showed sufficient data on isophorone exposure (more than 50 samples) that could be used to calculate statistics were printing, and reproduction of documents (100 results; mean, 0.99 mg/m^3; range, 0.15–12 mg/m^3), metallurgy (83 results; mean, 0.65 mg/m^3; range, 0.05–21 mg/m^3), manufacture of metal products (62 results; mean, 0.65 mg/m^3; range, 0.05–10 mg/m^3), and manufacture of electrical equipment (57 results; mean, 0.62 mg/m^3; range, 0.04–3 mg/m^3). Of these measurements, 88% had been taken before 2001.

Compliance measurements are also available from the United States Occupational Safety and Health Administration (OSHA) (OSHA, 2021) and discussed in Lavoué et al. (2013). Between 1984 and 2020, 755 personal isophorone measurements varying in duration between 11 and 880 minutes were collected. Of the 8% of measurements that were made after 2000, isophorone was not detected in ~30%. The isophorone measurements made between 1984 and 2020 ranged from < 0.015 ppm [< 0.09 mg/m^3] (the smallest reported detected value) to 40 ppm [230 mg/m^3] (interquartile interval, < 0.015–0.6 ppm [< 0.09–3.4 mg/m^3]). The three most visited industries were: commercial printing, not elsewhere classified (n = 239; median < 0.015 ppm [< 0.09 mg/m^3]; 90th percentile, 2.3 ppm [13 mg/m^3]); plastics products, not elsewhere classified (n = 150; median < 0.015 ppm; 90th percentile, 1.1 ppm [6.3 mg/m^3]); and blank books and loose-leaf binders (n = 41; median, < 0.015 ppm [< 0.09 mg/m^3]; 90th percentile, 1.9 ppm [11 mg/m^3]).

Table 1.4 summarizes the results of the identified literature on occupational exposure measurements in specific workplaces.

A NIOSH health hazard evaluation was conducted during two visits in 1977 to a metals coating company near Chicago, Illinois, USA (NIOSH, 1978b). Personal breathing zone (PBZ) and area-sample air measurements were collected in three coating lines and two reclaimed-solvent areas using charcoal sampling tubes and analysed using GC. Isophorone was below the LOD in several air measurements collected in one reclaimed-solvent area and 1.0 ppm [5.74 mg/m^3] in the second area. Isophorone and several other solvents were measured for workers carrying out different tasks on three coating lines during the first visit and on one coating line during the

Table 1.4 Occupational exposure to isophorone measured in workplace air

Occupational group, job type, location, and date	Monitoring method	Analytical method (LOD)	No. of samples	Mean (range)	Median (IQR)	Comments	Reference
Vinyl coating process in metals finishing company, Illinois, USA, 1977	Indoor PBZ air measurements, September 1977	GC of organic vapour charcoal sampling tubes (0.01 mg per tube)	15	NR (< 0.05–1.5 ppm) [< 1.60–8.61 mg/m^3]	ND (ND to 0.75 ppm) [ND to 4.30 mg/m^3]	7 samples, ND; tended to be due to short (< 3 h) sampling times	NIOSH (1978b)
	Indoor PBZ air measurements, November 1977		4	1.00 ppm (0.64–1.31 ppm) [5.74 mg/m^3 (3.67–7.52 mg/m^3)]	1.02 ppm (0.64–1.31 ppm) [5.85 mg/m^3 (3.67–7.52 mg/m^3)]		
Screen printer in specialty screen-printing operation, Pennsylvania, USA, 1978	Short-term and full-shift PBZ air measurements	GC of organic vapour charcoal sampling tubes (0.03 mg per tube)	7	NR (< 0.07–25.7 ppm) [< 0.40–148 mg/m^3]	ND (ND to 0.30 ppm) [ND to 1.72 mg/m^3]	All samples collected from same screen-printer; highest concentration was from short-term cleaning operation	NIOSH (1979)
Screen printing process using gloss vinyl inks in specialty decal company, Georgia, USA, 1982	Full-shift PBZ or area air measurements collected at flow rate of 50 or 100 cm^3/min, and 200 cm^3/min for short-term measurement	GC of organic vapour charcoal sampling tubes (0.01 mg per tube)	8	NR ([< 0.16]–3.4 ppm) [< 0.89–19.5 mg/m^3]	[1.3 ppm (ND to 2.15 ppm)] [7.5 mg/m^3 (ND to 14.4 mg/m^3)]	Flow rate of 100 cm^3/min gave highest concentrations	NIOSH (1983)
Plastic-product manufacturers, China, NR	Indoor area samples from 10 facilities	GBZ/T (method name) 160.55–2007 (NR)	10	[0.267 mg/m^3] (0.0065–2.1 mg/m^3)	0.65 × 10^{-2} mg/m^3 [0.65–0.725 × 10^{-2} mg/m^3]	Very little information given about methods; unclear whether 0.0065 mg/m^3 represents a measured value or LOD	Cai et al. (2019)

Table 1.4 (continued)

Occupational group, job type, location, and date	Monitoring method	Analytical method (LOD)	No. of samples	Mean (range)	Median (IQR)	Comments	Reference
Screen-printing process using high-isophorone inks and solvents, USA, 1982	Short-term (50–90 min) PBZ measurements, printing press	GC of organic vapour charcoal sampling tubes (NR)	18	23 ppm (SD, 5.4) [132 mg/m^3 (SD, 31)]	NR	Exposure calculated on TWA basis, but time frame unclear	Samimi (1982)
	Automatic dryer		19	9.5 ppm (SD, 3.3) [55 mg/m^3 (SD, 19)]	NR		
	Manual drying		15	15 ppm (SD, 4.1) [86 mg/m^3 (SD, 24)]	NR		
	Paint mixing		12	17.8 ppm (SD, 5.5) [102 mg/m^3 (SD, 32)]	NR		
	Screen wash		14	8.3 ppm (SD, 5.6) [48 mg/m^3 (SD, 32)]	NR		
Printing and reproduction of documents, France, 1987–2021	Personal samples, median duration, 127 min (range, 61–276 min)	Variable; INRS standard methods	100	0.99 mg/m^3 (range, 0.15–12 mg/m^3)	0.60 mg/m^3		INRS (2021)
Metallurgy, France, 1987–2021	Personal samples, median duration, 242 min (range, 70–450 min)		83	0.65 mg/m^3 (range, 0.05–21 mg/m^3)	0.25 mg/m^3		
Manufacture of metal products, France, 1987–2021	Personal samples, median duration, 230 min (range, 156–371 min)		62	0.69 mg/m^3 (range, 0.05–10 mg/m^3)	0.25 mg/m^3		
Manufacture of electrical equipment, France, 1987–2021	Personal samples, median duration, 118 min (range, 65–214 min)		57	0.62 mg/m^3 (range, 0.04–3 mg/m^3)	0.4 mg/m^3		

Table 1.4 (continued)

Occupational group, job type, location, and date	Monitoring method	Analytical method (LOD)	No. of samples	Mean (range)	Median (IQR)	Comments	Reference
Commercial printing, USA, 1984–2020	Personal OSHA compliance measurements varying from 11 to 360 min, from 20 companies	Variable; OSHA standard methods; smallest reported detected value, 0.015 ppm	239		[< 0.015 ppm (< 0.015–0.80 ppm); 90th percentile, 2.3 ppm; converted to < 0.086 mg/m^3 (< 0.086–4.59 mg/m^3); 90th percentile, 13.2 mg/m^3]	Calculated by Working Group using only measurements with duration > 10 min	OSHA (2021)
Plastics products, USA, 1984–2020	Personal OSHA compliance measurements varying from 13 to 389 min, from 14 companies		150		[< 0.015 ppm (< 0.015–0.61 ppm); 90th percentile, 1.1 ppm; converted to < 0.086 mg/m^3 (< 0.086–3.50 mg/m^3; 90th percentile, 6.3 mg/m^3]	Calculated by Working Group using only measurements with duration > 10 min	
Blank books and loose-leaf binders, USA, 1984–2020	Personal OSHA compliance measurements varying from 26 to 153 min, from 3 companies		41		[< 0.015 ppm (< 0.015–1.1 ppm); 90th percentile, 1.9 ppm; converted to < 0.086 mg/m^3 (< 0.086–6.3 mg/m^3; 90th percentile, 10.9 mg/m^3]	Calculated by Working Group using only measurements with duration > 10 min	

GC, gas chromatography; INRS, Institut national de recherche et de sécurité; IQR, interquartile range; LOD, limit of detection; min, minute; ND, not detectable; NR, not reported; OSHA, Occupational Safety and Health Administration; PBZ, personal breathing zone; ppm, parts per million; SD, standard deviation; TWA, time-weighted average.

second visit. [Ventilation was present, but its effectiveness was unclear.] Most of the general area samples in the coating lines contained non-detectable concentrations of isophorone. A PBZ sample for the finish coater collected over 4 hours contained non-detectable concentrations of isophorone. Time-weighted average (TWA) concentrations for exposure to isophorone were estimated at 1.5 ppm [8.61 mg/m^3] for the prime coater, and 0.75 [4.30 mg/m^3] and 0.97 ppm [5.57 mg/m^3] for the finish coater, in short-term samples collected over 1–1.5 hours. PBZ samples collected over 5.5–6 hours showed non-detectable concentrations of isophorone for the prime coat operator and, in three out of four samples, for the finish coat operator (the fourth sample contained isophorone at a concentration of 0.74 ppm [4.25 mg/m^3]). Short-term samples collected over a period of less than 35 minutes for the unwind operator, rewind operator, and finish coat operator all contained non-detectable concentrations of isophorone in the PBZ samples. The coating line for a different product was evaluated on the second visit and showed a mean isophorone concentration of 1.0 ppm [5.74 mg/m^3] in PBZ samples across the various tasks. [The Working Group noted that sampling times were generally longer during the second visit, which may have improved the ability to detect isophorone in the PBZ samples.]

