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DIACETYL EXPOSURE AS A PNEUMOTOXIC FACTOR: A REVIEW

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ABSTRACT

Diacetyl (2,3-butanedione) is a natural ingredient in foodstuffs which is not generally regarded health risk to consumers. Nevertheless, when manufactured for use as a synthetic flavouring/additive in processed foods (e.g. microwave popcorn), it poses a human health threat at the workplace. Its pneumotoxic action consists of inflammation, obstruction and restriction in the distal respiratory tract. One of the factors causing bronchiolitis obliterans is also recognised to be diacetyl. The scientific literature mostly describes human exposure to diacetyl in factory settings where functional disorders and structural changes of the respiratory system have been recorded, particularly bronchiolitis obliterans. Moreover, differential diagnosis shows pathological changes in the distal respiratory tract and in the pneumotoxic actions of diacetyl.

Key words: *food flavourings, additives, diacetyl, bronchiolitis obliterans*

STRESZCZENIE

Diacetyl (2,3-butandion) jako naturalny składnik żywności nie wydaje się stwarzać zagrożenia dla zdrowia konsumentów. Związek ten będąc syntetycznym dodatkiem do żywności przetworzonej jest czynnikiem szkodliwym dla zdrowia pracowników zatrudnionych przy jego syntezie i stosowaniu w produkcji prażonej kukurydzy do mikrofalówek. Pneumotoksyczne działanie tego związku manifestuje się zmianami zapalnymi, obturacyjnymi i restrykcyjnymi, szczególnie w dystalnych drogach oddechowych. Diacetyl uznano za czynnik etiologiczny zarostowego zapalenia oskrzelików. Na podstawie piśmiennictwa przedstawiono narażenie na diacetyl w warunkach przemysłowych, zaburzenia czynnościowe i zmiany strukturalne w układzie oddechowym u osób narażonych, ze szczególnym uwzględnieniem zarostowego zapalenia oskrzelików. Ponadto zwrócono uwagę na diagnostykę różnicową zmian patologicznych w dystalnych drogach oddechowych oraz na mechanizmy pneumotoksycznego działania diacetylu.

Słowa kluczowe: *środki aromatyzujące do żywności, diacetyl, zarostowe zapalenie oskrzelików*

INTRODUCTION

Diacetyl (2,3-butanedione; CAS: 431-03-8) is natural ingredient of butter, caramel, beer, coffee, cocoa, honey, vegetable oil, whisky, brandy and some other foodstuffs. It arises during primary milk maturation or in the manufacture of butter or margarine [1, 6] and is synthesised from methyl ethyl ketone and by special fermentation of glucose *via* methylacetylcarbinol [26, 38]. Diacetyl imparts an aroma/flavour similar to other diketones such as 2,3-pentanodione, 2,3-hexanodione and 2,3-heptanodione used in liquid form, pastes or powders to intensify such food flavour/aroma. It is a foodstuff ingredient of butter flavourings [5]. Synthetic diacetyl is used in the manufacture of popcorn, chips (i.e. french fries), confectionery, dairy products that include cheese, sour cream, mayonnaise, sauces, marinades and other processed foods and beverages where it imparts a buttery taste and aroma to foodstuffs [31, 37].

In order to fortify the natural odour of milk, the final concentrations of diacetyl used are 1-3 mg/kg. In microwave popcorn, levels of diacetyl used to range 1-25%, however this has now been decreased or indeed replaced by other substances with similar properties. Most confectionery flavouring contain 1% diacetyl [31]. In USA, France, Belgium, Norway and Sweden diacetyl is permitted as an additive to foodstuffs and in the EU it is manufactured on a large scale in Italy and the UK.

The physico-chemical properties of diacetyl are given in Table 1.

