Recommendation from the Scientific Committee on Occupational Exposure Limits for Diacetyl

SCOEL/SUM/149
June 2014

Employment, Social Affairs and Inclusion

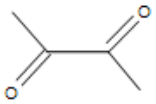
Table of Contents

Recommendation from the Scientific Committee on Occupational Exposure Limits for Diacetyl

8-hour TWA:	0.02 ppm (0.07 mg/m^3)
STEL:	0.10 ppm (0.36 mg/m^3)
BLV:	Not assigned
Additional categorisation:	-
Notation:	-

This evaluation is an update of the SCOEL Recommendation from 2010 and is based on OSHA (2007a), Harber *et al* (2006), IUF (2007), NTP (2007) and other sources as referenced. A final literature search was performed in January 2013.

1. Substance identification, physico-chemical properties

Name:	Diacetyl
Synonyms:	2,3-Butanedione; butane-2,3-dione; dimethyl glyoxal; 2,3-diketobutane; 2,3-dioxobutane
Molecular formula:	$C_4H_6O_2$
Structural formula:	
CAS No.:	431-03-8
Molecular weight:	86.09 g/mol
Boiling point:	88 °C
Melting point:	-2.4 °C
Vapour pressure (25 °C):	7.6 kPa
Water solubility (25 °C):	200 g/l
Log P_{ow}:	-1.34
Relative density (20 °C):	0.99 g/cm^3 at 20 °C
Flash point:	6 °C
Conversion factors: (20 °C, 101.3kPa)	1 ppm = 3.58 mg/m^3 1 mg/m^3 = 0.279 ppm

EU classification: No harmonised classification

2. Occurrence/use and occupational exposure

2.1. Occurrence and use

Diacetyl is found in bay leaves and other plant oils, beer, butter, coffee, vinegar and other food products and is also a metabolite of acetaldehyde in mammals. The substance is synthesised industrially and used as an artificial flavouring in a wide range of frozen and snack foods (including microwave popcorn and potato/corn chips), confectionery, baked goods, dairy products including processed cheese, sour cream and cottage cheese, commercial baking mixes, icings, salad dressings, sauces, marinades and other

processed foods and beverages (IUF 2007, NTP 2007). Diacetyl imparts an artificial butter or creamy flavour to these products.

Exposure is widespread in food manufacturing industries in which workers handle diacetyl in the liquid form and are potentially exposed to diacetyl as vapours, fumes or adsorbed on particles, in the manufacturing process or at various stages of production (NTP 2007, IUF 2007). High exposures with consequent adverse health effects in exposed workers have been reported during the manufacture of microwave butter-flavoured popcorn, a process that uses high concentrations of diacetyl relative to other identified uses.

Cigarette smoke contains 300–430 µg diacetyl per cigarette (Fujioka and Shibamoto 2006). Diacetyl has also been found in $PM_{2.5}$ particles emitted from motor vehicles (Rao *et al* 2001).

2.2. Occupational exposure

In Missouri, workers in a microwave popcorn production plant were evaluated by NIOSH (Kreiss *et al* 2002). Nine workers formerly employed in the plant had developed fixed airways obstructive lung disease (Plant A, Appendix 2). Investigations into respiratory exposures at this plant carried out in 2000 showed that workers in the microwave production areas were exposed to particulates (largely salt and oil/grease aerosols) and a range of organic vapours from flavourings (Kullman *et al* 2005). At qualitative sampling for volatile organic compounds (VOCs) in the air, over 100 different compounds in the microwave area were detected. The predominant compounds identified in the microwave mixing room included the ketones diacetyl, methyl ethyl ketone, acetoin and 2-nonanone, and acetic acid. Diacetyl, the predominant ketone in the plant, was present in concentrations ranging from below detectable limits to 98 ppm, with a mean of 8.1 ppm (standard deviation, SD, 18.5 ppm). The average ketone concentrations were highest in the microwave mixing room where the 10 area samples had a mean diacetyl concentration of 37.8 ppm (SD 27.6 ppm) and a mean acetoin concentration of 3.9 ppm (SD 4.3 ppm) (Kullman *et al* 2005).

Besides diacetyl, other diketones (e.g. 2,3-pentanedione, 2,3-hexanedione and 2,3-heptanedione) are also used in butter flavouring mixtures (Day *et al* 2011).

Further investigations by NIOSH showed that respiratory symptoms and airways obstruction were more frequent in oil and flavourings mixers with longer work histories and in packaging[1]-area workers near non-isolated tanks of oil and flavourings (Kanwal *et al* 2006). Even short-term peak flavouring exposures were reported to present a risk of lung damage, and average 8-hour diacetyl exposures as low as 0.02 ppm were measured in a work area where bronchiolitis obliterans (for a description of the disease, see Section 3.5.1) occurred in workers mixing butter flavourings with heated oil (Kanwal *et al* 2006, Kreiss 2007). In this case, peak exposures exceeded 80 ppm (Kanwal *et al* 2006). During flavour manufacture, the compounding of powder and liquid products caused the highest exposure levels (Martyny *et al* 2008).

In popcorn plants, exposure in the mixing room is characterised by high exposures and high peak levels due to the batch mixing operations of the flavouring ingredients, which comprise soybean oil, salt, butter flavourings and colouring agents. The mixing operations with flavourings are the primary point source for the release of volatile organic gases and vapours into plant air. In the packing line, popcorn and flavourings are automatically added to the popcorn bags, and afterwards the bags are sealed, labelled and automatically enclosed in plastic wrap (Kullman *et al* 2005).

[1] "Packing" or "packaging" are synonymously used in the publications.

To evaluate the toxicological effects, one therefore has to consider different possible metrics for exposure: average, peak and cumulative. Peak exposures are common in the mixing room, whereas exposures in the packing line are much more uniform.

Implementation of recommended exposure controls in recent years has resulted in lower exposure levels (Harber *et al* 2006). Between 2001 and 2003, exposure controls were instituted in the Missouri plant. In the mixing room, average diacetyl concentrations fell from about 38 ppm in 2001 to 0.46 ppm in 2003; in packaging, the average concentration was reduced from 1.69 to 0.002 ppm (NIOSH 2003); for details see Kanwal *et al* 2011. Another report indicated reduction in peak exposures during mixing, from 462 to 0.97 mg/m^3 (Pendergrass 2004).

2.3. Methods of exposure monitoring and analysis

Different methods have been developed over time for measuring acetoin and diacetyl concentrations in workplace air.

NIOSH Method 2557 was first used for determination of airborne diacetyl concentrations in several key studies conducted by this institute (Kanwal *et al* 2011, Kreiss *et al* 2002, Lockey *et al* 2009). Diacetyl was collected on carbon molecular sieve sorbent tubes, followed by extraction with acetone/methanol (99:1) and analysis by gas chromatography with a flame ionisation detector (GC-FID) (Cox-Ganser *et al* 2011, Pendergrass 2004); the limit of detection (LOD) may be as low as 2.3 ppb (8.3 µg/m^3). Later, it was discovered that this method may underestimate the diacetyl concentrations (Ashley *et al* 2008) depending on the diacetyl concentration, air humidity, sampling storage duration and to a minor extent on the sampling flow rate. For example, the laboratory recovery at 0.5 ppm diacetyl varied from less than 10 to 100 %, depending on air humidity. High diacetyl concentrations showed highest recoveries and were less influenced by air humidity. Based on laboratory studies, a mathematical correction procedure was developed. The procedure does, however, not account for a potential influence of other airborne chemicals on recovery (Cox-Ganser *et al* 2011), neither can the correction method reliably correct concentrations below the detection limit (for further discussion, see Table B in Appendix 1). Furthermore, the NIOSH method does not capture particulate or particulate bound diacetyl (White *et al* 2010).

OSHA then developed the PV2118 method to improve storage stability performances, which was subsequently replaced by the fully validated OSHA methods 1012 and 1013 (OSHA 2008a,b). Method 1013 is streamlined for monitoring low ppm levels, and method 1012 is optimised for ppb levels. In both methods, two 600 mg silica gel sorbent tubes are used in series with a recommended sampling time of 3 hours (9 l) and ethyl alcohol/water (95:5) for sample extraction. In method 1013, GC-FID is used; the LOD is 0.0031 ppm (0.011 mg/m^3) for acetoin and 0.0034 ppm (0.012 mg/m^3) for diacetyl. In method 1012, acetoin and diacetyl are derivatised using *O*-pentafluorobenzyl hydroxylamine hydrochloride (PFBHA) and analysed by gas chromatography using an electron capture detector (GC-ECD); the LOD is 0.447 ppb (1.61 µg/m^3) for acetoin and 0.389 ppb (1.37 µg/m^3) for diacetyl.

More recently, the UK Health & Safety Laboratory has developed a simple and reliable method for measurement of occupational exposure to diacetyl in the presence of other organic compounds. Diacetyl and the other volatile organic compounds are collected on Chromosorb—106 (C-106) sorbent tubes and analysed by thermal desorption and gas chromatography-mass spectrometry (GC-MS). A sampling time of 4 hours allows detection of less than 0.1 ppb (Pengelly 2012).

Due to its limitations, the NIOSH Method 2557 should not be used to measure airborne diacetyl in future studies (Cox-Ganser *et al* 2011). The evaluation by SCOEL of the NIOSH method was exclusively to obtain exposure-response relationships from epidemiological studies where the NIOSH method had been used for exposure assessment.

SCOEL did an attempt to obtain information on the order of magnitude of the bias of the NIOSH Method 2557. Results with biased (NIOSH) and unbiased (OSHA) methods were available from simultaneously conducted field studies in two facilities that mixed and formulated food flavourings (Ashley *et al* 2008). The unbiased methods (OSHA method PV2118 and modified OSHA method PV2118 (Ashley *et al* 2008, OSHA 2007b) had, however, higher detection rates than the NIOSH method, the latter method showing many results below the detection limit where the unbiased methods detected diacetyl in the air (Appendix 1). Thus, from this field study, it was not possible to establish a fixed correction factor for adjustment of results obtained with the NIOSH method. However, it was possible to obtain an idea about the magnitude of the bias of the NIOSH method by comparing results on airborne diacetyl levels that were detectable by both methods. This approach applies only to high concentration levels where least bias is expected (Cox-Ganser *et al* 2011). The comparison suggests that a single result obtained by means of the NIOSH method may underestimate the true concentration by a factor of 1–13 (Appendix 1). The mean bias was around a factor of 3–4.

It is noted that in the high-concentration range, correction by a factor of 3 may both under- and overestimate the bias of results obtained with the NIOSH method, depending on the many parameters discussed above. The correction factor reported by White *et al* (2011) was approximately 2, which is reasonably close to the factor of 3 derived by SCOEL.

