Australian Government
Department of Health
Australian Industrial Chemicals Introduction Scheme

Acetylpropionyl and diacetyl

Evaluation statement

14 January 2022

Table of contents

Contents

AICIS evaluation statement

Subject of the evaluation

Acetylpropionyl and diacetyl

Chemicals in this evaluation

Name	CAS registry number
2,3-Butanedione	431-03-8
2,3-Pentanedione	600-14-6

Reason for the evaluation

The Evaluation Selection Analysis indicated a potential risk to human health.

Parameters of evaluation

The chemicals are listed on the Australian Inventory of Industrial Chemicals (the Inventory).This evaluation is a human health risk assessment for all identified industrial uses of the chemicals including use in e-cigarette liquids.

Summary of evaluation

Summary of introduction, use and end use

There is currently no specific information about the introduction, use and end use of acetylpropionyl or diacetyl in Australia.

Based on international use information, acetylpropionyl and diacetyl are used as fragrance or flavour ingredients in consumer products including perfumes, cleaning and washing agents and e-cigarette liquids. Diacetyl has a reported use in hair styling products at concentrations of 0.1–1%. Acetylpropionyl has reported use in air-fresheners at concentrations of 0.1–5%. The chemicals have non-industrial applications as food flavorings.

Human health

Summary of health hazards

The critical health effects for risk characterisation include:

- systemic acute effects from oral exposure
- local effects including serious eye damage and skin sensitisation
- systemic effects following repeated inhalation exposure (irreversible lung damage)
- potential carcinogenicity following repeated inhalation exposure

Diacetyl and acetylpropionyl are water soluble chemicals and are expected to be well absorbed following oral and inhalation exposure. Diacetyl is reported to be endogenous in humans and both chemicals are anticipated to be readily metabolised. At high concentrations, these chemicals are expected to be reduced to the corresponding diol and conjugated with glucuronic acid prior to excretion.

Based on the available data, diacetyl and acetylpropionyl are expected to have low to moderate acute oral toxicity (median lethal dose LD50 = 250–3400 mg/kg bw), low acute dermal toxicity (LD50 >2000 mg/kg bw) and low acute inhalation toxicity (median lethal concentration LC50 2250–5200 ppm; 4 hours).

Based on the available data diacetyl and acetylpropionyl are not expected to be irritating to the skin, although in one briefly described study 100% diacetyl applied to intact or shaved skin of rabbits under occlusive conditions resulted in skin irritation. Based on limited available data, the chemicals in this group are considered to cause serious eye damage.

Diacetyl and acetypropionyl are considered to be weak skin sensitisers based on animal data. This is supported by observations in humans and in silico data.

Diacetyl and acetylpropionyl are expected to cause serious systemic health effects following repeated inhalation exposure. The effects of both chemicals in the nasal cavities and lungs are similar in both mice and rats, and are supported by observations from human epidemiological studies. Repeated oral and dermal exposure are not expected to result in adverse effects. The Joint FAO/WHO expert Committee on Food Additives (JEFCA) evaluated the risk from dietary exposure to diacetyl and acetylpropionyl, and concluded that they do not cause safety concern when used as flavoring agents at current levels (Maximised Survey-derived Daily intake (MSDI) EU 2200 microgram/*capita*/day) (JECFA 2000; EFSA 2011).

Although diacetyl and acetylpropionyl are considered to have genotoxic potential in vitro, due to their rapid metabolism, they are not expected to be genotoxic in vivo. Diacetyl gave mixed results in in vitro genetic toxicity studies and negative results in the only available in vivo mammalian erythrocyte micronucleus test. Acetylpropionyl was negative in an in vitro bacterial reverse mutation assay

Based on the weight of evidence, diacetyl and acetylpropionyl may be carcinogenic following inhalation exposure. Sustained cytotoxicity and cell proliferation resulting from chronic diacetyl exposure in combination with the reported formation of DNA adducts are likely to contribute to the induction of respiratory tumours. Although data are not available for acetylpropionyl, the damage to nasal cavities and lungs of mice and rats is similar to that reported for diacetyl, and is expected to occur via a similar mechanism. The data are not sufficient to classify the chemicals for carcinogenicity.

Health hazard classification

The chemicals satisfy the criteria for classification according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UNECE 2017) for hazard classes relevant for work health and safety as follows. This does not consider classification of physical hazards and environmental hazards.

Health hazards	Hazard category	Hazard statement
Acute toxicity – oral	Acute Tox. 4	H302: Harmful if swallowed
Serious Eye Damage	Eye damage 1	H318: Causes serious eye damage
Sensitisation	Skin Sens. 1B	H317: May cause allergic skin reaction
Specific target organ toxicity (repeat exposure)	STOT Rep. Exp. 1	H372: Causes damage to lungs through prolonged or repeated inhalation exposure

Summary of health risk

Public

Based on the available use information, the public may be exposed to the chemicals:

- by inhaling vapours and aerosols following use of non-nicotine liquids in e-cigarette devices
- by direct skin contact during use of cosmetic products
- by incidental skin and eye contact during use of domestic products
- by inhaling aerosols

Given the concentrations expected to be in use in personal care and domestic products, toxicology data showing limited sensitisation potential at low concentrations, and satisfactory margin of exposure (MOE) for inhalation exposure found by Health Canada (see **Supplementary Information – Human Exposure** section) there are no identified risks to the public from these uses. Higher exposures have been calculated for use in e-cigarettes (see **Supplementary Information – Human Exposure** section).

Use of e-cigarettes has increased rapidly since their introduction in the mid-2000s (Barrington-Trimis et al. 2014). E-cigarette devices are designed to heat e-cigarette liquids (frequently contain flavouring compounds dissolved in propylene glycol and/or glycerine) creating aerosol that penetrates deeply into the lungs. Given the short period that these products have been available on the market the long term safety and health effects associated with e-cigarette use and exposure are unknown. However, available evidence suggests that regular use of e-cigarettes is likely to have adverse health consequences (CSIRO 2018; NHMRC). As of February 2020, 2807 cases of vaping-associated pulmonary illness and 68 associated deaths were reported in the US (CDC). This is further supported by numerous case reports detailing vaping-associated pulmonary illness and include a recently reported case of vaping associated bronchiolitis obliterans (Landman et al. 2019).

Although many of these flavourings are ‘generally recognised as safe’ for use in food, inhalation data is generally limited and not considered as part of these food use assessments. Flavouring ingredients diacetyl and acetylpropinoyl are described as causing respiratory irritation, irreversible lung damage and have adverse effects on cell viability and function (Muthumalage et al. 2017; NICNAS 2019; Park et al. 2019).

Although causative agents and mechanisms of non-nicotine e-cigarette induced respiratory injury are still being determined, considering the well-established link between occupational diacetyl inhalation and the development of obstructive lung disease, evidence indicates that use of diacetyl and acetylpropionyl in non-nicotine e-cigarette fluids poses a risk to the public that requires management (see **Recommendation** section).

Workers

During product formulation, dermal, ocular and inhalation exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemicals at lower concentrations could also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of preparation and work practices employed. Good hygiene practices to minimise incidental oral exposure are expected to be in place.