Industrial scale of microwave popcorn production is multi-stage. Sweet corn seed is stored in silos for a maximum of 2 months followed by sieving, air purification and roasting. By automated means, the product

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Table 1. Physico-chemical properties of diacetyl [1, 31, 37]

Molecular formula	$C_4H_6O_2$
Structural formula	CH_3-CO-CO-CH_3
Appearance	Yellowish green liquid
Sensory qualities	Buttery odour, similar to benzoquinone or chlorine, odour threshold in water 4 x 10^{-3} mg/l, in air 0.323 mg/m^3 x 10^{-3}, taste threshold: butter - 1 mg/kg, in milk – 1.4 x 10^{-2} – 2.9 10^{-2} mg/kg, in water - 5.4 x 10^{-3} mg/l
Molecular mass	86.09
Melting point	-1.2°C
Boiling point	88.0°C (1013 hPa)
Density	1.1 (water = 1)
Vapour pressure	7.6 kPa (25°C)
Saturated vapour density	3.0 (air = 1)
Concentration of saturated steam	268,500 mg/m^3
Ignition temperature	27.0°C (closed cup)
Auto-ignition temperature	365.0°C
Limits of flammability	2.4-13.0% vol. (in air)
Partition coefficient (log P_{ow})	-1.34
Solubility	Soluble in water at 200 g/l (15°C), and in benzene, tetrachloromethane, acetone, propylene glycol, glycerol and ethanol.

is then packed into polyethylene bags together with flavourings that contain diacetyl; with all these processes taking place in premises where the flavourings are also mixed. Flavourings consist of soya oil, salt, butter flavourings and food colourings which are mixed together at 64 - 66°C. When ready, the liquid form mixture is transferred into storage at temperatures >51°C [22]. Because of the relatively low boiling-point of diacetyl (88°C) but its high vapour pressure (7.6 kPa at 25°C), this compound very readily permeates the air atmosphere at the workplace.

Human exposure to diacetyl only ever becomes toxic under industrial conditions. During its synthesis however, which occurs at temperatures ~360°C under enclosed conditions, there is no present of exposure; this only happens when the reactor is opened. Within the Dutch chemicals industry, operators where diacetyl was manufactured were exposed to 1.8 – 351 mg/m^3 or 3 – 396 mg/m^3 in more specific tasks. In addition to diacetyl, it was found that in such places the workplace air also contained around 0.4 – 29 mg/m^3 of acetaldehyde [38]. It should note that emission of diacetyl from liquid or paste forms is more intense than from powders. In the USA microwave popcorn plants, exposure to diacetyl was from below the limits of detection of the analytical method (LOD) to 350.8 mg/m^3. The mean arithmetic concentrations ± standard deviation (M ± SD) was 29 ± 66.2 mg/m^3 whilst those in the microwave mixing rooms were 135.3 ± 98.8 mg/m^3 [22]. In Poland, the average air concentrations of diacetyl found at the manufacture of confectionary were on average 51 mg/m^3 [15]. However, the maximum admissible concentration (MAC) value for diacetyl has not yet been established. In the EU, an occupational exposure limit (OEL) for this compound is recommended at level of 0.352 mg/m^3 [37], whereas the ACGIH in USA proposes using threshold limit value - time weighted average (TLV-TWA) and threshold limit value - short-term exposure limit (TLV-STEL) values of 0.04 mg/m^3 and 0.07 mg/m^3, respectively [1].

It has been found that there are over 100 different volatile organic compounds (VOCs) in the microwave area where microwave popcorn is manufactured, and the flavourings are in storage. These mostly included the ketons: diacetyl, butanone, 3-hydroxybutanone (acetoin), 2-nonanone and acetic acid. The average diacetyl concentration reached 125.5 (9.6 – 325.4) mg/m^3. For machine operators this value was 5.6 (0.86 - 18.4) mg/m^3 and for those packaging 6.8 (1.5-17.7) mg/m^3; all other places were below 2.0 mg/m^3. In addition, aerosols of salt and oil were found at mean concentrations of 0.13 ± 0.11 mg/m^3 [22]. For quality control purposes, the opening of microwave popcorn bags released 780 µg diacetyl/bag into the air as well as other VOCs [36]. Thus, the actual human exposure to flavouring ingredients is in general mixed. Tobacco smoke contains 300-430 µg diacetyl/cigarette [8].