At low concentrations, the bias may be more pronounced and especially important when a considerable number of samples have concentrations below the LOD. In the study of Lockey *et al* (2009), 49 % of the samples were below the LOD, and these concentrations were set to LOD/2 in the statistical analyses (White 2011). The same procedure was adopted by Kanwal *et al* (2011) and by NIOSH (2011) in the evaluation of the data of Kreiss *et al* (2002) where about 40 % of the samples (NIOSH 2011) had diacetyl levels below the LOD. The Ashley *et al* (2008) field study provides some insight into the potential bias introduced by replacing a concentration below the LOD by LOD/2. The authors stated that "--it was deemed inappropriate to adjust by dividing by 2 or the square root of 2—". In Table B (Appendix 1), it appears that the average underestimation is by a factor of 20 for concentrations below the LOD with a range from 4.2 to 295.

3. Health significance

Diacetyl may react with the guanidine group of arginine, forming open-chain and cyclised adducts which may cause specific auto-antibody responses (Mathews *et al* 2010). Also, diacetyl may react with cysteine forming cyclic derivatives (thiazole, thiazoline, oxazole and pyrazine) (Marchand *et al* 2011). Diacetyl can cause protein cross-linking (Kovacic and Cooksy 2010) and DNA adducts (see Section 3.6). Diacetyl has an electron affinity that is comparable to quinones and dinitrophenol. The two adjacent carbonyl groups favour delocalisation of the electron of the radical anion. Thus, diacetyl has been used to stabilise electrons in biological systems and for example, enhanced the response of bacterial spores to X-rays. In general, reactive carbonyl species may cause formation of reactive oxygen species (Kovacic and Cooksy 2010).

3.1. Toxicokinetics

3.1.1. Human data

No information on experimental toxicokinetics of diacetyl in humans was available in the open literature. Based on the structure and reported physicochemical characteristics, the substance can be predicted to be readily absorbed, widely distributed, metabolised and excreted. The primary route of exposure is predicted to be inhalation, following exposure to diacetyl vapour.

A considerable amount of diacetyl may be absorbed through the skin, based on the predicted absorption obtained from the Centers for Disease Control and Prevention Skin Permeation Calculator (CDC 2013). The calculator allows three different estimates, all based on the molecular weight (86), the log of the octanol-water partition coefficient (-1.34) and the diacetyl concentration in water, which is considered to be 990 mg/ml as this is the density of pure diacetyl. The three estimates ranged from 0.014 to 0.173 mg/hour/cm^2, corresponding to 28–346 mg/hour for a 2 000 cm^2 surface area exposure.

3.1.2. Animal data

A computational fluid dynamics-physiologically based pharmacokinetic model was used to compare diacetyl absorption and tissue concentrations in the rat and the human respiratory tracts. In rats, extensive enzymatic metabolism occurred in the mucosal tissue throughout the entire airways; these reactions could be described by two Michaelis-Menten pathways. One had a high affinity (K_m: 10 µM) and a low capacity, whereas the other had a low affinity (K_m: 6 500 µM) and a high capacity. At 1 ppm airborne exposure (tissue concentration < 20 µM), the low affinity pathway would not be anticipated to be quantitatively important. Clearance from the mucosa was dominated by enzymatic metabolism, and reaction with arginine played a negligible role due to slow reaction, which does not rule out a slow accumulation of arginine adducts. In rats, the initial whole body uptake was 78 % at 5 ppm and 62 % at 22 ppm, i.e. the diacetyl uptake efficiency was higher at the lower exposure levels as the *in situ* metabolism is important in controlling the overall uptake. Because of saturation of the metabolic pathways, increased distal penetration occurred at high concentrations. It was calculated that in rats, at 1 ppm, less than 2 % of inspired diacetyl penetrates through the small bronchi and enters the bronchioles. In humans, nose-breathing at rest was estimated to result in 8 % penetration through the small bronchi to the bronchioles, which slightly increased at mouth-breathing at rest. At mouth-breathing during light exercise, it was predicted that 24 % penetrated through the small bronchi to the bronchioles. The bronchiolar tissue diacetyl concentration at 1 ppm was estimated to be even more increased in humans as compared to the estimated concentration in rats (0.002 µM). The tissue concentration was estimated to be 5-fold higher than in the rat in nose-breathing humans at rest, 7-fold higher in mouth-breathing humans at rest, and 20- to 40-fold higher at mouth-breathing with light exercise (Gloede *et al* 2011). Overall, this suggests an assessment factor of 40 for extrapolating atmospheric doses causing bronchiolar damage in rats to humans.

The interaction between diacetyl (100 ppm) and butyric acid (30 ppm) was studied in the isolated upper respiratory tract of anesthetised rats where diacetyl is metabolised by diacetyl reductase of which butyric acid is a potent inhibitor. For diacetyl alone, the uptake was 36 %, whereas in the presence of butyric acid it was statistically significantly reduced to 31 %. The butyric acid uptake (> 90 %) was modelled to inhibit diacetyl reductase by 50–75 %. Thus, inhibition of the upper airway metabolism may increase penetration of diacetyl to the lower airways where its toxicity can be expressed (Morris

and Hubbs 2009). It is noted that the butyric acid concentration in this study was fairly high in terms of eye and upper airways irritation.

As reported by NTP (2007), a single dose of radiolabelled [^{14}C]-diacetyl (1.58, 15.8 or 158 mg/kg [0.0184, 0.184 or 1.84 mmol/kg]) administered to male Fischer 344 rats via intragastric gavage resulted after 72 hours in the exhalation of 82.0, 72.7 and 54.3 % of the dose, respectively, as carbon dioxide. In urine, the excreted amounts were 6.86, 15.7 and 34.1 %, respectively. At the high dose, elimination via volatile organics in breath and faeces was very low (maximum of 0.8 and 2.25 %, respectively). In the carcass and tissues, 6–7 % of the dose was recovered. At all tested levels, diacetyl was rapidly metabolised and excreted; excretion of radioactivity in urine, faeces and expired breath accounted for 86–87 % of the total dose recovered in 24 hours (RTI 1997).

Diacetyl is a metabolite of acetaldehyde in mammals. The metabolism of diacetyl, acetoin (3-hydroxy-2-butanone) and 2,3-butanediol, all metabolites of acetaldehyde, was investigated in rat liver homogenates, liver perfusion, and *in vivo* experiments. Diacetyl and acetoin were rapidly reduced to 2,3-butanediol, but there was very little oxidation of acetoin and 2,3-butanediol to diacetyl. Acetoin and 2,3-butanediol were more readily accumulated in the brain than diacetyl (Otsuka *et al* 1996).

Overall, diacetyl metabolism along the respiratory tract plays an important role in lowering the penetration of diacetyl to the bronchiolar level. The metabolism is most efficient in the low-dose range. Due to differences in toxicokinetics, an assessment factor of 40 is needed for extrapolating diacetyl air levels causing damage to small airways in rats to humans.

3.1.3. Biological monitoring

No information on biological monitoring of workers was available in the open literature.

3.2. Acute toxicity

3.2.1. Human data

Exposure to high atmospheric concentrations may cause central nervous system (CNS) depression (IPCS 2007). A 36-year old never-smoking man with normal lung function and normal serum α1-antitrypsin prepared an urgently needed flavouring product containing diacetyl, and worked with the heated mixture for several hours in a day. During the last few hours of the shift his eyes became sore and reddened, and a sticky conjunctival secretion developed soon thereafter. Treatment was with steroid and antihistamine; resolution required several days. Although spirometric tests were normal 3 months later, measurements after 9 months were suggestive of small airway disease. For example, the forced expiratory flow between 25 and 75 % of the vital capacity (FEF_{25-75}) was 30 % of the predicted value (Hendrick 2008).

3.2.2. Animal data

Inhalation

In a study on the acute inhalation toxicity of diacetyl vapour, rats were exposed for 4 hours to levels of 2 250, 5 200 or 23 900 ppm. All animals died at the two highest exposure levels, with evidence of respiratory tract injury (BASF 1993). The LC_{50} was estimated to be 2 250–5 200 ppm. Exposure to 23 900 ppm diacetyl resulted in atelectasis and oedema of the lungs, bronchial oedema and hydrothorax. Microscopic findings in the mid- and high-dose animals included hyperaemia of the lung, moderate

emphysema, centrilobular hypertrophy of hepatocytes and degenerative changes and necrosis in renal proximal tubules.

In a US NIOSH acute inhalation toxicity study (Hubbs *et al* 2002), rats were exposed for 6 hours to vapours generated by heating an artificial butter flavouring and examined 1 day after exposure. GC-MS analysis showed that the vapours consisted of a complex mixture of various organic gases with the major components being diacetyl, acetic acid, acetoin and acetoin dimers, butyric acid, 2-nonanone and δ-alkyl lactones. Diacetyl was used as a marker of exposure intensity, and at diacetyl levels of 285–371 ppm, extensive respiratory damage was observed, characterised by multifocal, necrotising bronchitis, mainly in the mainstem bronchus. Alveoli were unaffected. At levels of 203–371 ppm, necro-suppurative rhinitis was observed, affecting all four levels of the nose. Within the two posterior nasal levels (T3 and T4), necrosis and inflammation was principally localised to the nasopharyngeal duct.

Hubbs and co-workers also investigated the acute inhalation toxicity of pure diacetyl vapour in rats at concentrations of up to 365 ppm (time-weighted average, TWA), either as 6-hour continuous exposures or as 4 brief, intense exposures over 6 hours. A separate experimental group inhaled a single pulse of approximately 1 800 ppm diacetyl (92.9 ppm, 6-hour TWA). Rats were necropsied 18–20 hours after exposure. Exposure to diacetyl vapours resulted in epithelial necrosis and inflammation in the nose, larynx, trachea and bronchi. Bronchi were affected at diacetyl concentrations of 294.6 ppm or greater; the trachea and larynx were affected at diacetyl concentrations of 224 ppm or greater. Both pulsed and continuous exposure patterns caused epithelial injury, with the nasal epithelium being the most sensitive. The authors concluded that the no observed adverse effect concentration (NOAEC) for inhaled diacetyl was less than 92.9 ppm (Hubbs *et al* 2008).

Nasal pathological effects of diacetyl (240 ppm) and 2,3-pentanedione (112–354 ppm) were studied in rats following a single 6-hour exposure. Diacetyl and 2,3-pentanedione (241 ppm) caused similar necrotising rhinitis and lesions of the trachea. Bronchial lesions were recorded in one single rat in each of the air (control), diacetyl and 2,3-pentanedione group (1/9, 1/6 and 1/5, respectively). Lesions induced by 2,3-pentanedione indicated apoptosis (TUNEL assay and caspase 3 activation) (Hubbs *et al* 2012).

Other routes

The acute oral LD_{50} of diacetyl in rats has been reported to lie between 3 000 and 3 400 mg/kg (Colley *et al* 1969). Lower values of 1 580 mg/kg in the rat, 250 mg/kg in the mouse and 990 mg/kg in the guinea pig were reported by NTP, which also reported a dermal LD_{50} of > 5 000 mg/kg in the rabbit (NTP 2007).

