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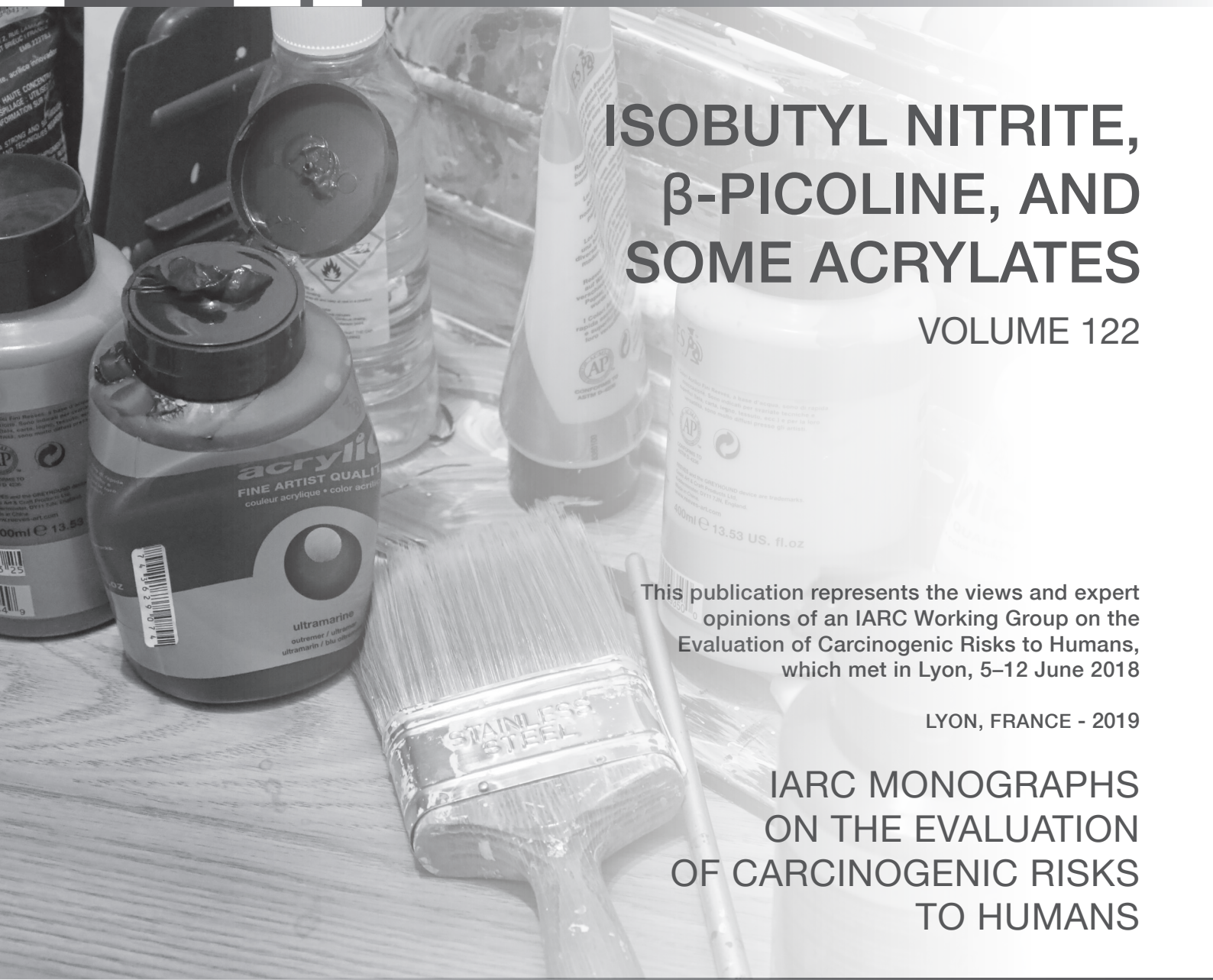

ISOBUTYL NITRITE, β-PICOLINE, AND SOME ACRYLATES

VOLUME 122

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International Agency for Research on Cancer

World Health Organization

ISOBUTYL NITRITE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

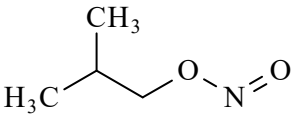
Chem. Abstr. Serv. Reg. No.: 542-56-3

IUPAC systematic name: 2-methylpropyl nitrite

Other names and abbreviations: IBN; iso-butyl nitrite; nitrous acid; isobutyl ester; nitrous acid; 2-methylpropyl ester

From Royal Society of Chemistry (2018).

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_4H_9NO_2$

Relative molecular mass: 103.12

1.1.3 Chemical and physical properties

Description: colourless to pale yellow liquid

Stability: stable; flammable volatile liquid; gradually decomposes in water; incompatible with acids, alcohols, strong bases, and strong oxidizing agents

Boiling point: 66–67 °C (experimental)

Flash point: –21 °C (experimental)

Density: 0.87 g/mL (experimental)

Refractive index: 1.373

Relative density (water = 1): 0.87 g/cm^3

Vapour pressure: 10 mm Hg [1.3 kPa] at 20 °C

Water solubility: slightly soluble and gradually decomposed by water: 935.9 mg/L, that is, < 1 mg/mL (estimated)

Conversion factor: 1 ppm = 4.22 mg/m^3 (at 1 atm and 25 °C).

1.1.4 Technical products and impurities

Analysis of commercially available isobutyl nitrite revealed a purity of only 63%. The major impurity was isobutyl alcohol, formed as a result of degradation of the parent isobutyl nitrite (Maickel, 1988).

1.2 Production and use

1.2.1 Production process

Isobutyl nitrite is synthesized by reacting isobutyl alcohol with sodium nitrite in dilute sulfuric acid (NTP, 1996).

1.2.2 Production volume

No data on production volumes were available to the Working Group. Isobutyl nitrite is one of the alkyl nitrites, commonly known as

Table 1.1 Representative methods for the analysis of isobutyl nitrite

Sample matrix	Assay procedure	Limit of detection (μg/mL)	Reference
Blood and commercial liquids	GC-FID with headspace injection	0.05	Vogt et al. (2015)
Adulterated coffee drinks	GC-EI/MS	0.06	Bal et al. (1988); Seto et al. (2000)
Rat and human blood samples	GC-ECD	0.001	Kielbasa et al. (1999)
Human blood and urine	GC-FID with headspace injection, in addition to cryogenic oven trapping	0.01 for blood; 0.005 for urine	Watanabe-Suzuki et al. (2003)

ECD, electron capture detection; EI/MS, electron ionization mass spectrometry; FID, flame ionization detection; GC, gas chromatography

"poppers". The quantity of poppers ordered online from countries where they are legal, such as China, Poland, South Africa, and the United Kingdom, has recently been growing (GINAD, 2018).

1.2.3 Use

Isobutyl nitrite, like other poppers, is mainly used for its psychoactive effects; its vasodilator properties are experienced as a cerebral "rush" (Dixon et al., 1981). Poppers are illegal in many countries (e.g. Australia, Canada, and France); isobutyl nitrite and other poppers are therefore commonly marketed as air freshener or deodorizer in some clubs and head shops, and online (Jeon et al., 2016). Poppers have become popular recreational drugs among men who have sex with men since it is claimed that they prolong the sense of sexual excitement (Shesser et al., 1981).

In the 1970s in the USA, isobutyl nitrite and other poppers were widely marketed in discotheques and sex and drug paraphernalia shops under trade names such as "Rush", "Bolt", "Hardware", "Quick Silver", and "Satan's Scent". An average bottle contained 10–15 mL of liquid comprising about 90% volatile alkyl nitrites, together with small quantities of the corresponding alcohol and vegetable oil to reduce volatility (Shesser et al., 1981).

Other reported minor uses of isobutyl nitrite include as an intermediate in the synthesis of aliphatic nitrites, nail polish removers, video head cleaners, fuels, and jet propellants (NTP, 1996).

1.3 Measurement and analysis

A summary of analytical methods reported for isobutyl nitrite is provided in Table 1.1. As a volatile compound, the most reliable method for analysis of isobutyl nitrite is based on gas chromatography followed by flame ionization or electron capture detection. The limits of detection of the methods fall within the range 0.001–0.060 μg/mL.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

The Working Group did not identify any reports of involuntary population exposure caused by background environmental levels of isobutyl nitrite in outdoor air, water, dust, soil, or wildlife. This is mainly attributable to the usage profile and physicochemical properties of this chemical, especially its instability and rapid degradation in air and water (NTP, 1996; McLaughlin et al., 2007).

1.4.2 Exposure in the general population

Humans are exposed to isobutyl nitrite mainly through inhalation and, to a lesser extent, ingestion. Exposure occurs via intentional

administration of poppers liquids for recreational purposes. Upon inhalation, users experience transient euphoria, and enhanced sexual excitement and performance (Schwartz & Peary, 1986; Haverkos & Dougherty, 1988).

Poppers are popular among men who have sex with men, with 60% of this population group in Australia admitting to trying poppers (Krilis et al., 2013; Rewbury et al., 2017). Their use as a party drug is also increasing among heterosexual and younger people (Smith & Flatley, 2013), with about 1.1% of the general population in the UK reporting using poppers at least once per year; poppers are now the fourth most popular recreational drug after cannabis, cocaine, and ecstasy (Pebody, 2011). The use of poppers decreased substantially in the 1980s in the USA. For example, the proportion of high school seniors reporting ever having used nitrites declined from approximately 10% in the class of 1979 to less than 2% in the class of 1992. In the Multicenter AIDS Cohort Study, reports of popper use during the 6 months before interview in men who have sex with men decreased from approximately 66% in 1984 to approximately 35% in 1989. This reduction may be attributed to the decreased availability of poppers because of federal bans, and to increased awareness of the adverse effects of nitrites within this community (Haverkos & Drotman, 1996).

1.4.3 Occupational exposure

Occupational exposure may occur during manufacture; however, the Working Group found no information on occupational exposure to isobutyl nitrite.

1.5 Regulations and guidelines

An occupational exposure limit for isobutyl nitrite has been derived by the American Conference of Governmental Industrial Hygienists as a ceiling value of 1 ppm (ACGIH, 2017). The same limit is in place in Belgium and Canada (Ontario) (IFA, 2018).