Occupational exposure controls implemented in the USA during 2000-3 led to decrease diacetyl levels at the workplace air. When the flavourings are mixed, peak diacetyl levels were reduced from 462 to 0.97 mg/m^3 [34]. In turn, average concentrations of this compound at the places where mixing occurs, machines are operated and the popcorn product is packaged, have been reduced from 205, 9.9 and 2.9 mg/m^3 in 2000 to 10.3 mg/m^3 or below LOD in 2003, respectively [17].

ACUTE TOXIC EFFECTS

Repeated exposures to diacetyl at single instances or at short intervals lead to pronounced irritation to the eyes, respiratory tract and skin. Symptoms include persistent cough, muco-purulent secretion from the respiratory tract, wheezing, dyspnoea/breathlessness, fatigue, mild fever, generalized body aches and skin rash. The substance can also cause central nervous system (CNS) depression, sometimes leading to a loss of consciousness [14, 37]. A case study on a 36 year old never-smoking man with normal lung function and normal serum α-1 antitrypsin activity, but exposed to diacetyl for several hours revealed sore and reddened his eyes, painful eyes and eyelids, together with a sticky conjunctival secretion. Spirometry was normal after 3 months of exposure, but

when repeated 6 months later, showed decease the flow rate of the midportion of the expiratory spirogram (the $FEF_{25\%–75\%}$) attaining 30% of the predicted value, thus indicating altered small-airway function [12].

CHRONIC TOXIC EFFECTS - OBLITERATIVE BRONCHIOLITIS

Occupational exposure to diacetyl-containing food flavourings has led to respiratory disease including obstruction of the small airways [3, 4, 16, 21-24] frequently coupled with persistent dry cough and breathlessness after exertion [25], together with spirometric changes [4, 16, 18, 22-24, 33, 38] (Table 2). Bronchiolitis obliteranswas found in 5/184 persons exposed to diacetyl during its synthesis or in microwave popcorn manufacture. This exposure was however mixed because the air at the workplace also contained acetoin, acetaldehyde and acetic acid [4, 38].

The incidence of bronchiolitis obliterans, often named constructive bronchiolitis or obliterative bronchiolitis, is a rather rare but irreversible disease of the lungs, where obstruction occurs in the distal air passages [11, 40]. The bronchioles become inflamed, exhibit submucosal fibrosis and fibrous tissue proliferation in capillary adventitia and adjacent interalveolar septa [4]. Centrifugal scarring can lead to more frequent obstruction of the small-airways and then complete blockage.

These obstructions arise through excess fibroblast proliferation and accumulation of collagen deposits. A loss of lung tissue elasticity resulting from damage to collagen and elastin fibres, as well as secondary atonia of the lung parenchyma causes the peripheral bronchi to collapse and increases air flow resistance in the bronchiole ends; which explains the symptoms of shortness of breath upon exertion. Clinical symptoms are a dry cough and dyspnoea, particularly during expiration that can either appear progressively or suddenly.

Blocked airways prevent the rapid emptying of the distal part of the respiratory tract during expiration [11]. This leads to excessive lung aeration resulting in ‘air-trapping’ pockets which become visible during radiology [4, 38]. The next stage in the progression, is an increase in total lung capacity (TLC) and other the volumes of the lungs, termed hyperinflation. Such changes are permanent and are not reversible by drugs that dilate the bronchi [11]. Factors responsible for these changes can be irritants like chlorine, sulphur dioxide, phosgene or ammonia [20].