3.3. Irritancy and corrosivity

3.3.1. Human data

Workers exposed to butter flavouring vapours in popcorn manufacturing facilities reported eye, skin and nasal irritation (Kanwal 2003, Kanwal and Martin 2003, as reported in NTP 2007). Atmospheric concentrations of diacetyl measured in one such facility reached up to 98 ppm (Plant A, Appendix 2), while in another facility at which such effects were reported, the maximum measured concentration of diacetyl was 1.1 ppm (Kanwal and Martin 2003).

3.3.2. Animal data

Diacetyl is reported to be a severe skin and eye irritant in rabbits (NTP 2007).

In a study examining the sensory irritation potency of diacetyl in a mouse bioassay, a 2-hour exposure to diacetyl resulted in concentration-dependent irritation in all parts of the respiratory tract (Larsen *et al* 2009). The sensory irritation response was short-lasting with an RD_{50} value of 966 ppm in the first part (0–10 min) of the exposure period. Later on, airflow limitation and pulmonary irritation developed. The NOAECs for each effect were above 100 ppm, and the authors estimated that initiation of sensory irritation in humans would occur above 20 ppm. The authors also reported that repeated exposure to high levels of diacetyl decreased the sensory irritation warning signal in mice, suggesting that the irritant effects of diacetyl can be particularly insidious (Larsen *et al* 2009).

3.4. Sensitisation

3.4.1. Human data

No irritation was observed after a 48-hour closed-patch test on human subjects, and no sensitisation reaction resulted from maximisation testing of 29 human volunteers using a 2 % diacetyl dilution in petrolatum (NTP 2007).

3.4.2. Animal data

Diacetyl is reported to have sensitising properties in animal studies (Anderson *et al* 2007). The sensitisation potential of diacetyl, glyoxal, methyl glyoxal and glycolaldehyde was assessed using quantitative structure-activity relationship programs. All four compounds were predicted to be sensitisers using Derek and NIOSH logistic regression, while TOPKAT 6.2 predicted all compounds except methylglyoxal to be sensitisers. The four compounds were also tested in a combined irritancy and local lymph node assay (LLNA). All compounds except glyoxal were found to be irritants and all tested positive in the LLNA with EC3 values ranging from 0.42 to 1.9 % (Anderson *et al* 2007).

3.5. Repeated dose toxicity

3.5.1. Human data (inhalation)

Exposure to butter flavouring fumes and/or vapours during food manufacture has been associated with the development of diverse respiratory conditions. Several employees have been diagnosed with a more serious condition known as bronchiolitis obliterans (Akpinar-Elci *et al* 2004, Kreiss *et al* 2002, van Rooy *et al* 2007 and 2009).

Bronchiolitis obliterans, also named constrictive bronchiolitis or obliterative bronchiolitis is a condition of irreversible, fixed airways obstruction characterised by narrowing of the bronchiolar lumen by submucosal fibrosis or fibrous tissue in the adventitia or adjacent alveolar septa. The constriction of the lumen lacks intraluminal granulation plugs as seen in organising pneumonia. There are various causes of bronchiolitis obliterans, including exposure to gases (e.g. chlorine, NO_2). In the medical history of such cases, the disease has normally been triggered by accidental spills. In bronchiolitis obliterans associated with diacetyl exposure, a history of spills is most often lacking.

The exact diagnosis of bronchiolitis obliterans is made histologically. There are radiological changes in high-resolution computed tomography (HRCT) compatible with bronchiolitis obliterans, including hyperinflation with a mosaic pattern of attenuation. The demonstration of fixed airways obstruction (i.e. reduced forced expiratory volume in 1

second, FEV_1) together with typical HRCT findings is mostly the basis for the clinical diagnosis. However, lung biopsies have shown that despite these findings, the histological picture does not necessarily confirm the clinical diagnosis (Akpinar-Elci *et al* 2004, Kreiss *et al* 2002, van Rooy *et al* 2007). It is therefore advisable to save the term bronchiolitis obliterans for histologically verified cases (Galbraith and Weill 2009). When fixed airways obstruction occurs together with demonstrated radiological changes, the diagnosis of bronchiolitis obliterans syndrome has recently come into use (Akpinar-Elci *et al* 2004, van Rooy *et al* 2007 and 2009). In the absence of radiological changes, fixed airways obstruction should be used.

A range of diseases displaying fixed airways obstruction, including asthma, chronic obstructive pulmonary disease (COPD) with and without emphysema, hypersensitivity pneumonitis and organising pneumonia have been associated with exposure to butter flavouring preparations, but scientific evidence of their association with exposure to diacetyl is looser than for bronchiolitis obliterans (Kreiss and Hubbs 2010).

Because bronchiolitis obliterans is a rare disease, some workers exposed to diacetyl may have been potentially misdiagnosed with asthma, bronchitis, emphysema and/or pneumonia. The loss of pulmonary function associated with severe bronchiolitis obliterans is by definition permanent (OSHA 2007a) and has in several cases resulted in the death of affected subjects (Egilman *et al* 2007) or individuals being placed on lung transplantation lists (Akpinar-Elci *et al* 2004, Parmet and von Essen 2002). However, less severe lung obstruction (or restriction) was much more frequent (Kreiss *et al* 2002), as shown in Appendix 2.

In 1994, one case of bronchiolitis obliterans was observed in a packaging worker at a microwave popcorn manufacturing plant (Kreiss *et al* 2002). Additional cases of bronchiolitis obliterans or obstructive lung disease in other microwave popcorn manufacturing plants, including at least 3 deaths, were subsequently reported (IUF 2007, Kreiss *et al* 2002, NTP 2007).

In subsequent studies, a subclinical decline in lung function also appears associated with diacetyl exposure, which further contributes to the hazard characterisation. In 2008, the California Department of Public Health conducted a cross-sectional study in 16 flavour manufacturing plants. The study comprised 467 workers with exploitable questionnaires and acceptable quality spirometry comprising FEV_1 and forced vital capacity (FVC). Occurrence of respiratory symptoms (dyspnoea, chronic bronchitis, asthma, cough and wheeze) was at similar or lower rates as compared to the general US population. The prevalence ratio (PR: number of observed/number of expected cases) was obtained from the working population and expected cases estimated from the general US population. The prevalence ratio (2.7, 95 % CI: 1.2–6.4; 5 observed cases/1.8 expected cases) for severe lung obstruction (percent predicted FEV_1< 50 %) was higher among the workers; the observed cases had never smoked. Especially, young workers (age < 40 years) showed a high excess of severe obstruction [PR (95 % CI): 15 (5–44)]. The number of workers with less severe obstruction was not different from the expected number. Obstruction was most prevalent (5.3 %) in companies using > 800 pounds of diacetyl annually compared to the prevalence (1.2 %) in companies using less diacetyl. The prevalence of obstruction in workers currently doing any production task was 4.5 %, compared to 2.0 % in production support workers, and 2.3 % in office workers (Kim *et al* 2010).

In another study, the California Department of Public Health obtained spirometric test results from workers in 20 flavouring manufacturing companies from April 2004 to August 2009. Interpretable data were available from 671 workers; 23 % had abnormal results at one or more tests, including 4.9 % with airway obstruction. Serial spirometric

test results from 416 workers showed an abnormally fast decline in FEV_1 in 7.3 and 9.6 % of the workers, respectively, depending on the quality of the spirometry data. Workers in companies using > 800 pounds of diacetyl in 2006 had an abnormal FEV_1 decline rate that was 2–3 times greater than workers in companies using less diacetyl (Kreiss *et al* 2012).

In 2008–2009, US NIOSH studied high-quality lung function tests in 106 employees in a flavouring manufacturing facility in Indiana. Mean tenure (range) was 16.2 (0.2–36) years. The diacetyl concentrations were obtained by unbiased methods (OSHA PV2118 and 1012), which showed a geometric mean 8-hour time-weighted concentration in the range < 0.001–1.9 ppm and with a maximum of 2.9 ppm. There were also exposures to other compounds. Abnormal spirometry was observed among 32 % of employees. Restrictive patterns were observed among 30 subjects (28 %), which was mild, moderate, moderately severe and severe among 22, 6, 1 and 1 employees, respectively. Any obstructive pattern was observed in 3 %; 2 employees had mild and 1 had moderate obstruction. A severe mixed pattern (obstructive + restrictive) was observed in 1 employee. The prevalence of restriction was 3.8 times that in the US population. Among 70 employees with high quality spirometric test, 13 (19 %) had an excessive FEV_1 decline and abnormalities were apparently progressive. The annual decline in FEV_1 was 2.8 times greater and the abnormal decline occurred about 7 times more frequently in the highly exposed compared with employees in other areas. The observed restrictive reactions may be due to other compounds than diacetyl, which appeared mainly associated with obstructive abnormalities (Kreiss *et al* 2011).

The raw data used by Kreiss *et al* (2011) were re-analysed by the Paustenbach group. They also found an increased prevalence of restriction (29 %). The prevalence risk [odds ratio (OR): 3.3] was significantly increased compared to the US population. However, the prevalence of restriction was not related to exposure levels. Thus, 27 % of the highly exposed and 33 % of the low-exposed had airway restriction. Neither tenure in a work area with high potential for exposure (OR: 0.97) nor tenure in liquid compounding (OR: 0.99) was associated with increased prevalence. Similarly, no increase was observed in those who had ever worked in a job with high potential for exposure (OR: 0.84) or ever worked in a liquid compounding area (OR: 0.72). Additionally, the decrease in lung function (FEV_1 and FVC) was analysed by general estimating equation (GEE) models, considered statistically superior to multiple linear regression models used by NIOSH. The GEE models did not indicate an association between lung function decline and tenure at the facility [$p = 0.46$ (FEV_1) and 0.90 (FVC)], tenure in work areas with high potential for exposure ($p = 0.13$ and 0.40, respectively) or tenure in liquid compounding ($p = 0.56$ and 0.997, respectively). The authors questioned the US population (mainly driven by urban centre populations) being an appropriate control group for the worker cohort, which consisted mainly of males aged 30–60 years, of which about a third were obese, and many had farming exposure. Therefore, workers in the group with low potential for exposure were considered more appropriate for comparison, which gave the conclusion that "many years of exposures to flavouring chemicals in this workplace, including diacetyl, were not found to produce an increased risk of abnormal (mainly restrictive) spirometric findings" (Ronk *et al* 2013).