The use of isobutyl nitrite (in poppers) has been prohibited in the European Union since 2007 (European Union, 2006). In the UK, the Advisory Council on the Misuse of Drugs declared that alkyl nitrites (poppers) do not fall within the scope of the current definition of a "psychoactive substance" in the Psychoactive Substances Act 2016, and are therefore legal (ACMD, 2016). Poppers are illegal in the USA, but they have low priority for drug enforcement agencies (GINAD, 2018).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Inhalation

Groups of 60 male and 60 female B6C3F$_1$ mice (age, 6 weeks) were exposed to isobutyl nitrite (purity, ≥ 97%; major impurity, isobutyl alcohol) by whole-body inhalation at 0 (controls), 37.5, 75, or 150 ppm, 6 hours per day (plus time to achieve 90% of the target concentration after the beginning of vapour generation, T_{90}, 10 minutes), 5 days per week for 103 weeks (NTP, 1996). A total of 7–10 males and 9–10 females from each group were evaluated at 15 months for alterations in haematology, histology, and clinical chemistry parameters. For the remaining rats, after 104 weeks (2 years), the survival of the exposed male mice was similar to that of controls, and body weights of exposed males were similar to those of controls. The survival rate of females at

Table 3.1 Studies of carcinogenicity with isobutyl nitrite in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start[a] No. of surviving animals	Incidence (%) of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 104 wk NTP (1996)	Inhalation (whole-body exposure) Isobutyl nitrite, ≥ 97% None 0 (control), 37.5, 75, 150 ppm, 6 h/d (+T_{90} = 10 min), 5 d/wk, 103 wk 50, 50, 50, 53 37, 35, 35, 30	*Lung* Bronchioloalveolar adenoma 7/50* (14%), 12/50 (24%), 13/49 (27%), 17/53** (32%) Bronchioloalveolar adenoma (multiple) 0/50, 3/50 (6%), 3/49 (6%), 5/53* (9%) Bronchioloalveolar carcinoma 1/50 (2%), 6/50 (12%), 5/49 (10%), 4/53 (8%) Bronchioloalveolar adenoma or carcinoma (combined) 8/50* (16%), 16/50 (32%), 16/49** (33%), 19/53*** (36%) Alveolar epithelial hyperplasia 0/50, 4/50, 7/49*, 13/53* *Thyroid* Follicular cell adenoma 1/50* (2%), 0/50, 0/50, 5/53 (9%) Follicular cell carcinoma 0/50, 1/50 (2%), 0/50, 0/53 Follicular cell adenoma or carcinoma (combined) 1/50* (2%), 1/50 (2%), 0/50, 5/53 (9%) Follicular cell hyperplasia 8/50, 17/50*, 12/50, 20/53**	 *P = 0.005 (trend), **P = 0.011; logistic regression test *$P \leq 0.05$, logistic regression test NS *P = 0.006 (trend), **P = 0.039, ***P = 0.008; logistic regression test *$P \leq 0.01$, logistic regression test *P = 0.004 (trend), logistic regression test NS *P = 0.011 (trend), logistic regression test *$P \leq 0.05$, **$P \leq 0.01$; logistic regression test	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range) for 2-yr inhalation studies with control groups for: bronchioloalveolar adenoma or carcinoma (combined), 170/773 (22.0 ± 8.7%; 10–42%); bronchioloalveolar carcinoma, 55/773 (7.1 ± 5.9%; 0–16%); thyroid follicular cell adenoma, 13/763 (1.7 ± 1.5%; 0–4%); thyroid follicular cell adenoma or carcinoma (combined), 13/763 (1.7 ± 1.5%; 0–4%); thyroid follicular cell carcinoma, 0/763

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[a] No. of surviving animals	Incidence (%) of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 104 wk NTP (1996)	Inhalation (whole-body exposure) Isobutyl nitrite, ≥ 97% None 0 (control), 37.5, 75, 150 ppm, 6 h/d (+T_{90} = 10 min), 5 d/wk, 103 wk 51, 51, 50, 50 32, 42, 36, 37	*Lung* Bronchioloalveolar adenoma 4/51* (8%), 14/51** (27%), 7/50 (14%), 17/50*** (34%)	*P = 0.005 (trend), **P = 0.028, ***P = 0.002; logistic regression test	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range) for 2-yr inhalation studies with control groups for: bronchioloalveolar adenoma, 53/761 (7.0 ± 3.3%; 0–14%); bronchioloalveolar adenoma or carcinoma (combined), 75/761 (9.9 ± 3.7%; 0–16%); bronchioloalveolar carcinoma, 23/761 (3.0%; 0–6%)
		Bronchioloalveolar carcinoma 2/51 (4%), 2/51 (4%), 2/50 (4%), 2/50 (4%)	NS	
		Bronchioloalveolar adenoma or carcinoma (combined) 6/51* (12%), 15/51 (29%), 9/50 (18%), 19/50** (38%)	*P = 0.005 (trend), **P = 0.003; logistic regression test	
		Bronchioloalveolar adenoma (multiple) 0/51, 2/51, 1/50, 2/50	NS	
		Alveolar epithelial hyperplasia 0/51, 2/51, 9/50*, 8/50*	*$P \leq 0.01$, logistic regression test	

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[a] No. of surviving animals	Incidence (%) of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (M) 6 wk 104 wk NTP (1996)	Inhalation (whole-body exposure) Isobutyl nitrite, ≥ 97% None 0 (control), 37.5, 75, 150 ppm, 6 h/d (+T_{90} = 10 min), 5 d/wk, 103 wk 46, 46, 46, 46 17, 23, 36, 28	*Lung* Bronchioloalveolar adenoma 0/46*, 3/46 (7%), 12/46** (26%), 13/46*** (28%)	*P < 0.001 (trend), **P = 0.003, ***P = 0.002; logistic regression test	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range) for 2-yr inhalation studies with control groups for bronchioloalveolar adenoma or carcinoma (combined), 22/493 (4.5 ± 3.8%; 0–10%)
		Bronchioloalveolar carcinoma 1/46* (2%), 2/46 (4%), 1/46 (2%), 6/46** (13%)	*P = 0.015 (trend), **P = 0.040; logistic regression test	
		Bronchioloalveolar adenoma or carcinoma (combined) 1/46* (2%), 5/46 (11%), 13/46** (28%), 15/46*** (33%)	*P < 0.001 (trend), **P = 0.001, ***P < 0.001; logistic regression test	
		Bronchioloalveolar adenoma (multiple) 0/46, 1/46 (2%), 0/46, 3/46 (7%)	NS	
		Alveolar epithelial hyperplasia 5/46, 8/46, 26/46*, 31/46*	*P ≤ 0.01, logistic regression test	

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[a] No. of surviving animals	Incidence (%) of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (F) 6 wk 104 wk NTP (1996)	Inhalation (whole-body exposure) Isobutyl nitrite, ≥ 97% None 0 (control), 37.5, 75, 150 ppm, 6 h/d (+T_{90} = 10 min), 5 d/wk, 103 wk 46, 45, 46, 46 29, 35, 31, 33	*Lung* Bronchioloalveolar adenoma 0/46*, 2/45 (4%), 2/46 (4%), 10/46** (22%)	*P < 0.001 (trend), **P = 0.001; logistic regression test	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range) for 2-yr inhalation studies with control groups for bronchioloalveolar adenoma or carcinoma (combined), 4/492 (0.8 ± 1.4%; 0–4%)
		Bronchioloalveolar carcinoma 0/46, 1/45, 0/46, 1/46	NS	
		Bronchioloalveolar adenoma or carcinoma (combined) 0/46*, 3/45 (7%), 2/46 (4%), 11/46** (24%)	*P < 0.001 (trend), **P < 0.001; logistic regression test	
		Bronchioloalveolar adenoma (multiple) 0/46, 0/45, 0/46, 2/46 (4%)	NS	
		Alveolar epithelial hyperplasia 3/46, 10/45*, 11/46*, 30/46**	*P ≤ 0.05, **P ≤ 0.01; logistic regression test	

d, day; F, female; GLP, good laboratory practice; h, hour; M, male; min, minute; NS, not significant; ppm, parts per million; SD, standard deviation; T_{90}, time to achieve 90% of the target concentration after the beginning of vapour generation; wk, week; yr, year

[a] ~10 animals per group were used for haematological testing

37.5 ppm was significantly greater than that of the control group, and the group exposed at the highest dose (150 ppm) had a lower body weight than controls. Necropsies were performed on all animals and all major organs were investigated by light microscopy.

A significantly increased incidence of bronchioloalveolar adenoma and of bronchioloalveolar adenoma or carcinoma (combined) of the lung was found in male and female exposed mice. In male mice, the incidence of bronchioloalveolar adenoma was increased in the group exposed at the highest dose with a significant positive trend ($P = 0.005$): the incidence was 7/50, 12/50, 13/49, and 17/53 ($P = 0.011$) for exposure at 0, 37.5, 75, and 150 ppm, respectively. There was also a significant increase in the incidence of bronchioloalveolar adenoma (multiple) in the group exposed at the highest dose (5/53 vs 0/50 for controls, $P \leq 0.05$). The incidence of bronchioloalveolar adenoma or carcinoma (combined) was also significantly increased (with a significant positive trend; $P = 0.006$) in the males exposed at the intermediate and highest doses: 8/50 (16%), 16/50 (32%), 16/49 (33%, $P = 0.039$), and 19/53 (36%, $P = 0.008$). In female mice, the incidence of bronchioloalveolar adenoma at 4/51, 14/51 ($P = 0.028$), 7/50, and 17/50 ($P = 0.002$) for exposures at 0, 37.5, 75, and 150 ppm, respectively, was significantly increased with a significant positive trend ($P = 0.005$). The incidence of bronchioloalveolar adenoma (multiple) was 0/51, 2/51, 1/50, and 2/50, respectively. The incidence of bronchioloalveolar adenoma or carcinoma (combined) was also significantly increased in the females exposed at the highest dose, with a significant positive trend ($P = 0.005$), with an incidence of 6/51 (12%), 15/51 (29%), 9/50 (18%), and 19/50 (38%, $P = 0.003$), respectively. In 2-year inhalation studies by the National Toxicology Program (NTP), the incidence of bronchioloalveolar adenoma or carcinoma (combined) in historical controls was 170/773 (22.0%; range, 10–42%) in male B6C3F$_1$ mice and 75/761 (9.9%; range, 0–16%) in female B6C3F$_1$ mice. A significant increase in the incidence of alveolar epithelial hyperplasia was also found in male and female mice at the intermediate and highest doses.