Given the critical systemic long-term effects, the chemicals could pose a risk to workers. Control measures to minimise dermal, ocular and inhalation exposure are needed to manage the risk to workers (refer to **Recommendation** section).

Conclusions

The conclusions of this evaluation are based on the information described in this statement. Obligations to report additional information about hazards under section 100 of the Industrial Chemicals Act 2019 apply.

The Executive Director is satisfied that the identified human health risks can be managed within existing risk management frameworks provided all requirements are met under environmental, workplace health and safety and poisons legislation as adopted by the relevant state or territory. The proposed means of managing the risks identified during this evaluation are set out in the **Recommendations** section.

Recommendations

Public health

Recommendation to Department of Health

It is recommended that the delegate of the Secretary for Poisons Scheduling list the chemicals in the Poisons Standard (the Standard for the Uniform Scheduling of Medicines and Poisons—SUSMP).

It is recommended that to manage the potential risk associated with the use of these chemicals, the entry prohibits their use in products intended to be inhaled (e.g. non-nicotine e-cigarette fluids).

Consideration should be given to the following:

- these chemicals are shown to cause irreversible lung damage following repeated inhalation exposure and have carcinogenic potential
- diacetyl and acetylpropionyl are prohibited ingredients in nicotine vaping products (TGA 2021b)
- the likely widespread use of these chemicals in e-cigarette products available in Australia.

Workers

Recommendation to Safe Work Australia

It is recommended that Safe Work Australia (SWA) update the Hazardous Chemical Information System (HCIS) to include classifications of these chemicals relevant to work health and safety.

Information on managing identified risks

The information in this report including recommended hazard classifications, should be used by persons conducting a business or undertaking (PCBU) at the workplace (such as an employer) to determine the appropriate controls under the Model Work Health and Safety Regulations.

Control measures that could be implemented to manage the risk arising from occupational exposure to the chemical include, but are not limited to:

- using closed systems or isolating operations
- using local exhaust ventilation to prevent the chemicals from entering the breathing zone of any worker
- minimising manual processes and work tasks through automating processes
- adopting work procedures that minimise splashes and spills
- cleaning equipment and work areas regularly
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Measures required to eliminate,or manage risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used.

These control measures may need to be supplemented with:

- Conducting health monitoring for any worker who is at significant risk of exposure to the chemical, if valid techniques are available to monitor the effect on the worker's health.
- Conducting air monitoring to ensure control measures in place are working effectively and continue to do so.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

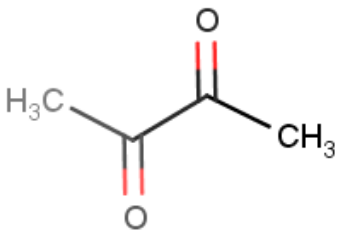
Model codes of practice, available from the Safe Work Australia website, provide information on how to manage the risks of hazardous chemicals in the workplace, prepare an SDS and label containers of hazardous chemicals. Your Work Health and Safety regulator should be contacted for information on Work Health and Safety laws and relevant Codes of Practice in your jurisdiction.

Supporting information

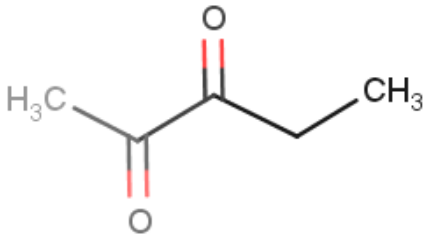
Grouping rationale

The chemicals in this group are C4-C5 α-diketones, diacetyl and acetylpropionyl. The chemicals are structurally similar and are expected to have analogous toxicological hazards.

Chemical identity

Chemical name	2,3-butanedione
CAS No.	431-03-8
Synonyms	diacetyl 2,3-dioxybutane dimethyl diketone
Structural formula	
Molecular formula	C4H6O2
Molecular weight (g/mol)	86.09
SMILES	O=C(C(=O)C)C
Chemical description	-

Chemical name	2,3-pentanedione
CAS No.	600-14-6
Synonyms	acetylpropionyl

Structural formula	
Molecular formula	C5H8O2
Molecular weight (g/mol)	100.12
SMILES	O=C(C(=O)CC)C
Chemical description	-

Relevant physical and chemical properties

Chemical name	2,3-butanedione
Physical form	yellow to yellow-green liquid
Melting point	-2.4°C
Boiling point	88°C
Vapour pressure	6.9 kPa at 20°C
Water solubility	200 g/L at 25°C
log K_{ow}	-1.34 (predicted)

Chemical name	2,3-pentanedione
Physical form	yellow to yellow-green liquid
Melting point	-52.0°C
Boiling point	110-112°C
Vapour pressure	2.8 kPa at 20°C,
Water solubility	60 g/L at 15°C
log K_{ow}	-0.850 (predicted)

Introduction and use

Australia

No comprehensive information is available on the introduction and industrial use of acetylpropionyl and diacetyl in Australia. These chemicals have been identified as components of e-cigarette liquids in one Australian study (NICNAS 2019).

Acetylpropionyl has a reported non-industrial use as a flavour and fragrance in pharmaceutical products in Australia, where the total concentration as a flavour and fragrance is less than 5% and 1%, respectively (TGA 2021a).

International

The following international uses have been identified through the:

- Consumer Product Information Database (DeLima Associates)
- European Union Registration, Evaluation and Authorisation of Chemicals (REACH)
- European Commission Cosmetic Ingredients & Substances (CosIng) database
- Substances in Preparations in Nordic countries (SPIN) database
- Environment and Climate Change Canada Health Canada
- United States Environmental Protection Agency (US EPA) Chemical and Product Categories database (CPCat).

These chemicals have reported use as flavourings in consumer products including e-cigarette fluids.

Acetylpropionyl and diacetyl are listed on the International Fragrances Association (IFRA) transparency list and have reported cosmetic and domestic uses as fragrance ingredients. These include cosmetic and personal care products such as perfumes, cleaning and washing agents, air fresheners (at reported concentrations of 0.1–5% acetylpropionyl) and scented candles.

Diketones, including acetylpropionyl and diacetyl have reported commercial uses as solvents in colouring agents (e.g. paints, inks and lacquers) and lubricants.

Diacetyl has reported site limited use as an intermediate in the production of other chemicals.

Acetylpropionyl and diacetyl have reported non-industrial uses as food flavourings, in pharmaceuticals and cigarette additives.

Existing Australian regulatory controls

AICIS

No specific controls are currently available for the chemical.

Public

Diacetyl and acetylpropionyl are listed in Schedule 1 of the Therapeutic Goods (Standard for Nicotine Vaping Products) (TGO 110) Order 2021. Schedule 1 substances must not be added as ingredients to nicotine vaping products (TGA 2021b).

Workers

The chemicals are not listed on the HCIS (SWA).

No specific controls are currently available for the chemicals. In 2019 and 2020 Safe Work Australia reviewed and recommended the following new workplace exposure standards for diacetyl and acetylpropionyl, as shown below. At the time of publication of this evaluation statement, these values were yet to be finalised (SWA 2019; SWA 2020).