NIOSH measured occupational exposures to isophorone and other solvents in several screen-printing operations in the USA between 1978 and 1984. [The Working Group noted that these investigations were generally triggered by workers' reports of nausea, headache, or eye and nose irritation.] Measurements were made in these studies using PBZ or area air sampling onto charcoal tubes, which were then analysed using GC.

Several PBZ samples were collected in 1977 at a small specialty screen-printing operation in Pittsburgh, Pennsylvania, USA (NIOSH, 1979). Of seven samples, all except two had non-detectable concentrations of isophorone (0.30 ppm [1.72 mg/m^3] during screen printing in an unventilated area and 25.7 ppm [148 mg/m^3] in a very short-term sample while cleaning the screens [ventilation effectiveness was unclear]; the LOD was 0.3 mg per sample.) [The Working Group noted this was more than five times the short-term ceiling limit value in effect at that time, 5 ppm [28.7 mg/m^3].]

In 1980, NIOSH (1981) measured isophorone exposures in a company in Ridgefield, New Jersey, USA, employing 54 workers to screen-print, cut, laminate, and sew decals. Isophorone was a component of the printing ink and was also used directly in spray bottles as a "reducer" and on the printing screens as an anti-static coating. [The Working Group noted that employees using these sprays reported acute respiratory and neurological symptoms.] Isophorone was detected in the PBZ air of only the screen printers: their full-shift TWA (8 hours) concentrations were 0.7 and 14 ppm [4.0 and 80.4 mg/m^3]. It was noted that the ventilation was poorly designed.

In a small specialty printing company in Augusta, Georgia, USA, PBZ and area air samples were collected in 1982 for two screen-printing workers using gloss vinyl inks containing 35–40% isophorone (NIOSH, 1983). Ventilation was considered poorly designed. The median air concentration across eight PBZ and area samples was 1.3 ppm [7.5 mg/m^3], with the highest concentrations noted near the drying racks (area sample, 2.5 ppm [14 mg/m^3]) and while printing decals (PBZ sample, 3.4 ppm [20 mg/m^3]).

Lastly, NIOSH (1984) investigated exposures to isophorone and other solvents at a silk-screen printing vinyl-wallcovering manufacturer in Chicago, USA, in 1984. Isophorone (a solvent in the "retarder") was not detected during screen printing operations at this facility. [The Working Group noted that the full-shift air sampling rates were lower than those used in previous NIOSH studies, which could have affected the detection limit, which was not given for isophorone.]

A study focusing on isophorone exposures among screen-printing workers was carried out in a 34 000 ft² [3160 m²] facility in the USA [location unspecified] (Samimi, 1982). Isophorone was a main constituent of the inks and ink thinners, ranging from 10% to 75% among the various products, which were used to screen-print plastic, paper, or metal sheets at this mostly unventilated workplace. The product was dried in a ventilated dryer or hung to dry in a [presumably unventilated] room. It was noted that isophorone exposures were highest for workers involved in press operations, drying operations, ink formulation, and screen cleaning. The authors collected 78 short-term (50–90 minutes) PBZ air samples using charcoal tubes among workers expected to have highest solvent exposures. [The Working Group noted that the sampling and analytical methods used in this study were similar to those used in the above series of NIOSH studies.] TWA PBZ exposure concentrations were generally highest among printing press operators (mean, 23 ppm [132 mg/m³] and workers involved in paint mixing (mean, 17.8 ppm [102 mg/m³]), but mean concentrations for all workers were above 8 ppm [46 mg/m³]. It was also noted that PBZ concentrations were higher than corresponding area samples. [The Working Group noted that this is a typical finding for many solvents, aerosols, and particulates studied in occupational settings.]

[The Working Group noted that it was unclear how representative of current exposure conditions these 40-year-old studies of screen-printing workers are. However, it is notable that a recent publication identified isophorone as "the most widely used screen-printing ink solvent (comprising 75% of the total solvent)" (Kiurski et al., 2016).]

A small study was carried out of indoor air in a paper and cardboard printing company in Slovakia in 2015 (Vilcekova & Meciarova, 2016). Quantitative measurements were made for total VOCs in a location with a floor area of 144.3 m². Although no quantitative exposure levels were measured for individual VOCs, qualitative analysis was done for isophorone and 20 other VOCs by sampling with a zNose 4300 electronic nose in four cycles ["cycle" was not defined] (Meciarova et al., 2014). Total VOC concentrations fluctuated throughout the day and were typically above 40 mg/m³, with the highest levels (spiking above 120 mg/m³) seen in the latest part of the 8-hour day. Isophorone was one of the three most commonly occurring individual VOCs, appearing in all four cycles.

Cai et al. (2019) measured isophorone and several other ketones and aldehydes in workplace air of the production workspaces of 10 large-scale plastic-product manufacturers in China. Air concentrations for seven of the plants were reported as 0.0065 mg/m³ [the Working Group interpreted this to be the LOD], and the highest concentration measured at a plant was 2.1 mg/m³. [The Working Group noted that few details were provided about the sampling and analytical methods used or about the facilities themselves in this study. All measured values were noted by the authors to be well below occupational exposure limits in China.]

In addition to the printing and coating operations described above, isophorone has occasionally been reported in office settings. A recent study by Davis et al. (2019) found isophorone to be emitted in 8% of 3D printers tested. A NIOSH investigation (NIOSH, 2014) of a large government office complex in the USA in 2011 noted that grab samples had been historically collected for isophorone, but no information was provided on whether isophorone was detected.

1.4.3 Exposure of the general population

Only one study reporting quantitative measurements of isophorone in the general population was available. A case–control study (Hanai et al., 2012; Table 1.2) measured isophorone in the headspace of urine samples from

20 lung cancer cases and 20 healthy controls for the purpose of identifying novel diagnostic markers of lung cancer. A mean concentration of 130 nM [18 µg/L] was reported among control participants, and concentrations ranged from 39 to 1412 nM [5.4–195 µg/L]. It has been reported that the most likely routes of isophorone exposure in the general population are the inhalation of contaminated air and the ingestion of contaminated drinking-water (ATSDR, 2018), as well as direct contact with lacquers, paints, inks, and adhesives (US EPA, 2000), and exposure as a bystander during professional spraying and agrochemical use (ECHA, 2022). [The Working Group noted that while exposure to chemicals and products containing high concentrations of isophorone is probably higher in occupational settings, exposure to such products also occurs among hobbyists in the general population. However, there are substantial gaps in the published data on environmental exposures. The relative importance of exposure to other sources of isophorone described in this section (food, food packaging, and inflatables) remains to be quantified. It was further noted that, in many populations, isophorone exposure via drinking-water may be higher among people drinking bottled water due to migration from plastic bottles.]

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) Occupational exposure limits

The American Conference of Governmental Industrial Hygienists (ACGIH, 2001) recommended a 15-minute ceiling limit of 28 mg/m^3 for isophorone. There is no occupational exposure limit for the European Union, although occupational exposure limits have been defined by some European Union countries. This is the case for Germany, which has a MAK value (maximum workplace concentration) of 11 mg/m^3 (IFA, 2021b). European Union Council Directives state that pregnant or breast-feeding workers and persons under age 18 years may not be occupationally exposed to isophorone (Council directive 92/85/EEC; Council Directive 94/33/EC; European Council, 1992, 1994). Table 1.5 summarizes the current occupational exposure limits for isophorone in some countries with an 8-hour time-weighted average (TWA), or 15-minute short-term or ceiling limits (IFA, 2021b). In the USA, NIOSH has established an "immediately dangerous to life and health" (IDLH) limit for isophorone of 200 ppm on the basis of acute inhalation toxicity data in humans (NIOSH, 1994).

(b) Environmental exposure limits

In the USA, there were insufficient data for derivation of minimal risk levels (MRL) for inhalation exposure. For oral exposure of intermediate and long-term duration, MRLs of 3 mg/kg per day and 0.2 mg/kg per day, respectively, were derived – the latter based on lesions of the liver, kidney, and gastrointestinal tract in mice (ATSDR, 2018). The US EPA recommended ambient water quality criteria (AWQC) for human health are 34 µg/L for consumption of water and aquatic organisms and 1800 µg/L for consumption of aquatic organisms only (US EPA, 2015). Low reliability guideline trigger values of 120 µg/L for freshwater and 130 µg/L for marine water were derived in Australia and New Zealand (Australian and New Zealand Environment and Conservation Council, 2000).

