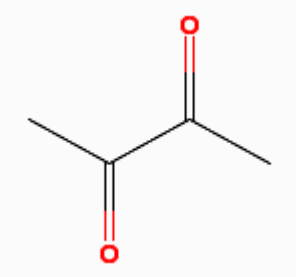
DIACETYL

CAS number: 431-03-8

Synonyms: Biacetyl; 2,3-Butanedione; Dimethylglyoxal; Dimethyl diketone; 2,3-Diketobutane

Molecular formula: $C_4H_6O_2$/$CH_3COCOCH_3$

Chemical structure:



TLV–TWA, 0.01 ppm (0.04 mg/m^3)

TLV–STEL, 0.02 ppm (0.07 mg/m^3)

A4 — Not Classifiable as a Human Carcinogen

TLV® Recommendation

A TLV–TWA of 0.01 ppm (0.04 mg/m^3) is recommended for occupational exposure to diacetyl. Several cases of bronchiolitis obliterans-like illness were reported among microwave popcorn workers exposed to diacetyl used as a flavoring agent in 2002 (Parmet et al., 2002; Akpinar-Elci et al., 2002; MMWR, 2002). The continued investigation of this issue identified what the authors considered to be a case of bronchiolitis obliterans-like illness in a mixer employee at a popcorn manufacturing plant that had a mean exposure for mixer employees estimated by area sampling, as 0.2 ppm, and by personal sampling as 0.02 ppm (Kanwal et al., 2006). Exposures in the mixing rooms of popcorn manufacturing plants were likely heterogeneous, and the worker may have had short-term peak exposures much higher than this, possibly greater than 80 ppm (NIOSH, 2004). Lockey et al. (2009) identified employment as a mixer employee prior to the introduction of powered air purifying respirators (PAPR), and an estimated cumulative exposure level of 0.8 ppm-years or greater to be associated with an increased risk of decreased FEV_1 on lung function testing. The same paper reported that workers outside the mixing rooms of popcorn manufacturing plants had no identifiable reduction in mean FEV_1 at mean levels of exposure of 0.01–0.07 ppm. However, in other papers there have been reports of bronchiolitis obliterans-like illness occurring in workers outside the mixing room type environment.

A study of induced sputum in microwave popcorn production workers also reported changes in inflammatory markers in sputum at mean levels of exposure ranging from approximately 0.03–0.8 ppm (Akpinar-Elci et al., 2005).

Short-term peak exposures may be potentially important in the genesis of this illness, therefore a TLV–STEL of 0.02 ppm is assigned. There is insufficient evidence to recommend a Skin or a SEN notation. An A4, Not Classifiable as a Human Carcinogen, carcinogenicity notation is assigned based on there being only a single study in mice with conflicting evidence (Stoner et al., 1973).

TLV® Basis

Lung damage (Bronchiolitis obliterans-like illness).

Chemical and Physical Properties

Molecular weight: 86.1
Specific gravity: 0.98
Melting point: -2.4°C
Boiling point: 88.0°C
Vapor pressure: 57 torr (7.6 kPa) at 25°C
Saturated vapor concentration: 75,000 ppm
Flash point: 6.0°C
Flammable limits: not available
Autoignition temperature: 365.0°C
Solubility: 20 g/100 ml in water at 25°C
Octanol/water partition coefficients: -1.34 log (K_{ow})
Conversion factors at 25°C and 760 torr:
1 ppm = 3.52 mg/m^3, 1 mg/m^3 = 0.284 ppm

Major Sources of Occupational Exposure

Diacetyl is a naturally occurring substance. It is found in some foods including butter, caramel, coffee, cocoa, and honey. It has been identified in a number of dairy products other than butter (e.g., cheese, yogurt, milk). It is also present in a number of flowers and plant extracts, as well as in aromatic components of tobacco smoke (NTP, 2007). It occurs in fermented products such as beer and wine (and likely contributes to the buttery flavor of chardonnay wines).