A group of 135 workers from the microwave popcorn production plant in Missouri was evaluated by NIOSH in 2000. These workers had 2.6 times the expected rates of chronic cough and shortness of breath, according to comparisons with the national data, and twice the expected rates of physician-diagnosed asthma and chronic bronchitis. Overall, the workers had 3.3 times the expected rate of airway obstruction; those who had never smoked had 10.8 times the expected rate. Workers directly involved in the production of microwave popcorn had higher rates of shortness of breath on exertion and skin

problems that had developed since they started work than workers in other parts of the plant (Kreiss *et al* 2002). As indicated above (Section 2.2), workers were exposed to an aerosol and a range of organic vapours from flavourings (Kullman *et al* 2005). VOCs detected in the air in the microwave area included diacetyl, methyl ethyl ketone, acetoin, 2-nonanone and acetic acid. Diacetyl, the predominant ketone in the plant, was present in concentrations ranging from below the LOD (< 0.01 ppm) to 98 ppm, with a mean of 8.1 ppm. A bronchiolitis obliterans syndrome had developed in about 40 % (5/13) of the workers exposed in the mixing room with a mean exposure concentration of 32 ppm and peak exposures of 1 230 ppm, whereas in the packing area with a mean exposure of 2 ppm, 3–4 % (4/121) had developed a bronchiolitis obliterans syndrome (Appendix 2) (this was the basis of the SCOEL 2010 evaluation). However, decreased lung function was also recorded and forms the basis of the present Recommendation. Thus, there was a strong relation between quartiles of estimated cumulative exposure to diacetyl and the prevalence and extent of airway obstruction (IUF 2007, Kreiss *et al* 2002). A 4.5 % reduction of predicted FEV_1% was observed in the second quartile (0.65–4.5 ppm-years), followed by reductions of 8.9 and 12.5 % in the third (4.5–11 ppm-years) and fourth (≥ 11 ppm-years) quartiles, respectively. Kreiss *et al* (2002) concluded that the excess rates of lung disease and lung function abnormalities as well as the relation between exposure and outcomes in this working population indicated that these people probably had occupational bronchiolitis obliterans caused by the inhalation of volatile butter-flavouring ingredients. A NOAEC of 0.65 ppm-years cumulative exposure can be derived from this study (uncorrected diacetyl air measurements obtained by the NIOSH Method 2557). In the plant, the median employment duration was 3.4 years (range: 0.1–17.6).

A more recent study showing exposure-response relationships was conducted in the Missouri plant (Kanwal *et al* 2011), where eight follow-up surveys were conducted in the period November 2000–July 2003; exposure assessments were performed with the NIOSH Method 2557 and results were corrected when exposure concentrations were above detection levels. Workers were divided into those who started working before implementation of control measures in November 2000 (group 1, n = 146 with 6 years of employment at the last survey), and those who started after November 2000 (group 2, n = 227 with 6 months of employment at the last survey). In the mixers, the mean (maximum) exposures in the years 2000, 2001, 2002 and 2003 were 57 (147), 11 (109), 5 (17) and 1.6 (13) ppm, respectively. In the packing line machine operators, corresponding exposures were 2.8 (8.1), 1.7 (4.6), 0.6 (1.6), and < 0.004 (0.007) ppm, respectively, and in the quality control laboratory workers 0.8 (1.5), 1.0 (3.5), 0.3 (0.7) and < 0.1 (0.5) ppm, respectively.

A combined subgroup consisting of mixers, maintenance and quality control workers was compared with a corresponding subgroup of packing line workers. In group 1 (highest past exposure, longer tenure), the first subgroup (n = 23) showed obstructive spirometry both at the first and the last survey (30.4 %, 7/23), whereas among workers in the packing line (n = 40), 20 % (8/40) showed obstruction at the first survey and 15 % (6/40) at the last survey. In the first subgroup, the mean percent predicted FEV_1 at the first and the last survey was 85.8 % and 83.4 %, respectively. In the packing line workers, the mean values were 87.0 and 85.7 %, respectively. In workers with ≥ 2 tests, the mean change in FEV_1 was -71.0 ml/year in the first subgroup and -43.5 ml/year in the packing line workers. Workers for which ≥ 3 tests were present showed a mean change in FEV_1 of -58.7 ml/year and -44.6 ml/year in the first subgroup (n = 22) and in packing line workers (n = 39), respectively. The authors concluded that there were no statistically significant changes in the prevalence of airway obstruction or in mean percent predicted FEV_1 over time in either subgroup, nor in the difference in mean annual change in FEV_1 between the subgroups. In group 2 (no past exposure, shorter tenure), the first subgroup (n = 6) showed obstruction on spirometry in 33.3 % (2/6) and 16.7 %

(1/6), respectively, in the first and last survey. In both surveys, the mean percent predicted FEV_1 was 95 % in the first subgroup and ≥ 96 % in the packing line workers (n = 72–73). Workers with ≥ 2 tests showed a mean change of FEV_1 of -162.7 ml/year in the first subgroup and -83.0 ml/year in the packing line workers (n = 72). In workers with ≥ 3 tests, the changes in the two subgroups were +17.0 ml/year (n = 4) and -21.3 ml/year (n = 31), respectively. The authors concluded that there were no significant changes in the prevalence of airway obstruction or in mean percent predicted FEV_1 from the first to the last test in either of the group 2 subgroups. Group 1 (highest past exposure, longer tenure) and group 2 (no past exposure, shorter tenure) workers with ≥ 3 spirometric tests and a decline of FEV_1 of > 300 ml and/or 10 % from the first to the last test were compared. For all workers in the two groups, the prevalences were 19/87 (22 %) and 3/40 (8 %), respectively. For those working as mixers after November 2000, prevalences were 4/5 (80 %) and 0/4 (0 %), respectively. For those working in the quality control laboratory after November 2000, prevalences were 2/8 (25 %) and 0/1 (0 %), respectively. From this study, it is apparent that the diacetyl associated decline in lung function was most conspicuous in group 1 with the highest past exposure and the longest exposure duration. The abnormal lung function in group 1 workers remained mostly unchanged over time, consistent with an irreversible lung disease process (Kanwal *et al* 2011).

A recent cross-sectional study provides exposure-response relationships from a different cohort. The estimates were based on data from 725 employees in four other microwave popcorn production plants studied from 2005 to 2006 (Lockey *et al* 2009). The employees consisted of non-Asian males, Asian males, non-Asian females and Asian females, comprising 400, 52, 208 and 65 workers, respectively. In the four groups, mixers exposed before introduction of pre-powered air-purifying respirators (pre-PAPR) comprised 24, 7, 8 and 0, respectively, and the number of exposed mixers after introduction of PAPR were 16, 1, 3 and 0, respectively. Personal breathing zone concentrations were obtained by means of the NIOSH Method 2557, considered to have a LOD of 0.007 ppm at the set airflow; reported values were not corrected and are thus considered to be underestimated. The mean exposure levels were similar in the four plants for non-mixers (0.014–0.074 ppm), whereas pre-PAPR mixers had higher exposures (0.057–0.86 ppm). Exposures in mixers after introduction of PAPR (0.015–0.044 ppm) were similar to non-mixers. Based on the distribution of exposures, the cumulative diacetyl exposure was dichotomised at high (≥ 0.8 ppm-years) and low (< 0.8 ppm-years) levels; the selected cut point was at the top exposures in workers performing non-mixing, quality assurance, intermittent mixing and PAPR mixing. Numerous of the pre-PAPR mixing workers (n = 39) had much higher exposure levels. Those exposed above the cut-off point had decreased FEV_1 as percentage of the predicted value estimated from the US population, both in non-Asian males (-10.3 %) and Asian males (-12.7 %). Only exposures ≥ 0.8 ppm-years were associated with decreased FEV_1. The non-Asian male workers in the pre-PAPR mixer group had an 8-fold increased risk of an obstructive pattern (95 % CI: 2-29); the value was not affected by removing individuals with a pre-employment history of asthma. The 0.8 ppm-years cut-off-value was defined arbitrarily and it cannot be excluded that the true NOAEC in this population is higher; this value should, therefore, be considered as a lower boundary of the NOAEC.

A comprehensive documentation of the exposure assessment used in the Lockey *et al* (2009) study was published later on. The exposure assessment comprised the largest published data set of diacetyl measurements with a single method (White *et al* 2010). It comprised 639 full-shift breathing zone measurements of which 50.9 % were above the LOD. White (2011) also published exposure data corrected for analytical recovery. At high absolute humidity and long day-to-extraction values (up to 6 days), it was

estimated that a new corrected value may be as much as 20-fold greater than the original concentration value. However, samples obtained under conditions of low humidity and short days-to-extraction (2–3 days) changed little. Corrected arithmetic mean values were roughly double those in the original papers. Thus, in the four plants, the arithmetic means in non-mixers (maximum in parenthesis) were 0.069 (1.98), 0.123 (1.93), 0.042 (1.36) and 0.045 (1.00) ppm, respectively. Corresponding values in mixers were 0.94 (4.20), 0.84 (7.67), 0.12 (0.40) and 2.70 (11.7) ppm, respectively. No corrected cumulative exposure was given, but it can reasonably be assumed that the cut-off point used by Lockey *et al* (2009) would correspond to a corrected value of 1.6 ppm-years.

Besides studies conducted in plants where exposure is to complex artificial butter flavouring vapours and aerosols, one study conducted in the Netherlands examined workers more specifically exposed to diacetyl. Van Rooy and co-workers examined workers from a chemical plant that produced diacetyl between 1960 and 2003 (van Rooy *et al* 2007 and 2009). Diacetyl was produced by oxidation of 2,3-butylene glycol into acetoin, which was further oxidised into diacetyl. Acetaldehyde and acetic acid were side-products of the reaction. Diacetyl production took place in a completely closed system at elevated temperature. Process operators were only exposed to the reaction components at the end of the production process and did not have exposures to heated products. Van Rooy and co-workers used historical exposure data to classify all workers into three exposure groups with varying exposure profiles to diacetyl, based on frequency and level of exposure, process operators forming the highest exposure group. Exposure monitoring was done using cartridges containing silica gel coated with dinitrophenylhydrazine and analysed by GC (van Rooy *et al* 2007). The air concentrations of diacetyl, as determined by area sampling, ranged in general from 1.8 to 351 mg/m^3 and from 3 to 396 mg/m^3 for specific tasks. Acetaldehyde air concentrations ranged from 0.4 to 29 mg/m^3. Control measures taken in 2001, with the aim of enclosing the process, led to a reduced exposure for both diacetyl (geometric mean change from 10.0 to 5.8 mg/m^3) and acetaldehyde (geometric mean change from 7.6 to 0.7 mg/m^3). Four cases consistent with a bronchiolitis obliterans syndrome in the highest exposure group of 102 process operators were identified, three of which were lifelong non-smokers. A cumulative diacetyl dose-response relationship could not be demonstrated (van Rooy *et al* 2009). The authors concluded that exposure to an agent during diacetyl production appears to be responsible for causing a bronchiolitis obliterans syndrome in chemical process operators, consistent with the suspected role of diacetyl in downstream food production (van Rooy *et al* 2007 and 2009).

Bronchiolitis obliterans was also diagnosed in 2 workers involved in flavour manufacture. Neither of the workers was employed in the microwave-popcorn industry; both workers had handled pure diacetyl as well as other chemicals involved in the manufacturing process (CDC 2007).

Although diacetyl is thought to be the primary contributor to respiratory disease in popcorn manufacturing plants, workers in production areas were also exposed to high concentrations of other ketones, other VOCs, and respirable dust. Therefore, diacetyl may not be the only factor contributing to bronchiolitis obliterans; e.g. tannins have also been proposed as a causal factor (Kreiss *et al* 2002). As indicated from rat studies with 2,3-pentanedione, also other diketones may cause bronchiolitis obliterans (Morgan *et al* 2012). In the publication of van Rooy *et al* (2007) relating to manufacture of diacetyl, potential additional exposures included acetoin, acetaldehyde and acetic acid. These substances are present also in the popcorn manufacturing plants.