Diacetyl

0.04 mg/m^3 (0.01 ppm) time weighted average (TWA)

0.07 mg/m^3 (0.02 ppm) short term exposure limit (STEL)

Acetylpropionyl

0.083 mg/m^3 (0.02 ppm) TWA

International regulatory status

Exposure standards

The following international exposure standards were identified for diacetyl (Chemwatch):

Exposure limits of 0.04 mg/m^3 (0.01 ppm) TWA and 0.07–0.36 mg/m^3 (0.02-0.1 ppm) STEL/MAK have been identified in different countries such as Belgium, Canada, Colombia, Germany, Ireland, Italy, Sweden, Switzerland and the United Kingdom.

The American Conference of Governmental Industrial Hygienists (ACGIH) recommended a threshold limit value (TLV) of 0.04 mg/m^3 (0.01 ppm) TWA and 0.083 mg/m^3 (0.02 ppm) STEL with a notation for lung damage (bronchiolitis obliterans-like illness) (ACGIH, 2013). The National Institute for Occupational Safety and Health (NIOSH) has a recommended exposure limit (REL) of 0.005 ppm (as a TWA for up to 8 hours/day 40 hours/week), an action level of 0.0026 ppm and a STEL of 0.025 ppm (NIOSH, 2016).

The following international exposure standards were identified for acetylpropionyl (Chemwatch):

Exposure limits of 0.04 mg/m^3 (0.01 ppm) TWA and 0.083–0.16 mg/m^3 (0.02-0.04 ppm) STEL/MAK have been identified in Germany and Sweden.

The National Institute for Occupational Safety and Health (NIOSH) has a recommended exposure limit (REL) of 0.0093 ppm (as a TWA for up to 8 hours/day 40 hours/week) and a STEL of 0.031 ppm (NIOSH 2016).

Canada

Diacetyl and acetylpropionyl are listed in the 'Industry Guide to Vaping Products Subject to the Canada Consumer Product Safety Act' as substances with known inhalation risks that should never be added to vaping substances (Health Canada).

United Kingdom

Diacetyl and acetylpropionyl are listed in 'Advice on ingredients in nicotine containing liquids in electronic cigarettes and refill containers' as substances not permitted as ingredients in e-cigarette liquids (UK MHRA).

Human Exposure

Public

Although Australian use data are not available for diacetyl and acetylpropionyl, internationally the chemicals are widely used in e-cigarette flavours considered appealing to teenagers and young adults. Of 51 "appealing" e-cigarette flavours (e.g. tutti frutti, cotton candy and caramel popcorn) surveyed, diacetyl and acetylpropionyl were detected in 76% and 45% of samples, respectively (Allen et al. 2016). In separate studies, diacetyl and acetylpropionyl were identified in 46% and 19% of sampled e-liquids (n = 146) by headspace analysis (LeBouf et al. 2018). E-cigarette fluids containing these chemicals are expected to be widely available in Australia. While analysis of Australian customs data identified 35 unique introducers, it is expected that a significant proportion of e-cigarette liquid introductions are not captured in this data (e.g. introduced for personal use, or as part of low-value customs imports) (NICNAS 2019). It is also likely that e-cigarette liquids are mixed in Australia from available flavour ingredients.

The maximum concentration of diacetyl and acetylpropionyl in e-cigarette liquids is reported as 11 mg/mL and 1.0 mg/mL, respectively (NICNAS 2019). The maximum concentration of diacetyl and acetylpropionyl in e-cigarette vapours is reported as 3.7 and 1.1 µg/m^3 (1.05 and 0.28 ppb), respectively (Klager et al. 2017; NICNAS 2019). Analysis of the e-cigarette liquids and aerosol samples indicated that both chemicals are readily aerosolised (Farsalinos et al. 2015). The median exposure to diacetyl and acetylpropionyl (calculated assuming a consumer vapes 3 mL/day) in e-cigarette liquids was reported as 56 µg/day (interquartile range 26–278 µg/day) and 91 µg/day (interquartile range 20–432 µg/day), respectively (Farsalinos et al. 2015). In a separate study, based on the assumptions that a consumer vapes 3.4 mL of a refill fluid containing diacetyl (0.32 mg/mL) and a transfer rate of diacetyl to the aerosol of 100%, it was calculated that a consumer would be exposed to 1.09 mg of diacetyl/day, equivalent to 85.83 ppb/8 hour average (Omaiye et al. 2019). This exceeds the NIOSH 8hr-REL of 5 ppb (see **International regulatory status** section) for adult workers. In a separate study, various parameters were used to model daily intake. These included a range of values for concentration of diacetyl in e-cigarette vapour (mg/m^3; C_{puff}), absorption factor (AF), inhalation rate (m^3/hour), exposure time (hours/day), exposure frequency (days/year), exposure duration (years) and body weight (BW, kg). Average daily doses (ADD) of 1.04–5.2 and 0.47–3.34 mg/kg/day (using an AF of 100%) were calculated for teen (BW 45 kg) and adult (BW 70 or 100 kg) exposure, respectively. The non-cancer hazard quotient (HQ) was calculated as a ratio of ADD to the NIOSH REL benchmark dose (BMD; 0.0175 mg/kg/day). Hazard quotients of less than 1 were not calculated under any of the modelled scenarios. This indicates an excess risk for both teens and adults in the development of non-carcinogenic morbidities compared to non-e-cigarette users (White et al. 2021). No exposure standards are available for the general public.

The public may be exposed to the chemicals in personal care and domestic products. The margin of exposure (MOE) for inhalation exposure to diacetyl in hair styling products was calculated as 703–5625 based on an lowest observed adverse effect concentration (LOAEC) of 45 mg/m^3. The MOE for inhalation exposure to acetylpropionyl in air fresheners was calculated as 364–12750 based on an no observed adverse effect concentration (NOAEC) of 51 mg/m^3. These MOEs were considered adequate to account for uncertainties in databases, and indicate low risk of adverse effects in these exposure scenarios (Environment and Climate Change Canada Health Canada 2019).

Health hazard information

Toxicokinetics

Diacetyl and acetylpropionyl are water soluble chemicals and are expected to be well absorbed via the oral and inhalational routes. Diacetyl, and its metabolites, acetoin (3-hydroxy-2-butanone; CAS 513-86-0) and 2,3-butanediol (CAS 513-85-9), are reported to be endogenous in humans with plasma concentrations of 0.25-0.75, 2.2 and 5–10 µM, respectively. In cats, diacetyl and acetoin are reported to be formed following metabolism of pyruvate by pyruvate decarboxylase (EFSA 2011).

In rats and mice, orally administered aliphatic acyclic α-diketones are rapidly absorbed from the gastrointestinal tract (WHO 1999). Following oral administration, diacetyl was found in the liver, kidney and brain (Otsuka et al, 1996). In humans, this class of chemicals is expected to be metabolised by alpha-hydroxylation and subsequent oxidation to the corresponding ketocarboxylic acid. The acid may then undergo oxidative decarboxylation to yield carbon dioxide and the corresponding carboxylic acid, which may be completely metabolised via the fatty acid pathway and citric acid cycle. At high concentrations, these chemicals are expected to be reduced to the corresponding diol and conjugated with glucuronic acid for excretion (WHO, 1999). Following oral single doses of 1.58, 15.8 or 158 mg/kg bw ^{14}C-diacetyl in rats, major portions of the chemical were excreted as carbon dioxide (54–82%) or in urine (7–34%). Urinary excretion increased with increased dosage, supporting the hypothesis that the metabolic pathway leading to the formation of carbon dioxide is saturated at higher doses (NTP 2018).