[The Working Group noted that the incidences of lung bronchioloalveolar adenoma or carcinoma (combined) in males exposed at 75 ppm and in females exposed at 150 ppm were significantly increased compared with controls, and the incidences in all exposed groups of females exceeded the upper bound of the range for historical controls from 2-year NTP inhalation studies. In addition, alveolar epithelial hyperplasia occurred in all exposed groups of males and females (this lesion was absent in controls), and the incidence in males and females exposed at 75 and 150 ppm was significantly greater than that in the controls. The Working Group acknowledged that the increase in the incidence of bronchioloalveolar adenoma or carcinoma (combined) of the lung in female mice was mainly driven by the increase in the incidence of bronchioloalveolar adenoma: the incidence of the carcinoma was 2/51, 2/51, 2/50, and 2/50 (4%), respectively; the incidence of bronchioloalveolar carcinoma in historical controls in NTP inhalation studies was 23/761 (3.0%, range, 0–6%) in female B6C3F$_1$ mice. However, the Working Group considered the increased incidences of bronchioloalveolar adenoma and bronchioloalveolar adenoma or carcinoma (combined) in male and female mice to be related to treatment because of: (i) the strength of the statistical evidence; (ii) the increased multiplicity of bronchioloalveolar adenomas in exposed male and female mice; (iii) the comparison with the historical controls from NTP 2-year inhalation studies; and (iv) the increased incidence of alveolar epithelial hyperplasia in both sexes, supporting a continuum (the so-called adenoma–carcinoma sequence).]

In male mice, a significant positive trend ($P = 0.004$) in the incidence of follicular cell adenoma of the thyroid was found, with an

incidence of 1/50 (2%), 0/50, 0/50, and 5/53 (9%) for exposure at 0, 37.5, 75, and 150 ppm, respectively. One male mouse exposed at the lowest dose developed a follicular cell carcinoma of the thyroid. There was a significant positive trend (P = 0.011) in the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid, with incidence of 1/50 (2%), 1/50 (2%), 0/50, and 5/53 (9%), respectively. In male mice, the incidence of follicular cell hyperplasia of the thyroid was 8/50, 17/50 ($P \leq 0.05$), 12/50, and 20/53 ($P \leq 0.01$). There was no hepatomegaly in treated male mice. In historical controls in NTP 2-year inhalation studies, the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid in male mice was 13/763 (1.7%; range, 0–4%); no follicular cell carcinoma of the thyroid was observed in 763 male historical controls. [Follicular cell adenoma of the thyroid occurred with a significant positive trend in male mice, and the incidence in males exposed at 150 ppm was marginally (non-significantly) greater than that in the controls (1/50 at 0 ppm vs 5/53 at 150 ppm). Follicular cell neoplasms of the thyroid are relatively uncommon in male mice, as demonstrated by the rate in NTP historical controls. In the present study, the increase in the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid was accompanied by an increase in the incidence of follicular cell hyperplasia of the thyroid. Considering the rarity of these neoplasms in male mice and the increased incidence of follicular cell hyperplasia of the thyroid in exposed males, the increased incidence of follicular cell adenoma or carcinoma (combined) of the thyroid may have been related to exposure to isobutyl nitrite. [The Working Group noted that this was a well-conducted study that complied with good laboratory practice.]

3.2 Rat

Inhalation

Groups of 56 male and 56 female Fischer 344 rats (age, 6 weeks) were exposed to isobutyl nitrite (purity, ≥ 97%; major impurity, isobutyl alcohol) by whole-body inhalation at 0 (controls), 37.5, 75, or 150 ppm, 6 hours per day (plus T_{90}, 10 minutes), 5 days per week for 103 weeks (NTP, 1996). A total of 10 males and 10 females from each group were evaluated at 15 months for alterations in haematology, histology, and clinical chemistry parameters. For the remaining rats, the survival rates of males exposed at 75 and 150 ppm were significantly greater than those of controls. The body weights of male and female rats exposed at 150 ppm were lower than those of the controls. Necropsies were performed on all animals and all major organs were investigated by light microscopy.

A significantly increased incidence of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, and of bronchioloalveolar adenoma or carcinoma (combined) of the lung was found in exposed male rats. In male rats, the incidence of bronchioloalveolar adenoma was increased for the groups exposed at the intermediate and highest doses, with a significant positive trend ($P < 0.001$); the incidence was 0/46, 3/46 (6%), 12/46 (26%, P = 0.003), and 13/46 (28%, P = 0.002) for exposure at 0, 37.5, 75, and 150 ppm, respectively. The incidence of bronchioloalveolar carcinoma – 1/46 (2%), 2/46 (4%), 1/46 (2%), and 6/46 (13%, P = 0.040) – was increased in the group exposed at the highest dose, with a significant positive trend (P = 0.015). The respective incidence of bronchioloalveolar adenoma or carcinoma (combined) was 1/46 (2%, P for trend, < 0.001), 5/46 (11%), 13/46 (28%, P = 0.001), and 15/46 (33%, $P < 0.001$).

In female rats, the incidence of bronchioloalveolar adenoma – 0/46, 2/45, 2/46, and 10/46 (P = 0.001) – was significantly increased in the

group exposed at the highest dose, with a significant positive trend ($P < 0.001$). The incidence of bronchioloalveolar carcinoma was 0/46, 1/45, 0/46, and 1/46, respectively. The incidence of bronchioloalveolar adenoma or carcinoma (combined) was also significantly increased with a significant positive trend ($P < 0.001$); incidence was 0/46, 3/45 (7%), 2/46 (4%), and 11/46 (24%, $P < 0.001$) for exposure at 0, 37.5, 75, and 150 ppm, respectively. For historical controls in NTP 2-year inhalation studies, the incidence of bronchioloalveolar adenoma or carcinoma (combined) was 22/493 (4.5%; range, 0–10%) in males and 4/492 (0.8%; range, 0–4%) in females.

For preneoplastic lesions, there was a significant increase in the incidence of alveolar epithelial hyperplasia in male rats exposed at 75 and 150 ppm, and in female rats exposed at all concentrations.

[The Working Group concluded that the increased incidence of bronchioloalveolar adenoma and of adenoma or carcinoma (combined) in exposed male and female rats, and of bronchioloalveolar carcinoma in male rats, was related to treatment. The incidence of bronchioloalveolar adenoma or carcinoma (combined) in female rats exposed at 37.5 ppm (7%), male rats exposed at 75 ppm (28%), and male (33%) and female rats (24%) exposed at 150 ppm were clearly not within the NTP historical range for control animals. An increased incidence of alveolar epithelial hyperplasia was also observed in all exposed groups of male and female rats (except in females exposed at 75 ppm, and only significant in males exposed at 150 ppm) at the 15-month interim evaluation, and in all exposed groups of male and female rats (all significant with the exception of males exposed at 37.5 ppm) in the 2-year study. The occurrence of alveolar epithelial hyperplasia and the increased incidences of lung epithelial neoplasms in an apparent continuum (the so-called adenoma–carcinoma sequence), along with a non-significant increase in the number of rats with multiple adenomas supporting this continuum, were considered by the Working Group as evidence of carcinogenic activity in male and female rats.]

[The Working Group noted that this was a well-conducted study that complied with good laboratory practice.]

4. Mechanistic and Other Relevant Data

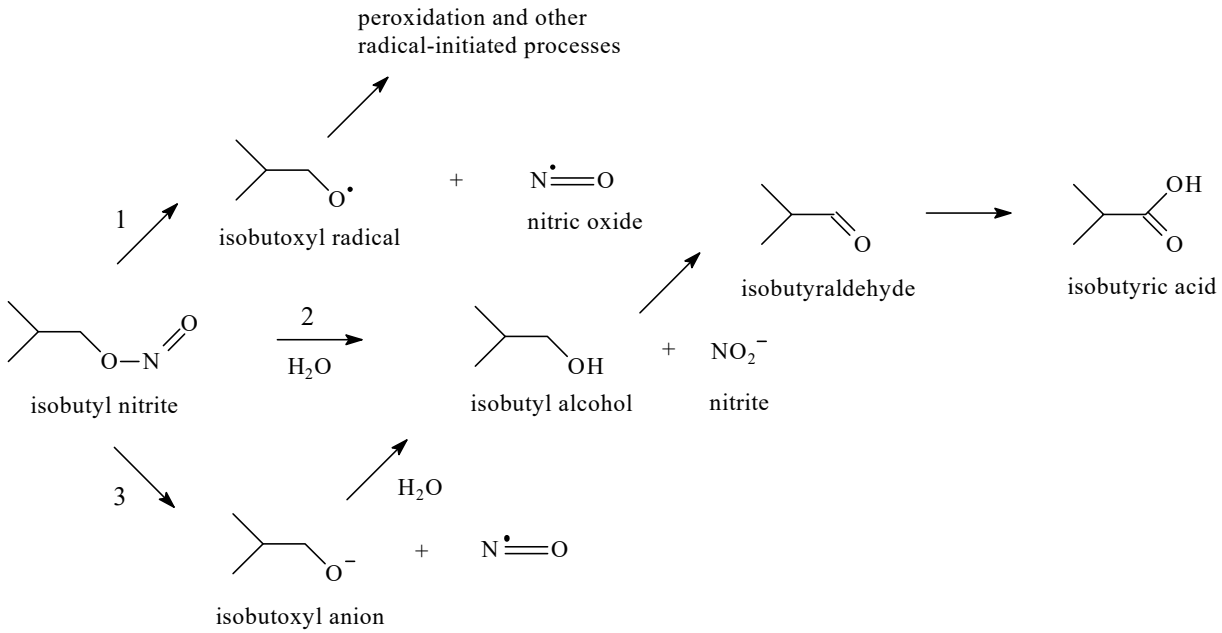
4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Data on the absorption, distribution, and excretion of isobutyl nitrite in humans were not available to the Working Group; however, oral or inhalation exposure to the compound induces methaemoglobinaemia (see Section 4.3.1) and has vasodilating effects, indicating that absorption occurs in humans. The degradation of isobutyl nitrite in human blood at 37 °C in vitro has been reported to follow first-order kinetics, with a half-life (1.2 ± 0.2 minutes) comparable to that obtained in rat blood, but the products were not characterized in this study (Kielbasa et al., 1999).

Isobutyl nitrite is generally regarded to undergo hydrolytic decomposition in vivo (Fig. 4.1), yielding nitrite and isobutyl alcohol (NTP, 1996). Watanabe-Suzuki et al. (2003) confirmed the presence of isobutyl alcohol in the blood of three men who inhaled isobutyl nitrite for 2 minutes. Isobutyl alcohol concentrations within the range 0.35–0.75 µg/mL were observed at time zero, and declined to 0.06–0.10 µg/mL after 10 minutes. Isobutyl nitrite was not detected in any of the blood samples. The formation of isobutyl alcohol from isobutyl nitrite was rapid in vitro (< 10 minutes) in human urine and

Fig. 4.1 Proposed metabolic pathways of isobutyl nitrite, accounting for the species detected in human and/or animal models



1. Spontaneous homolytic cleavage of the N–O bond; 2. Hydrolysis; 3. Enzymatic reduction
Compiled by the Working Group

whole-blood matrices spiked with isobutyl nitrite (10 nmol/mL).