3.5.2. Animal data

Inhalation route

The respiratory toxicity of diacetyl has been studied in rats. As rats are obligate nose breathers, the scrubbing effect of the upper airways was by-passed by intratracheal instillation of a single dose (125 mg/kg in an instilled volume of 200 µl). Within 7 days, rats developed severe intraluminal polypoid fibrosis or concentric fibrosis in the bronchioles. Airway resistance increased, and dynamic and static compliance decreased. The Clara cell secretory protein decreased markedly, and its distribution in the airway epithelium indicated severe epithelial disorganisation. Inflammatory cells were mainly neutrophils and macrophages (Palmer *et al* 2011). In a study in C57BL/6 mice, oro-pharyngeal aspiration to bypass the nose (400 mg/kg diacetyl in a 50 µl aqueous solution) was used. Effects recorded 4 days later included foci of fibrohistiocytic proliferation with little or no inflammation at the junction of the terminal bronchiole and alveolar ducts. The fibrohistiocytic lesions were usually composed of a mixture of spindle cells and histiocyte-like cells. No fibrohistiocytic lesions were observed at the dose of 100 mg/kg (Morgan *et al* 2008).

NTP/NIEHS has carried out a number of repeated dose inhalation studies in C57BL/6 mice (Morgan *et al* 2008). In one study, male C57BL/6 mice were whole-body exposed to diacetyl vapour 6 hours/day for 5 days at levels of 0 (n = 7), 200 (n = 10) or 400 ppm (n = 15). The diacetyl exposed mice showed severe, dose-related changes in the nasal epithelium, larynx and large airways. Two of the mice exposed to 400 ppm were found dead and 9 animals were killed in a moribund condition after 3 exposures. Histopathological examination of the animals at this exposure concentration showed necrotising rhinitis, necrotising laryngitis and bronchitis. At 200 ppm, 3 animals were killed after 2 exposures and 3 animals after 3 exposures, with similar histopathological changes, although laryngeal damage was less severe. When the duration of exposure was reduced to 1 hour/day for 2 or 4 weeks at exposure levels of 0, 100, 200 or 400 ppm, no deaths occurred. Nasal and laryngeal toxicity was less marked, but peribronchial and peribronchiolar lymphocytic inflammation was observed. A similar pattern was observed with intermittent high-dose exposures at 1 200 ppm (15 min, twice a day, 4 weeks).

The investigators also exposed male C57BL/6 mice (5 per group) in a whole-body exposure chamber at diacetyl vapour levels of 0, 25, 50 or 100 ppm, 6 hours/day, 5 days/week for 6 or 12 weeks. A further set of animals was allowed a 6-week post exposure recovery period before examination. All mice survived the treatment period. In the 100-ppm group, suppurative rhinitis with chronic active inflammation, foci of respiratory mucosal ulceration and/or necrosis, and moderate squamous metaplasia were seen in the nasal epithelium after 6 and 12 weeks of exposure, accompanied by atrophy of the olfactory epithelium. Inflammatory changes also extended to some of the smaller airways and bronchioles in 3 of the 5 mice. Inflammatory changes, metaplasia, and olfactory epithelial atrophy were present, with decreased severity, in the mice exposed to 50 ppm diacetyl. Nasal lesions recorded in the groups exposed to 50 and 100 ppm were more severe after 12, as compared to 6, months of exposure (severity scores 32 vs. 48, and 77 vs. 98, respectively). The nasal lesions were less severe after 6 weeks recovery. Inflammation and squamous metaplasia were relatively minor in the 25-ppm group.

Peribronchial lymphocytic inflammation was also noted after 12 weeks of exposure in 4 of the 5 mice exposed to 50 ppm, and in 2 of the 5 mice exposed to 25 ppm, but the inflammation in these animals was minimal to mild and was not accompanied by epithelial atrophy or denudation. Lung lesions were not more severe after 12 than after 6 months, but persisted 6 weeks after cessation of exposure (Table 6 in Morgan *et al* 2008). The NOAEC for peribronchiolar lymphocytic inflammation was 25 ppm and the

lowest observed adverse effect concentration (LOAEC) was 50 ppm. The NOAEC for nasal effects was below 25 ppm. As the changes at this exposure level were relatively minor, 25 ppm can be taken as a LOAEC.

The authors concluded that exposure to diacetyl vapour results in a pattern of injury that replicates features of human bronchiolitis obliterans (Morgan *et al* 2008). This conclusion has, however, been challenged by others (e.g. Finley *et al* 2008), who have queried the rationale for the diacetyl exposure regimens used in these animal studies and their relevance to the actual worker exposure concentrations measured in the sentinel microwave popcorn packaging plant.

Finley *et al* (2008) have also commented on the fact that the pattern of respiratory damage produced by pure diacetyl in rodents is limited to changes in the nasal and respiratory epithelium and does not progress to obstructive lung disease and/or deep lung damage, supporting the hypothesis that other agents may be responsible for or contribute to the lung damage seen in workers exposed to butter flavour fumes. The toxicokinetic data produced by Gloede *et al* (2011) (Section 3.1.2) contribute, however, to explain these species differences.

Other routes

A 90-day oral toxicity study in rats has been carried out, at dose levels of 0, 10, 30, 90 or 540 mg/kg/day by gavage (Colley *et al* 1969). No adverse effects were noted in the three lowest dose groups. At the highest dose of 540 mg/kg/day, rats showed a decreased body weight gain, an increase in water consumption, increased blood leukocytes and an increase in relative weights of liver, kidney and adrenal and pituitary glands. There was macroscopic and microscopic evidence of severe irritancy in both the glandular and non-glandular parts of the stomach. The no-effect-level in this study was 90 mg/kg/day.

3.6. Genotoxicity

3.6.1. In vitro

Diacetyl forms covalent adducts with 2-deoxyguanosine in an acellular system *in vitro* (More *et al* 2012). Diacetyl has been reported to give weakly positive results in the Ames test in *Salmonella typhimurium* strains TA100, 102 and 104, indicating a potential to induce frame-shift mutations (Bjeldanes and Chew 1979, Dorado *et al* 1992, Marnett *et al* 1985). A positive result was also obtained in *Escherichia coli* strain WP2 uvra (Kato *el al* 1989), but no evidence of mutagenicity was demonstrated in the SOS-chromotest using *E. coli* PQ37 (von der Hude *et al* 1988). Diacetyl induced sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) AUXB1 cells (Tucker *et al* 1989). Diacetyl induced a mutagenic response in the L5178Y mouse lymphoma mutation assay in the presence of human liver S9 for activation, although only at cytotoxic levels. According to the authors, the increase in the frequency of small colonies in the assay with diacetyl indicated that it causes damage to multiple loci on chromosome 11 in addition to functional loss of the thymidine kinase locus (Whittaker *et al* 2008).

3.6.2. In vivo – human data

No data on genotoxic effects in humans were available.

3.6.3. In vivo – animal data

Diacetyl has been reported to show promoting and initiating activities in the rat stomach mucosa (Furihata *et al* 1985). It was administered in a single intragastric dose where the

maximum dose corresponded to about half of the LD_{50} followed by investigation of the pyloric mucosa. In the pyloric mucosa, the ornithine decarboxylase activity (ODC) was maximum, and dose-dependently increased, 16 hours after the administration, (25- and 100-fold at 500 and 1 500 mg diacetyl/kg, respectively). ODC is the rate limiting step in the polyamine synthesis; polyamines promote cell growth. Also DNA synthesis was maximum, and dose-dependently increased, 16 hours after the administration. At 300 mg/kg of diacetyl, the DNA synthesis was increased about 10-fold. The authors considered the increase in ODC and DNA synthesis to represent promoting activity. The unscheduled DNA synthesis (UDS) was used to evaluate direct DNA damage in the non-S phase. To distinguish non-S phase DNA repair from diacetyl induced S phase (replicative) DNA synthesis, hydroxyurea (HU) was used to suppress the S phase DNA synthesis to a level corresponding to that in non-treated animals (Furihata and Matsushima 1987). UDS was significantly increased in the diacetyl groups (≥ 500 mg diacetyl/kg) compared to the zero time level. It is noted that the used doses caused glandular stomach mucosal inflammation.

A negative result was obtained in the mouse micronucleus test at dose levels up to 500 mg/kg (NTP 2007), indicating that diacetyl does not induce systemic genotoxic effects.

3.7. Carcinogenicity

3.7.1. Human data

There were no data on carcinogenicity in humans.

3.7.2. Animal data

There were no data from long-term animal experiments. Diacetyl was tested in a subchronic 24-week study for its potential to induce primary lung tumours in male and female strain A mice. Diacetyl was administered once per week by intraperitoneal injection with a total dose of either 1.7 or 8.4 g/kg. The results of an initial study showed an increase in lung tumours associated with diacetyl as compared with controls. However, a repeat study showed a similar incidence in diacetyl treated and control mice (Stoner *et al* 1973).

3.8. Reproductive toxicity

3.8.1. Human data

No human data were available on the reproductive toxicity of diacetyl.

3.8.2. Animal data

Fertility

No specific investigations of effects on fertility in animals were available.

Developmental toxicity

Groups of 25–27 Syrian golden hamsters, 21–24 CD-1 mice, and 21–23 albino Wistar rats were given a solution containing 90 % diacetyl by gavage on days 6–10 of gestation for hamsters and on days 6–15 of gestation for mice and rats. The doses for all species were 16, 74, 345 and 1 600 mg/kg bw per day. No effects were seen on maternal survival, weight, reproductive parameters or on foetal survival or macroscopic appearance of external, skeletal, or soft tissues (WHO 1999).

4. Recommendation

In humans, airborne exposure to diacetyl in industries using butter flavouring agents has been associated with subclinical alterations of lung function and with fixed airway obstruction that may progress to a life-threatening bronchiolitis obliterans or bronchiolitis obliterans syndrome. Animal studies support the view that diacetyl can be the causative agent, although other compounds including other diketones are also present in butter flavouring mixtures (Day *et al* 2011). As indicated from rat studies with 2,3-pentanedione, such compounds may also cause bronchiolitis obliterans (Morgan *et al* 2012). Furthermore, other compounds may impair the local metabolism of diacetyl in the upper airways, thereby increasing its penetration to the bronchiolar level. SCOEL considers diacetyl as being able to cause subclinical to severe fixed airway obstruction, which is the critical health effect for recommending an OEL. Symptoms such as cough and shortness of breath are considered secondary to the obstructive lung disease. In the 2010 evaluation, SCOEL recommended an 8-hour TWA of 0.1 ppm. In other evaluations, 8-hour TWA recommended OELs range from 0.005 (NIOSH 2011) to 0.2 ppm (Maier *et al* 2010). In addition, NIOSH proposes a 15-min short term exposure limit (STEL) at 25 ppb.

In the present update, SCOEL evaluated several approaches to derive a recommended OEL (Appendix 3).