Systemic uptake of ^{14}C-diacetyl from the lung following intratracheal instillation and oropharyngeal aspiration was evaluated in male Sprague Dawley(SD) rats (n = 6), and male B6C3F mice (n = 6) at 100 mg/kg and 157 mg/kg, respectively. Blood and plasma were collected after 24 hours. In rats, 0.88% and 0.66% of the administered dose was reported in blood and plasma, respectively. In mice, 0.38% and 0.17% of the administered dose was reported in blood and plasma, respectively (Fennel et al. 2015).

Diacetyl uptake efficiency was evaluated in a surgically isolated rat upper respiratory tract model. Uptake efficiency was reported as moderate (25–75%) relative to water soluble acids such as acetic acid (95%) (Morris and Hubbs 2009). Metabolism was approximately 4 fold greater in nasal olfactory tissue than nasal respiratory or tracheal tissues. Extrapolation to humans using computational fluid dynamic physiologically based pharmacokinetic (CFD-PBPK) models suggests nasal scrubbing to be less efficient in humans than in rats. As a result, diacetyl may penetrate the intrapulmonary airways to a greater degree in humans than rats. Furthermore, the concentration of diacetyl reaching the bronchi was estimated as 1.5 fold greater in humans (who are commonly mouth breathing e.g. as during physical activity and e-cigarette use) compared to rats (which are obligate nose-breathers) (Morris and Hubbs 2009).

Acute toxicity

Oral

Based on limited data, diacetyl and acetylpropionyl have low to moderate acute oral toxicity warranting hazard classification (see **Recommendations** section).

The reported median lethal dose (LD50) values of diacetyl following oral exposure in various species include (ACGIH 2013; NTP 2018):

- rat 1580–3400 mg/kg bw
- guinea pig 990 mg/kg bw
- mouse 250 mg/kg.

Gross pathological and microscopic evaluation revealed severe irritation of the glandular and non-glandular regions of the stomach (ACGIH 2013; NTP 2018).

An LD50 value of 3000 mg/kg was reported for acetylpropionyl following oral exposure in rats. Further details were not available (REACHb).

Dermal

Based on limited data, diacetyl and acetylpropionyl have low acute dermal toxicity.

The LD50 values of diacetyl and acetylpropionyl in rabbits were reported as >5000 mg/kg and 2500 mg/kg, respectively (ACGIH 2013; MAK 2020)

Inhalation

Based on the available data, diacetyl and acetylpropionyl have low acute inhalation toxicity.

The median lethal concentration (LC50) of diacetyl was reported to be 2250–5200 ppm (7922–18309 mg/m^3) in rats. Reported signs of toxicity at the highest dose (23 9000 ppm) included atelectasis and oedema of the lungs, bronchial oedema and hydrothorax (SCOEL 2014).

Male SD rats were exposed to vapour from butter flavouring containing average diacetyl concentrations of 0 mg/m^3 (n = 19), 203 ppm (715 mg/m^3, low, n = 6), 285 ppm (1004 mg/m3, medium, n = 4), 352 ppm (1239 mg/m^3, high, n = 6) and 371 ppm (1306 mg/m^3, high, pulsed exposure ranging from 72-940 ppm, n = 3) for 6 hours. Other major components of the butter flavouring included acetic acid, acetoin, butyric acid, acetoin dimers and 2-nonanone. Reported signs of toxicity included necrotic changes in the nasal and respiratory epithelium in all rats in the medium and high dose groups, and one rat in the low dose group. Nasal and bronchoavelor lavage showed changes consistent with an inflammatory response. The authors concluded that the no observed effect level for a 6 hour exposure lay below the levels used in the experiment (Hubbs et al. 2002). In a follow up study, male Hla:(SD)CVF rats (n=6/group) were exposed to diacetyl vapour at 0 ppm, 99/120 ppm (349/423 mg/m^3, low), 195/224 ppm (687/789 mg/m^3, mid) or 295/356 ppm (1035/1253 mg/m^3, high) for 6-hours. This resulted in epithelial necrosis and inflammation in the trachea and larynx in the mid dose group and epithelial necrosis and inflammation in bronchi in the high dose group (Hubbs et al. 2008)

Male SD rats were exposed to 125 mg/kg diacetyl by intratracheal instillation (bypassing the nose) resulting in the development of bronchial and bronchiolar fibrotic lesions consistent with bronchiolitis obliterans (Palmer et al. 2011). In a separate study, male C57B1/6 mice were exposed to 0, 100, 200 or 400 mg/kg diacetyl by oropharyngeal aspiration (bypassing the nose). In the high dose group, this resulted in foci of fibrohistiocytic proliferation with little or no inflammation at the junction of the terminal bronchiole and alveolar duct (Morgan et al. 2008). Together these data suggest that rats are more susceptible than mice to diacetyl induced bronchiolitis obliterans.

Male Hla:(SD)CVF rats (n = 6/group) were exposed to 112, 241, 318 or 354 ppm (459, 987, 1302 or 1450 mg/m^3) acetylpropionyl or 240 ppm (845 mg/m^3) diacetyl vapour for 6 hours. Breathing abnormalities including breathing through the mouth, and breathing noise were reported 18 hours after the end of the exposure period in the 318 and 354 ppm acetylpropionyl groups. Significantly, increased necrosis and apoptosis of the respiratory and olfactory epithelium of the nose were reported in all acetylpropionyl exposed groups. The histopathological changes induced by exposure to ~240 ppm diacetyl or acetylpropionyl were reported as similar (Hubbs et al. 2012; MAK Commission 2020).

In an in vitro primary normal human bronchial epithelial (NHBE) cell model that utilised an air-liquid interface to mimic in vivo airway characteristics, exposure to diacetyl and acetylpropionyl (48 hours, n = 3, in culture medium at concentrations of 25 and 100 ppm, respectively) resulted in significant reduction in the number of ciliated cells. Observations that ciliogenesis was impaired were supported by transcriptomic profiling (Park et al. 2019).

Corrosion/Irritation

Skin irritation

There is limited information on the skin irritation effects of these two chemicals. Based on the weight of evidence, diacetyl and acetylpropionyl are not considered to be skin irritants.

In a combined skin irritation and local lymph node assay (LLNA) performed similar to OECD TG 429, female BALB/c mice (n = 5/group) were exposed topically to increasing concentrations (1.25, 2.5 and 5.0% v/v in 4:1 acetone/olive oil) of diacetyl on the dorsal surface of each ear (25 µL/ear) for 3 consecutive days. Irritation was evaluated by measuring the thickness of the ear pinnae prior to treatment, and 24 hours following the last treatment. Diacetyl induced significant ear swelling at concentrations of ≥2.5%. However, increases were less than 25% and; therefore, not specifically associated with excessive irritation. In a follow up study conducted according to the same protocol, female BALB/c mice (n=5/group) were exposed topically to increasing concentrations (12.5, 25 and 50% v/v in acetone) of C4−C7 α-diketones, including diacetyl and acetylpropionyl. Diacetyl and acetylpropionyl did not induce significant ear swelling. The positive control (2,4-dinitrofluorobenzene, 0.3%) resulted in an average increase of 129% (Anderson et al. 2013).