Bronchiolitis obliterans is diagnosed by histopathology of a lung biopsy [4]. Whenever respiratory tract blockages are coupled with changes observed by radiology, using high resolution computed tomography (HRCT), then this condition is defined as bronchiolitis obliterans syndrome – BOS [4, 9, 39]. If there are no radiological changes observed then it is recommended

Table 2. Epidemiological findings for chronic effects of diacetyl on the respiratory system

No.	Study type	Subject numbers studied	Diacetyl concentration (mg/m^3)	Study results	References
Flavouring manufacture for foodstuffs					
1.	Cross-sectional	34	0.11 - 0.80 (PS)	Decreased FEV_1 or TLV values, lung obstruction. BO, decreased FEV_1 or TVC values.	[16] [38]
2.	Longitudinal	175	3.04 - 404.5 (PS) 1.83 - 356.9 (AS)	Increased neutrophils, chronic cough, bronchial asthma.	[39]
Microwave popcorn manufacturing					
3.	Case report series	3	Not available	Decreased FEV_1 or TVC values.	[33]
4.	Cross-sectional	117	2.0 – 115.5 (PS)	Lung obstruction, dysopnoea , fatigue, skin irritation.	[21]
5.	Case report series	9	6.8 - 115.6 (AS)	BO, decreased FEV_1 or TVC values.	[4]
6.	Cross-sectional	108	0.09 - 26.9 (AS)	Lung obstruction, increased neutrophils-OR: 3.8 (1.3 – 11.5).	[3]
7.	Cross-sectional	135	0.09 - 26.9 (AS)	Increased muscle tone of Chest.	[2]
8.	Cross-sectional	537	0.72 - 4.3 (AS) 0.07 - 3.6 (PS)	Decreased FEV_1 or FVC values, dyspnoea, chronic cough, wheezing.	[18]
9.	Cross-sectional	3	< LOD	Decreased FEV_1 or FVC values, lung obstruction.	[23]
10.	Longitudinal	725	1.25 - 3.08 (PS)	Decreased FEV_1 or FVC values, lung obstruction.	[24]

AS – area sampling
PS – personal sampling
LOD – detection limit
BO – bronchiolitis obliterans confirmed by histology and radiology
FEV_1 – the forced expiratory volume in 1 second
FVC – the forced vital capacity

that the concept of 'fixed airways obstruction' be used. A non-invasive method of evaluation the levels of obstructive changes in the airways is spirometry. Here, a forced expiratory volume in 1 second (FEV_1) value below 60% of the predicted value, as well as lowered forced vital capacity (FVC) and the ratio of these measures (FEV_1/FVC) indicate fixed airflow obstruction of the distal respiratory tract [9]. Because this condition is rare, there is a potential for mis-diagnosing it as either bronchial asthma, bronchitis, emphysema or pneumonia.

There are a number of differences between bronchiolitis obliteransand other more common obstructive lung diseases such as asthma or chronic obstructive pulmonary disease (COPD). For example, in asthma, the degree of airway obstruction expressed by the FEV_1/FVC ratio is not long lasting and alters from day to day. Furthermore, FEV_1 values return to normal when treating asthma with short-term bronchiole dilators. Moreover, COPD nearly always results in decreased diffusion capacity of the lungs for carbon dioxide (CO_2) together with excessive reactivity of respiratory tract. These described symptoms are not characteristic features of bronchiolitis obliterans. This condition can be distinguished from fibrotic changes of the lung, such as those in idiopathic pulmonary fibrosis or asbestosis by means of impairment of air flow but not FVC value. Notwithstanding, during the early disease stage, the TLC value is raised however, when fibrotic lung changes occur, this indicator becomes lowered [11].