A study in humans demonstrated that isobutyl alcohol is metabolized to isobutyraldehyde and isobutyric acid in vivo (Rüdell et al., 1983). This is consistent with the demonstration that alcohol and aldehyde dehydrogenase enzymes from human liver mediate the conversion of isobutyl alcohol to isobutyraldehyde and isobutyric acid in vitro (Ehrig et al., 1988).

4.1.2 Experimental systems

In male Sprague-Dawley rats exposed to isobutyl nitrite at 900 ppm by inhalation for 45 minutes, there was a rapid systemic absorption and elimination of the compound; steady-state concentrations (~290 ng/mL) were reached within 15 minutes, and declined monoexponentially with a half-life of 1.4 ± 0.2 minutes upon cessation of the exposure (Kielbasa et al., 1999). Shorter half-lives, consistent with enzymatic degradation, were observed in biological fluids (rat whole blood and rat plasma) compared with phosphate buffer (Kielbasa et al., 1999).

The pharmacokinetics of isobutyl nitrite and its primary metabolite, isobutyl alcohol, were investigated more completely in male Sprague-Dawley rats after inhalation and intravenous infusion (Kielbasa & Fung, 2000a). The pharmacokinetic parameters of isobutyl nitrite appeared invariable over time; regardless of the rate of infusion, the half-life and volume of distribution were determined to be 1.3 ± 0.2 minutes and 5.8 ± 0.4 L/kg, respectively. After the intravenous infusion, the systemic clearance of isobutyl nitrite in rats was 3.0 ± 0.3 L/kg per minute. Isobutyl nitrite was almost completely metabolized to isobutyl alcohol (98% conversion), the concentration of which declined monoexponentially with a half-life of 5.3 minutes upon termination

of the infusion. A similar half-life was found for isobutyl alcohol when given by intravenous bolus. Urinary excretion of isobutyl alcohol was very low (0.49 ± 0.01% of the administered dose after an intravenous bolus at 50 mg/kg), and no evidence of glucuronide or sulfate conjugates was found. [The Working Group noted that oxidation to isobutyraldehyde and isobutyric acid may have occurred faster than phase II conjugation.] The bioavailability of isobutyl nitrite upon inhalation was estimated to be 43%, suggesting that a first-pass effect may occur in the lung. The pharmacokinetics of isobutyl nitrite appeared to be independent of the route of administration; in contrast, compared with intravenous exposure, the half-life of isobutyl alcohol decreased by approximately four times after inhalation of isobutyl nitrite (from 5.3 min to 1.5 min, $P < 0.001$). The change in the disposition of isobutyl alcohol might be related to release of nitric oxide from isobutyl nitrite, with ensuing alteration of the blood flow to the lung due to relaxation of smooth muscle (Kielbasa & Fung, 2000a).

In male Sprague-Dawley rats, apparent steady-state blood levels were achieved during exposure and were proportional to exposure concentration, from 0.05 ± 0.03 μM at 23 ppm to 3.53 ± 0.35 μM at 1177 ppm (Kielbasa & Fung, 2000b).

Isobutyl nitrite was extensively metabolized to isobutyl alcohol when male Sprague-Dawley rats were exposed by inhalation or intravenous infusion (Kielbasa & Fung, 2000a). When given by intravenous infusion to New Zealand White rabbits of both sexes, isobutyl nitrite generated dose-dependent increments of nitric oxide in exhaled air that were correlated with dose-dependent decreases in systemic blood pressure (Cederqvist et al., 1994).

It is generally assumed that hydrolytic cleavage of isobutyl nitrite generates nitrite and isobutyl alcohol, whereas homolytic cleavage yields the nitric oxide and isobutoxyl radicals. Although the reactive nitric oxide is associated with the vasodilating effect, the isobutoxyl radical may initiate peroxidation reactions (NTP, 1996). Consistent with this mechanism, it has been demonstrated that isobutyl nitrite, diluted in air at concentrations of up to 900 ppm, undergoes spontaneous decomposition under normal room light, generating nitric oxide at approximately 115 ppm (Soderberg et al., 2000).

As an alternative to homolytic cleavage, nitric oxide production from isobutyl nitrite may stem from metabolic reduction. [The Working Group noted that the reductive process will presumably also produce the isobutoxyl anion, which will be readily protonated to isobutyl alcohol.] Bovine vascular subfractions had significant catalytic activity for generation of nitric oxide, which was inhibited by heating and irradiation, consistent with enzymatic conversion to nitric oxide in vascular smooth muscle. Moreover, the major generation of nitric oxide was associated with the cytosol, and a minor and distinct activity generating nitric oxide was identified in the microsomal fraction (Kowaluk & Fung, 1991). In a later study, xanthine oxidase from bovine milk catalysed the reduction of isobutyl nitrite to nitric oxide in vitro in the presence of xanthine under anaerobic conditions; in a process following Michaelis–Menten kinetics, the production of nitric oxide compared with that of urates had a molar ratio of 2:1 (Doel et al., 2000).

4.1.3 Modulation of metabolic enzymes

After a single exposure of adult male BALB/c mice to isobutyl nitrite at 900 ppm by inhalation for 45 minutes, a significant reduction in the hepatic activities of cytochrome P450-mediated 3-cyano-7-ethoxycoumarin deethylation (81.5%), glutathione *S*-transferase (GST; 74.7%), and carboxylesterase (25.2%) (Turowski et al., 2007). Under the same conditions, C57BL/6 mice had corresponding, although smaller, decreases in these hepatic enzyme activities. When assessed

in C57BL/6 mice, the enzyme activities returned to control levels 24 hours after exposure. Similar decreases in hepatic enzyme activities also occurred after repeated exposure of C57BL/6 mice to isobutyl nitrite at 900 ppm for 45 minutes per day for 6 days. A follow-up mechanistic investigation in vitro, using purified rat liver GST, demonstrated that a 10-second exposure to isobutyl nitrite at 22 mM (but not to sodium nitrite at 22 mM) caused an immediate decrease in GST activity that further intensified, but not linearly, over a longer exposure (60 minutes). The addition of glutathione at 5 mM before exposure to isobutyl nitrite prevented GST inactivation, regardless of exposure time (10 seconds or 60 minutes). In contrast, GST inactivation could not be reversed by glutathione addition after exposure to isobutyl nitrite, which indicated irreversible protein oxidation. Comparative experiments investigating the exposure of GST to different nitric oxide donors indicated that GST inactivation by isobutyl nitrite was not associated with *S*-nitrosylation of the protein or disulfide formation, but rather with tyrosine nitration (Turowski et al., 2007).

In an earlier study, a rat homologue of human γ-glutamyltranspeptidase-related enzyme, which cleaves the γ-glutamyl peptide bond of glutathione, was found to be highly expressed in lung tumours during the inhalation of isobutyl nitrite at 75 or 150 ppm for 6 hours per day, 5 days per week for 2 years (NTP, 1996). Elevated expression of the human γ-glutamyltranspeptidase-related enzyme was also found in normal lung tissue from an animal exposed to isobutyl nitrite compared with a normal unexposed lung (Potdar et al., 1997).

4.2 Mechanisms of carcinogenesis

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016) in the following order: is genotoxic; induces chronic inflammation; is immunosuppressive; alters cell proliferation, cell death, or nutrient supply; and multiple characteristics (e.g. microarrays). Insufficient data were available for evaluation of the other key characteristics of carcinogens.

4.2.1 Genetic and related effects

Isobutyl nitrite has been evaluated for genotoxicity and related potential in a variety of assays. Table 4.1, Table 4.2, and Table 4.3 summarize the studies considered the most representative of the genetic and related effects of isobutyl nitrite.

(a) Humans

No data on exposed humans were available to the Working Group.

In vitro, the ability of isobutyl nitrite to induce nuclear DNA damage was assessed in primary cultures of human lung cells using the comet assay (Robbiano et al., 2006). The cells were obtained from apparently healthy areas of lung fragments discarded during surgery for pulmonary carcinoma or adenocarcinoma. Isobutyl nitrite at 3.90–31.25 μM (purity, 95%) did not induce DNA damage (tail length and tail moment) in lung cells from two male donors (one former smoker and one smoker), whereas a dose-dependent increase was observed in one male donor (former smoker).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1

Male Sprague-Dawley rats given a single dose of isobutyl nitrite, corresponding to half the median lethal dose (LD_{50}), by gastric intubation had statistically significant increased DNA damage in the lung but not in the liver or kidney, as measured by the comet assay (Robbiano et al., 2006).

Isobutyl nitrite was tested using test for micronucleus formation in samples of peripheral blood from male and female $B6C3F_1$ mice exposed by

Table 4.1 Genetic and related effects of isobutyl nitrite 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, B6C3F$_1$ (M, F)	Peripheral blood; normochromatic erythrocytes	+	150 ppm (M), 75 ppm (F)	Inhalation, 6 h/d, 5 d/wk for 13 wk	Purity, ≥ 93%	NTP (1996)
DNA strand breaks	Rat, Sprague-Dawley (M)	Lung	+	606 mg/kg	Gastric intubation, single dose in olive oil at 0.01 mL/g bw	Purity, 95%	Robbiano et al. (2006)
DNA strand breaks	Rat, Sprague-Dawley (M)	Liver and kidney	–	606 mg/kg	Gastric intubation, single dose in olive oil at 0.01 mL/g bw	Purity, 95%	Robbiano et al. (2006)

bw, body weight; d, day; F, female; h, hour; HID, highest ineffective dose; LED, lowest effective dose; M, male; ppm, parts per million; wk, week
[a] +, positive; –, negative; the level of significance was set at $P < 0.05$ in all cases

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

End-point	Species, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks	Rat, Sprague-Dawley, lung	+	NT	7.8 µM	Purity, 95%	Robbiano et al. (2006)
Mutation	Mouse, lymphoma L5178Y	+	+	75.9 µM	Purity, NR	Dunkel et al. (1989)
Sister-chromatid exchange	Chinese hamster ovary	+	+	50 µg/mL (– S9); 160 µg/mL (+ S9)	Purity, ≥ 93%	NTP (1996)
Chromosomal aberrations	Chinese hamster ovary	+	+/–	16 µg/mL (– S9); 1081 µg/mL (+ S9)	Purity, ≥ 93%	NTP (1996)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant from rat liver
[a] +, positive; +/–, equivocal (variable response in several experiments within an adequate study); the level of significance was set at $P < 0.05$ in all cases