Application of 100% diacetyl to intact or shaved skin of rabbits under occlusive conditions resulted in severe skin irritation (MAK Commission 2016). Similarly, application of 100% acetylpropionyl on rabbit skin resulted in moderate skin irritation (MAK Commission 2020). No further details were provided.

Eye irritation

Based on limited data, diacetyl and acetylpropionyl are considered to cause serious eye damage.

In an eye irritation study reporting on a Draize test (details not provided), diacetyl was instilled into one eye each of three female New Zealand White (NZW) rabbits. The eyes were observed at 1, 4, 24, 48, 72, 96 hours and 7, 14 and 21 days post treatment. Diacetyl was described as corrosive with observed effects not reversible within 21 days (Sugai et al. 1990).

Sensory irritation

Studies in mice suggest that diacetyl is a sensory irritant. Sensory irritation is the result of the chemical stimulating the trigeminal nerve endings in the cornea and nasal mucosa, which evokes a stinging or burning sensation in the eyes and upper respiratory tract (nose and throat). This is a receptor-mediated mode of action and occurs at relatively low concentrations. Sensory irritation is different to eye and skin irritation used for hazard classification, and also different from the irritation leading to cytotoxicity. The latter is a result of physical damage to the cells, whereas sensory irritation is a nerve response. While there is clear evidence of irritation responses, sensory irritation is not considered to be specific target organ toxicity (STOT) under GHS.

In a study evaluating the airway irritation potency of diacetyl, male BALB/cJ mice (n = 7 or 8/dose) were exposed to diacetyl vapour at concentrations of 191–1154 ppm (679-4063 mg/m^3) for 2 hours. Breathing pattern analysis including ‘time of break’ between inhalation and exhalation (as a marker of sensory irritation), rapid shallow breathing and time of inspiration, were assessed. Decreased respiratory rate and increased ‘time of break’, indicated sensory irritation, which occurred quickly and reached a maximum within the first 10-20 minutes of the exposure period. The concentration depressing the respiratory rate by 50% (RD50) and the extrapolated threshold (RD0; ~NOEL) were calculated as 966 and 108 ppm (3401 and 380 mg/m^3), respectively (Larsen et al, 2009). When extrapolated to humans the authors noted that ‘no important sensory irritation’ is expected in humans at concentrations ≤20 ppm (≤70 mg/m^3). As a result, the authors concluded that sensory irritation is unlikely to offer a warning signal protective against bronchiolitis obliterans (Larsen et al. 2009).

Observation in humans

In a 48 hour patch test in human volunteers, 2% diacetyl in petrolatum did not result in skin irritation (n = 29). Further details are not available (MAK Commission 2016).

Acute respiratory effects have been documented in workers following inhalation of butter flavourings. The concentration of diacetyl in the ambient air was up to 345 mg/m^3 (98 ppm). Effects included nasal, ocular, throat and skin irritation (MAK Commission 2016).

Sensitisation

Skin sensitisation

Based on the available data, diacetyl and acetylpropionyl are considered to be skin sensitisers and warrant hazard classification (see **Recommendations** section).

In a combined skin irritation and local lymph node assay (LNNA) similar to OECD TG 429, female BALB/c mice (n = 5/group) were exposed topically to increasing concentrations (1.25, 2.5 and 5.0% v/v in 4:1 acetone/olive oil) of diacetyl. The reported concentration producing a threefold increase in lymphocyte proliferation (EC3) was 1.9% (Anderson et al., 2007). In a follow up study conducted according to the same protocol, female BALB/c mice (n=5/group) were exposed topically to increasing concentrations (12.5, 25 and 50% v/v in acetone) of C4-C7 α-diketones including diacetyl and acetyl propionyl. Similar to all of the chemicals assayed, diacetyl and acetylpropionyl were reported to have weak sensitisation potential with reported EC3 values 15.4% and 17.9%, respectively. Due to the significant variation in response compared to the earlier study, batches of diacetyl from 3 separate suppliers were assayed, EC3 values of 2.5%, 14.2% and >25% were reported. Following chromatographic analysis a contaminant was identified, the concentration of which was reported as inversely proportional to the EC3 value (Anderson et al. 2013).

In a LLNA assay in mice, the reported stimulation indices (SI) were 1.4, 2.8 and 5.2 after treatment with concentrations of 5%, 10% and 25% diacetyl. A three fold increase in lymphocyte proliferation (EC3) was reported at concentrations of 12.6%, indicating weak sensitisation potential. No further details of the test including the number of animals used were available (Roberts et al. 1990).

In a LLNA assay similar to OECD TG 429, BALB/c mice were exposed topically to increased concentrations (1, 2.5, 5, 10 and 25%) of acetylpropionyl. A three fold increase in lymphocyte proliferation (EC3) was reported at concentrations of ≥5% indicating weak sensitisation potential (REACHb).

The chemicals have structural alerts for protein binding based on the mechanistic profiling functionality of the Organisation for Economic Co-operation and Development (OECD) QSAR Application Toolbox (OECD QSAR Toolbox). Haptenation reactions of carbonyl compounds may occur following reaction with primary amine groups (e.g. lysine) and guanidino groups (e.g. arginine) (Roberts et al. 1999). This is supported by observations in vivo, in which diacetyl has been shown to bind to arginine residues of proteins including haemoglobin and albumin (Fennel et al. 2015).

Respiratory sensitisation

No data are available to evaluate respiratory sensitisation.

Observation in humans

In maximisation tests in human volunteers, 2% diacetyl (n = 29), 4% acetylpropionyl (n = 25), in petrolatum did not induce sensitisation. Structurally related 2,3-hexanedione (4%, n = 25) did not induce sensitisation, while a 5% preparation of 2,3-heptanedione induced a response in 2 of 25 volunteers. Further details are not available (MAK Commission 2016; MAK Commission 2020).

In a patch test, “very little” diacetyl on a moistened 1 cm^2 cloth, was applied to the skin of a female patient. The patient was reported to present with oedematous dermatitis that occurred on the face and hands after application of perfume and when exposed to tobacco smoke, wood smoke and coffee. An intense reaction was reported after 2 hours, after which the patch was removed. The reaction was reported to have persisted for several days. No reactions were reported in the control group of four volunteers. In a separate study, a patch test with 1% diacetyl resulted in a reaction after 24 and 48 hours in a patient with eczematous vesicular skin changes. The condition was reported to have developed 3–4 weeks after starting work at a confectionary plant and would recur following contact with

flavour mixtures. No reaction occurred in the control group of 60 volunteers (MAK Commission 2016).

Repeat dose toxicity

Oral

Based on the available data, diacetyl and acetylpropionyl are not expected to cause serious systemic health effects following repeated oral exposure.