When screening for early signs of bronchiolitis obliterans, the $FEF_{25-75\%}$ value is recommended coupled with both the diffusing capacity of the lung for carbon monoxide (D_{LCO}) and lung volumes which are highly regarded diagnostic features of this condition [4, 38]. When diagnosing airway inflammation, the bronchoalveolar lavage (BAL) is used. Amongst other things, this procedure provides a profile of inflammatory cells and interleukin concentrations of IL-6 and Il-8; these being mediators of inflammation [11]. Salivary tests can also yield relevant information, where workers exposed to food flavourings show increased levels of neutrophils ($>1.63x10^5$ ml^{-1}) with odds ratio (OR) 3.8 (95% CI: 1.3-11.5) as well as increased IL-8 and eosinophil cationic protein (ECP) concentrations [3].

Bronchiolitis obliterans can indeed lead to death or qualify for a lung transplant. However milder symptoms of obstructed or restricted airways are usually seen in those persons exposed to diacetyl. Studies performed on workers at an USA microwave popcorn factory showed 9/450 persons (aged 27 - 51 years) suffering from bronchiolitis obliterans, of whom only 2 were also confirmed histologically. Five out of these nine workers were employed as mixers of flavourings. Most subjects had never smoked cigarettes and the lengths of employment varied between 1 – 17 years. Those who had worked from 5 months to 9 years experienced coughing, dyspnoea and wheezing. TEV_1 values were 14.0 – 66.8% of expected whilst HRCT showed significant bronchial thickening. Once the exposures had stopped then all persons recovered normal lung function within 2 years [4].

TOXICOKINETICS AND MECHANISMS OF PNEUMOTOXIC ACTION

Diacetyl is a hydrophilic substance and is readily absorbed by the upper respiratory tract; as observed in rat studies [29]. The pharmacokinetics of diacetyl using a physiologically-based pharmacokinetic (PBPK) modelling, indicates that inhaling diacetyl penetrates more deeply in those persons breathing through their mouths than in rats breathing through their noses. Uptake of this substance by the upper respiratory mucosa was clearly intensified by its metabolism.

Mucociliary clearance of diacetyl was dominated by its biotransformation and slow reaction with arginine. The absorption efficiency in rats was greater at lower levels of exposure. At higher exposures, the enzymes metabolizing diacetyl become saturated and hence levels of the parent compound are increased which pass into the distal regions of the respiratory tract. It has been calculated that when rats are exposed to 3.58 mg/m^3 diacetyl concentration, only 2% reach the bronchi; for persons breathing through their noses this value is 8%. However in the latter case, when breathing via the mouth then this amount becomes slightly increased.

During mild exercise and whilst breathing through the mouth, 24% of the received diacetyl dose passes into the bronchi. The calculated amount of diacetyl present in the bronchial tissue of an exposed person, at rest, breathing through their nose is 5 times higher than in rats but 7 times higher when breathing through the mouth, but in the latter becomes 20 – 40 times higher during mild exercise [10, 29]. Concentration differences in the distribution of diacetyl within the respiratory tract for humans and rodents provoke differences in the localisation of any pathological changes. Whilst in the former these occur mainly in the distal regions, in rodents they affect the upper respiratory tract. Exposing rats or mice to relatively high concentrations of diacetyl results in fibrinopurulent inflammation or necrotic rhinitis of regions such as nose, larynx, trachea and bronchus together with the loss of microvilli and cilia of ciliated epithelium [13, 28].

Diacetyl, like acetoin and 2,3-butanediol is a metabolite of acetaldehyde. The former two are rapidly reduced in mammalian tissues to 2,3-butanediol which undergoes glucuronidation before being excreted. Acetoin is enzymatically formed through the pyruvate dehydrogenase complex or by a non-enzymatic reaction between ace-

taldehyde and pyruvate in the presence of thiamine. Within mammalian hepatic tissue or homogenates thereof, acetoin and 2-3-butanediol are very slowly oxidized to diacetyl and as a result they accumulate in other tissue e.g. brain [32]. Diacetyl is therefore an endogenous compound with a significant toxicological role. Its reduction is catalysed by nicotinamide nucleotide dependent diacetyl reductase present in mammalian tissue [30, 32], which is regarded as a detoxification mechanism. This reduction is inhibited by butyric acid which is present in foodstuffs as flavourings/additives. It has been demonstrated that the efficiency of absorbing diacetyl alone in the isolated upper respiratory tract of the rat is 36% of the received dose, but in the presence of butyric acid this value then significantly (statistically) decreases to 31%. Thus inhibiting diacetyl metabolism in the upper airways, may increase its transport to the bronchioles, where its toxicological effects are manifested [29].