Table 4.3 Genetic and related effects of isobutyl nitrite 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			
Drosophila melanogaster Canton-S wildtype (M)	Sex-linked recessive lethal mutations	–	NA	25 000 ppm by injection; 100 000 ppm by feeding	Purity, ≥ 93%	Woodruff et al. (1985)
Salmonella typhimurium TA1535	Reverse mutation	+	+	1000 µg/plate	Purity, NR	Quinto (1980)
Salmonella typhimurium TA100, TA1535	Reverse mutation	+/–	+	6666 µg/plate	Purity, 92.7%	Mortelmans et al. (1986)
Salmonella typhimurium TA1537	Reverse mutation	–	–	1000 µg/plate	Purity, NR	Quinto (1980)
Salmonella typhimurium TA98, TA1537	Reverse mutation	–	–	10 000 µg/plate	Purity, 92.7%	Mortelmans et al. (1986)
Salmonella typhimurium TA100, TA1535	Reverse mutation	+	+	3333 µg/plate	Purity, NR	Dunkel et al. (1989)
Salmonella typhimurium TA98, TA1537	Reverse mutation	–	–	10 000 µg/plate	Purity, NR	Dunkel et al. (1989)
Salmonella typhimurium TA1535	Reverse mutation	+	+	Saturated vapour, 190 µg/mL	Purity, NR	Mirvish et al. (1993)
Salmonella typhimurium TA100	Reverse mutation	NT	+/–	Saturated vapour, 190 µg/mL	Purity, NR	Mirvish et al. (1993)
Salmonella typhimurium TA98	Reverse mutation	–	–	Saturated vapour, 190 µg/mL	Purity, NR	Mirvish et al. (1993)
Salmonella typhimurium TA1535	Reverse mutation	+	+	260 µg saturated solution (2.6 mg/mL)	Purity, NR	Mirvish et al. (1993)
Salmonella typhimurium TA98	Reverse mutation	–	–	10 000 µg/plate	Purity, ≥ 93%	NTP (1996)
Salmonella typhimurium TA100	Reverse mutation	+/–	+	6666 µg/plate	Purity, ≥ 93%	NTP (1996)
Salmonella typhimurium TA1535	Reverse mutation	NT	+	1000 µg/plate	Purity, ≥ 93%	NTP (1996)
Salmonella typhimurium TA98, TA1537, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, Mix TA7001–7006	Reverse mutation	–	–	1000 µg/mL	Purity, NR	Gee et al. (1998)
Salmonella typhimurium FU100	Forward mutation	+	+	28 µg/mL (without metabolic activation); 500 µg/mL (with metabolic activation)	Purity, NR	Miller et al. (2005)

HIC, highest ineffective concentration; LEC, lowest effective concentration; M, male; NA, not applicable; NR, not reported; NT, not tested; ppm, parts per million

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); the level of significance was set at $P < 0.05$ in all cases

inhalation. Males and females displayed a significantly increased frequency of micronucleated normochromatic erythrocytes, with females being more sensitive (NTP, 1996).

(ii) Non-human mammalian cells in vitro

See Table 4.2

In primary lung cells from male Sprague-Dawley rats exposed to isobutyl nitrite at concentrations of 7.8–31.2 μM for 20 hours, DNA fragmentation, as measured by tail length and tail moment using the comet assay, was significantly increased by a dose-dependent amount (Robbiano et al., 2006).

Isobutyl nitrite was tested in the L5178Y *Tk*$^{+/-}$ mouse lymphoma assay at concentrations of up to 1.5 mM in the absence and presence of exogenous metabolic activation. Dose-dependent increases in mutant frequency were observed, both with and without S9. The presence of S9 reduced the toxicity of the compound by approximately one order of magnitude; however, the mutation frequency remained the same at comparable toxicity levels, with and without metabolic activation (Dunkel et al., 1989).

Isobutyl nitrite was also tested for sister-chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells using standard protocols. The results were positive for sister-chromatid exchanges in the absence and presence of rat liver S9, and positive for chromosomal aberrations in the absence of exogenous metabolic activation. In contrast, mixed results were obtained for chromosomal aberrations in the presence of S9 (two trials; the results were negative in one and weakly positive in another). The lowest effective concentrations for a positive response with both end-points were higher in the presence of S9 (NTP, 1996).

(iii) Non-mammalian experimental systems

See Table 4.3

When tested in vivo, isobutyl nitrite did not induce sex-linked recessive lethal mutations in the germ cells of Canton-S wildtype *Drosophila melanogaster* males exposed via feeding (100 000 ppm) or injection (25 000 ppm) (Woodruff et al., 1985).

Several studies have demonstrated mutagenic activity with isobutyl nitrite in the standard *Salmonella* assay when using tester strains sensitive to base-pair substitution mutations, such as TA100 and TA1535. Although the results in the absence of S9 activation were equivocal in some studies and positive in other reports, consistently positive results were obtained with the same strains in the presence of exogenous metabolic activation. Isobutyl nitrite was mutagenic in TA1535 in the absence and presence of S9 activation (Quinto, 1980). In contrast, isobutyl nitrite gave negative results in tester strains sensitive to frameshift mutations, such as TA97, TA98, and TA1537, both in the absence and presence of S9 activation (Quinto, 1980; Mortelmans et al., 1986; Dunkel et al., 1989; Mirvish et al., 1993; NTP, 1996). The saturated vapour was 11-fold more mutagenic in strain TA1535 than a saturated solution, a difference that was attributed to continuous replenishment of the hydrolysis-prone test compound by the vapour (Mirvish et al., 1993). The same study demonstrated that isobutyl nitrite in solution was more mutagenic by about 2.8-fold in TA1535 than sodium nitrite, suggesting that the compound is mutagenic per se and not as a result of hydrolytic conversion to nitrite. The other hydrolysis product, isobutyl alcohol, gave negative results in the same experiment.

A subsequent study used a modified, partially automated, liquid protocol and either individual *Salmonella his*$^{-}$ tester strains of the TA7000 series, each reverting by a specific base substitution mutation, or a mix of these strains that detected missense mutations. In contrast to the standard assays, isobutyl nitrite gave negative results in this protocol, both in the absence and presence of exogenous metabolic activation (Gee et al., 1998). More recently, isobutyl nitrite

gave positive results, both in the presence and absence of S9, in an assay for forward mutation in FU100, a *S. typhimurium* strain derived from TA100 and displaying resistance to 5-fluorouridine. The lowest effective concentration was about 18 times lower in the absence of S9 (Miller et al., 2005). [The Working Group noted that the TA7000 and the FU100 studies both used isobutyl nitrite in liquid suspension, and hydrolysis would be expected. The positive results with FU100 suggest that the assay for forward mutation may be more sensitive.]

4.2.2 Chronic inflammation

(a) Humans

No data for humans were available to the Working Group. Isobutyl nitrite is a known irritant (see Section 4.3).

(b) Experimental systems

Inhalation of isobutyl nitrite resulted in inflammatory changes in male and female Fischer 344/N rats and B6C3F$_1$ mice exposed at concentrations of up to 300 ppm for 13 weeks, 6 hours per day, 5 days per week (Gaworski et al., 1992; NTP, 1996).

Kielbasa & Fung (2000c) evaluated tissue levels and phosphorylation of nitric oxide synthase (NOS) enzymes in rat kidney, liver, lung, and spleen after a single exposure to isobutyl nitrite at either 109 or 1517 ppm by inhalation for 4 hours. Increased expression of inducible NOS, nitrotyrosine, and phosphotyrosine immunoreactive proteins were observed in the liver and kidney of rats exposed at 1517 ppm, but not in the lung or spleen. These data contrast with those of Soderberg et al. (1996a), who showed that alveolar macrophages from mice exposed to isobutyl nitrite at 900 ppm demonstrated elevated inducible NOS production after inhalation for 45 minutes per day for 14 days.

Soderberg & Ponnappan (2002) examined the formation of nitrotyrosine in the murine macrophage cell line RAW 267.4 and in peritoneal macrophages obtained from C57BL/6 mice exposed in vivo to isobutyl nitrite at 900 ppm for 45 minutes per day for 5 days. Inhibition of mitochondrial respiration was only observed in cultured RAW cells at isobutyl nitrite concentrations that induced significant cytotoxicity (> 25 mM). Reduced nitrotyrosine formation was observed in RAW cells exposed to isobutyl nitrite at 6 mM compared with unexposed controls. Similar results were obtained when inactivated peritoneal macrophages from mice exposed to isobutyl nitrite at 900 ppm as described above were used to investigate nitrotyrosine formation. When activated macrophages were used, the changes were less consistent; some proteins demonstrated reduced nitrotyrosine formation and some demonstrated increased nitrotyrosine formation compared with controls, and some proteins did not show any change in nitrotyrosine formation (Soderberg & Ponnappan, 2002). [The Working Group noted that this study in vitro indicated that peroxynitrite formation does not contribute to the observed effects.]

4.2.3 Immunosuppression

(a) Humans

No data on exposed humans were available to the Working Group.

Only a few studies described modulation of immune function after exposure in vitro to structurally related nitrites. After inhalation of amyl nitrite, a decrease in natural killer cell activity (~30%) was observed in the peripheral blood, although no significant changes in cell proliferation in response to stimulation with phytohaemaglutinin, concanavalin A, or pokeweed mitogen were noted (Dax et al., 1991). In contrast, an increased response to T-cell mitogens in peripheral blood lymphocytes was associated with self-reported use of inhaled nitrites (Ross & Drew, 1991).

In vitro, isobutyl nitrite significantly suppressed blastogenesis, natural killer cell function, antibody-dependent cell-mediated cytotoxicity, and interferon production in peripheral blood leukocytes (Hersh et al., 1983). Lymphocytes exposed to isobutyl nitrite at a concentration of 0.5% in cell culture for 72 hours demonstrated reduced cell proliferation in response to phytohaemaglutinin, concanavalin A, or pokeweed mitogen. Antibody-dependent cell-mediated cytotoxicity in lymphocytes and monocytes and adherence in monocytes were also inhibited when the cells were cultured in the presence of 0.5% isobutyl nitrite.