Isophorone is a hazardous substance. According to the harmonized classification and labelling implemented in the European Union (Classification, Labelling and Packaging Regulation), isophorone is classified as a suspected human carcinogen on the basis of limited evidence of carcinogenicity in human studies and limited evidence of carcinogenicity in animal studies, i.e. carcinogen, category 2; acute toxicity, category 4; and is subject to several substance control regulations on general product

Table 1.5 Occupational exposure limits for isophorone in various countries

Country	8-hour TWA		Short-term (15 min)		Ceiling		Reference
	ppm	mg/m³	ppm	mg/m³	ppm	mg/m³	
Argentina	5	25	5	25			NICNAS (2013)
Australia					5	28	IFA (2021b)
Austria	2	11	2	11			IFA (2021b)
Belgium					5	28	IFA (2021b)
Canada – province of Ontario					5		IFA (2021b)
Canada – province of Quebec					5	28	IFA (2021b)
Chile					5	28	NICNAS (2013)
China						30	IFA (2021b)
Denmark	5	25			5	25	IFA (2021b)
Finland	1	5.7					IFA (2021b)
France					5	25	IFA (2021b)
Germany – AGS[a]	2	11	4	22			IFA (2021b)
Germany – DFG[a]	2	11	4	22			IFA (2021b)
Greece	5	25	5	25			NICNAS (2013)
ILO and WHO	2[c]	11[c]	5	29			ILO & WHO (2020)
Ireland[b]			5	25			IFA (2021b)
New Zealand					5	28	IFA (2021b)
Norway					5	25	IFA (2021b)
Poland		5				10	IFA (2021b)
Republic of Korea					5	25	IFA (2021b)
Romania	4.42	25	8	50			IFA (2021b)
Singapore			5	28			IFA (2021b)
South Africa			5	25			NICNAS (2013)
Spain			5	29			IFA (2021b)
Sweden			5	30			IFA (2021b)
Switzerland	2	11	4	22			IFA (2021b)
United Kingdom			5	29			IFA (2021b)
USA – ACGIH					5	28	NIOSH (1994)
USA – Cal/OSHA	4	23					OSHA (2020)
USA – NIOSH	4	23					IFA (2021b)
USA – OSHA	25	140					IFA (2021b)

ACGIH, American Conference of Governmental Industrial Hygienists; AGS, Ausschuss für Gefahrstoffe (German Committee on Hazardous Substances); Cal/OSHA, California Division of Occupational Safety and Health; DFG, Deutsche Forschungsgemeinschaft; ILO, International Labour Organization; JSOH, Japan Society for Occupational Health; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; ppm, parts per million; TWA, time-weighted average.

[a] Inhalable fraction and vapour.

[b] 15 min reference period.

[c] MAK, the maximum concentration in the workplace air which generally does not have known adverse effects on the health of the employee nor cause unreasonable annoyance (e.g. by a nauseous odour) during 8 h daily, assuming an average work week of 40 h.

safety, including medical devices and a ban from use in any cosmetic products and food contact materials in the European Union (ECHA, 2021b).

1.5.2 Reference values for biological monitoring of exposure

No reference values related to isophorone biological monitoring were available.

1.6 Quality of exposure assessment in key epidemiological studies of cancer

There was one case–control study on brain and other central nervous system (CNS) cancers available for review by the Working Group. The study was nested within a cohort of workers at three facilities manufacturing semi-conductor and electronic storage devices in the USA (Rodrigues et al., 2020). Further details on the exposure assessment for this study were also provided in Rodrigues et al. (2019).

Details on the selected domains of the exposure assessment review for these studies are summarized in Table S1.6 (Annex 1, Supplementary material for isophorone, Section 1, Exposure Characterization, available from: https://publications.iarc.fr/611).

1.6.1 Exposure assessment methods

The exposure assessment for the study by Rodrigues et al. (2020) was completed by conducting site visits at the three facilities and compiling more than 700 000 documents with site-specific job, task, and process information, and an industrial hygiene database with more than 10 000 samples across 31 chemicals and dusts of interest (including isophorone). Exposure was assigned by group exposure matrices and time period ("manufacturing era" to record periods where processes remained relatively stable) using division, department, and job title coupled with the sampling data, which were used to assign a mean exposure.

1.6.2 Critical review of exposure assessment methods

The exposure assessment for the study by Rodrigues et al. (2020) was well described for the cohort overall, and it was a key strength that such detailed and extensive site-specific information and a large database of industrial hygiene measurements were available to assign mean exposure by work group and over manufacturing eras. In the earlier work, the authors identified a confidence for each exposure estimate (Rodrigues et al., 2019). [The Working Group noted that although confidence estimates were apparently available, they were not used in the later analysis (Rodrigues et al., 2020).] The number of samples used for isophorone in particular was not reported (although availability of at least 20 samples across all three facilities was a criterion for inclusion) and so the strength of the exposure assessment for isophorone exposure with respect to hygiene data was difficult to evaluate. In addition, all 126 836 employees were categorized into 1 of 10 exposure groups per manufacturing era (three eras per facility), so there was likely to be substantial heterogeneity within each group. Job histories were only available for the parts of workers' careers that were spent at these facilities; this may not be a weakness if workers typically spent most or all of their career in these facilities, but if they spent significant time periods employed in other jobs, there could be an issue with exposure misclassification. An additional weakness was that industrial hygiene measurements were not randomly collected, such that their generalizability across the combination of chemical, facility, exposure group, and era was unclear.

2. Cancer in Humans

2.1 Cohort study

See Table 2.1.

Rodrigues et al. (2020) conducted a case–control study nested in a cohort of workers at three facilities manufacturing semiconductor and electronic storage devices located in East Fishkill in New York, Burlington in Vermont, and San José in California, USA. The study evaluated associations between CNS cancer and exposure to 31 agents of interest, including isophorone.

Deceased cases with a malignant CNS neoplasm were identified via National Death Index records or from death certificates. Incident cases of CNS cancer and date of diagnosis were identified through record linkage with the NY and CA state cancer registries. Ten controls per case were selected using incidence density sampling, matched on year of birth, facility, sex, and race. Ten primary exposure groups (PEGs) were created on the basis of type of production, tasks performed, and work environment with potential for exposure to chemical and physical agents. Mean concentrations were estimated for each chemical agent in each PEG using industrial hygiene data from the three facilities. Changes in the work environment over time were accounted for by use of manufacturing eras (i.e. PEG-exposure matrix by era for each chemical) when exposures associated with work processes remained relatively stable. Cumulative exposures to each chemical were estimated using work history variables, including department and job title assigned to one of the 10 PEGs and work start and end date in each facility and division, and mean concentrations for each exposure matrix cell (based on chemical, PEG, and era). Odds ratios were estimated for the risk of CNS cancer (incidence and mortality combined) using conditional logistic regression models, controlling for the matching variables and stratifying by facility. Exposure categories were tertiles of mg/m^3-years and the reference category was employees with no exposure to the particular chemical. The 120 CNS cancer cases and 1028 controls who had worked only at one facility were mostly male (88%) and White (93%).

Among the 1137 workers (119 cases and 1018 controls) in the analyses with cumulative exposure tertile, 728 (64%) were ever exposed to isophorone, including 239 in the highest tertile (> 5.09 mg/m^3-years) and 78 of the 119 included cases. Odds ratios were non-statistically significantly elevated in the highest tertile of cumulative exposure for isophorone at two of the three facilities. At one of the facilities (San José, CA), odds ratios were elevated in all three tertiles of exposure, although none were statistically significant. None of the tests for trend for isophorone (including at the San José facility) were statistically significant. [A strength of the study was the quantitative exposure assessment with detailed work history information and historical industrial hygiene data, although as the authors pointed out, none of the 31 chemicals identified as carcinogens or possible carcinogens is specifically a known cause of CNS cancer in humans. Isophorone results were presented in a table but not described by the authors in the text, because only chemicals with largely elevated odds ratios were discussed. The authors cited the small number of exposed cases and controls as a limitation but did not conduct analyses with three facilities combined controlling for this matching variable. Only stratified analyses by facility controlling for other three matching variables were conducted to "evaluate the internal consistency of results".]

2.2 Evidence synthesis for cancer in humans

One epidemiological study (Rodrigues et al., 2020) has been conducted on the carcinogenicity of isophorone. The study was a case–control

Table 2.1 Cohort studies on exposure to isophorone and cancer

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rodrigues et al. (2020) East Fishkill (NY), Burlington (VT) and San José (CA), USA Deceased cases, 1965–1999; incident cases, 1976–1999 Nested case–control	Cases: 120 deceased cases of malignant neoplasms of the CNS were identified via National Death Index records or from death certificates. Incident cases of CNS cancer were identified through record linkage with the NY and CA state cancer registries. Population/eligibility characteristics: cases and controls nested in the cohort of 126 836 employees included in the 1965–1999 mortality study at all three facilities and 1976–1999 study of cancer incidence among 89 054 workers at NY and CA facilities Controls: 1028; 10 controls per case (alive at the time of the case's index date) were selected using incidence-density sampling from the cohort, matched on year of birth, facility, sex, and race Exposure assessment method: expert judgement; detailed and site-specific information obtained from site visits combined with hygiene measurement data to create work group-exposure matrices specific to manufacturing era	CNS, incidence	Cumulative exposure tertile, East Fishkill (NY) facility (OR): 0 > 0 to < 1.69 mg/m³-years 1.70–5.09 mg/m³-years > 5.09 mg/m³-years Trend-test *P* value, 0.73	 25 14 8 6	 1 1.33 (0.65–2.72) 0.70 (0.31–1.62) 1.05 (0.41–2.73)	Year of birth, sex, race	*Exposure assessment critique*: occupational histories are specific just to company jobs (information on other jobs held was not provided) but the impact of this is difficult to discern. There were no measurements specific to the worker, but industrial hygiene data for the specific locations was used to quantify exposure. A key strength was the detailed information on individual jobs. The main limitation is the potential for exposure misclassification, as the industrial hygiene samples were not randomly collected; instead, they were collected based on a combination of chemical, facility, exposure group, and era. It was unclear if this is particularly a problem for isophorone vs the other chemicals assessed. Regression models of isophorone and cumulative exposure tertile excluded 1 case and 10 controls, presumably for missing exposure information. *Other comments*: conducted sensitivity analysis with 5 yr exposure lag. *Strengths*: quantitative exposure assessment with detailed work history information and historical industrial hygiene data.
		CNS, incidence	Cumulative exposure tertile, Burlington (VT) facility (OR): 0 > 0 to < 1.69 mg/m³-years 1.70–5.09 mg/m³-years > 5.09 mg/m³-years Trend-test *P* value, 0.52	 8 2 4 3	 1 0.75 (0.15–3.71) 1.18 (0.32–4.39) 1.27 (0.27–6.06)	Year of birth, sex, race	