The mechanism by which bronchiolitis obliterans arises still remains unknown. It is suggested that diacetyl directly damages airway epithelia resulting from an induced inflammatory process and by carbonyl and oxidative stress caused by the generation of reactive dicarbonyl and reactive oxygen species [41]. The presence of two adjacent carbonyl groups in the diacetyl's carbon chain enhances the reactivity of these groups with protein amino groups. When such products are formed, this leads to excessive cytokine production and chronic states of inflammation. Another source of these products comes from cross-linking with structural proteins like collagen and laminin or they can be the result of sclerosis processes in the lungs, blood vessels or other tissue [27]. Furthermore, this leads to, amongst other effects, inhibition of muscle enolase [35] and pyruvate kinase in erythrocytes [19], key enzymes of glycolysis. Diacetyl also modifies arginine in the inner mitochondrial membrane, impairing its permeability [7]. Such disruption leads to an energy deficit and cell death.

It is postulated that the processes of repairing the distal respiratory tract occur during the uncontrolled phases of fibroblast and myoblast proliferation. This then leads to an accumulation of fibroblasts and collagen deposition, as well as scarring that is responsible for the partial or complete obstruction of bronchioles [11]. However, during the formation and development of bronchiolitis obliterans, symptoms of allergic pulmonary inflammation, asthma , diffuse interstitial fibrosis and granuloma can be excluded [11].

CONCLUSIONS

1. Diacetyl, as an ingredient of foodstuff flavourings/additives, is a pneumotoxic substance under specific workplace conditions.
2. Chronic exposure to this substance, particularly during the manufacture of microwave popcorn, leads to obstructive changes in the distal regions of the respiratory tract.
3. Diacetyl is one of the factors responsible for causing bronchiolitis obliterans.
4. Reducing the occupational exposure to diacetyl in industry seems to be the best method for preventing bronchiolitis obliterans.
5. The presence of diacetyl as a flavouring/additive in foodstuffs does not appear to constitute a health hazard to consumers.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. ACGIH, Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices. American Conference of Governmental Industrial Hygienists 2012.
2. *Akpinar-Elci M., StempleK.J., Elci O.C., Dweik R.A., Kreiss K., Enright P.L.*: Exhaled nitric oxide measurement in workers in a microwave popcorn production plant. Int J Occup Environ Health 2006; 12:106-110.
3. *Akpinar-Elci M., Stemple K.J., Enright P.L., Fahy J.V., Bledsoe T.A., Kreiss K., Weissman D.N.*: Induced sputum evaluation in microwave popcorn production workers. Chest 2005; 128:991-997. DOI: 10.1378/chest.128.2.991.
4. *Akpinar-Elci M., Travis W.D., Lynch D.A., Kreiss K.*: Bronchiolitis obliterans syndrome in popcorn production plant workers. Eur Respir J 2004;24:298-302. DOI: 10.1183/09031936.04.00013903.
5. *Boylstein R., Piacitelli C., Grote A., Kanwal R., Kullman G., Kreiss K.*: Diacetyl emissions and airborne dust from butter flavorings used in microwave popcorn production. J Occup Environ Hyg 2006; 3:530-535.
6. *Colley J., Gaunt L.F., Lansdown A.B.G., Grasso P.*: Acute and short-term toxicity of diacetyl in rats. Fd Cosmet Toxicol 1969; 7:571-582, quoted [1].
7. *Eriksson O., Fontaine E., Bernardi P.*: Chemical modification of arginines by 2,3-butanedione and phenylglyoxal causes closure of the mitochondrial permeability transition pore. J Biol Chem 1998; 273(20):12669-12674.
8. *Fujioka K., Shibamoto T.*: Determination of toxic carbonyl compounds in cigarette smoke. Environ Toxicol 2006;21:47-54.
9. *Galbraith D., Weill D.*: Popcorn lung and bronchiolitis obliterans: a critical appraisal. Int Arch Occup Environ Health 2009; 82:407-416. DOI: 10.1007/s00420-008-0337-x.
10. *Gloede E., Cichocki J.A., Baldino J.B., Morris J.B.*: A validated hybrid computional 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 2011; 123:231-246, quoted [1].