(b) Experimental systems

In female C57BL/6 mice, spleen cellularity was significant decreased (by 39%) after a single exposure by inhalation to isobutyl nitrite at 900 ppm for 45 minutes. Cell loss appeared to be nonspecific as the values of individual lymphocyte subpopulations were unchanged, and the numbers of leukocytes in the peripheral blood and resident peritoneal macrophages were also significantly reduced (Guo et al., 2000). In $B6C3F_1$ female mice exposed by inhalation to isobutyl nitrite at 37.5, 75, or 150 ppm for 6 hours per day, 5 days per week, for up to 15 weeks, there was a dose-related suppression of T-cell-dependent antibody responses in the spleen (Ratajczak et al., 1995). Although splenic atrophy was observed, there were no differences in the relative number of leukocyte subpopulations in the spleen. T-cell proliferation, natural killer cell activity, and infection with *Klebsiella pneumoniae* were not affected by exposure to isobutyl nitrite. A dose-related increase in interferon-induced hydrogen peroxide production in vitro by cultured alveolar macrophages isolated from female $B6C3F_1$ mice was present in the third week of exposure, but not at the termination of the study at 15 weeks (Ratajczak et al., 1995). Persistence of the immune alterations was shown in female $B6C3F_1$ mice that were allowed to recover for 2 weeks after exposure to isobutyl nitrite at 37.5, 75, or 150 ppm for 6 hours per day, 5 days per week for up to 15 weeks. The numbers of antibody-forming cells in the spleen remained decreased, although spleen cellularity returned to control levels (Ratajczak et al., 1995).

Several studies from one laboratory demonstrated immunosuppressive effects of isobutyl nitrite in C57BL/6 mice. A single 45-minute exposure to isobutyl nitrite at 900 ppm produced transient anaemia in female C57BL/6 mice (Soderberg et al., 1996a). Erythrocyte counts, haemoglobin, and haematocrit levels (erythrocyte volume fraction) were reduced by 7%, but recovered to above normal levels 24 hours later. Blood leukocyte counts were also reduced 24 hours after exposure. In mice exposed to isobutyl nitrite at 900 ppm in an inhalation chamber for 45 minutes per day, for 14 days, the number of peripheral blood leukocytes was reduced by 32% but the number of erythrocytes was increased by 7% (Soderberg et al., 1996a, b). The numbers of bone marrow and spleen burst-forming units-erythroid were increased approximately twofold, although the numbers of colony-forming units-granulocyte/macrophage were decreased by about 50%. A reduction in the production of myeloid colony-stimulating activity was observed in bone marrow stromal cells after exposure to isobutyl nitrite. A single exposure to isobutyl nitrite depleted blood cells including erythrocytes, but single and repeated exposure to isobutyl nitrite stimulated erythropoiesis and maintained suppression of myelopoiesis (Soderberg et al., 1996b).

Soderberg & Barnett (1991) found that female C57BL/6 mice exposed to isobutyl nitrite at 900 ppm for 45 minutes per day for 14 days demonstrated consistent suppression of antibody responses after immunization with T-dependent antigen sheep erythrocytes. T-cell proliferation was also significantly inhibited. Dose-related suppression of the antigen-specific antibody response for both immunoglobulin M and G

occurred in male and female B6C3F$_1$ mice at concentrations of 750 ppm and above (Soderberg & Barnett, 1993). Exposure to isobutyl nitrite at 600 ppm increased antibody responsiveness. This biphasic response was reproducible and was not due to non-specific cell proliferation. No differences in the levels of suppression between males and females were observed, consistent with the study reported by Ratajczak et al. (1995) where normal immune responses returned 5–7 days after the final exposure. Soderberg (1994) specifically assessed end-points associated with T-cell function in female C57BL/6 mice exposed to isobutyl nitrite at 900 ppm for 45 minutes per day for 14 days. Cytotoxic T-lymphocyte activity against P815 mastocytoma cells was reduced by 36%, and T-cell proliferation after mitogenic stimulation or co-culture with allogenic leukocytes was reduced by 37% and 51%, respectively. Production of interleukin-2 (IL-2) in vitro from isolated and cultured splenic lymphocytes from exposed C57BL/6 mice was similar to that of air controls, and activated T-lymphocytes isolated from these same mice responded normally in vitro when treated with exogenous IL-2 (Soderberg, 1994). In normal T-cells co-cultured with an undefined accessory cell population from irradiated spleen cells, T-cell proliferation was inhibited in the presence of accessory cells from mice exposed to isobutyl nitrite. Exposure of female C57BL/6 mice to isobutyl nitrite at 900 ppm by inhalation reduced the number of recoverable peritoneal exudate cells, impaired the ability of peritoneal macrophages from these mice to kill P815 tumour cells in vitro, and reduced the levels of nitric oxide produced in these cells after stimulation with lipopolysaccharides. The reduction in tumoricidal activity was still observed in macrophages isolated from these mice 7 days after the cessation of treatment, but recovered to normal levels 2 weeks after treatment was stopped. The production of tumour necrosis factor-α by peritoneal macrophages and natural killer cell activity were unaffected by isobutyl nitrite exposure in these studies. Production of the proinflammatory cytokine IL-1β was significantly reduced after exposure of female C57BL/6 mice to isobutyl nitrite at 900 ppm for 5 days or 14 days (Soderberg et al., 2004). Lotzová et al. (1984) showed that inhalation exposure to isobutyl nitrite suppressed natural killer cell activity by approximately 60% in female B6D2F$_1$ mice. [The Working Group noted that it was not possible to estimate the doses achieved in this study as mice were exposed to 100% compound in an open system.]

In contrast to the studies reporting immunosuppression described in this section, isobutyl nitrite did not alter sheep erythrocyte-stimulated antibody production or T-lymphocyte mitogenesis after stimulation by phytohaemaglutinin, concanavalin A, pokeweed mitogen, and lipopolysaccharide in male and female BALB/c mice exposed via inhalation at 20, 50, or 300 ppm for 6.4 hours per day, 5 days per week, for up to 18 weeks (Lewis et al., 1985).

In mammalian cells in vitro, Hersh et al. (1983) demonstrated that isobutyl nitrite, at a concentration of 0.05% and 0.01%, significantly reduced the production of α,β-interferon in C3H/HeJ-derived mouse embryo fibroblasts stimulated with poly(I)-poly(C). [The Working Group noted that this study in vitro indicated that nitric oxide formation does not contribute to the observed effects.]

4.2.4 Altered cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

After exposure to isobutyl nitrite at 300 ppm for 6.5 hours per day, 5 days per week, for up to 18 weeks, BALB/cAnNCrlBR mice showed decreased thymus weight (females), decreased liver weight (males), decreased leukocyte counts

(males), and mild focal hyperplasia and vacuolization of the epithelium lining of bronchi and bronchioles of the lungs (males and females) (Lynch et al., 1985). However, changes in organ weight and haematology were not accompanied by any observed histological changes.

Hyperplasia of the bronchiolar and nasal turbinate epithelium was seen in male and female Fischer 344/N rats and B6C3F$_1$ mice after exposure to isobutyl nitrite vapours at up to 400 ppm (rats) or 600 ppm (mice) for 6 hours per day, 5 days per week, in the NTP 13-week (rats) or 14-week (mice) studies (Gaworski et al., 1992). Lymphocytic atrophy was seen in the spleen and thymus of mice. Higher concentrations resulted in mortality in rats (600 ppm or higher) and mice (800 ppm). The 13-week exposures resulted in respiratory system changes, including increased lung weights in rats and female mice exposed at 300 ppm, hyperplasia of the nasal mucosa in male rats exposed at 275 ppm and in female rats exposed at 150 ppm, and hyperplasia of the lung epithelium in male mice exposed at ≥ 150 ppm and in female mice exposed at ≥ 75 ppm (Gaworski et al., 1992).

In the 2-year NTP bioassay, hyperplasia of the alveolar epithelium was evident in rats exposed at 37.5 ppm or more, and in mice exposed at 75 ppm or more (NTP, 1996).

Exposure to isobutyl nitrite by inhalation upregulated the expression of vascular endothelial growth factor (VEGF) protein and mRNA, as well as expression of VEGF receptor 2 (VEGFR-2), VEGFR-3, Smad-5, and Smad-7 in the liver of C57BL/6 mice (Tran et al., 2003).

Exposure to isobutyl nitrite in vitro induced expression of VEGF in macrophage cells (Tran et al., 2003).

4.2.5 Multiple key characteristics

Upregulation of VEGF, VEGFR-3, Smad-5, and Smad-7 was demonstrated in a study of low-density arrays used to examine the effect of exposure to isobutyl nitrite on the expression of 23 cancer- and angiogenesis-related genes in mouse tissues (Tran et al., 2005). Various statistical methods yielded concordant results for the most significant genes, namely VEGF, VEGFR-3, Smad-5, and Smad-7. Reverse-transcription polymerase chain reaction confirmed VEGF upregulation as observed via gene arrays.

4.3 Other adverse effects

4.3.1 Humans

There are case reports showing methaemoglobinaemia in humans after ingestion or inhalation of isobutyl nitrite (Covalla et al., 1981; Shesser et al., 1981; Schwartz & Peary, 1986; O'Toole et al., 1987; Bradberry et al., 1994; Pruijm & de Meijer, 2002; Jansen et al., 2003; Lindenmann et al., 2006). In cases of intoxication with isobutyl nitrite, hypotension (Shesser et al., 1981; Lindenmann et al., 2006) as well as visual loss and maculopathy (Pece et al., 2004; Davies et al., 2012; Pahlitzsch et al., 2013) were reported.

Some cases also showed irritant contact dermatitis (Schwartz & Peary, 1986), or tracheobronchitis and/or irritation of the tracheobronchial tree (Covalla et al., 1981; Schwartz & Peary, 1986).

4.3.2 Experimental systems

Concentrations of methaemoglobin were elevated in male and female mice exposed to isobutyl nitrite at 50 and 300 ppm (Lynch et al., 1985).

Hypotension was seen after exposure of rats by inhalation and of rabbits by intravenous infusion (Kielbasa & Fung, 2000a). In rabbits exposed to isobutyl nitrite, there was an association between generation of nitric oxide and hypotension in vivo (Cederqvist et al., 1994).

4.4 Data relevant to comparisons across agents and end-points

4.4.1 High-throughput screening programmes

High-throughput screening data generated by the Toxicity Forecaster (ToxCast) and Toxicity Testing in the 21st Century (Tox21) research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013) were considered in the assessment of the six chemicals reviewed in *IARC Monographs* Volume 122 (isobutyl nitrite, β-picoline, methyl acrylate, ethyl acrylate, 2-ethylhexyl acrylate, and trimethylolpropane triacrylate). The United States Environmental Protection Agency (EPA) has systematically analysed more than three million concentration–response chemical assay pairs from ToxCast and Tox21. The resulting concentration–response models and activity calls were released to the public via the Interactive Chemical Safety for Sustainability ToxCast Dashboard and by downloadable files, including a data analysis pipeline (tcpl R package) and a database (invitrodb_v3) (EPA, 2017a, 2018). The underlying concentration–response data, analysis decision logic and methods, concentration–response model outputs, activity calls, and activity caution flags were also provided (Filer et al., 2017). For the six chemicals considered in the present volume, four were tested in ToxCast and in Tox21 assays and the other two solely in Tox21 assays.