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rodrigues et al. (2020) East Fishkill (NY), Burlington (VT) and San José (CA), USA Deceased cases, 1965–1999; incident cases, 1976–1999 Nested case–control (cont.)		CNS, incidence	Cumulative exposure tertile, San José (CA) facility (OR):			Year of birth, sex, race	*Limitations*: small number of exposed cases (including highly exposed cases in analysis by facility) and controls; stratified analyses by facility only and no analyses with all three facilities together; potential co-exposures to other occupational agents was also of concern.
			0	8	1		
			> 0 to < 1.69 mg/m³-years	13	1.45 (0.56–3.71)		
			1.70–5.09 mg/m³-years	13	1.60 (0.61–4.18)		
			> 5.09 mg/m³-years	15	1.18 (0.46–2.99)		
			Trend-test *P* value, 0.33				

CA, California; CI, confidence interval; CNS, central nervous system; NY, New York; OR, odds ratio; vs, versus; VT, Vermont.

study on exposure to 31 occupational agents and fatal and incident CNS cancers nested in a cohort of employees at three facilities. Cumulative occupational exposure to isophorone was estimated based on work history variables and estimates of mean concentrations from industrial hygiene data for 10 PEGs. Approximately 64% of the 1137 participants included (cases and controls) were ever exposed to isophorone (78 of 199 included cases). There were some weak positive associations observed in some categories of cumulative exposure in analysis stratified by three facilities, although none was significant and there was no trend. Although detailed and quantitative exposure estimation was performed, limitations included small numbers of exposed cases, including highly exposed cases in analysis by facility. Potential co-exposure to other occupational agents was also of concern.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

Oral administration (gavage)

In a well-conducted study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6C3F$_1$ mice (age, 6–8 weeks) were given isophorone (purity, ≥ 94%) at a dose of 0, 250, or 500 mg/kg body weight (bw) in corn oil, for the control group and the groups at the lower and higher dose, respectively, by gavage, 5 days per week, for 103 weeks (NTP, 1986). At study termination, survival was 16/50, 16/50, and 19/50 in males, and 26/50, 35/50, and 34/50 in females, for the control group and the groups at the lower and higher dose, respectively. In females, there was a significant positive trend in survival in females, and the survival rates at both doses were significantly higher than that of the controls. In males, the survival rates of the treated groups were similar to that of the control group. A significant decrease in body-weight gain was observed in females at the higher dose (about 5% lower at the end of the exposure period compared with controls). No significant difference in body weight was observed in females at the lower dose or in males at either dose. All mice (except one missing male mouse in the control group) underwent complete necropsy, and histopathological examination was performed on all gross lesions, and main tissues and organs.

In male mice, there was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) (P = 0.027, incidental tumour trend test; P = 0.025, Cochran–Armitage trend test), with incidence being significantly increased at the higher dose: control, 18/48 (37%); lower dose, 18/50 (36%); and higher dose, 29/50 (58%); P = 0.033, Fisher exact test. The incidence of hepatocellular adenoma was 6/48, 7/50, and 13/50, and the incidence of hepatocellular carcinoma was 14/48 (29%), 13/50 (26%), and 22/50 (44%), for the control group and the groups at the lower and higher dose, respectively. The incidence of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined) in males at the higher dose exceeded the upper bound of the ranges observed in historical controls in this laboratory – hepatocellular carcinoma, 218/1034 (mean ± standard deviation, 21.1 ± 7.6%); range, 8.3–36%; and hepatocellular adenoma or carcinoma (combined), 335/1034 (32.4 ± 9.4%); range, 14–50%. There was a significant positive trend in the incidence of fibrosarcoma of the subcutis (P = 0.044, life-table trend test; P = 0.019, incidental tumour trend test; P = 0.023, Cochran–Armitage trend test), with the increase in incidence being significant at the higher dose: control, 3/48; lower dose, 4/50; higher dose, 10/50; P = 0.042, Fisher exact test; P = 0.009, incidental tumour test. The incidence of fibroma of the subcutis was 0/48, 2/50, and 3/50, for the control group and the groups

Table 3.1 Studies of carcinogenicity in experimental animals exposed to isophorone

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6–8 wk 103 wk NTP (1986)	Oral administration (gavage) Isophorone, ≥ 94% Corn oil 0, 250, 500 mg/kg bw 1×/day, 5 days/wk, 103 wk 50, 50, 50 16, 16, 19	*Liver* Hepatocellular adenoma 6/48, 7/50, 13/50 Hepatocellular carcinoma 14/48, 13/50, 22/50 (44%) Hepatocellular adenoma or carcinoma (combined) 18/48, 18/50, 29/50 (58%)* *Subcutis* Fibroma 0/48, 2/50, 3/50 Fibrosarcoma 3/48, 4/50, 10/50 (20%)*	NS NS *P* = 0.027, incidental tumour trend test; *P* = 0.025, Cochran–Armitage trend test **P* = 0.033, Fisher exact test NS *P* = 0.044, life-table trend test; *P* = 0.019, incidental tumour trend test; *P* = 0.023, Cochran–Armitage trend test **P* = 0.042, Fisher exact test; *P* = 0.009, incidental tumour test	Principal strengths: well-conducted study that complied with GLP; used multiple doses; used males and females; adequate number of animals per group; adequate duration of exposure and observation Principal limitations: one control animal missing Other comments: survival rates of treated animals were similar to those of controls Historical controls: hepatocellular carcinoma, 218/1034 (21.1% ± 7.6%; range, 8.3–36%); hepatocellular adenoma or carcinoma (combined), 335/1034 (32.4% ± 9.4%; range, 14–50%); integumentary system (skin and subcutis: fibroma, fibrosarcoma, neurofibrosarcoma or sarcoma, combined), 70/1040 (6.7% ± 6.5%; range, 0–22%); subcutis fibrosarcoma, 28/1040 (2.7% ± 4.0%; range, 0–16.7%); lymphoma or leukaemia (combined) of the lymphohaematopoietic system, 132/1040 (12.7% ± 5.9%; range, 2.1–27.1%); and lymphoma of the lymphohaematopoietic system, 126/1040 (12.1% ± 5.1%; range, 2.1–22.0%)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6–8 wk 103 wk NTP (1986) (cont.)		*Integumentary system (skin and subcutis)*: fibroma, fibrosarcoma, neurofibrosarcoma, or sarcoma (combined) 6/48, 8/50, 14/50 (28%)*	*P* = 0.034, incidental tumour trend test; *P* = 0.033, Cochran–Armitage trend test **P* = 0.048, Fisher exact test; *P* = 0.050, incidental tumour test	
		Lymphohaematopoietic system: malignant lymphoma 7/48, 18/50 (36%)*, 5/50	**P* = 0.013, Fisher exact test	
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6–8 wk 103 wk NTP (1986)	Oral administration (gavage) Isophorone, ≥ 94% Corn oil 0, 250, 500 mg/kg bw 1×/day, 5 days/wk, 103 wk 50, 50, 50 26, 35, 34	No significant increase in tumour incidence in treated animals		Principal strengths: well-conducted study that complied with GLP; used multiple doses; used males and females; adequate number of animals per group; adequate duration of exposure and observation Other comments: significant positive trend in survival

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 6–7 wk 103 wk NTP (1986)	Oral administration (gavage) Isophorone, ≥ 94% Corn oil 0, 250, 500 mg/kg bw 1×/day, 5 days/wk, 103 wk 50, 50, 50 33, 33, 14	*Kidney* Tubular cell adenoma 0/50, 0/50, 2/50 Tubular cell adenocarcinoma 0/50, 3/50, 1/50 Tubular cell adenoma or tubular cell adenocarcinoma (combined) 0/50, 3/50 (6%), 3/50 (6%)* *Preputial gland*: carcinoma 0/50, 0/50, 5/50 (10%)*	 NS NS $P = 0.014$, life-table trend test; $P = 0.034$, incidental tumour trend test *$P = 0.025$, life-table test $P = 0.002$, life-table trend test; $P = 0.019$, incidental tumour trend test; $P = 0.006$, Cochran–Armitage trend test *$P = 0.028$, Fisher exact test; $P = 0.012$, life-table test	Principal strengths: well-conducted study that complied with GLP; used multiple doses; used males and females; adequate number of animals per group; adequate duration of exposure and observation Principal limitations: accidental kill by gavage errors was 4/50, 5/50, and 6/50 Other comments: there was a significant trend for decreased survival, and the survival rate at 500 mg/kg bw was decreased compared with controls Historical controls: tubular cell adenoma or tubular cell adenocarcinoma (combined) of the kidney, 4/1091 (0.4%); preputial gland tumours, 38/1094 (3.5%; range, 0–14%); and preputial gland carcinoma, 19/1094 (1.7%)
Full carcinogenicity Rat, F344/N (F) 6–7 wk 103 wk NTP (1986)	Oral administration (gavage) Isophorone, ≥ 94% Corn oil 0, 250, 500 mg/kg bw 1×/day, 5 days/wk, 103 wk 50, 50, 50 30, 23, 20	No significant increase in tumour incidence in treated animals		Principal strengths: well-conducted study that complied with GLP; used multiple doses; used males and females; adequate number of animals per group; adequate duration of exposure and observation Principal limitations: accidental kill by gavage errors was 1/50, 6/50, and 14/50 Other comments: survival rates were similar to those of controls

bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; NS, not significant; wk, week.