Because diacetyl can cause mild to life-threatening airway obstruction, SCOEL selected a conservative approach (approach 2). SCOEL did not follow the NIOSH (2011) approach (approach 4) because of concerns about the robustness of the exposure data on which the assessment was based (Appendix 1). SCOEL accepted the view that a sensitive group may exist (NIOSH 2011), although it may also be an artefact due to biased exposure assessment. Furthermore, SCOEL accepted that there is uncertainty about the importance of the genotoxicity of diacetyl. By the SCOEL approach, a best estimate NOAEC of 0.05 ppm is derived from the study by Kreiss *et al* 2002 (0.65 ppm-years divided by 40 years multiplied with a correction factor of 3 for analytical bias). Using an additional uncertainty factor of 2 for possible sensitive groups and rounding lead to a recommended 8-hour TWA-OEL of 0.02 ppm (0.07 mg/m^3).

The promotion effect in rat stomach mucosa was recorded at high exposure levels (≥ 300 mg/kg). Exposure at the recommended OEL is considerably lower (0.07 mg/m^3 x 10 m^3/70 kg = 0.01 mg/kg) and the dose reaching the target site in the lungs even smaller. The promotion effects observed by Furihata *et al* (1985) are therefore considered of little concern and covered by the conservative extrapolation of approach 2.

A STEL is needed to prevent adverse health effects (mainly respiratory damage) which may arise due to peaks in exposure not controlled by the above TWA limit. However, the data-set on diacetyl does not allow deriving a scientifically based STEL. SCOEL applies, therefore, an arbitrary assessment factor of 5 on the TWA-OEL and recommends a STEL value of 0.10 ppm (0.36 mg/m^3).

Although the potential for dermal absorption of diacetyl exists, a skin notation is not warranted as the adverse effects are seen in the respiratory tract and are due to inhalation. Diacetyl is a severe skin and eye irritant, but not considered a skin sensitiser in humans. Thus, care should be taken to avoid dermal exposure when handling bulk quantities of diacetyl.

Diacetyl can be measured by unbiased methods at low concentrations, indicating that measurement difficulties are not foreseen at the recommended OEL and STEL.

The present Recommendation was adopted by SCOEL on 11 June 2014.

5. References

Akpinar-Elci M, Travis WD, Lynch DA, Kreiss K (2004). Bronchiolitis obliterans syndrome in popcorn production plant workers. Eur Respir J 24:298-302.

Anderson SE, Wells JR, Fedorowicz A, Butterworth LF, Meade BJ, Muson AE (2007). Evaluation of the contact and respiratory sensitization potential of volatile organic compounds generated by simulated indoor air chemistry. Toxicol Sci 97(2):355-363.

Ashley K, McKernan LT, Burroughs E, Deddens J, Pendergrass S, Streicher RP (2008). Field evaluation of diacetyl sampling and analytical methods. J Occup Environ Hyg 5:D111–D116.

BASF (1993). Study on the acute inhalation toxicity LC50 of diacetyl FCC as a vapour in rats. Project No. 1310247/927010, cited in NTP 2007.

Bjeldanes LF, Chew H (1979). Mutagenicity of 1,2-dicarbonyl compounds: maltol, kojic acid, diacetyl and related substances. Mutat Res 67(4):367-371.

CDC, Centers for Disease Control and Prevention (2007). Fixed obstructive lung disease among workers in the flavor-manufacturing industry--California, 2004-2007. MMWR Morb Mortal Wkly Rep 56(16):389-393.

CDC, Centers for Disease Control and Prevention Skin Permeation Calculator (2013). http://www.cdc.gov/niosh/topics/skin/skinPermCalc.html (accessed 1 February 2013).

Colley J, Gaunt LF, Lansdown ABG, Grasso P, Gangolli SD (1969). Acute and short-term toxicity of diacetyl in rats. Food Cosmet Toxicol 7:571-582.

Cox-Ganser J, Ganser G, Saito R, Hobbs G, Boylstein R, Hendricks W, Simmons M, Eide M, Kullman G, Piacitelli C (2011). Correcting diacetyl concentrations from air samples collected with NIOSH method 2557. J Occup Environ Hyg 8:59-70.

Day G, LeBouf R, Grote A, Pendergrass S, Cummings K, Kreiss K, Kullman G (2011). Identification and measurement of diacetyl substitutes in dry bakery mix production. J Occup Environ Hyg 8:93-103.

Dorado L, Ruiz Montoya M, Rodriguez Mellado JM (1992). A contribution to the study of the structure-mutagenicity relationship for α-dicarbonyl compounds using the Ames test. Mutat Res 269(2):301-306.

Egilman D, Milloux C, Valentin C (2007). Popcorn-worker lung caused by corporate and regulatory negligence: an avoidable tragedy. Int J Occup Environ Health 13:85-98.

Finley BL, Flake GP, Kirby PJ, Palmer SM (2008). Comments on respiratory toxicity of diacetyl. Toxicol Sci 106:429-432.

Fujioka K, Shibamoto T (2006). Determination of toxic carbonyl compounds in cigarette smoke. Environ Toxicol 21:47-54.

Furihata C, Yoshida S, Matsushima T (1985). Potential initiating and promoting activities of diacetyl and glyoxal in rat stomach mucosa. Jpn J Cancer Res 76:809-814.

Furihata C, Matsushima T (1987). Use of in vivo/in vitro unscheduled DNA synthesis for identification of organ-specific carcinogens. Crit Rev Toxicol 17:245-277.

Galbraith D, Weill D (2009). Popcorn lung and bronchiolitis obliterans: a critical appraisal. Int Arch Occup Environ Health 82:407–416

Gloede E, Cichocki JA, Baldino JB, Morris JB (2011). A validated hybrid computational fluid dynamics-physiologically based pharmacokinetic model for respiratory tract vapor absorption in the human and rat and its application to inhalation dosimetry of diacetyl. Toxicol Sci 123:231-246.

Harber P, Saechao K, Boomus C (2006). Diacetyl-induced lung disease. Toxicol Rev 25(4):261-272.

Hendrick DJ (2008). "Popcorn worker's lung" in Britain in a man making potato crisp flavouring. Thorax 63:267-268.

Henschler D (1984). Exposure limits: history, philosophy, future developments. Ann Occup Hyg 28:79-92.

Hubbs AF, Battelli LA, Goldsmith WT, Porter DW, Frazer D, Friend S, Schwegler-Berry D, Mercer RR, Reynolds JS, Grote A, Castranova V, Kullman G, Fedan JS, Dowdy J, Jones WG (2002). Necrosis of nasal and airway epithelium in rats inhaling vapors of artificial butter flavouring. Toxicol Appl Pharmacol 185(2):128-135.

Hubbs AF, Cumpston AM, Goldsmith WT, Battelli LA, Kashon ML, Jackson MC, Frazer DG, Fedan JS, Goravanahally MP, Castranova V, Kreiss K, Willard PA, Friend S, Schwegler-Berry D, Fluharty KL, Sriram K (2012). Respiratory and olfactory cytotoxicity of inhaled 2,3-pentanedione in Sprague-Dawley rats. Am J Pathol 181:829-844.

Hubbs AF, Goldsmith WT, Kashon ML, Frazer D, Mercer RR, Battelli LA, Kullman GJ, Schwegler-Berry D, Friend S, Castranova V (2008). Respiratory toxicologic pathology of inhaled diacetyl in Sprague-Dawley rats. Toxicol Pathol 36:330-44.

IPCS, International Programme for Chemical Safety (2007). 2,3-Butanedione. International Chemical Safety Cards, ICSC:1168.

IUF, International Union of Food, Agricultural, Hotel, Restaurant, Catering, Tobacco and Allied Workers' Associations (2007). Flavouring ingredient diacetyl linked to deadly lung disease. http://www.iuf.org/cgi-bin/dbman/db.cgi?db=default&ww=1&uid=default&ID=4675&view_records=1&en=1.

Kanwal R (2003). Letter to Frank Morrison, Nebraska Popcorn, Clearwater, Nebraska, regarding findings from February 2002. Health Hazard Evaluation (HHE) at plant dated July 2, 2003. HETA 2002-0089. http://www.cdc.gov/niosh/hhe/reports/pdfs/2002-0089-letter.pdf.

Kanwal R, Martin SB, Jr (2003). Letter to Keith Heuermann, BK Heuermann Popcorn, Inc., Phillips, Nebraska, regarding findings from August 2001. Health Hazard Evaluation (HHE) at plant dated May 13, 2003. HETA 2001-0517. http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0517-letter.pdf.

Kanwal R, Kullman G, Piacitelli C, Boylstein R, Sahakian N, Martin S, Fedan K, Kreiss K (2006). Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. J Occup Environ Med 48(2):149-157.

Kanwal R, Kullman G, Fedan KB, Kreiss K (2011). Occupational lung disease risk and exposure to butter-flavoring chemicals after implementation of controls at a microwave popcorn plant. Public Health Rep 126:480-494.

Kato F, Araki A, Nozaki K, Matsushima T (1989). Mutagenicity of aldehydes and diketones. Mutat Res 216:366-367.

Kim JL, Materna BL, Prudhomme JC, Fedan KB, Enright PL, Sahakian NM, Windham GC, Kreiss K (2010). Industry-wide medical surveillance of California flavor manufacturing workers: cross-sectional results. Am J Ind Med 53:857-865.

Kovacic P, Cooksy AL (2010). Electron transfer as a potent cause of diacetyl toxicity in popcorn lung disease. Rev Environ Contam Toxicol 204:133-148.

Kreiss K, Fedan KB, Nasrullah M, Kim TJ, Materna BL, Prudhomme JC, Enright PL (2012). Longitudinal lung function declines among California flavoring manufacturing workers. Am J Ind Med 55:657-668.

Kreiss K, Gomaa A, Kullman G, Fedan K, Simoes EJ, Enright PL (2002). Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. New Eng J Med 347(5):330-338.

Kreiss K (2007). Flavoring-related bronchiolitis obliterans. Curr Opin Allergy Clin Immunol 7(2):162-167.

Kreiss K, Hubbs A (2010). Letter to the Editor: RE: Galbraith D and Weill D (2009). Popcorn lung and bronchiolitis obliterans: a critical appraisal. Int Arch Occup Environ Health 83(4):467-469.

Kreiss K, Piacitelli C, Cox-Ganser J (2011). Lung function (spirometry) testing in employees at a flavorings manufacturing plant – Indiana. National Institute of Occupational Safety and Health. Health hazard evaluation (HHE) report, HETA 2008-0155-3131, June 2011.

Kullman G, Boylstein R, Jones W, Piacitelli C, Pendergrass S, Kreiss K (2005). Characterization of respiratory exposures at a microwave popcorn plant with cases of bronchiolitis obliterans. J Occup Environ Hyg 2(3):169-178.

Larsen ST, Alarie Y, Hammer M, Nielsen GD (2009). Acute airway effects of diacetyl in mice. Inhal Toxicol 21:1123-1128.