11. *Harber O., Saechao K., Boomus C.*: Diacetyl-induced lung disease. Toxicol Rev 2006;25(49):261-272.
12. *Hendrick D.J.*: „Popcorn worker's lung" in Britain in a man making potato crips flavouring. Thorax 2008; 63:267-268.
13. *Hubbs A.F., Goldsmith W.T., Kashon M.L., Frazer D., Mercer R.R., Battelli L.A., Kullman G.J., Schwegler-Berry D., Friend S., Castranova V.*: Respiratory toxicologic pathology of inhaled diacetyl in Sprague-Dawley rats. Toxicol Path 2008; 36:330-344. DOI: 10.1177/0192623307312694.
14. IPCS, International Programme on Chemical Safety. International Chemical Safety Cards. ICSC: 1168. 2,3-Butanedione 2007.
15. *Jeżewska A.*: Personal information, CIOP, 2007 (data not published).
16. *Kanwal R., Kullman G.*: HETA 2006-0303-3043, Report on severe fixed obstructive lung disease in workers at a flavoring manufacturing plant. Health Hazard Evaluation Report. National Institute for Occupational Safety and Health (NIOSH), Cincinnati, OH 2007, quoted [25].
17. *Kanwal R., Kullman G., Fedon K.B., Kreiss K.*: Occupational lung disease risk and exposure to butter-flavoring chemicals after implementation of controls at a microwave popcorn plant. Public Health Rep 2011; 126:480-494.
18. *Kanwal R., Kullman G., Piacitelli C., Boylstein R., Sahakian N., Martin S., Fedan K., Kreiss K.*: Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. J Occup Environ Med 2006;48(2):149-157. DOI: 10.1097/01.jom.0000194152.48728.fb.
19. *Kilinc K., Özer N.*: Irreversible inactivation of human erythrocyte pyruvate kinase by 2,3-butanedione. Arch Biochem Biophys 1984;230(1):321-326.
20. *King T.E., jr.*: Bronchiolitis. In: *Schwarz M.I., King T.E., Jr*. eds. Interstitial Lung Disease. 3rd Edn. Hamilton--London, B.C. Decker, Inc., 1998.
21. *Kreiss K., Gomaa A., Kullman G., Fedan K., Simoes E.J., Enright P.L.*: Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. N Eng J Med 2002; 347(5):330-338.
22. *Kullman G., Boylstein R., Jones W., Piacitelli C., Pendergrass S., Kreiss K.*: Characterization of respiratory exposures at a microwave popcorn plant with cases of bronchiolitis obliterans. J Occup Environ Hyg 2005; 2:169-178. DOI: 10.1080/15459620590923091.
23. *Kullman G., Sahakian N.*: HETA 2006-0195-3044: Yatsko's Popcorn, Sand Coulee, Montana. NIOSH Health Hazard Evaluation Report. National Institute of Occupational Safety and Health (NIOSH), Cincinnati, OH 2007, quoted [25].
24. *Lockey J.E., Hilbert T.J., Levin L.P.*: Airway obstruction related to diacetyl exposure at microwave popcorn production facilities. Eur Resp J 2009; 34(1):63-71. DOI: 10.1183/09031936.00050808.
25. *Maier A., Kohrman-Vincent M., Parker A., Haber L.T.*: Evaluation of concentration-response options for diacetyl in support of occupational risk assessment. Reg Toxicol Pharmacol 2010; 58:285-296. DOI: 10.1016/j.yrtph.2010.06.011.