at the lower and higher dose, respectively. In the subcutis, one sarcoma was observed at the higher dose, and one neurofibrosarcoma was observed in controls. In the skin, the incidence of fibroma was 2/48, 1/50, and 0/50, for the control group and the groups at the lower and higher dose, respectively; and one neurofibrosarcoma was observed at the lower dose. There was a significant positive trend ($P = 0.034$, incidental tumour trend test; $P = 0.033$, Cochran–Armitage trend test) in the incidence of mesenchymal tumours (fibroma, fibrosarcoma, neurofibrosarcoma or sarcoma, combined) of the integumentary system (skin and subcutis, combined) with the incidence being significantly increased at the higher dose: control, 6/48 (12%); lower dose, 8/50 (16%); and higher dose, 14/50 (28%); $P = 0.048$, Fisher exact test; $P = 0.050$, incidental tumour test. In addition, the incidence of mesenchymal tumours (fibroma, fibrosarcoma, neurofibrosarcoma, or sarcoma, combined) of the integumentary system in male mice at the higher dose exceeded the upper bound of the range observed in historical controls in this laboratory – 70/1040 (6.7 ± 6.5%); range, 0–22%. There was a significant increase in the incidence of malignant lymphoma of the lymphohaematopoietic system at the lower dose: control, 7/48 (15%); lower dose, 18/50 (36%); higher dose, 5/50 (10%); $P = 0.013$, Fisher exact test. The incidence of malignant lymphoma of the lymphohaematopoietic system at the lower dose exceeded the upper bound of the range observed in historical controls in this laboratory – 126/1040 (12.1 ± 5.1%); range, 2.1–22.0%.

In female mice, there was no significant increase in tumour incidence in any of the treated groups compared with controls (NTP, 1986).

[The Working Group noted this was a well-conducted GLP study that used multiple doses, an adequate number of animals per group, an adequate duration of exposure and observation, and males and females.]

3.2 Rat

3.2.1 Oral administration (gavage)

In a well-conducted GLP study, groups of 50 male and 50 female F344/N rats (age, 6–7 weeks) were given isophorone (purity, ≥ 94%) at a dose of 0, 250, or 500 mg/kg bw in corn oil, for the control group and the groups at the lower and higher dose, respectively, by gavage, 5 days per week, for 103 weeks (NTP, 1986). There was a significant negative trend in survival in males; survival at study termination was: 33/50, 33/50, and 14/50 in males, and 30/50, 23/50, and 20/50 in females, for the control group and the groups at the lower and higher dose, respectively. The survival rate was significantly decreased in males at the higher dose. Gavage errors accounted for all of the 36 accidental deaths of male and female rats. Deaths related to gavage error increased with dose in females. A decrease in body weight was observed in males and females at the higher dose (7% lower at the end of the exposure period). All rats underwent complete necropsy, and histopathological examination was performed on all gross lesions, and main tissues and organs.

In male rats, there was a significant positive trend in the incidence of tubular cell adenoma or tubular cell adenocarcinoma (combined) of the kidney ($P = 0.014$, life-table trend test; $P = 0.034$, incidental tumour trend test), with incidence being significantly increased at the higher dose: control 0/50, lower dose, 3/50 (6%); higher dose, 3/50 (6%); $P = 0.025$, life-table test. The incidence of tubular cell adenoma or carcinoma (combined) of the kidney in both treated groups (3/50, 6%) was higher by 15-fold than the incidence reported for historical controls in this laboratory (4/1091, 0.4%). [The range of incidence of kidney tubular cell tumours in the historical control groups was not reported.] The incidence of tubular cell adenoma of the kidney was 0/50, 0/50, and 2/50, and the incidence of tubular cell adenocarcinoma of the kidney was 0/50, 3/50,

and 1/50, for the control group and the groups at the lower and higher dose, respectively. There was a significant positive trend in the incidence of carcinoma of the preputial gland (P = 0.002, life-table trend test; P = 0.019, incidental tumour trend test; P = 0.006, Cochran–Armitage trend test), with the incidence being significantly increased at the higher dose – control, 0/50; lower dose, 0/50; and higher dose, 5/50 (10%); P = 0.028 Fisher exact test; P = 0.012, life-table test. The incidence of preputial cell carcinoma in historical controls was 19/1094 (1.7%). [The range of incidence of preputial gland carcinoma in historical control groups was not reported.]

Regarding non-neoplastic lesions in male rats, the incidence of tubular cell hyperplasia of the kidney was 0/50, 1/50, and 4/50, for the control group and the groups at the lower and higher dose, respectively.

In female rats, there was no significant increase in tumour incidence in any of the treated groups compared with controls (NTP, 1986).

[The Working Group noted that this was a well-conducted GLP study that used multiple doses, an adequate number of animals per group, an adequate duration of exposure and observation, and males and females. The Working Group also noted the high number of accidental gavage-related deaths in males and females in this study.]

3.2.2 Inhalation

Two groups of 10 male and 10 female Wistar rats [age not reported; body weight, approximately 140 g at 2 weeks before exposure] were exposed by inhalation (whole-body exposure) to isophorone at a concentration of 0 (control) or 250 ppm [1413 mg/m^3] for 6 hours per day, 5 days per week, for 18 months. In treated rats, slight conjunctivitis and irritation of the nasal mucosa with a bloody discharge were observed. Frequent haemorrhages were found with oedema in the alveoli of the lungs, and microvacuolization was found in the liver. Tumour incidence was not reported (Dutertre-Catella, 1976). [The Working Group noted that this study was inadequate for the evaluation of the carcinogenicity of isophorone in experimental animals due to incomplete reporting, and lack of details regarding the study design, postmortem evaluation, and tumour incidence.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of isophorone has been assessed in one well-conducted GLP study in male and female B6C3F$_1$ mice (NTP, 1986) and in one well-conducted GLP study in F344/N rats (NTP, 1986) treated by oral administration (gavage), and in one inhalation (whole-body exposure) study in male and female Wistar rats (Dutertre-Catella, 1976).

In the GLP study in male and female B6C3F$_1$ mice treated by gavage, there was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) in male mice, with the incidence being significantly increased at the higher dose. There was a significant positive trend in the incidence of fibrosarcoma of the subcutis in male mice, with the incidence being significantly increased at the higher dose. There was a significant positive trend in the incidence of mesenchymal tumours (fibroma, fibrosarcoma, neurofibrosarcoma or sarcoma, combined) of the integumentary system (skin and subcutis, combined) in male mice, with the incidence being significantly increased at the higher dose. A significantly increased incidence of malignant lymphoma of the lymphohaematopoietic system in male mice was also reported at the lower dose. In female mice, there was no significant increase in tumour incidence in any of the treated groups compared with controls (NTP, 1986).

In the GLP study in male and female F344/N rats treated by gavage, there was a significant

positive trend in the incidence of tubular cell adenoma or tubular cell adenocarcinoma (combined) of the kidney in male rats, with the incidence being significantly increased at the higher dose. There was a significant positive trend in the incidence of carcinoma of the preputial gland, with the incidence being significantly increased at the higher dose. In female rats, there was no significant increase in tumour incidence in any of the treated groups compared with controls (NTP, 1986).

The one inhalation study in Wistar rats was judged to be inadequate for the evaluation of the carcinogenicity of isophorone in experimental animals (Dutertre-Catella, 1976).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Only one study was performed to study dermal absorption of isophorone in human skin. Low permeability (< 20%) was demonstrated, and no potential to damage the skin was observed after dermal application of isophorone (25 mg/mL) for 60 minutes (Fasano & McDougal, 2008). Urinary isophorone was detected at higher concentrations in 20 lung cancer patients than in 20 healthy control volunteers (Hanai et al., 2012). [The Working Group noted that there was pharmacokinetic disposition, but the significance of this study was not clear.]