Diacetyl is also synthesized and used as a food additive and is mainly used as a flavoring agent where it imparts a butter-like taste. It has been used as a flavoring for popcorn, but also for margarine, candies, and a wide range of other products. Other uses for diacetyl include: reactant/starting material in chemical or biochemical reactions; analytical reagent; antimicrobial/preservative; modifier of radiation response for chemical and biological systems; and photoinitiator/photosensitizer in polymerizations (NTP, 1994).

Animal Studies

Acute/Subacute

ORAL

Jenner et al. (1964) reported an oral LD_{50} of 1580 mg/kg in rats, and 990 mg/kg in guinea pigs. An oral LD_{50} of 250 mg/kg in mice was reported, along with the above figures, in an NTP report published in 1994.

In one study, both an acute toxicity and a short-term feeding study were reported (Colley et al., 1969). For the acute toxicity study groups of five adult SPF derived CFE rats were given diacetyl either by oral intubation or by intraperitoneal injection. After treatment, animals were observed for up to 14 days and then autopsies were carried out on selected survivors (details of how they were selected were not given). The authors reported that at high doses, death followed soon after convulsions. The estimated LD_{50} for oral dosing was 3.40 g/kg for male rats and 3.00 g/kg for female rats, whereas for intraperitoneal dosing the LD_{50} was 0.40 g/kg for male rats and 0.64 g/kg for female rats. In the short-term feeding study, groups of 15 male and 15 female SPF derived CFE rats were given doses of diacetyl for 90 days, after which all were killed and autopsies performed. The doses used corresponded to 0, 10, 30, 90 or 540 mg diacetyl/kg/day. Food consumption was similar in all groups but weight gain was less in the group given the highest dose of diacetyl. This group also consumed more water. Organ weights were also lower in this group, except for the adrenal gland, which was increased. Some hematological changes were also seen at the highest doses with this group having a lower hemoglobin concentration, lower packed cell volume, and an increased reticulocyte count, along with an increased leucocyte count, predominantly due to an increase in neutrophils. Effects were more marked in males than females. At autopsy, sloughing and ulceration of the gastric epithelium was seen in all 15 male rats and 14 out of 15 female rats given the highest dose of diacetyl. No changes were seen in the animals fed lower doses. No other histological differences were identified between test and control animals. The authors reported a no-observed-adverse-effect-level (NOAEL) of 90 mg/kg/day.

DERMAL

A report to the Research Institute for Fragrance Materials (RIFM) was quoted by Opdyke (1979) as reporting an acute dermal LD_{50} in rabbits of > 5.0 g/kg.

INHALATION

A number of papers have reported on the inhalation toxicology of diacetyl. Hubbs et al. (2002) reported a study during which male Sprague-Dawley rats were exposed for six hours to the vapor from butter flavoring, one of the major constituents of

which was diacetyl. Exposure groups comprised control (0 ppm,19 rats); low-exposure (203 ppm, six rats); middle-exposure (285 ppm, four rats); high-constant-exposure (352 ppm, six rats); and, high-pulsed exposure (371 ppm, ranging 72–940 ppm, three rats). Rats were euthanized the day after exposure with all but one of the middle-exposure group of animals undergoing histopathological examination, and nasal and bronchoalveolar lavage being performed on most animals. All of the middle- and high-exposure rats developed necrotic changes in the nasal and respiratory epithelium, as well as one of six in the low-exposure group. Nasal lavage and bronchoalveolar lavage showed changes consistent with an inflammatory response. The authors concluded that the no effect level for a six-hour exposure to butter flavoring vapor lies below the levels used in this experiment.