Lockey JE, Hilbert TJ, Levin LP, Ryan PH, White KL, Borton EK, Rice CH, McKay RT, LeMasters GK (2009). Airway obstruction related to diacetyl exposure at microwave popcorn production facilities. Eur Respir J 34:63-71.

Maier A, Kohrman-Vincent M, Parker A, Haber LT (2010). Evaluation of concentration-response options for diacetyl in support of occupational risk assessment. Regul Toxicol Pharmacol 58:285-296.

Marchand S, Almy J, de Revel G (2011). The cysteine reaction with diacetyl under wine-like conditions: proposed mechanisms for mixed origins of 2-methylthiazole, 2-methyl-3-thiazoline, 2-methylthiazolidine, and 2,4,5-trimethyloxazole. J Food Sci 76:C861-C868.

Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN (1985). Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mulat Res 148(1-2):25-34.

Martyny JW, van Dyke MV, Arbuckle S, Towle M, Rose CS (2008). Diacetyl exposures in the flavor manufacturing industry. J Occup Environ Hyg 5:679-688.

Mathews JM, Watson SL, Snyder RW, Burgess JP, Morgan DL (2010). Reaction of the butter flavorant diacetyl (2,3-butanedione) with N-α-acetylarginine: a model for epitope formation with pulmonary proteins in the etiology of obliterative bronchiolitis. J Agric Food Chem 58:12761-12768.

More SS, Raza A, Vince R (2012). The butter flavorant, diacetyl, forms a covalent adduct with 2-deoxyguanosine, uncoils DNA, and leads to cell death. J Agric Food Chem 60:3311-3317.

Morris JB, Hubbs AF (2009). Inhalation dosimetry of diacetyl and butyric acid, two components of butter flavoring vapors. Toxicol Sci 108:173-183.

Morgan DL, Flake GP, Kirby PJ, Palmer SM (2008). Respiratory toxicity of diacetyl in C57BL/6 mice. Toxicol Sci 103(1):169-180.

Morgan DL, Jokinen MP, Price HC, Gwinn WM, Palmer SM, Flake GP (2012). Bronchial and bronchiolar fibrosis in rats exposed to 2,3-pentanedione vapors: implication for bronchiolitis obliterans in humans. Toxicol Pathol 40:448-465.

NIOSH, National Institute for Occupational Safety and Health (2003). HETA 2002-0408-2915, Agrilink Foods Popcorn Plant, Ridgway, Illinois, Health Hazard Evaluation (HHE) Report. Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health, October 2003. Report no. 2002-0408-2915, Cincinnati, OH.

NIOSH, National Institute for Occupational Safety and Health (2011). Criteria for a recommended standard. Occupational exposure to diacetyl and 2,3-pentanedione. External review draft, August 12, 2011.

NTP, National Toxicology Program (2007). Chemical information review document for artificial butter flavoring and constituents. Diacetyl [CAS No. 431-03-8] and acetoin [CAS No. 513-86-0]. Supporting nomination for toxicological evaluation by the National Toxicology Program, January 2007. Prepared by Integrated Laboratory Systems, Inc. Research Triangle Park, NC Under Contract No. N01-ES-35515 for National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, US Department of Health and Human Services Research Triangle Park, NC.

OSHA, Occupational Safety and Health Administration (2007a). Hazard communication guidance for diacetyl and food flavorings containing diacetyl.
http://www.osha.gov/dsg/guidance/diacetyl-guidance.html
(accessed February 2014).

OSHA, Occupational Safety and Health Administration (2007b).
https://www.osha.gov/dsg/guidance/101707-diacetyl-meeting-notes.html
(accessed 12 December 2008).

OSHA, Occupational Safety and Health Administration (2008a). Sampling and analytical methods. Method No. 1012.
http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html
(accessed November 2008).

OSHA, Occupational Safety and Health Administration (2008b). Sampling and analytical methods. Method No. 1013.
http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html
(accessed September 2008).

Otsuka M, Mine T, Ohuchi K, Ohmori S (1996). A detoxication route for acetaldehyde: metabolism of diacetyl, acetoin, and 2,3-butanediol in liver homogenate and perfused liver of rats. J Biochem 119(2):246-251.

Palmer SM, Flake GP, Kelly FL, Zhang HL, Nuget JL, Kirby PL, Foley JF, Gwinn WM, Morgan DL (2011). Severe airway epithelial injury, aberrant repair and bronchiolitis obliterans developments after diacetyl instillation in rats. PLoS ONE 6(3):e17644.

Parmet AJ, von Essen S (2002). Rapidly progressive, fixed airway obstructive disease in popcorn workers: a new occupational pulmonary illness? J Occup Environ Med 44:216-218.

Pengelly I (2012). Assessment of the occupational exposure to diacetyl contained in food flavorings and cooking products. Poster at the British Occupational Hygiene Society (BOHS) Annual Conference, Cardiff, 24-26 April 2012.

Pendergrass SM (2004). Method development for the determination of diacetyl and acetoin at a microwave popcorn plant. Environ Sci Technol 38:858-861.

Rao X, Kobayashi R, White-Morris R, Spaulding R, Frazey P, Charles MJ (2001). GC/ITMS measurement of carbonyls and multifunctional carbonyls in PM2.5 particles emitted from motor vehicles. J AOAC Int 84:699-705.

Ronk CJ, Hollins DM, Jacobsen MJ, Galbraith DA, Paustenbach DJ (2013). Evaluation of pulmonary function within a cohort of flavorings workers. Inhal Toxicol 25:107-117.

RTI, Research Triangle Institute (1997). Final report on disposition and excretion of [14C]2,3-butadione in male rats following oral administration. RTI Study No. 64U-6855. RTI Protocol No. RTI-6855-06. Report from RTI, Research Triangle Park, NC, to National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Schachter EN (2002). Popcorn worker's lung. N Engl J Med 347:360-361.

Stoner GD, Shimkin MB, Kniazeff AJ, Weisburger JH, Weisburger EK, Gori GB (1973). Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumour response in Strain A mice. Cancer Res 33:3069-3085.

Tucker JD, Taylor RT, Christensen ML, Strout CL, Hanna ML, Carrano AV (1989). Cytogenetic response to 1,2-dicarbonyis and hydrogen peroxide in Chinese hamster ovary AUXBI cells and human peripheral lymphocytes. Mutat Res 224(2):269-279.

van Rooy FG, Rooyackers JM, Prokop M, Houba R, Smit LA, Heederik DJ (2007). Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. Am J Respir Crit Care Med 176(5):498-504.

van Rooy FGBGJ, Smit LAM, Houba R, Rooyackers J, Heederik DJJ (2009). A cross-sectional study of lung function and respiratory symptoms among chemical workers producing diacetyl for food flavourings. Occup Environ Med 66:105-110.

von der Hude W, Behm C, Gurtler R, Basler A (1988). Evaluation of the SOS chromotest. Mutat Res 203(2):81-94.

White KL (2011). Correction to microwave popcorn plant diacetyl exposure data. J Occup Environ Hyg 8:D25-D26.

White KL, Heikkila K, Williams R, Levin L, Lockey JE, Rice C (2010). Diacetyl exposures in four microwave popcorn plants. J Occup Environ Hyg 7:185-193.

Whittaker P, Clarke JJ, San RHC, Begely TH, Dunkel VC (2008). Evaluation of the butter flavouring chemical diacetyl and a fluorochemical paper additive for mutagenicity and toxicity using the mammalian cell gene mutation assay in L5178Y mouse lymphoma cells. Food Chem Toxicol 46:2928-2933.

WHO, World Health Organization (1999). FAO/WHO Joint Expert Committee on Food Additives; WHO Food Additives Series 42: Safety evaluation of aliphatic acyclic and alicyclic alpha-diketones and related alpha-hydroxyketones. IPCS, International Programme on Chemical Safety, World Health Organization, Geneva.

Appendix 1. Comparison of methods of analysis for diacetyl in air

In a field study, the two OSHA methods, PV2118 and a modified version of PV2118, gave virtually similar results and results were not significantly influenced by humidity or temperature (Ashley *et al* 2008, OSHA 2007b).

Results from the NIOSH (LOD: 0.6 µg/sample and limit of quantification: 2 µg/sample) (Pendergrass 2004) and the modified OSHA methods are tabulated in Table A. The OSHA method has a higher detection rate than the NIOSH method.

Comparisons were only performed for samples in which diacetyl was detected by both methods (Tables I and II in Ashley *et al* 2008), which allowed the use of 15/24 and 5/30 data pairs. This lends a limited credit to the ratio calculated from the values of the remaining data pairs, which are listed below.

Table A. Comparison of results obtained by NIOSH and OSHA methods.

Table No. as of Ashley *et al* 2008	Detected levels of airborne diacetyl (ppm)		Ratio B/A
	NIOSH 2557 (A)	OSHA modified PV2118 (B)	
I	0.04	0.11	2.75
I	0.02	0.04	2.00
I	0.07	0.08	1.14
I	0.04	0.12	3.00
I	0.1	0.15	1.50
I	0.01	0.04	4.00
I	0.42	0.54	1.29
I	0.08	0.16	2.00
I	0.02	0.16	8.00
I	0.17	0.35	2.06
I	0.02	0.07	3.50
I	0.39	0.48	1.23
I	1.05	1	0.95
I	0.05	0.37	7.40
I	0.02	0.04	2.00
II	0.05	0.63	12.60
II	0.57	1.58	2.77
II	0.08	0.14	1.75
II	0.53	6.33	11.94
II	0.03	0.03	1.00
Mean ratio (range):			3.64 (0.95–12.60)

Table B is an evaluation of bias from using the LOD divided by 2 as substitute for a concentration when the NIOSH 2557 method (estimated LOD: 0.6 µg/sample) showed no diacetyl in the air, whereas the OSHA PV2118 or modified OSHA did. Paired measurements were available from 54 simultaneous samples by the two methods (Ashley *et al* 2008). For 19 of these (35 %), the NIOSH method showed no diacetyl in the air.

Table B. Comparison of OSHA and corrected NIOSH results

Table No. as of Ashley *et al* 2008	Levels of airborne diacetyl (ppb)		Ratio A/B
	Detected with PV2118 or modified OSHA method (A)	Not detected with NIOSH 2557 and substituted by LOD/2 [a] (B)	
I	80	1.76	45
I	40	1.76	23
I	520	1.76	295
II	250	14.2	18
II	90	14.2	6.3
II	260	14.2	18
II	60	14.2	4.2
II	890	14.2	63
II	1 160	14.2	82
II	280	14.2	20
II	580	14.2	41
II	360	14.2	25
II	300	14.2	21
II	400	14.2	28
II	190	14.2	13
II	80	14.2	5.6
II	370	14.2	26
II	240	14.2	17
II	60	14.2	4.2
Mean ratio (range):	-	-	19.4 (4.2–295)

[a] *Table I:* samples collected for 8 hours at a flow rate of approximately 0.1 l/min, suggesting a LOD of 3.52 ppb. *Table II:* samples collected for 2 hours at a flow rate of approximately 0.05 l/min, suggesting a LOD of 28.4 ppb.

LOD: limit of detection.