4.1.2 Experimental systems

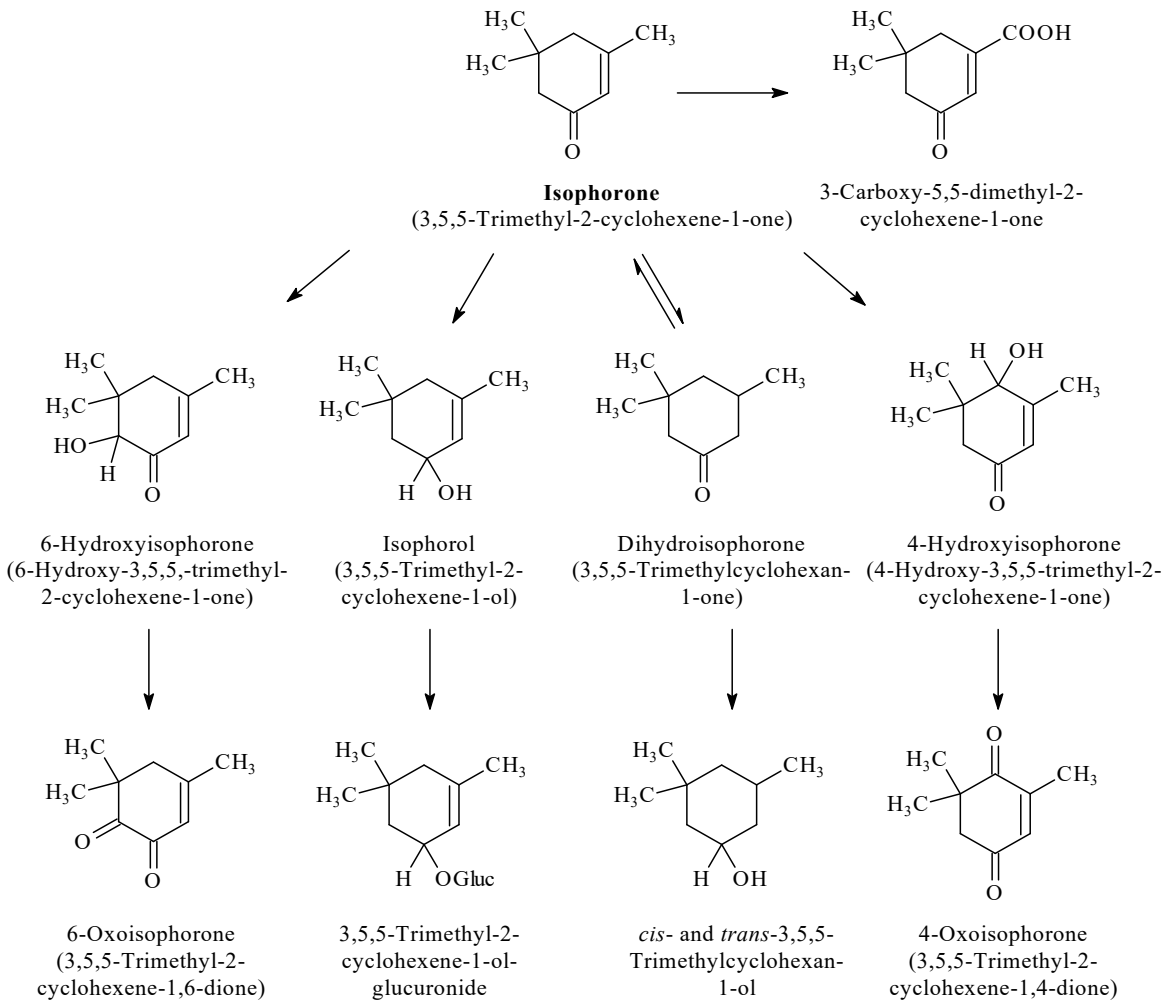
(a) Absorption and distribution

Studies in mice, rats, and rabbits show that isophorone is absorbed after inhalation, and after oral and dermal exposure (Dutertre-Catella et al., 1970, 1978; Dutertre-Catella, 1976). In six rats and one rabbit treated with isophorone (4 g/kg bw) by gavage, isophorone was detected 1 hour after treatment in the stomach, pancreas, and adrenal glands of both species, at concentrations ranging from 0.3 to 6.3 µg/g tissue wet weight. In six rats treated with isophorone (400 ppm, 2 mg/m^3) by inhalation for 4 hours, isophorone was detected in the kidneys, adrenal glands, liver, pancreas, and brain; levels in the kidneys were higher in males than in females. The presence of isophorone in all these organs decreased rapidly 1 hour after the end of the inhalation exposure (Dutertre-Catella, 1976). In another experiment in six rats treated by gavage, isophorone (1 g/kg bw) could not be detected in any organ 48 hours after treatment. In two rabbits treated with isophorone (1 g/kg bw) by gavage, most of the administered dose was detected in the blood at 10 minutes to 1 hour after treatment, with concentrations decreasing rapidly thereafter (to 50% of the administered dose at 3 hours and trace amounts at 21 hours) (Dutertre-Catella, 1976).

(b) Metabolism and excretion

Metabolites of isophorone have been identified in the urine of animals exposed to isophorone by oral administration (Dutertre-Catella et al., 1970, 1978; Dutertre-Catella, 1976). In rabbits and rats treated with isophorone (1 g/kg bw) by gavage, the following metabolites were identified in the urine: 3,5,5-trimethyl-2-cyclohexene-1-ol (isophorol) and its glucuronic conjugate; 3-carboxy-5,5-dimethyl-2-cyclohexene-1-one; 3,5,5-trimethylcyclohexan-1-one (dihydroisophorone); and *cis*- and *trans*-3,5,5-trimethylcyclohexan-1-ol (Dutertre-Catella, 1976; Dutertre-Catella et al., 1978). After isophorone ingestion, more dihydroisophorone and less isophorol was found in rat urine than in rabbit urine. Truhaut et al. (1973) identified isophorone in the urine of rats and rabbits given 3,5,5-trimethylcyclohexan-1-one (dihydroisophorone) (1 g/kg bw) by gavage (Truhaut et al.,

Fig. 4.1 Metabolic scheme for isophorone



Gluc, glucuronide.
Created by the Working Group.

1973). Dutertre-Catella (1976) and Dutertre-Catella et al. (1978) proposed that the metabolism of isophorone involves methyl oxidation to 3-carboxy-5,5-dimethyl-2-cyclohexene-l-one, reduction of the ketone group to isophorol, reduction of the ring double bond to dihydroisophorone, and dismutation of dihydroisophorone to *cis*- and *trans*-3,5,5-trimethylcyclohexan-1-ol (Dutertre-Catella, 1976; Dutertre-Catella et al., 1978). The metabolic pathways of isophorone are presented in Fig. 4.1. [The Working Group noted that the enzymes implicated in the metabolism of isophorone in rodents have not been identified.]

Studies in rats and rabbits suggest that urine is the predominant route of elimination of isophorone (Dutertre-Catella et al., 1970, 1978; Truhaut et al., 1973; Dutertre-Catella, 1976). After oral administration of isophorone, rats and rabbits excreted unchanged isophorone and metabolites in the urine, and unchanged isophorone in the expired air. Forty-eight hours after ingestion of dihydroisophorone (a metabolite of isophorone) by rats or rabbits, an estimated 50–70% of the

administered dose was present as glucuronic conjugates in the urine (Dutertre-Catella, 1976; Dutertre-Catella et al., 1978). [The Working Group noted that the rate and extent of excretion were not reported.]

4.2 Evidence relevant to key characteristics of carcinogens

4.2.1 Is genotoxic

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

Intraperitoneal injection of isophorone did not result in micronucleus formation in bone marrow cells (polychromatic erythrocytes) of male and female CD-1 mice (O'Donoghue et al., 1988).

(ii) Non-human mammalian cells in vitro

See Table 4.2.

Tests for unscheduled DNA synthesis in rat primary hepatocytes treated with isophorone were reported to give weakly positive results in two studies (Selden et al., 1994) [the Working Group noted the use of a non-standard protocol in one of the studies], although the same test system gave a negative result in a third study (O'Donoghue et al., 1988). Gene mutation studies in mouse L5178Y/*Tk*$^{+/-}$ lymphoma cells treated with isophorone (at concentrations ranging from 400 to 1500 µg/mL) gave one positive result without metabolic activation at non-cytotoxic concentrations (McGregor et al., 1988), three additional positive results without metabolic activation (NTP, 1986; Tennant et al., 1987; Honma et al., 1999b) [but the Working Group noted concerns over cytotoxicity and/or study design], and three studies gave negative or equivocal results with and without metabolic activation (O'Donoghue et al., 1988; Sofuni et al., 1996; Honma et al., 1999a). Tests for chromosome aberrations were reported to give positive results with and without metabolic activation in one study in Chinese hamster lung fibroblast cells (Matsuoka et al., 1996) [the Working Group noted that a non-standard protocol was used and no indication of cytotoxicity was provided], but negative results were reported in three additional studies in Chinese hamster ovary cells (with isophorone at concentrations up to 1600 µg/mL) with and without metabolic activation (NTP, 1986; Tennant et al., 1987; Gulati et al., 1989). In Chinese hamster ovary cells, tests for sister-chromatid exchange were reported to give positive results without metabolic activation in two studies (Tennant et al., 1987; Gulati et al., 1989) [the Working Group noted that no indication of cytotoxicity was provided], but results were negative with or without metabolic activation (with isophorone at concentrations of up to 1000 µg/mL) in a third study (NTP, 1986).

(iii) Non-mammalian experimental systems

See Table 4.3.

Isophorone did not induce micronucleus formation in a hen's egg test (Greywe et al., 2012). Isophorone exposure by feeding or injection gave negative results in the sex-linked recessive lethal test in *Drosophila melanogaster* (Foureman et al., 1994). Isophorone did not induce mutation in *Salmonella typhimurium* in the presence or absence of metabolic activation (Mortelmans et al., 1986; NTP, 1986; Tennant et al., 1987; Kubo et al., 2002).