Morgan et al. (2008) reported results of a study in which C57B1/6 mice were exposed to diacetyl vapors. Groups of mice were given both whole body exposures (for continuous exposure), and nose only exposures (for intermittent exposure—allowing immediate removal of exposure). Exposure groups comprised:

- Subacute: whole body exposure; six hours/day for up to five days at levels of 0 ppm (n = 7), 200 ppm (n = 10), or 400 ppm (n = 15).
- Subchronic: whole body exposures; six hours/day, five days a week, for six or 12 weeks at levels of 0 ppm, 25 ppm, 50 ppm, or 100 ppm (n = 5 per group).
- Intermittent low exposure: nasal only exposure; one hour per day, five days/week for two or four weeks at levels of 0 ppm, 100 ppm, 200 ppm, or 400 ppm (n = 5 per group).
- Intermittent high level exposure: nasal only exposure; 15 minutes, twice daily, five days/week, for two weeks at levels of 0 ppm and 1200 ppm (n = 5 per group).
- A further group of mice were given diacetyl by oropharyngeal aspiration in a single dose at levels of 0, 100, 200, or 400 mg/kg body weight.

Results of the subacute exposure study showed:

- In the 200 ppm-exposure group, a number of mice became moribund and had to be sacrificed prior to the end of the experiment (6 of 10).
- None of the 400 ppm-exposure group survived to the end of the experiment. Two died, and the remainder had to be sacrificed as they became moribund.
- Mice that became moribund showed acute necrotizing rhinitis and acute or erosive necrotizing laryngitis. Acute necrotizing bronchitis was also seen in the 400 ppm-exposure group. Changes seemed to be more severe in the upper respiratory tract than the lower respiratory tract.

Similar findings were seen in the intermittent low dose exposure group with a dose-related increase in respiratory system toxicity and inflammatory response (including the 100 ppm group), and in the intermittent high exposure group. A NOAEL for these mice was not determined for subacute exposures as the authors felt there were significant adverse effects at all dose levels tested. Results for the subchronic exposure group are discussed below.

Hubbs et al. (2008) reported findings from their study of inhalational toxicology of diacetyl in rats. In the first part of their study (experiment 1), a continuous six-hour exposure was administered to male Hla:(SD)CVF rats at one of four doses: control (0 ppm), low (99.3 ppm); medium (198.4 ppm); or high (294.6 ppm). In the second part (experiment 2), equivalent cumulative doses were administered as either four 15-minute pulses during a six-hour period, or as a continuous six-hour exposure. A further group was also given a single 15-minute pulse, which aimed to be equivalent to a 75 ppm continuous exposure assessed as a TWA over a six-hour period. Rats were euthanized 24–26 hours after the start of exposure. Results from experiment 1 revealed significant toxicity to the nasal epithelium in the medium and high exposure groups with what appeared to be predominantly a necrotizing suppurative rhinitis. Airway changes were seen in the lower airways of only two of six rats in the high-exposure group, with a necrotizing suppurative bronchitis. Electron microscopy of the tracheal bifurcation in the higher exposure groups also revealed changes in the surface morphology with loss of microvilli, decreased numbers of ciliated and mucus cells, flattening of the epithelium, and foci of denudation of the basement membrane. Results from experiment 2 suggested that the exposure pattern had a relatively minor effect on toxicity, pulsed exposures being associated with a lower pathology score only in the first part of the nose adjacent to the anterior nares at the low dose. Again, changes were greater in the upper respiratory tract than the lower, with damage extending to the

peripheral bronchi only at higher doses. The group given the single pulse with a peak of 1949 ppm showed some damage to the nasal epithelium adjacent to the anterior nares typical of a necrotizing and/or suppurative rhinitis, but no changes elsewhere in the nose or lower in the respiratory tract. While the continuous low level exposure at approximately 100 ppm in experiment 1 showed few changes in the nasal epithelium, similar exposures in experiment 2 did, as did the pulsed exposures at the levels equivalent to 100 ppm as a TWA. The authors conclude that the NOAEL for rats is below 100 ppm.

SENSITIZATION

One abstract to date has reported some evidence of skin sensitization in mice (Auttachoat et al., 2007). A full paper reporting these data could not be identified.