Appendix 2: Diacetyl exposure and development of lung disease

Plant	Place of exposure	Diacetyl concentrations (ppm), average (range)	No. of samples	No. of cases/ no. of exposed
Popcorn producing plants in the United States (main reference: Kanwal et al 2006)				
Plant A [a] (Missouri)	Mixing room	A: 30–40 (1–98) [b]	12	BO: 5/13 [a, c]
	Packing area	A: 1.9 (0.3–6.8)	22	BO: 4/115 [a, c]
	Quality control	A: 0.6 (0.3–0.9)	5	AO: 5/6 [a, c]
	All areas		-	AO: 21/116 [a] AR: 10/116 [a]
Plant B	Mixing room	A: 0.6 (0.4–1.0) P: 0.6 (0.4–0.7)	3 2	BO: 1/?
	Packing area	A: 0.7 (0.4–1.2) P: 0.5 (0.2–1.0)	9 8	-
Plan C	Mixing room	A: 0.4 (0.02–0.9) P: 0.03 (0.01–0.04)	2 2	-
	Packing area	A: 0.03 (0.01–0.05) P: 0.02 (0.01–0.04)	4 7	-
Plant D	Mixing room	A: 0.2 (< 0.001–0.6) P: 0.02 (< 0.001–0.05) [d]	3 5	BO: 1/?
	Packing area	A: 0.004 (< 0.001–0.03) P: 0.002 (< 0.001–0.009)	13 12	-
	Quality control	?: < 0.001	?	AO:0/3
Plant E	Mixing room	A: 0.6 (0.3–0.9) P: 0.4	2 1	-
	Packing area	A: 0.3 (0.2–0.4) P: 0.6 (0.3–1.2)	2 3	BO: 3/?
Plant F	Mixing room	A: 1.2 (0.5–2.7) P: 1.0 (0.2–2.0)	6 7	BO: 1/?
	Packing area	A: 0.2 (LOQ–0.03) P: 0.02 (LOQ–0.03)	18 24	-
	Quality control	?: 0.02 (< 0.01–0.03)	?	AO: 1/11
Diacetyl production in the Netherlands (van Rooy et al 2007)				
	Process operators	A: (0.6–100) Years: 1995–2003 [e] A: (1.6–2.8) Year: 2001 P: Peaks up to 113 [e]	30 [f]	BO: 4/102 [g]

A: area sampling, AO: airway obstruction, AR: airway restriction BO: bronchiolitis obliterans, P: personal sampling, <: below minimum quantifiable concentration (LOQ).

[a] In October 2000, there were approximately 135 employees in the Missouri plant. Of these, 116 participated in spirometric testing, 20 (17 %) of 117 workers who completed a questionnaire were not considered exposed. The ingredients of the flavourings included soybean oil, salt, butter flavouring and colouring agents. In the mixing room, one worker per shift opened the lid of an oil tank that was heated to approximately 54°C and where a peak diacetyl level of 1 230 ppm was measured (Kreiss *et al* 2002). The cases from the quality control workers were from Kanwal *et al* (2006) and Kreis *et al* (2002). Other cases were from Kreiss *et al* (2002).

[b] Reported area concentrations were 32.27 ppm (range: 1.34-97.94), N = 12 (Kreiss *et al* 2002); 37.8 ppm (range: 2.3–98), N = 10 (Table 4 in Kullman *et al* 2005) and 37.8 (range 1.3–97.9), N = 12 (Table 1 in Kanwal *et al* 2006).

[c] In the Missouri plant, one additional case of BO was observed later, so the total number of bronchiolitis obliterans cases among mixers was 5. In this plant, approximately 161 workers participated (Kanwal *et al* 2006) in a study and, considering 17 % as non-exposed, the number of exposed individuals is estimated to 134. Subtracting the 13 workers from the mixing room (Schachter 2002) and the 6 from the quality control area (Kanwal *et al* 2006, Kreiss *et al* 2002), suggests about 115 exposed workers in the packing area; this relatively uncertain value is used as an estimate of the number of exposed. However, it may be higher as 425 had been employed in the period 1992–2000 (Schachter 2002). Accepting 17 % as non-exposed (Kreiss *et al* 2002), 13 in the mixing room (Schachter 2002) and 6 in the quality control area (Kanwal *et al* 2006, Kreiss *et al* 2002), the number of exposed in the packing area could be as high as 425 x 0.83 – 13 – 6 = 334 (Kanwal *et al* 2006).

[d] Peak diacetyl air concentrations of over 80 ppm over several minutes (Kanwal *et al* 2006).

[e] Between the years 1960 and 2003, 206 workers were potentially exposed. Information was obtained from 175. Process operators (n = 102) were considered the highest exposed group. Discharge of diacetyl in containers had the highest exposure potential, 0.9–113 ppm. All identified bronchiolitis obliterans cases were among process operators.

[f] A total of 26 area samples and 4 personal task-based samples.

[g] Three cases of bronchiolitis obliterans were identified. However, one additional case was found after the study among 10 non-participants. Thus, at least 4 cases of bronchiolitis obliterans arose among the workers.

Appendix 3: Risk assessment approaches

Approach 1: Modified Haber's law

This model assumes that a threshold level of diacetyl exists, under which the human organism is able to cope with exposure. Thus, effects occurring at this or lower levels are reversible and no accumulation of adverse effects occur, whereas effects occurring above this level are irreversible. This can be illustrated from the classic study of Flury on hydrogen cyanide, where death in experimental animals follows a modified Haber's law [(exposure time x (concentration minus threshold) = Constant], for reference see Henschler 1984.

This approach was used in the previous evaluation conducted by SCOEL in 2010. A NOAEC for *respiratory symptoms* was derived directly from exposures lasting several years. For prevention of bronchiolitis obliterans, the concentration should be below 0.6 ppm obtained from personal sampling and below 0.3 ppm obtained by area sampling (Appendix 2, Plant E) as this value is from a packing area where the concentration is considered relatively even. However, subclinical alteration of *lung function* should also be prevented. The study by Kreiss *et al* (2002) provides the lowest NOAEC expressed as a cumulative dose (0.65 ppm-years) based on a decrease in lung function. As the median time since start of employment was 3.4 years (range: 0.1–17.6), this corresponds to a predicted no-effect level of 0.2 ppm. Both these values are, however, biased as the analytical method (NIOSH 2557) is sensitive to several workplace and storage parameters. An unbiased NOAEC was estimated by multiplying with a factor of 3 (Appendix 1, Table A), which suggests a value of about 0.6 ppm for initiation of a decrease in lung function. For bronchiolitis obliterans, the expected effect level would be about 1.8 ppm (3 x 0.6 ppm, personal sampling, Appendix 2, Plant E). In this model, the ratio between concentration causing bronchiolitis obliterans and the NOAEC for decrease in lung function corresponds to a factor of 3. This model does not take into account peak exposures that may have an important effect.

Approach 2: Unmodified Haber's law

In this approach, besides recognising that bronchiolitis obliterans and fixed airway obstruction are irreversible, severe and life threatening effects, some concern is also given to the possible genotoxic and carcinogenic activity of diacetyl. Epidemiological findings are, therefore, extrapolated to apply to any concentration of diacetyl, i.e. all damages are entirely (toxicodynamically) irreversible, the damages are proportional to the concentration rate (concentration/time), and adverse effects occur when a certain number of damages have occurred at a critical site. In this case, the unmodified Haber's law (concentration x time = constant) can be used for extrapolation.

The lowest NOAEC (0.65 ppm-years) for cumulative exposure is derived from the Kreiss *et al* (2002) study, in which NIOSH method 2557 was used. If Haber's law applies, the value can be extrapolated to a 40-year working life period, resulting in a NOAEC of 0.016 ppm for the 40-year period. To correct for the bias of the method, this value can be multiplied by a factor of 3, suggesting that an unbiased NOAEC would be about 0.05 ppm. From the study of Lockey *et al* (2009), a corrected NOAEC of 1.6 ppm-years can be derived, which corresponds to a 40-year extrapolated value of 0.04 ppm. Both values can be considered a lower boundary, as the many values below the detection limit from the NIOSH 2557 method may have caused a considerably greater bias (Appendix 1, Table B) than a factor of 3. Another bias is also possible as high concentrations may more efficiently exhaust protective constituents (e.g. antioxidant defence and reactions within

the mucous layer) and thus being able to induce a disproportionate increase in tissue concentrations at a critical site compared to the low concentration obtained by extrapolation to a 40-year period. Whether such biases exist and their potential magnitude can, however, not be accounted for. This model does not take into account peak exposures that may have an important effect.

Approach 3: Extrapolation from animals to humans

SCOEL also analysed exposure-response relationships in animal studies and extrapolated the results to a predicted NOAEC in humans. The relevant effect for human risk assessment is the bronchiolar effect, with a NOAEC of 25 ppm in a 12-week study in mice. Adjustment for differences in daily exposure length is 6/8 (exposure of animals 6 hours/day, 5 days/week), adjustment for toxicokinetic differences between rats and humans is 40 (Gloede *et al* 2011), and an extrapolation from rats to mice is arbitrarily set to 2. Since the mice study does not indicate a cumulative effect at 25 ppm (the lung effects did not increase by extending the exposure period from 6 to 12 weeks), a concentration-based OEL of 0.23 ppm can be recommended. However, if an adjustment (3/20) for sub-chronic (~3 month) to lifelong exposures (20 months) is introduced, the recommended OEL would be 0.035 ppm. The range 0.035–0.23 ppm can be considered to overestimate the toxicity to an unknown extent as the metabolism is relatively more efficient at low concentrations; high concentrations, used for extrapolation, allows a relatively higher fraction of inhaled diacetyl penetrating to the bronchiolar level. A potential difference in sensitivity between human and mice bronchiolar cells (toxicodynamic differences) cannot be accounted for; often a default value is in the range of 1–2.5.

Approach 4: The US NIOSH (2011) approach

NIOSH (2011) analysed exposure-response relationships from several plants, but due to limited consistency across plants, the data from the Missouri plant was selected for the risk assessment; this plant had the highest number of exposure assessments (about 600) compared to the other analysed plants. The predicted lung function (FEV_1 and FEV_1/FVC) and the lower limits of normal (LLofN; 5^{th} percentile) were from the general US population. Virtually all cases below LLofN were attributed either to diacetyl exposure or smoking. NIOSH observed that many cases arose after relatively short employment duration and some individuals in the exposed population were losing FEV_1 faster than others. For risk assessment, NIOSH used two non-threshold methods, the benchmark dose procedure and a life-table procedure. NIOSH accepted a risk of 1/1 000 for lung function below LLofN at a 45-year work-time exposure; the cumulative exposure metrics was used in the extrapolations. Based on a population with different susceptibility, NIOSH derived an 8-hour TWA value of 5 ppb and a 15-min short term exposure (STEL) of 25 ppb.

The NIOSH methods are not considered by SCOEL, as concerns were raised about the robustness of the extrapolation method where LOD/2 replaces values below the detection limit of the NIOSH 2557 method (Appendix 1, Table B).