In a 90 day sub-chronic oral toxicity study, SPF derived CFE rats received 0, 10, 30, 90 or 540 mg diacetyl/kg bw/day by oral gavage (n = 15/sex/dose). Food consumption was similar across groups; however, body weight was significantly reduced and water consumption significantly increased in the high dose group. Relative liver and kidney weights were reduced and adrenal weights were increased in the high dose group. Haematological changes in this group included decreased haemoglobin, and increased reticulocyte and leucocyte count. Sloughing and ulceration were reported in the gastric epithelium in all male rats and 14/15 female rats in the high dose group. No changes were reported in the lower dose groups. A no observed adverse effect level (NOAEL) of 90 mg/kg bw/day was established by the study authors (ACGIH 2013; Colley et al. 1969).

Dermal

No data are available to evaluate dermal repeated dose toxicity.

Inhalation

Based on the available data, diacetyl and acetylpropionyl are expected to cause serious systemic health effects, including the development of bronchiolitis obliterans, following repeated inhalation exposure and warrant hazard classification (see **Recommendations** section). Although pathogenesis of bronchiolitis obliterans is not well understood, epithelial injury, as described in both acute and chronic inhalation studies, appears to be a critical first step. In addition to sustained cytotoxicity and cell proliferation, some evidence suggests immunological injury of airway epithelium may play a key role. This may occur following haptenation reactions of diacetyl and acetylpropionyl with airway epithelium membrane proteins.

In a 105 week study, Wistar Han rats (50/sex/dose) were exposed to diacetyl vapour at concentrations of 0, 12.5, 25 or 50 ppm (0, 44, 88 or 176 mg/m^3) for 6 hrs plus T_{90} (time required to reach inhalation equilibrium; 12 minutes)/day, 5 days/week for 105 weeks (whole body inhalation). In the high dose group (50 ppm), survival was significantly reduced for male (44%) mice, but not female (60%) mice, compared to controls (72% and 68%, respectively). The authors reported the primary cause of early death in this group as inflammation of the lungs. No significant difference was reported in female rats. Mean body weights were significantly reduced in the 50 ppm treatment groups. Eye abnormalities were the most frequent clinical observation in both male and female rats at both 25 and 50 ppm. Severe suppurative inflammation was reported in the nose of almost all rats in the 50 ppm groups, and mild inflammation in most male and some female rats in the 25 ppm groups. Non-neoplastic lesions were reported in the respiratory and olfactory epithelium of the nose in the 25 ppm and 50 ppm groups. Lesions in the larynx, trachea and lungs were significantly increased in the 50 ppm groups. The most common lesions in the lungs included epithelial hyperplasia (characterised by increased epithelial height and cell density) in the bronchi and bronchioles of most rats in the 50 ppm groups. Chronic active inflammation of the cornea

was reported in rats in the 50 ppm (28 males, 31 females), 25 ppm (16 males, 23 females) and 12.5 ppm (6 males, 6 females) treatment groups (NTP 2018).

Similar effects were reported in B6C3F1 mice (50/sex/dose) exposed to diacetyl vapour using the same treatment protocol. In the high dose group (50 ppm), survival was significantly reduced for both male (50%) and female (34%) mice compared to controls (70% and 72%, respectively). The authors reported the primary cause of early death in these groups as inflammation of the upper respiratory tract (nose, larynx and/or trachea). Severe suppurative inflammation was reported in the nose of all mice in the 50 ppm groups (50/50 male and females), most mice in the 25 ppm groups (47/50 males, 50/50 females) and some mice in the 12.5 ppm groups (4/48 males, 20/50 females). Non-neoplastic lesions were reported in the respiratory and olfactory epithelium of the nose in the 25 and 50 ppm groups. Lesions in the larynx, trachea and lungs were significantly increased in the 50 ppm groups. The most common bronchial lesion in both male (34/50) and female (38/50) mice was minimal to moderate extrapulmonary bronchus epithelium regeneration (NTP 2018).

In a 90 day study, Wistar Han rats (10/sex/dose) were exposed to diacetyl vapour at concentrations of 0, 6.25, 12.5, 50 or 100 ppm (0, 22, 44, 176 or 352 mg/m^3) for 6 hrs plus T_{90} (10 minutes)/day, 5 days/week for 14 weeks (whole body inhalation). An additional group (10/sex/dose) was exposed to the chemical for 23 days for clinical pathology analysis. Mean body weights were significantly reduced in the 100 ppm treatment groups. Abnormal breathing was reported in 5/10 males and 5/10 females in the 100 ppm group. Haematological changes were reported on day 23, and at study termination including increased neutrophil counts in the 100 ppm females; and increased erythrocyte count, and packed cell volume in 100 ppm males and females. The authors hypothesised that the increased erythrocyte counts resulted from respiratory lesion induced hypoxia and mild secondary erythrocytes. Relative lung weights were significantly increased in 100 ppm females. Relative heart weights were increased in both males and females at 100 ppm. Significant decreases in absolute liver and thymus weights were reported in 100 ppm males and females. Treatment resulted in a significant increase in non-neoplastic lesions in the respiratory tract (nose, larynx, trachea and lung) of male and female rats in the 50 and 100 ppm treatment groups. These included necrosis, squamous metaplasia and hyperplasia of the respiratory epithelium (NTP 2018).

Similar effects were reported in B6C3F1 mice (10/sex/dose) exposed to diacetyl vapour using the same treatment protocol. This included haematological changes (increased neutrophil count; and decreased mean cell volume and mean cell haemoglobin were reported in 50 and 100 ppm males and 100 ppm female mice) and increased relative lung weights in 50 and 100 ppm males, and 100 ppm female mice. Minimal to moderate suppurative inflammation was reported in all males and females in the 50 and 100 ppm groups. Minimal to mild respiratory epithelium necrosis occurred in all mice in the 50 and 100 ppm groups, and 8/10 males and 4/10 females in the 25 ppm group (NTP 2018).

In a 90 day study, Wistar Han rats (10/sex/dose) were exposed to acetylpropionyl vapour at concentrations of 0, 6.25, 12.5, 50 or 100 ppm (0, 26, 51, 102, 205 or 409 mg/m^3) for 6 hrs, 5 days/week for 14 weeks (whole body inhalation). Abnormal breathing, sneezing and eye abnormalities were reported in the 50 and 100 ppm groups. Treatment resulted in a significant increase in non-neoplastic lesions in the respiratory tract (nose, larynx, trachea and lung) of male and female rats in the 25, 50 and 100 ppm treatment groups. These included squamous metaplasia and hyperplasia of the respiratory epithelium. Similar effects were reported in B6C3F1 mice (10/sex/dose) exposed to acetylpropionyl vapour using the same treatment protocol. The NOAEC for respiratory tract effects in both rats and mice was reported as 12.5 ppm (51 mg/m^3). The NOAEC for non-respiratory tract effects in mice was 25 ppm. (Environment and Climate Change Canada Health Canada 2019; NTP 2017).