OTHER STUDIES

Fedan et al. (2006) reported the effects of diacetyl on guinea pig isolated, perfused trachea. Diacetyl applied intraluminally at 3 mM increased reactivity to methacholine by approximately 10-fold, while concentrations of 10 mM inhibited responses to methacholine completely (possibly due to direct toxicity). Relaxation responses were unaffected, which the authors interpreted as the diacetyl showing no effect on smooth muscle. The authors concluded that diacetyl exerts a toxic effect on the airway epithelium leading to airway hyper-reactivity (at least *in vitro*) and loss of the epithelial protective barrier function.

Subchronic

Morgan et al. (2008) reported results of a study in which C57B1/6 mice were exposed to diacetyl vapors (*see* Animal Inhalation Studies on page 2 for details). The study included both subacute and subchronic exposure groups with the subchronic group receiving whole body exposures six hours a day, five days a week, for six or 12 weeks at levels of 0 ppm, 25 ppm, 50 ppm, or 100 ppm. Body weights of mice exposed to 100 ppm were less than controls throughout the exposure and post-exposure period, but those of the 25 ppm and 50 ppm were not significantly different to controls. Histopathology of the mice revealed suppurative rhinitis and active inflammatory changes in the 100 ppm exposure group, with less marked and relatively minor changes in the 50 ppm and 25 ppm exposure groups. In the lungs, all mice (5/5 with six weeks exposure, 5/5 with 12 weeks exposure) in the 100 ppm exposure group showed evidence of airway toxicity and inflammation with necrosis, ulceration, squamous metaplasia, and peribronchial lymphocytic infiltration. Similar but less marked evidence of inflammation was seen in some of the mice in the 50 ppm (9/10 with peribronchial lymphocytic inflammation) and 25 ppm (5/10 with peribronchial lymphocytic inflammation) exposure groups. A NOAEL for these mice for subchronic exposure was not determined as the authors felt there were significant adverse effects at all dose levels tested.

Chronic/Carcinogenicity

A single study was identified that reported the effects of a number of food additives (including diacetyl) and chemotherapeutic agents in inducing pulmonary tumors in mice. In a first series of tests, 40 A/He female mice in two groups were given intraperitoneal injections three times weekly for eight weeks with diacetyl in water at doses of either 1.70 g/kg or 8.40 g/kg. Animals were euthanized at 24 weeks and the lungs fixed and then examined for tumors (by counting visible tumors on the lung surface with some being examined histopathologically). The number of tumors occurring in the lungs of treated animals was compared with controls who had received only the vehicle. In this first series, a significantly greater number of lung tumors occurred in the mice given the highest dose of diacetyl than occurred in the controls. The authors then repeated the experiment in a second series of tests on 30 female and 30 male mice, 15 of each gender receiving the same doses as above. The diacetyl was reassayed prior to this second experiment. In the second series, no difference in tumor numbers was seen between treated and control mice. Unequivocally positive results were obtained in the same experiments with a positive control (urethan) and with some of the chemotherapeutic agents tested. The authors do not discuss why the two test series for diacetyl may have differed, but they concluded that 'Cinnamyl anthranilate was the only positive of the 41 food additives tested, implying they were more convinced by the second than the first series of results.

Genotoxicity

In a report by Bjeldanes and Chew (1979), the mutagenicity of diacetyl was tested against two test strains of *Salmonella typhimurium*, TA98 and TA100.

A dose-dependent mutagenic activity was seen for diacetyl with the TA100 strain, but not for the TA98 strain. The addition of a liver microsomal preparation made no difference to the results. Marnett et al. (1985) reported that diacetyl was also mutagenic in the *Salmonella typhimurium* TA104 strain.

In a more recent report, diacetyl at concentrations of 100–250 µg/ml was found to be highly mutagenic in the L5178Y mouse lymphoma cell assay when activated with human liver S9 (Whittaker et al., 2008).