Chemicals with a very low relative molecular mass (< 150) generally have only low affinity for biomolecular interactions because of limited free energy for binding (Hopkins et al., 2004). Four of the six chemicals considered in the present volume – isobutyl nitrite, β-picoline, methyl acrylate, and ethyl acrylate – have a relative molecular mass of less than 150. Screening in vitro at the concentrations used in ToxCast and Tox21 (typically 100 μM or less) may therefore be inadequate to detect receptor-type molecular interactions that do not rely on chemical reactivity. The four compounds with a low relative molecular mass also have high vapour pressures, which could lead to a loss of sample during storage and/or testing, and therefore failure to reach expected active concentrations.

The Tox21 and ToxCast in vitro assays were selected to cover a broad range of potential biological activity and are not specifically focused on carcinogenesis. The Working Group of *IARC Monographs* Volume 112 therefore mapped the 821 assay end-points available at that time to the key characteristics of known human carcinogens, yielding consensus assignments of 263 assay end-points mapped to 7 of the 10 key characteristics or to the category "other" (IARC, 2017; Chiu et al., 2018); this was later updated to 291 in *IARC Monographs* Volume 119 (IARC, 2018). New assay end-points added to Tox21 and ToxCast projects since that determination were reviewed and 57 additional assay end-points were added to the mapped key characteristics, resulting in 348 in total (including the category "other"); however, these six chemicals were only tested in 304 of these assays. The assay end-points used, the activity call, and the mapping to key characteristics are available are available as supplemental material to the present volume (Annex 1). The key characteristics, as well as number of assays included in Volume 122 and a brief description, are provided below.

1. *Is electrophilic or can be metabolically activated*: 1 assay end-point, that is, cytochrome P450 biochemical activity assays including aromatase

2. *Is genotoxic*: 10 assay end-points consisting of cellular TP53 induction and DNA repair-sensitive cellular assays

3. *Alters DNA repair or causes genomic instability*: 0 assay end-points

4. *Induces epigenetic alterations*: 5 assay end-points including biochemical assays targeting histone deacetylases and other

enzymes modifying chromatin, as well as assays for cellular transcription factors involved in epigenetic regulation

5. *Induces oxidative stress*: 13 assay end-points, all cellular assays, targeting nuclear erythroid-related factor 2 (NRF2) and/or the antioxidant responsive element (ARE) and other stress-related transcription factors, as well as protein upregulation in response to reactive oxygen species

6. *Induces chronic inflammation*: 47 assay end-points measuring protein expression levels in primary human cells in complex environments

7. *Is immunosuppressive*: 0 assay end-points

8. *Modulates receptor-mediated effects*: 95 assay end-points targeting nuclear receptors (including aryl hydrocarbon receptor) in cellular assays for transactivation, and receptor dimerization and nuclear translocation, as well as biochemical radioligand binding assays and coregulatory recruitment assays

9. *Causes immortalization*: 0 assay end-points

10. *Alters cell proliferation, cell death, or nutrient supply*: 100 assay end-points measuring cytotoxicity or general development using a wide variety of assay formats in cell lines, primary human cells, and developing zebrafish larvae.

In addition, there are 35 assay end-points classified as "Other" that measure biological activity against targets not readily classifiable with respect to the 10 key characteristics.

4.4.2 Outcomes for chemicals tested

The specific assays tested, mapping to the key characteristics, and the activity calls are available as supplemental material to the present volume (Annex 1). Table 4.4 lists the number of assays tested and the number of positive findings for each key characteristic and each chemical.

Brief summaries of potentially significant outcomes for each chemical tested are provided below (see also Table 4.4).

(a) Isobutyl nitrite

Isobutyl nitrite (CAS No. 542-56-3) was inactive in all 116 of the Tox21 programme assay end-points mapped to the key characteristics of carcinogens. Chemical quality control (QC) information was available for the Tox21 chemical library sample solution; it was graded "D" because of a purity of less than 50%, and a comment that "the sample has decomposed to the alcohol" was included (NIH, 2017). The chemical has a predicted vapour pressure of 2.13 mm Hg [1.3 kPa] and an experimental boiling point of 66.8 °C (EPA, 2017b). [The Working Group noted that there may have been limited ability to detect bioactivity in the Tox21 assays because of the low relative molecular mass of the chemical, 103.1, which may limit biomolecular interactions at the concentrations tested, and the poor analytical chemistry analysis for the tested sample.]

(b) β-Picoline

β-Picoline (CAS No. 108-99-6) was found to be bioactive in 13 of 266 ToxCast and Tox21 assay end-points mapped to the key characteristics. In two assays mapped to "induces oxidative stress" (ATG_NRF2_ARE_CIS and ATG_MRE_CIS) marginal activity was shown only at the highest concentration tested (200 μM). The result of one assay mapped to "modulates receptor-mediated effects" was called positive, but concentration–response curve-fit warning flags clearly showed this to be a bad fit and therefore a false-positive call. Five assays mapped to "alters cell proliferation, cell death, or nutrient supply" were called active. Two were transcription factor activation assays (ATG_AP_1_CIS and ATG_Xbp1_CIS) that showed marginal activity only at the highest concentration tested (200 μM). The other three

Table 4.4 Summary of activity of agents reviewed in *IARC Monographs* Volume 122 and tested in ToxCast and/or Tox21 high-throughput screening assays

Key characteristic	Isobutyl nitrite	β-Picoline	Methyl acrylate	Ethyl acrylate	2-Ethylhexyl acrylate	Trimethylolpropane triacrylate
1. Is electrophilic or can be metabolically activated	0 out of 1 assays[a]	0 out of 1 assays	0 out of 1 assays[a]	NA	0 out of 1 assays	1[b] (0) out of 1 assays
2. Is genotoxic	0 out of 9 assays	0 out of 10 assays	0 out of 10 assays	0 out of 9 assays	0 out of 10 assays	9 out of 10 assays
3. Alters DNA repair or causes genomic instability	NA	NA	NA	NA	NA	NA
4. Induces epigenetic alterations	0 out of 1 assays	0 out of 5 assays	0 out of 5 assays	0 out of 1 assays	0 out of 5 assays	2 out of 5 assays
5. Induces oxidative stress	0 out of 1 assays	2 out of 13 assays	0 out of 8 assays	0 out of 4 assays	1 out of 13 assays	5 out of 13 assays
6. Induces chronic inflammation	0 out of 1 assays	0 out of 47 assays	0 out of 2 assays	0 out of 1 assays	0 out of 47 assays	0 out of 47 assays
7. Is immunosuppressive	NA	NA	NA	NA	NA	NA
8. Modulates receptor-mediated effects	0 out of 39 assays	1[b] (0) out of 94 assays	0 out of 75 assays	0 out of 39 assays	4[b] (0) out of 95 assays	33 out of 76 assays
9. Causes immortalization	NA	NA	NA	NA	NA	NA
10. Alters cell proliferation, cell death or nutrient supply	0 out of 64 assays	5[b] (2) out of 96 assays	1[b] (0) out of 72 assays	0 out of 64 assays	8 out of 100 assays	71 out of 96 assays
Total number of assays mapped to key characteristics	116	266	173	118	271	248

NA, not applicable: no assays in ToxCast and/or Tox21 were determined to be applicable to the evaluation of the indicated key characteristic; ToxCast/Tox21, Toxicity Forecaster and Toxicity Testing in the 21st Century research programmes of the government of the USA

[a] Indicates the number of positive results out of the number of assays mapped to key characteristics of carcinogens, as listed in supplemental Table 1 (see Annex 1)

[b] Indicates an active call in an assay (i.e. "hit") which was determined to be most likely a false positive artefact upon review of the assay parameters and dose-response data by the Working Group [the number in parentheses reflects the true number of biological hits in the opinion of the Working Group]

were viability assays, each with significant curve-fitting warning flags that indicated likely false-positive results. Finally, there are five assays from the "other" category considered active, all from the Attagene (ATG) transcription factor activation assay platform; all were marginally active only at the highest concentration tested (200 μM). The analytical QC of the tested sample solution was not available. 3-Methyl pyridine has an experimental vapour pressure of 6.05 mm Hg (EPA, 2017c). [The Working Group noted that the relative molecular mass of the chemical is 93.1, which may limit biomolecular interactions at the concentrations tested, and that volatilization of the sample may have affected actual sample concentration.]

(c) Methyl acrylate

For methyl acrylate (CAS No. 96-33-3), active hit calls were made for only 1 of 173 ToxCast and Tox21 assays mapped to the key characteristics. The single active call was for a cell viability assay, but multiple curve-fit warning flags were associated with the results, indicating a false-positive finding. The chemical QC analysis of the solution used in Tox21 showed that the expected structure was not detected and no significant impurities were observed at the time of analysis. Methyl acrylate has an experimental vapour pressure of 86.6 mm Hg and a boiling point of 80.0 °C (EPA, 2017d). [The Working Group noted that the relative molecular mass of the chemical is 86.1, which may limit biomolecular interactions at the concentrations tested, and that sample volatility may have contributed to the lack of expected structure noted in the analytical QC of the Tox21 sample solution.]

(d) Ethyl acrylate

There were no active hit calls in any of the 118 Tox21 assays mapped to the key characteristics tested with ethyl acrylate (CAS No. 140-88-5). The analytical chemistry determination of the sample solution tested in Tox21 was not available. The experimental vapour pressure of ethyl acrylate was reported as 38.6 mm Hg and the boiling point as 99.5 °C (EPA, 2017e). [The Working Group noted that the relative molecular mass of the chemical is 100.1, which may limit biomolecular interactions at the concentrations tested, and that sample volatility may have limited chemical exposure in the assay.]