4.2.2 Evidence relevant to other key characteristics

(a) Humans

No data were available to the Working Group.

Table 4.1 Genetic and related effects of isophorone in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Mouse, CD-1 (M, F)	Bone marrow, polychromatic erythrocytes	–	0.54 mL/kg [500 mg/kg bw]	Intraperitoneal injection, mice killed after 12, 24, and 48 h	Only one dose tested	O'Donoghue et al. (1988)
DNA binding	Mouse, $B6C3F_1$ (M, F)	Liver, kidney	–	500 mg/kg bw	Gavage, mice killed after 24 h	Non-standard assay, only one dose tested	Thier et al. (1990)
DNA binding	Rat, F344 (M, F)	Liver, kidney	–	500 mg/kg bw	Gavage, rats killed after 24 h	Non-standard assay, only one dose tested	Thier et al. (1990)

bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male.

[a] –, negative

Table 4.2 Genetic and related effects of isophorone in non-human mammalian cells in vitro

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Unscheduled DNA synthesis	Rat, primary hepatocytes	–	NT	0.2 µL/mL [185 µg/mL]	GLP study (6 concentrations tested, biological triplicates, positive control).	O'Donoghue et al. (1988)
Unscheduled DNA synthesis	Rat, primary hepatocytes	(+)	NT	5.75 mM [795 µg/mL]	Non-standard protocol (5 concentrations tested, biological triplicates).	Selden et al. (1994)
Unscheduled DNA synthesis	Rat, primary hepatocytes	(+)	NT	5 mM [691 µg/mL]	Standard protocol (only one concentration tested, no biological triplicates).	Selden et al. (1994)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	(+)	NT	1200 µg/mL	Positive only at concentration with cytotoxicity > 80%; positive controls not included.	NTP (1986)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	(+)	NT	400 µg/mL	No indication of cytotoxicity; number of experiments, NR.	Tennant et al. (1987)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	+	NT	800 µg/mL	Well-conducted study (5 concentrations tested, biological triplicates, positive control).	McGregor et al. (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	–	–	1300 µg/mL	Only one experiment; positive controls included.	O'Donoghue et al. (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	+/–	–	1500 µg/mL	Probably negative.	Sofuni et al. (1996)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	–	+/–	1500 µg/mL	Probably negative, positive (+S9) in only one laboratory; the mutation frequency with isophorone was < 2 times the spontaneous mutation frequency.	Honma et al. (1999a)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	(+)	NT	1500 µg/mL	Non-standard protocol with long-term treatment; only one experiment.	Honma et al. (1999b)
Chromosome aberrations	Chinese hamster, ovary cells	–	–	1000 µg/mL		NTP (1986)
Chromosome aberrations	Chinese hamster, ovary cells	–	–	1600 µg/mL	No indication of cytotoxicity or the number of experiments performed.	Tennant et al. (1987)
Chromosome aberrations	Chinese hamster, ovary cells	–	–	1600 µg/mL		Gulati et al. (1989)

Table 4.2 (continued)

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Chromosome aberrations	Chinese hamster, lung cells	(+)	(+)	1250 µg/mL –S9 1500 µg/mL +S9	Non-standard protocol; no indication of cytotoxicity.	Matsuoka et al. (1996)
Sister-chromatid exchange	Chinese hamster, ovary cells	–	–	1000 µg/mL		NTP (1986)
Sister-chromatid exchange	Chinese hamster, ovary cells	(+)	NT	500 µg/mL	No indication of cytotoxicity; number of experiments, NR.	Tennant et al. (1987)
Sister chromatid exchange	Chinese hamster, ovary cells	(+)	–	500 µg/mL –S9 1600 µg/mL +S9	Positive in only one experiment (of two); no indication of cytotoxicity.	Gulati et al. (1989)

GLP, Good Laboratory Practice; HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant (liver); Tk, thymidine kinase.

[a] +, positive; –, negative; +/–, equivocal; (+) or (–), positive or negative in a study of limited quality.

Table 4.3 Genetic and related effects of isophorone in non-mammalian experimental systems

Test system species, strain	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Hen's egg	Micronucleus formation (blood erythrocytes)	–	NT	10 mg/65 g egg	Single and repeated treatments	Greywe et al. (2012)
Drosophila melanogaster	Sex-linked recessive lethal mutations	–	NT	2000 µg/mL (feeding) or 12 500 µg/mL (injection)		Foureman et al. (1994)
Salmonella typhimurium	Mutation	–	–	10 000 µg/plate	No indication of the strain tested	Tennant et al. (1987)
Salmonella typhimurium TA98 and TA100	Mutation	–	–	1 mM [138.21 µg/mL]		Kubo et al. (2002)
Salmonella typhimurium TA98, TA100, TA1537, and TA1538	Mutation	–	–	10 000 µg/plate		Mortelmans et al. (1986)
Salmonella typhimurium TA98, TA100, TA1535, and TA1537	Mutation	–	–	10 000 µg/plate		NTP (1986)

LEC, lowest effective concentration; HIC, highest ineffective concentration; NT, not tested.

[a] –, negative.

(b) Experimental systems

Regarding electrophilicity, there was no covalent binding of isophorone or its metabolites to DNA in the liver or kidney of Fischer 344 rats and B6C3F$_1$ mice 24 hours after administration of isophorone by gavage (500 mg/kg/bw) (Thier et al., 1990; see Table 4.1).

Regarding oxidative stress, a single dose of isophorone (500 mg/kg/bw) administered by intraperitoneal injection to male Sprague-Dawley rats caused significant depletion of hepatic, testicular, and epididymal glutathione (Gandy et al., 1990). Using a bacterial (*Escherichia coli)* reporter assay expressing 14 different stress response genes, isophorone (0.6–36 g/L) significantly induced the expression of catalase-peroxidase (*KatG*) (Nobels et al., 2011).

Regarding immunosuppression, leukopenia without any change in differential or erythrocyte counts was observed in male Sprague-Dawley rats exposed to isophorone (67 or 90 ppm) for 4 hours (Brondeau et al., 1990).

Regarding immortalization, a transformation study was performed in BALB/c-3T3 mouse cells (Matthews et al., 1993). In one experiment, isophorone at up to 1.34 mM did not induce cell transformation. In two additional experiments, cell transformation was observed at 0.5 and 2.67 mM. [The Working Group noted that inconsistencies between the three experiments confounded interpretation of the study.]

Regarding alterations in cell proliferation, cell death, or nutrient supply, in studies in B6C3F$_1$ mice and F344/N rats exposed to isophorone (250 and 500 mg/kg bw per day) by gavage for 103 weeks (see Section 3), there was a slight increase in the incidence of renal tubular cell hyperplasia of a single tubule in the kidney [which is a rare lesion] and a significant increase [P = 0.028] in the incidence of epithelial hyperplasia of the renal pelvis in both treated groups of male rats compared with controls (Bucher et al., 1986; NTP, 1986). [The Working Group noted that there was no dose-dependent effect on the incidence of renal pelvis hyperplasia. The Working Group also noted that there were no effects on renal histopathology in 16-day and 13-week studies of isophorone administered at higher dose levels (Bucher et al., 1986; NTP, 1986).]

4.2.3 High-throughput in vitro toxicity screening data evaluation

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 130 was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). Isophorone was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes of the US EPA and the United States National Institutes of Health. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2021). A supplementary table (Annex 2, Supplementary material for Section 4, Mechanistic Evidence, web only; available from: https://publications.iarc.fr/611) provides a summary of the findings including the assay name, the corresponding key characteristic, the resulting "hit calls" both positive and negative, and any reported caution flags for isophorone. The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (available from: https://gitlab.com/i1650/kc-hits) using the US EPA ToxCast and Tox21 assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for the present monograph. Findings and interpretations from these high-throughput assays for isophorone are discussed below.

After mapping against the key characteristics of carcinogens, the ToxCast/Tox21 database contained 291 assays in which isophorone was tested. Of these, it was found to be active and without caution flags in seven assays relevant to the key characteristics of carcinogens. [The Working Group noted that the cytotoxic limit for isophorone is 14.8 μM.]

Isophorone was active in five assays mapped to key characteristic 8 (KC8), "modulates receptor-mediated effects". It was active in one assay related to G protein-coupled receptor (GPCR) binding activity in human platelets with a half-maximal activity concentration (AC_{50}) of 22.7 μM. In HepG2 cells, isophorone activated the nuclear receptor subfamily 1, group I, member 2 (NR1I2) (AC_{50}, 48.7 μM); the nuclear receptor subfamily 1, group H, member 3 (NR1H3) (AC_{50}, 36 μM); the nuclear receptor subfamily 1, group H, member 2 (NR1H2) (AC_{50}, 63.3 μM); and the retinoid X receptor, β (RXRB) (AC_{50}, 51.4 μM).

In addition, isophorone was active in two assays mapped to KC10, "alters cell proliferation, cell death, or nutrient supply", in HEK293 cells. The assay measurements were performed 32 and 40 hours after exposure, with AC_{50}s of 40.93 and 36.74 μM, respectively.