Absorption, Distribution, Metabolism, and Excretion

There have been relatively few published studies on the pharmacokinetics of diacetyl. Diacetyl is a water-soluble chemical and was well absorbed on its inhalation in the upper respiratory tract of anesthetized rats (Morris and Hubbs, 2009). A physiologically-based pharmacokinetic (PBPK) model developed by the researchers indicated that inspired diacetyl would penetrate more deeply into the airways of mouth-breathing humans than nose-breathing rats. Respiratory epithelial uptake was apparently enhanced by metabolism of the chemical *in situ. In vivo* and *in vitro* experiments with rats demonstrated that diacetyl is readily reduced to acetoin (3-hydroxy-2-butanone) and to a lesser extent to 2,3-butanediol (Otsuka et al., 1996). Both of these metabolites could be oxidized to a limited degree back to ketones. Diacetyl, acetoin, and 2,3-butanedione were found in the liver, kidney, and brain of rats dosed orally with any one of the compounds. Glucuronide conjugates of 2,3-butanediol were identified in rat and human urine. Reduction of diacetyl was shown to be catalyzed by diacetyl reductase, a ubiquitous enzyme in rats and humans (Nakagawa et al., 2002). Reduction represents a detoxification pathway, as the parent compound appears to be the primary toxic moiety.

Diacetyl has been proposed to cause acute and chronic injury and inflammation through the interrelated mechanisms of carbonyl stress and oxidative stress (Wondrake et al., 2002). The presence of two juxtaposed carbonyl groups on a carbon chain enhances the reactivity of each group with amino groups of proteins. Accumulation of the glycation end-products leads to aberrant production of inflammatory cytokines and chronic inflammatory diseases. These end products also result in cross-linking of proteins such as collagen and laminin, eventually resulting in sclerosis in the lung, blood vessels, and other tissues (Miller and Gerrard, 2005). Reduction potentials for diacetyl and its glycation products favor catalytic redox cycling with oxygen, giving rise to oxidative damage through generation of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, and hydroperoxides (Kovacic and Cooksy, 2005). ROS and reactive carbonyls, especially dicarbonyls, have been implicated in not only chronic inflammatory diseases like bronchiolitis, but diabetes, adverse effects of alcohol, cancer, and aging (Wondrake et al., 2002).

Human Studies

Food components, such as oils and flavorings, have been reported to be associated with a disease resembling bronchiolitis obliterans prior to the first reporting of this association in microwave popcorn workers (Simpson et al., 1985; NIOSH, 1986). The first reports of microwave popcorn workers developing respiratory problems were published in 2002 (Parmet et al., 2002; Akpinar-Elci et al., 2002; MMWR, 2002). A total of eight former workers with an illness resembling bronchiolitis obliterans were identified from a single plant in Missouri. The cases occurred among mixer employees (4 of 13) and microwave packaging workers (4 of 276). No cases were reported from other areas of the plant. Mixer employees were mixing soybean oil, salt, and flavorings into a large heated tank. Initial reports identified approximately 100 volatile organic compounds (VOC) in the plant air, of which diacetyl was measured with a geometric mean concentration of 18 ppm in the room where the mixing tank was located.

In a 2002 paper, Kreiss et al. reported the results of a cross-sectional survey of current employees and environmental conditions from the same microwave popcorn production plant where the previous cases of bronchiolitis obliterans-like illness had been identified. Health data collected included respiratory symptoms using a standardized questionnaire, spirometric measurements of lung function, carbon monoxide diffusing capacity, and chest X-ray. Comparisons for answers to questions on symptoms were made with National Health and Nutrition Examination Survey (NHANES) III data. Area sampling for diacetyl was performed in addition to area and personal sampling for a number of other airborne contaminants. This allowed an estimate of exposure in each job, which was then used to estimate cumulative exposure for each worker