(e) 2-Ethylhexyl acrylate

For 2-ethylhexyl acrylate (CAS No. 103-11-7), active hit calls were made for 13 of 271 ToxCast and Tox21 assay end-points mapped to the key characteristics. One assay end-point for "induces oxidative stress" (ATG_NRF2) was active with an AC_{50} (the concentration at which the half-maximal response along a sigmoid curve is produced) of 101 μM, along with a tumour protein TP53 activation assay mapped to "is genotoxic" (ATG_p53_CIS), active at 116 μM. Orthogonal assays for NRF2 and TP53 in Tox21 were inactive. Four other active calls were mapped to key characteristic 8, "modulates receptor-mediated effects", consisting of estrogen receptor (ER) α and ERβ activation, retinoic acid receptor (RAR) activation, and progesterone receptor (PR) activation (OT_ERa_EREGFP_0120, TOX21_ERb_BLA_Antagonist, TOX21_RAR_LUC_Agonist, and TOX21_PR_BLA_Antagonist). The ERα assay curve fit was not flagged, but was not a monotonic response as would be expected for a receptor-modulated effect. In addition, the same assay but with a 4-hour incubation (OT_ERa_EREGFP_0480) rather than 2-hour incubation was completely inactive. [The Working Group noted that this was probably a false-positive result.] Both the ERβ and the PR curve fits had multiple warning flags and appeared to be false positives. The RAR response was marginal at the highest concentration tested; however, orthologous assays for RARα, RARβ, and RARγ in the ATG platform were all inactive. There were 8 active assays mapped to "alters cell proliferation, cell death, or nutrient supply". Three of these had

poor curve fits as evidenced by warning flags and visual inspection. There were cytotoxic responses seen in four primary human cell culture models that included smooth muscle cells, dermal fibroblasts, and endothelial cells (BSK_CASM3C_SRB, BSK_hDFCGF_Proliferation, BSK_hDFCGF_SRB, and BSK_3C_Proliferation). Potencies were 24–33 μM (AC_{50}). The dermal fibroblast cell cultures were shown to be particularly sensitive to oxidative stress (Kleinstreuer et al., 2014). The analytical QC analysis of the Tox21 sample solution indicated that the expected structure was present, but only at 5–30% of the expected concentration. [The Working Group noted that the low concentration may suggest volatility.]

(f) Trimethylolpropane triacrylate

For trimethylolpropane triacrylate (CAS No. 15625-89-5), there were 126 active calls for 283 ToxCast and Tox21 assay end-points mapped to the key characteristics. It was active against one assay mapped to "is electrophilic or can be metabolically activated" (TOX21_Aromatase_Inhibition); however, the corresponding cell viability assay (TOX21_Aromatase_Inhibition_viability) was active at the same concentrations, which would support the theory that the effects were due to cytotoxicity. There were nine positive assays mapped to "is genotoxic". Two of these were related to DNA repair (TOX21_DT40_100 and TOX21_DT40_657); however, activity was equivalent at the wildtype cell line (TOX21_DT40), consistent with general cytotoxicity being responsible for the activity (Nishihara et al., 2016). The assay for DNA damage (TOX21_ELG1_LUC_Agonist) was active with an AC_{50} of 5.2 μM. As this is a gain-of-signal reporter gene assay, it is less prone to cytotoxic effects that artefactually decrease the reporter signal. It was also active in an assay for TP53 activation (TOX21_p53_BLA) five times out of five tests (the TP53 assay was repeated over time to examine potential effects of chemical degradation), another gain-of-signal reporter gene assay. The AC_{50}s fell within the range 15–101 μM. One other assay mapped to genotoxicity (TOX21_H2AX_HTRF_CHO) was positive, with an AC_{50} of 11.5 μM. Trimethylolpropane triacrylate was also considered active in five assays mapped to "induces oxidative stress", with AC_{50} values within the range 2–19 μM. It was active in 33 assays mapped to "modulates receptor-mediated effects". The most potent effect was seen for the xenobiotic pregnane X receptor (PXR) where it was active in two assays with AC_{50} values of 0.77 and 0.78 μM (ATG_PXRE_CIS and ATG_PXR_TRANS). For other receptor assays, there was a consistent pattern of partial agonist activity just before a large loss of effect at cytotoxic concentrations. Because of the confounding effects of cytotoxicity, the interpretation of receptor modulation effects, other than for PXR, is challenging. Trimethylolpropane triacrylate was active in 71 assays mapped to "alters cell proliferation, cell death, or nutrient supply". Sixty-eight of these were categorized as cytotoxicity or apoptosis with an average AC_{50} of 4.72 ± 2.73 μM for both cell lines and primary human cells. Two additional assays indicated upregulation of growth factor or growth factor receptor in primary human cells (BSK_hDFCGF_EGFR and BSK_KF3CT_TGFb1). The final assay showed upregulation of the AP1 transcription factor (TOX21_AP1_BLA_Agonist), but the curve fit was flagged because of activity at a single concentration and obvious confounding by cytotoxicity. There were two positive assay results linked to "induces epigenetic alterations" (ATG_Pax6_CIS and ATG_Sp1_CIS), but both activities were much higher than the average cytotoxicity concentrations and therefore considered not biologically significant. Finally, there were five positive assay results not mapped to any of the key characteristics but to an "other" category. Three of these (ATG_EGR_CIS, ATG_NFI_CIS, and ATG_Oct_MLP_CIS) were activated transcription factor responses for proteins characterized as being involved in cell differentiation. The ATG_SREBP_CIS assay was

also activated, an end-point associated with low cellular sterol levels for precursors of cholesterol biosynthesis. The last of these "other" activities was TOX21_TSHR_Agonist, an assay for activation of thyroid-stimulating hormone receptor that could also respond to increased levels of cyclic adenosine monophosphate. As for the majority of assays in other categories, however, all assays in the "other" category had AC_{50} values above the average cytotoxicity potency. [The Working Group noted that trimethylolpropane acrylate was highly cytotoxic and that interpretation of bioactivity in vitro in the micromolar concentration range was likely to be confounded by nonspecific effects.] The chemical QC determination of the sample solution tested with Tox21 showed the expected structure, but purity was less than 50%.

4.4.3 Overall considerations

In summary, trimethylolpropane triacrylate showed bioactivity in 126 of 283 assays, of which 248 were mapped to the key characteristics of carcinogens (119 showed bioactivity). Nine active assays were mapped to genotoxicity, although in two cases bioactivity occurred at concentrations inducing cytotoxicity in other assays. Additionally, 71 assays were mapped to "alters cell proliferation"; these were predominantly cytotoxicity assays with an average potency of 5 μM. Finally, there were 33 assays mapped to "modulates receptor-mediated effects", with the most potent effect against the xenobiotic receptor PXR; other receptor effects were at cytotoxic concentrations.

Data from high-throughput toxicity testing programmes were considered uninformative for the other compounds tested. Isobutyl nitrite was inactive in all of the 116 assays mapped to the key characteristics and had poor analytical chemistry results, probably because of chemical volatility. For β-picoline, there was weak support for oxidative and cellular stress responses based on marginal bioactivity in a few assays, but chemical volatility may have limited the chemical exposure in these assays. For methyl acrylate, there was bioactivity in only 1 of 173 assays. Ethyl acrylate was inactive in all 118 assays, but volatility may have limited the chemical exposure in these assays. 2-Ethylhexyl acrylate showed 13 active assays out of 271 mapped to the key characteristics, but most of these results were inconsistent and not considered significant, with the exception of cytotoxicity noted in four primary human cell culture models.

5. Summary of Data Reported

5.1 Exposure data

Isobutyl nitrite is an alkyl nitrite. It is mainly used in "poppers", consumed as a recreational drug for their psychoactive effects. Poppers are illegal in many countries, and as a result are commonly sold as air fresheners or deodorizers. Other minor uses of isobutyl nitrite include as an intermediate in the synthesis of aliphatic nitrites, nail polish removers, video head cleaners, fuels, and jet propellants. There were no available data on the production volume of isobutyl nitrite. There was evidence of the increased online purchase of poppers from countries where their use is legal. Human exposure to isobutyl nitrite occurs mainly through intentional inhalation. No quantitative data on environmental concentrations or occupational exposure to isobutyl nitrite were identified.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

There was one well-conducted good laboratory practice inhalation study of isobutyl nitrite in male and female mice. In males, isobutyl nitrite significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma and of bronchioloalveolar adenoma or carcinoma (combined) of the lung, and of follicular cell adenoma and of follicular cell adenoma or carcinoma (combined) of the thyroid gland. In females, isobutyl nitrite significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma and of bronchioloalveolar adenoma or carcinoma (combined) of the lung.

There was one well-conducted good laboratory practice inhalation study of isobutyl nitrite in male and female rats. In males, isobutyl nitrite significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, and of bronchioloalveolar adenoma or carcinoma (combined) of the lung. In females, isobutyl nitrite significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma and of bronchioloalveolar adenoma or carcinoma (combined) of the lung.

5.4 Mechanistic and other relevant data

Studies on the absorption, distribution, or excretion in humans were not available, but methaemoglobinaemia and the vasodilating effects of isobutyl nitrite in humans indicate that absorption occurs. Isobutyl nitrite undergoes hydrolytic decomposition in humans, yielding nitrite and isobutyl alcohol.

Rapid systemic absorption and elimination were observed in rats exposed by inhalation, with a short half-life (~1 minute) regardless of route of administration. Isobutyl nitrite is extensively metabolized in rats and rabbits, and isobutyl alcohol was identified in rats. Nitric oxide, found in the exhaled air of exposed rabbits, can be formed by reduction of isobutyl nitrite or by homolytic cleavage of the nitric oxide bond, which also yields the isobutoxyl radical.

Regarding the key characteristics of carcinogens, there is *moderate* evidence that isobutyl nitrite is genotoxic. Results were generally positive, but there were few studies available. In one study of human lung cells in vitro, dose-dependent induction of DNA damage was detected by the comet assay in cells from one of three donors. There was increased DNA damage in rat lung but not in rat liver or kidney in one study, and a test for micronucleus formation in mice in vivo gave positive results for DNA damage. In the few studies in rodent cells in vitro, isobutyl nitrite gave positive results in tests for mutations, sister-chromatid exchanges, and chromosomal aberrations. In the Ames test, isobutyl nitrite gave positive results in strains sensitive to base substitutions, but negative results in strains sensitive to frameshift mutations.

Isobutyl nitrite is a known irritant that causes nonspecific inflammatory responses at the exposure site in humans and rodents. There is *moderate* evidence that isobutyl nitrite is immunosuppressive. No data from studies of isobutyl nitrite in exposed humans were available, and the few studies of structurally related nitrites were equivocal. A single study using human peripheral blood cells in vitro demonstrated suppressed lymphocyte blastogenesis, natural killer cell function, antibody-dependent cell-mediated cytotoxicity, and interferon production in isolated leukocytes. Dose-dependent suppression of antigen-specific antibody production occurred in most, but not all, strains of mice exposed to isobutyl nitrite via inhalation, and was shown to persist after cessation of exposure. Suppression of other indicators of immune function (including natural killer cell and cytotoxic T-lymphocyte activity) was reported in mice, but results were inconsistent.

In cases of intoxication in humans, methaemoglobinaemia, hypotension, visual effects, and irritant contact dermatitis were reported. Methaemoglobinaemia was observed in rats and rabbits, and hypotension linked to nitric oxide generation was reported in rabbits.

Hyperplasia of the lung was observed in chronically exposed rodents.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of isobutyl nitrite.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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