In a sub-acute study, male Wistar Han rats were exposed to diacetyl and acetylpropionyl at nominal concentrations of 0, 100, 150 or 200 ppm (diacetyl: 0, 352, 528 or 704 mg/m^3; acetylpropionyl: 410, 614, 818 352 mg/m^3) for 6 hrs/day, 5 days/week (whole body) for 12 exposures. Half of the animals were sacrificed the day after the treatment (post exposure group). The remaining half (recovery group) were sacrificed after a further 2 weeks. Early mortality was reported in rats exposed to 200 ppm diacetyl (8/20) and acetylpropionyl (12/20). Causes of mortality was reported to be related to respiratory tract necrosis, ulceration and inflammation. Body weights of rats exposed to 150 or 200 ppm of either chemical were decreased at all time points, with animals exposed to 200 ppm of either chemical continuing to lose weight during the recovery period. Absolute lung weights of the exposed animals did not vary significantly from the control group. Bronchial lesions occurred at similar incidence and severity in the diacetyl and acetylpropionyl exposed groups. Bronchial fibrosis occurred at 150 and 200 ppm, in post-exposure as well as recovery groups with fibrosis scores of 1.6 and 4.0 and the average number of bronchial fibrotic lesions of 6 and 25 in the diacetyl and acetylpropionyl groups, respectively. In the recovery group, airway resistance increased significantly in diacetyl exposed rats and lung compliance was significantly decreased in the diacetyl and acetylpropionyl exposed groups. Necrosis and ulceration of bronchial epithelium were significantly reduced following a 2 week recovery period. However, these animals still exhibited similar incidence and severity of fibrosis when compared to the post exposure group. (Morgan et al. 2016). The results indicate that diacetyl and acetylpropionyl are of similar reactivity.

In a sub-acute inhalation study, Wistar Han rats (n=6/group) and B6C3F1 mice (n=6/group) were exposed to acetylpropionyl at nominal concentrations of 0, 50, 100, or 200 ppm (0, 204, 409, 819 mg/m^3) for 6hrs/day 5 days/week (whole body) for up to 2 weeks. In study 2 (male rats and mice only), broncho-alveolar lavage fluid (BALF) was collected after 1, 3, 5, or 10 days of exposure. In study 1, mortalities were reported in 4/6 male and 1/6 female rats in the high dose group after one exposure. In study 2, all rats survived; however, mortalities were reported for 2/6 mice in the high dose group on day 6 and day 9 of exposure. The concentration of neutrophils in the BALF were elevated in the high dose male rats and mice after 5 and 10 exposures, respectively. Histopathological changes including mucosal inflammation and olfactory epithelium atrophy were reported in noses of rats and mice at all exposure levels and increased in incidence and severity with increasing concentration. All surviving high concentration male and female rats developed bronchial fibrosis (Morgan et al. 2012).

Male C57B1/6 mice were exposed to diacetyl by oropharyngeal aspiration (bypassing the nose) using a number of exposure profiles relevant to workplace conditions (Morgan et al. 2008). These included:

(a) Sub-acute exposure to 0 (n = 7), 200 (704 mg/m^3, n = 10) or 400 ppm (1408 mg/m^3, n = 15) for 6 hours/day for 5 days. In the 200 ppm group 6/10 mice became moribund and had to be sacrificed prior to the end of the study. In the 400 ppm group 2 mice died and the remaining mice were sacrificed as they became moribund. Acute necrotising rhinitis and laryngitis were reported in moribund animals. Acute necrotising bronchiolitis was reported in the 400 ppm group.

(b) Intermittent low exposure (nasal only) for 1 hr/day, 5 days/week, for 2−4 weeks at 100, 200 and 400 ppm (354, 704, 1408 mg/m^3; n = 5/group) resulted in peribronchial and peribronchioloar lymphocitic inflammation in some animals in the low dose and all animals in the high dose groups.

(c) Intermittent high exposure for 15 minutes, twice per day, 5 days/week for 2 weeks at 1200 ppm (4225 mg/m^3, n = 5) resulting in peribronchial and peribronchiolar lymphocytic inflammation.

(d) Sub-chronic exposure for 6 hours/day, 5 days/week, for 6 or 12 weeks at 0, 25, 50 or 100 ppm (0, 88, 176, 352 mg/m^3; n = 5/group) resulting in moderate nasal injury and lymphocytic bronchiolitis accompanied by epithelial atrophy.

A no observed adverse effect concentration (NOAEC) was not determined for any exposure profile, as significant effects were reported at all concentrations tested.

Observation in humans

Diacetyl is used widely in food industries where occupational exposure has been associated with respiratory impairment and the development of bronchiolitis obliterans (characterised by changes in pulmonary function that are associated with scarring and constriction of small airways) (Holden and Hines 2016; NIOSH 2016; NTP 2018).

Numerous case reports and cross-sectional surveys are available detailing the development of work related bronchiolitis obliterans in workers at microwave popcorn manufacturing, coffee processing, flavouring manufacturing and chemical manufacturing facilities. Exposure conditions were reported to vary widely. For example, at 6 microwave popcorn plants investigated between 2000 and 2003, diacetyl air concentrations ranged from 0.63 to 57.2 ppm (2.2-201 mg/m^3) in the mixing areas, and 0.019 to 3.0 ppm (0.067-11 mg/m^3) in packaging areas. It was noted that workers may have also been exposed to other chemicals including acetoin and acetaldehyde (NTP 2018).

Symptoms in affected workers included progressive shortness of breath on exertion; chronic non-productive cough; wheezing; skin, eye, nose and throat irritation. Analysis of 6 cross-sectional NIOSH surveys conducted at microwave popcorn manufacturing facilities indicated increased risk of fixed air-way obstructions was present at exposures as low as 0.02 ppm (0.07 mg/m^3)(Kanwal et al. 2006).

Using this data NIOSH conducted a quantitative risk assessment based on the impairment findings from a representative popcorn manufacturing facility. This utilised a benchmark dose (BMD) procedure to estimate the excess prevalence of respiratory impairment (reduced forced expiratory volume in one second (FEV_1) less than the lower limit of normal) as a function of prior exposure history. The BMD was defined as the exposure that resulted in the maximum allowable increase in impairment over a set time period (45 years). Using this model, an excess risk of 1 in 1000 corresponded to approximately 0.001-0.005 ppm diacetyl (3.5-17.5 µg/m^3). This analysis formed the basis of the NIOSH recommended REL (see **International regulatory status – Exposure standards**) (NIOSH 2016).

Genotoxicity

Based on available data the chemicals in this group are considered to have genotoxic potential in vitro; however, due to their rapid metabolism they are not expected to be genotoxic in vivo.