based on the time spent performing each job. Exposure to diacetyl was then categorized in quartiles for analytic purposes. Mean (range) exposures to diacetyl were 0.04 ppm (≤ 0.25 ppm) for the plain popcorn packaging line, bag printing area, offices, and outside area, 0.56 ppm (0.33–0.89 ppm) for quality control or maintenance, 1.88 ppm (0.26–6.80 ppm), and 32.27 ppm (1.34–97.94 ppm) in the mixing room. Current workers reported 2.8 to 3.3 times the prevalence of respiratory symptoms as those in the NHANES study, with prevalence ratios, if anything, being higher in nonsmokers. Symptom prevalence was higher in areas of the plant with higher exposure, but symptoms still occurred in areas of the plant with only low levels of exposure with 50% in the plain popcorn packaging line, bag printing area, offices, and outside area reporting "mucous membrane irritation." Overall, workers had 3.3 times the expected rate of airways obstruction, and workers who had never smoked, 10.8 times. Airway obstruction on spirometry also increased with increasing cumulative diacetyl exposure, but average FEV_1 was below 100% predicted even among the lowest exposure group. The majority of individuals with airflow obstruction did not demonstrate reversibility on testing with a bronchodilator.

Further details of a total of nine cases occurring in this plant were published by Akpinar-Elci et al. in 2004. Identified cases all worked in the mixing room or in the microwave popcorn packaging area. No exposure data were presented in this paper except that exposure levels were quoted for these workers from the previous paper by this group (Kreiss et al., 2002).

The exposure levels in this same plant were further reported in a later paper by Kullman et al. (2005). The overall mean diacetyl exposure level reported was 8.1 ppm (standard deviation (SD) 18.5 ppm) while the geometric mean (GM) exposure was 0.71 ppm, with a geometric standard deviation (GSD) of 14.4 ppm. The highest mean (37.8 ppm, SD 27.6) and geometric mean (26.0 ppm, GSD 3.03) exposure was in the mixing room, but diacetyl was detected even in areas away from microwave popcorn production, including the offices. Mean exposure in the microwave popcorn packaging area was 2.05 ppm (SD 1.69), GM 1.59 ppm (GSD 2.22). Cases reported above had all been in the mixing area and the microwave popcorn packaging area. The authors reported that in addition to diacetyl a number of other VOCs were present in the workplace including acetoin, methyl ethyl ketone, 2-nonanone, and acetic acid. However, they also commented that no obvious exposure to known hazardous substances recognized to be associated with a respiratory illness such as that described, were identified at levels above occupational exposure guidelines.

NIOSH medical and environmental survey data from six microwave popcorn plants, including the index plant giving rise to the reports above, was published by Kanwal et al. (2006). All current workers at the six plants were invited to participate in the medical survey, which consisted of a questionnaire that collected, among other data, information on symptoms, and spirometry. The environmental survey data included information gleaned from both area and personal samples at the six plants. Workers mixing flavorings reported a significant excess of some respiratory symptoms and had a lower mean % predicted FEV_1 than employees that were never mixer employees. This held true even if data from the index plant were omitted. Similar findings were identified for microwave packaging workers. Five of six quality control workers at the index plant who sampled popcorn from the production line were also found to have fixed airways obstruction, although no other plant reported similar findings. The other plants all had lower measurable concentrations of diacetyl in their quality control labs (mean concentration for the index plant 0.6 ppm). Maintenance workers were also found to have an excess of symptoms and fixed airways obstruction, again this seeming to be largely, although not entirely, due to findings from the index plant. The mean diacetyl concentrations in the mixing rooms of the included plants estimated from area sampling varied from 0.2 ppm to 37.8 ppm, and mean concentrations in the microwave packaging areas varied from 0.004 ppm to 1.9 ppm. Mean results of personal sampling from the mixing areas of the different plants varied from 0.02 ppm to 1 ppm, while those for the packaging areas varied from 0.002 ppm to 0.6 ppm. No data for personal samples was available for the index plant. The plant, which had given rise to the initial index cases, had the highest reported concentrations of diacetyl in both mixing and packaging areas, while a plant with both general and local exhaust ventilation had the lowest concentrations of diacetyl in both mixing and packaging areas. The authors further reported that even at the plant with lowest levels of exposure, a suspected case with fixed airways obstruction was found among the mixing room staff, leading the authors to conclude that the LOAEL from their data was 0.2 ppm based on area sampling, and 0.02 ppm based

on personal sampling from the mixing room at that plant.