26. Merck, The Merck Index, an Encyclopedia of Chemicals, Drugs, and Biologicals, 14th ed., Merck & Co., Inc. Whitehouse Station, NJ, USA 2006, p. 504.
27. *Miller A.G., Gerrard J.A.*: Assessment of protein function following cross-linking by α-dicarbonyls. Ann N Y Acad Sci 2005; 1043:195-200.
28. *Morgan D.L., Flake G.P., Kirby P.J., Palmer S.M.*: Respiratory toxicity of diacetyl in C57BI/6 mice. Toxicol Sci 2008; 103(1):169-180.DOI: 10.1093/toxsci/kfn016.
29. *Morris J.B., Hubbs A.F.*: Inhalation dosimetry of diacetyl and butyric acid, two components of butter flavoring vapors. Toxicol Sci 2009; 108:173-183, quoted [1].
30. *Nakagawa J., Ishikura S., Asami J.*: Molecular characterization of mammalian dicarbonyl/L-xylulose reductase and its localization within the kidney. J Biol Chem 2002; 277:17883-17891.
31. NIOSH, Criteria for a Recommended Standard Occupational Exposure to Diacetyl and 2,3-Pentanedione. Department of Health and Human Services. Centers for Disease Control and Prevention. National Institute for Occupational Safety and Health. August 12, 2011.
32. *Otsuka M., Mine T., Ohuchi K., Ohmori S.*: A detoxication route for acetaldehyde: metobolism of diacetyl, acetoin, and 2,3-butanediol in liver homogenate and perfused liver of rats. J Biochem 1996; 119(2):246-251.
33. *Parmet A.J., von Essen S.*: Rapidly progressive, fixed airway obstructive disease in popcorn workers: a new occupational pulmonary illness? J Occup Environ Med 2002; 44:216-218.
34. *Pendergrass S.M.*: Method development for the determination of diacetyl and acetoin at a microwave popcorn plant. Environ Sci Technol 2004; 38(3):858-861.
35. *Pietkiewicz J., Wolny M.*: Inactivation of enolase from carp (*Cyprinus carpio*) muscle by 2,3-butanedione. Biochem Int 1991;23(1):69-74.
36. *Rosati J.L., Krebs K.A., Liu X.*: Emissions from cooking microwave popcorn. Crit Rev Food Sci Nutr 2007;47:701-709.
37. SCOEL, Recommendation from the Scientific Committee on Occupational Exposure Limits for diacelyt. SCOEL/SUM/149 February 2010.
38. *van Rooy F.G.B.G.J., Rooyockers J.M., Prokop M., Houba R., Smit L.A.M., Heederik D.J.J.*: Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. Am J Respir Crit Care Med 2007;176:498-504. DOI: 10.1164/rccm.200611-16200c.
39. *van Rooy F.G.B.G.J., Smit L.A.M., Houba R., Zaat V.A.C., Rooyackers J.M., Heederik D.J.J.*: A cross-sectional study of lung function and respiratory symptoms among chemical workers producing diacetyl for food flavourings. Occup Environ Med 2009; 66:105-110. DOI: 10.1136/oem.2008.039560.
40. *Visscher D.W., Myers J.L.*: Bronchiolitis. The Pathologist's perspective. Proc Am Thorac Soc 2006;3:41-47. DOI: 10.1513/pats.200512-124JH.
41. *Wondrake G.T., Cervantes-Laurean D., Roberts M.J.*: Identification of α-carbonyl scavengers for cellular protection against carbonyl stress. Biochem Pharmacol 2002; 63:361-373.

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