In vitro

In vitro studies with diacetyl gave mixed results:

- Mixed results were reported in a bacterial reverse mutation assay in *Salmonella typhimurium.* Positive results were reported for *S. typhimurium* TA97 with and without hamster and rat liver S9 mixes at concentrations of 10–1000 µg/plate. A reproducible dose related increase in the number of revertants was reported over the range 10−333 µg/plate; however, the increase was considered weak as it was less than 2 fold. Negative or equivocal responses were reported for *S. typhimurium* TA100, TA98, TA1535 (NTP 2018).
- Mixed results were reported in a bacterial reverse mutation assays in *S. typhimurium* and *Escherichia coli.* Positive results were reported in *S. typhimurium* TA97 without metabolic activation and *E. coli* WP2 *uvrA/*pKM101 (analogous to TA102) with and without metabolic activation at concentrations of 100–2000 µg/plate. Results in TA97 with metabolic activation were equivocal. Negative or equivocal responses were reported for *S. typhimurium* TA100 and TA98, with and without metabolic activation (NTP 2018).
- Mixed results were reported in a bacterial reverse mutation assay in *S. typhimurium.* Positive results were reported in *S. typhimurium* TA102 (base substitution strain) with and without rat liver S9 metabolic activation at concentrations in the range 0.17–17200 µg/plate. Negative responses were reported for *S. typhimurium* TA98 (frame shift strain) and TA100 (Aeschbacher et al. 1989).
- Positive results were reported in a sister chromatid exchange (SEC) assay in Chinese hamster ovary (CHO) AUXB1 cells following treatment for 20−22 hours at concentrations 125 and 250 µM. Up to 82% (at 125 µM) of SCE activity induced by diacetyl was blocked in the presence of 1mM bisulfite (reacts with carbonyl moieties) (Tucker et al. 1989).
- Positive results were reported in an in vitro mammalian forward mutation assay in mouse lymphoma L5178Y cells with human S9 metabolic activation at concentrations 100–250 µg/mL. However, the concentration required to achieve a 2 fold increase in mutations results in a 62% growth reduction (Whittaker et al. 2008).
- Negative results were reported in a chromosomal malsegregation assay in *Saccharomyces cerevisiae* D61.M exposed to diacetyl at 148–393 µg/mL. Cultures were tested at 28°C or under conditions of cold shock. Treatment did not induce chromosome loss, mitotic recombination or respiratory deficient mutants (Zimmermann and Mohr 1992).

In vitro studies with acetyl propionyl reported negative results:

- Negative results were reported in a bacterial reverse mutation assay in *S. typhimurium* TA98, TA100 and TA102 with and without rat liver S9 metabolic activation at concentrations of 0.009–900 µmol/plate (Aeschbacher et al. 1989). TA97 was not used in this study.

In vivo studies with diacetyl were reported negative results:

- In a mammalian erythrocyte micronucleus test, male B6C3F1/N mice (n=5/dose) were treated intraperitoneally with diacetyl once daily for 3 days at doses ranging from 7.8–500 mg/kg bw/day. The incidence of micronuclei in bone marrow polychromatic erythrocytes (PCEs) did not increase in any of the treated groups, indicating a lack of clastogenicity (NTP 2018).
- Following the 90 day inhalation studies in rats and mice (see Repeat Dose Toxicity section) peripheral blood samples were obtained and analysed for frequency of micronucleated PCEs and normochromatic erythrocytes (NCEs). The incidence of micronuclei in peripheral blood PCEs and NCEs did not increase in any of the treated

groups. The percentage of PCEs among circulating red blood cells was unaffected by the treatment (NTP 2018).

Diacetyl and acetylpropionyl contain structural alerts (α-dicarbonyls) for DNA binding via Schiff base formation as profiled by the OECD QSAR Toolbox v4.2. This is supported by in vitro studies where diacetyl has been shown to form 2-deoxyguanosine adducts in SH-SY5Y cells in a dose dependent manner (More et al. 2012).

Carcinogenicity

Based on the weight of evidence, diacetyl and acetylpropionyl are considered to have carcinogenic potential following inhalation exposure. Sustained cytotoxicity and cell proliferation resulting from chronic diacetyl or acetylpropionyl exposure in combination with the DNA binding potential may contribute to the induction of respiratory tumours. However, data are not sufficient for hazard classification.

In the 105 week inhalation study described above (see Repeat dose toxicity – inhalation), rats (50/sex/dose) were exposed to diacetyl vapour at concentrations of 0, 12.5, 25 or 50 ppm (0, 44, 88 or 176 mg/m^3) for 6 hrs plus T_{90} (12 minutes)/day, 5 days/week for 105 weeks (whole body inhalation). Three squamous cell carcinomas and one squamous cell papilloma of the nasal mucosa occurred in male rats exposed to 50 ppm diacetyl. Three squamous cell carcinomas of the nasal mucosa were reported in female rats exposed to 50 ppm diacetyl. No squamous cell carcinomas or papillomas were reported in the concurrent male or female controls, or in the NTP historical control database. The authors concluded that there was “some evidence of carcinogenic activity” in rats based on the incidence of these tumours.

In the 105 week mouse inhalation study described above (see Repeat dose toxicity – inhalation) adenocarcinomas occurred in the nasal cavity of two female mice exposed to 50 ppm diacetyl vapour. No nasal adenocarcinomas or other nasal neoplasms occurred in other treatment groups, chamber controls or in the NTP historical control database. The incidence of adrenal cortical adenoma was significantly increased in the 12.5 ppm male mice (8/49) compared to controls (2/50). However, the increase was within the historical control range and no exposure concentration response was observed. The authors concluded that there was “equivocal evidence of carcinogenic activity” in female mice and “no evidence of carcinogenic activity” in male mice based on the tumour incidence.

In a 24 week study, female A/He mice (n = 20) were treated with diacetyl (intraperitoneal) at concentrations of 1.7 or 8.4 mg/kg, once per week. A significant increase in the number of lung tumours per mouse was reported in the high dose treatment group. However, results were not replicated in a follow up study (15/sex/group) (Stoner et al. 1973).

In male Fischer 344 rats, diacetyl (oral gavage, 150–400 mg/kg) induced ornithine decarboxylase activity up to 100-fold after 16 hours. Treatment also induced a greater than 10 fold increase in DNA synthesis. The authors concluded that diacetyl has potential tumour promoting properties (Furihata et al. 1985).

Reproductive and development toxicity

Based on limited data, the chemicals in this group are not expected to cause specific adverse effects on development following oral exposure.

In a non-GLP compliant developmental toxicity study, pregnant rats (n = 21-23/dose), mice (n = 21-24/dose) and hamsters (n=21-25/dose) were administered diacetyl by gavage at 0, 16,

74.3, 345 or 1600 mg/kg bw/day on gestational days (GD) 6–15, 6–15 or 6–10, respectively. Treatment produced no adverse effects to survival, body weight, or reproductive parameters in the dams; or to foetal external, skeletal and soft tissues. A maternal and foetal NOAEL of 1600 mg/kg bw/day was reported (EFSA 2011).

Neurotoxicity

Based on limited data, the chemicals in this group are not expected to be neurotoxic.

In an acute toxicity study, male Hla(SD)CVF rats (n = 6) were exposed to 270 ppm acetylpropionyl vapour resulting in increased expression of inflammatory mediators IL-6 and NO-synthase-2, and decreased expression of the growth factor Vegf-A mRNA in various brain regions. Neuropathological injury was not observed in histopathological sections of the olfactory bulb (Hubbs et al. 2012).

Diacetyl has been reported to accelerate amyloid-β^{1-42} aggregation and increase amyloid-β induced cytotoxicity in vitro (More et al., 2012). However, as β-amyloid has been reported to interfere with the results of 3-(4,5-dimethylyhiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assays and as the cellular model relied on undifferentiated cells, these results cannot be extrapolated to humans (EFSA 2013).

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