Within this paper (Kanwal et al., 2006), the individual plants are identified by a letter, and reviewing the individual Health Hazard Evaluation (HHE) reports for these plants it is not possible to identify definitively which plant corresponds to which letter in the paper. However, both the occupational hygiene and health measurement results are described in greater detail in the individual HHE reports. For the site with the lowest exposure levels (NIOSH, 2004) results of air sampling for diacetyl were reported, sampling having been performed according to NIOSH method 2557. Five personal monitoring samples were obtained for workers in the mixing room at this plant. The results for these samples were 0.050 ppm; 0.030 ppm; 0.004 ppm; 0.005 ppm; and not detected. Results for the three area samples collected were 0.570 ppm; not detected; and not detected. Reviewing the health data for this site, prevalence ratios for the presence of airways obstruction on spirometry were not significantly increased for production workers, but detailed data for individual workers, or for the mixing room itself, were not presented. One mixer employee had clinical findings suspicious of bronchiolitis obliterans-like illness with evidence of fixed airways obstruction. From the information presented in the HHE report, it is not possible to comment further on the dose-response relationship for this suspected case.

A study of chemical workers manufacturing food flavorings at a plant in the Netherlands reported exposure and respiratory health outcomes among 175 workers (van Rooy et al., 2009). Comparison data were obtained from the Dutch section of the European Community Respiratory Health Survey. Cumulative exposure was estimated for workers based on exposure estimates and work history for use in modeling effects on lung function. Arithmetic mean exposure levels were 27.9 mg/m^3 or approximately 7.9 ppm. Geometric mean exposure levels were 8.1 mg/m^3, or approximately 2.3 ppm. Exposures up to 396 mg/m^3, approximately 112 ppm were associated with tasks such as tapping diacetyl containers. Workers in areas considered to be exposed to diacetyl were more likely to report respiratory symptoms than those in the comparison population. However, no clear effect of exposure on lung function was identified among these workers, workers with exposure prior to 1995, and those with greater cumulative exposure having, if anything, a higher FEV_1. These findings seem most likely to be due to selection bias, although the authors suggest that errors in exposure assessment may also have played a role.

A study of workers from four popcorn manufacturing plants previously studied by NIOSH provided some additional information on dose-response relationships among workers in trying to establish a LOAEL (Lockey et al., 2009). A survey was carried out on 725 current full-time employees at four microwave production plants of one employer. Mixer employees were compared with other workers in terms of spirometric measurements of lung function. External comparisons to NHANES III data were used in this report. Information on respiratory symptoms appears to have been collected but was not reported. Exposure assessments were available at the plants from personal sampling carried out by NIOSH and by the employers' own exposure monitoring program. A total of 646 personal breathing-zone samples were available for analysis. Exposure was estimated for each of five exposure groups, with the mixer employees who likely had the highest levels of exposure further subdivided in pre- and post-introduction of PAPRs. A protection factor of 25 was assumed for the use of PAPR. Mixer employees spent approximately 50% of their work time in the mixing room and 50% elsewhere. Cumulative exposure was then estimated by multiplying mean exposure for the exposure group by years of employment. Adjustments to these estimates were made to allow for the fact that mixers spent approximately 50% of their work time outside the mixing area. No adjustment was made for the possibility of historical exposures being higher than the currently measured exposures. Arithmetic (geometric) mean exposures for the nonmixers in the four plants were 0.031 (0.018) ppm, 0.074 (0.014) ppm, 0.027 (0.001) ppm, and 0.014 (0.003) ppm. The corresponding estimates for mixers were 0.678 (0.293) ppm, 0.384 (0.059) ppm, 0.057 (0.029) ppm, and 0.860 (0.230) ppm. Work as a pre-PAPR mixer employee was associated with a significantly lower FEV_1 as a percent of predicted value for both nonAsian males (-6.1%) and Asian males (-11.8%). High cumulative exposure to diacetyl (≥ 0.8 ppm-years) was also significantly associated with a lower percent predicted for nonAsian males (-10.3%), and Asian males (-12.7%). The authors concluded that working in a popcorn production plant outside the mixing room did not appear to be associated with an increased risk of a lower FEV_1 percent predicted; however, work as a pre-PAPR mixer employee, or a cumulative exposure ≥ 0.8 ppm-years, was

associated with evidence of airways obstruction.