4.3 Other relevant evidence

Several studies reported effects related to α_{2u}-globulin in the kidney of male rats. An increase in α_{2u}-globulin was reported in the kidneys of male Sprague-Dawley rats treated with isophorone (150 mg/kg bw per day) by gavage for 14 days (Saito et al., 1992). In addition, isophorone (207 mg/kg bw per day) administered by gavage for seven consecutive days caused an increase in urinary and renal α_{2u}-globulin and hyaline droplets in the renal proximal convoluted tubule epithelial cells of male Sprague-Dawley rats (Saito et al., 1996). In a 2-year study in male and female F334/N rats treated with isophorone by gavage (250 and 500 mg/kg bw per day), the incidence of nephropathy in treated and control male rats was similar, with greater severity in males at the lower dose, and an increase in the incidence of nephropathy in female rats compared with controls. In male rats only, there was also an increase in the incidence of mineralization of the renal tubule epithelial cells (most often found in the medullary collecting ducts and occurring coincidentally with lesions of chronic nephropathy); a significant increase in the incidence of epithelial hyperplasia of the renal pelvis; and an increase in the incidence of other non-neoplastic lesions (including a low incidence of hyperplasia that was described as confined to one tubule) and renal tumours (NTP, 1986). [The Working Group noted that α_{2u}-globulin has not been determined to be relevant to carcinogenesis in other organs besides the kidney.] Male NCI-Black-Reiter (NBR) rats, which do not synthesize α_{2u}-globulin, did not exhibit hyaline droplet formation and nephrotoxicity (necrosis, exfoliation, and regeneration of renal tubule epithelial cells) after exposure to isophorone (1000 mg/kg bw per day) by gavage for 4 days (Dietrich & Swenberg, 1991). [The Working Group noted that major limitations of this study were that a positive control (i.e. a strain of male rat that produces α_{2u}-globulin) was not included, and longer time-points were not evaluated.]

Lehman-McKeeman et al. (1990) reported that isophorone bound to α_{2u}-globulin extracted from the urine of male Sprague-Dawley rats and reduced its lysosomal degradation in vitro. Furthermore, Borghoff et al. (1991) determined that isophorone competed with 2,2,4-trimethylpentane for binding to α_{2u}-globulin in protein extracts isolated from the kidney of male Fischer 344 rats. [The Working Group noted that it has been suggested that the binding of isophorone to α_{2u}-globulin is reversible on the basis of modelling predictions (Borghoff et al., 1991) but this has not been conclusively demonstrated experimentally.]

IARC has established seven criteria that need to be fully met to conclude that an agent induces

tumours of the kidney by an α_{2u}-globulin-associated response (IARC, 1999). For isophorone, only one of the seven criteria was met, that is, identification of the accumulating protein as α_{2u}-globulin. The remaining six criteria were not met, specifically: (i) lack of genotoxic activity of the agent and/or metabolite (see Section 4.2.1) [the Working Group noted that there is mixed evidence for genotoxicity of isophorone in non-human mammalian experimental systems]; (ii) male rat specificity for nephropathy and renal tumorigenicity [the Working Group noted that there is no evidence for α_{2u}-globulin-dependent renal hyperplasia or tumorigenicity (using the NBR rat strain), and isophorone induced tumours at other sites in which α_{2u}-globulin has not been demonstrated to be relevant to carcinogenesis, i.e. the preputial gland in male rats and the liver, subcutis, and lymphohaematopoietic system in male mice (see Section 3)]; (iii) induction of the characteristic sequence of histopathological changes associated with α_{2u}-globulin accumulation [the Working Group noted that Saito et al. (1992, 1996) only showed increased α_{2u}-globulin accumulation and hyaline droplets, whereas NTP (1986) reported nephrotoxic effects (including increased incidence of tubular cell hyperplasia confined to a single tubule, and tumours) but no α_{2u}-globulin accumulation and hyaline droplets, and no increased injury to kidney proximal tubule epithelial cells that would be characteristic of α_{2u}-globulin nephropathy in 16-day, 13-week, or 2-year studies]; (iv) reversible binding of the chemical or metabolite to α_{2u}-globulin [the Working Group noted that reversible binding of isophorone has been predicted but not determined experimentally]; (v) induction of sustained increases in cell proliferation in the renal cortex; and (vi) similarities in dose–response relationships for the tumour outcome and for histopathological end-points associated with α_{2u}-globulin nephropathy. [The Working Group noted that the NTP (1986) study did not report α_{2u}-globulin accumulation and hyaline droplets so these end-points could not be correlated to kidney tumours in male rats.]

While such data can be informative in interpreting the relevance to humans of kidney tumours observed in rodents, the findings did not fulfil all criteria required in order to conclude that the induction of renal tumours by isophorone operates via a mechanism associated with α_{2u}-globulin in male rats (IARC, 1999).

5. Summary of Data Reported

5.1 Exposure characterization

Isophorone is a High Production Volume chemical that is widely used as a solvent and also as a chemical intermediate in the manufacture of a variety of products, including polymers and their precursors, lacquers, inks, paints, nitrocellulose finishes, and cleaning products. It is also used in the production of agrochemicals and is a constituent of certain pesticides. The widescale industrial use of isophorone permits its release into the environment, primarily through atmospheric release at urban and industrial centres, but also via industrial effluents. The most substantial human exposures to isophorone probably occur in occupational settings, particularly as airborne exposure during industrial processes using isophorone or products containing isophorone. In particular, printing and screen printing; coating, painting and spray painting; machine operating; plastics production; packaging; and cleaning may be notable sources of occupational exposure, although few recent quantitative data were available to the Working Group. Isophorone has been detected and quantified in a variety of environmental samples and products, notably drinking-water, pesticides, food items and food packaging, inflatable pool toys, and other polymer-based products. No quantitative data on exposure to isophorone in the general population were available to the Working Group.

5.2 Cancer in humans

The available evidence on cancer in humans consisted of a single study investigating the association between cancers of the central nervous system and exposure to isophorone. The study observed some weakly elevated risk estimates in some categories of cumulative exposure in analyses conducted by facility, but none of the elevations were statistically significant. No trend with increasing exposure category was present. The study was only weakly informative due to small numbers of exposed cases, in particular, small numbers of highly exposed cases, and the potential for co-exposures to other agents used in the workplace. The study did not permit a conclusion to be drawn about the presence or absence of a causal association between exposure to isophorone and cancer risk.

5.3 Cancer in experimental animals

Treatment with isophorone caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species.

Isophorone was administered by oral administration (gavage) in one study in $B6C3F_1$ mice. In males, isophorone caused an increase in the incidence of fibrosarcoma of the subcutis, mesenchymal tumours (fibroma, fibrosarcoma, neurofibrosarcoma, or sarcoma, combined) of the integumentary system (skin and subcutis, combined), hepatocellular adenoma or carcinoma (combined), and malignant lymphoma of the lymphohaematopoietic system.

Isophorone was administered by oral administration (gavage) in one study in F344/N rats. In males, isophorone caused an increase in the incidence of carcinoma of the preputial gland, and tubular cell adenoma or tubular cell adenocarcinoma (combined) of the kidney.

5.4 Mechanistic evidence

Only one study on the absorption, distribution, metabolism, and excretion of isophorone in humans was available; this study demonstrated poor dermal absorption. Studies in rodents and rabbits demonstrated that isophorone is rapidly absorbed via multiple routes and is excreted as parent compound and/or metabolites in the urine.

Overall, the mechanistic evidence that isophorone exhibits the key characteristics of carcinogens ("is genotoxic", "induces oxidative stress", "is immunosuppressive", and "alters cell proliferation, cell death, or nutrient supply") is suggestive but inconsistent across experimental systems. No studies relevant to the key characteristics were available in exposed humans or human cells in vitro. The mechanistic evidence on whether isophorone is genotoxic was inconsistent across non-human mammalian and non-mammalian experimental systems. One study on micronucleus formation in mice exposed to isophorone by intraperitoneal injection gave negative results. In one study in mammalian cells, there was a positive response without metabolic activation, but for several other studies in mammalian cells, results were negative or equivocal with and without metabolic activation. Isophorone with and without metabolic activation was not mutagenic in non-mammalian experimental systems (including bacteria) in multiple studies. Regarding the key characteristics "induces oxidative stress", "is immunosuppressive", and "alters cell proliferation, cell death, or nutrient supply", there was a paucity of available data. One study in rats provided evidence that isophorone induces oxidative stress, whereas in another study in rats isophorone caused leukopenia. Rare instances of renal tubular cell hyperplasia of a single tubule in the kidney and a significant increase in the incidence of epithelial hyperplasia of the renal pelvis were reported in male rats exposed to isophorone by gavage in a 2-year study. Regarding whether

isophorone exhibits the key characteristic "is electrophilic or metabolically activated", negative results were reported in one study on DNA binding in the liver and kidney of rodents treated with isophorone by gavage. Isophorone was largely inactive at non-cytotoxic concentrations in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of isophorone.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of isophorone.

6.3 Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

Isophorone is *possibly carcinogenic to humans (Group 2B)*.

6.5 Rationale

The Group 2B evaluation for isophorone is based on *sufficient evidence* for cancer in experimental animals. This *sufficient evidence* in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species. The evidence for cancer in humans was *inadequate*. There was only one study on cancers of the central nervous system, which was not sufficiently informative to permit a conclusion to be drawn about the presence or absence of a causal association due to small numbers of exposed cases, in particular, small numbers of highly exposed cases, in an analysis by facility. Potential co-exposure to other agents used in the workplace was also of concern. The mechanistic evidence was *limited* because the findings regarding key characteristics of carcinogens across experimental systems were suggestive, but inconsistent.

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