Confirmation that the process of preparing and opening the microwave popcorn bags, such as carried out by the quality control workers, could result in meaningful exposure to diacetyl and other agents was provided in a paper published in 2007 (Rosati et al.). The authors studied emissions during the process of popping and opening a bag of microwave popcorn by microwaving the popcorn in a chamber and collecting emissions during popping time and then afterwards when the bag was opened. The average bag of popcorn emitted 780 µg of diacetyl along with a wide range of other volatile organic compounds.

Additional data about the potential pro-inflammatory effects of diacetyl exposure comes from a study of induced sputum in microwave popcorn production workers (Akpinar-Elci et al., 2005). Induced sputum characteristics were compared between 59 workers in processes with high exposure (mixing, microwave packaging, maintenance, quality control) and 22 in processes with low exposure (office, polyethylene packaging, warehouse, outdoor). Mean exposures of the high exposure process workers varied through the year ranging from approximately 0.03 ppm to approximately 8.0 ppm. Workers in the high exposure group had significant increases in a number of inflammatory markers in induced sputum compared with workers in processes categorized as low exposure. These included neutrophil count, eosinophilic cationic protein and interleukin 8. While these are recognized pro-inflammatory markers, their importance in bronchiolitis obliterans-like illness is not well characterized.

Workers exposed to diacetyl in settings other than the manufacture of microwave popcorn have also been reported to have a bronchiolitis obliterans-like disease. van Rooy et al. (2007) reported three cases of fixed airways obstruction in workers who had previously worked in a plant that had produced diacetyl during the period of 1960–2003. One of these workers underwent lung biopsy and had findings of emphysema and chronic bronchiolitis reflecting non-specific small airway disease. The employer had some historical occupational hygiene measurements for the period during which diacetyl had been produced, comprising 26 area samples and 4 personal samples. Air concentrations ranged from 1.8 mg/m^3 to 351.0 mg/m^3 (0.38 ppm–73.71 ppm) for the area samples, and 0.4 mg/m^3 to 29.0 mg/m^3 (0.08 ppm–6.09 ppm) for the personal samples.

In a 2002 case report by Alleman and Darcey, an illness thought to be bronchiolitis obliterans organizing pneumonia (BOOP) in a worker handling spices and flavorings outside the microwave popcorn industry was published. The worker had been manually dumping bags of spice powder into a hopper as part of a potato chip manufacturing process. No information was available on exposure to diacetyl or other flavorings in use as the authors were not able to access the workplace. A later case report (Hendrick, 2008) identified another similar case of respiratory illness in a worker manufacturing potato crisp (chip) flavorings. Exposure to diacetyl was reported in this second case as it was identified as a component in the flavorings being manufactured. No quantitative exposure data were available for these cases.

A further paper reports the results of occupational hygiene sampling in 16 small and medium size flavor manufacturing companies employing from 10 to 130 employees (Martyny et al., 2008). Mean concentrations of diacetyl in area samples ranged from < 0.01 ppm to 3.71 ppm, while mean personal samples ranged from 0.03 ppm to 15.26 ppm. A large proportion of both area (61%) and personal (44%) samples were below the limit of detection (0.01 ppm).

In addition to a bronchiolitis obliterans-like illness, asthma (reversible airways obstruction) has been reported in three food production workers exposed to butter flavored oils used in popcorn manufacture (Sahakian et al., 2008). Diacetyl concentrations in air were below the detection limit for the 12 area and two personal samples collected, although diacetyl could be detected directly above the surface of the heated butter flavored oil.

TLV® Chronology

2011: *proposed:* TLV–TWA, 0.01 ppm; TLV–STEL, 0.02 ppm; A4, Not Classifiable as a Human Carcinogen

References

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