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# 2-Butoxyethanol (Ethylene glycol monobutyl ether)

## MAK Value Documentation, addendum – Translation of the German version from 2018

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# 2-Butoxyethanol[1)] (Ethylene glycol monobutyl ether)

## MAK Value Documentation

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## Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the carcinogenicity classification of 2-butoxyethanol [111-76-2]. In long-term studies, 2-butoxyethanol resulted in hepatocellular carcinomas, haemangiosarcomas and forestomach tumours in mice and phaeochromocytomas in rats and was classified in Carcinogen Category 4.
New studies indicate that the hepatic tumours and the phaeochromocytomas are consequences of the haemolysis which is caused by the metabolite butoxyacetic acid. The forestomach tumours in mice are judged to be irrelevant for humans. There are differences between rats and humans concerning the formation and the haemolytic potency of butoxyacetic acid, which renders humans much less susceptible for haemolysis than rats, although humans can develop signs of haemolysis after severe oral intoxication. However, the CNS-depression and the irritation caused by 2-butoxyethanol precludes that humans are exposed regularly to 2-butoxyethanol concentrations at the workplace which might result in significant haemolysis. Therefore, the Commission has removed 2-butoxyethanol from the Carcinogen Category 4.



## Keywords

2-butoxyethanol; ethylene glycol monobutyl ether; n-butoxyethanol; O-butyl ethylene glycol; butyl glycol; ethylene glycol n-butyl ether; ethylene glycol monobutyl ether; glycol butyl ether; monobutyl glycol ether; 3-oxa-1-hepatanol; mechanism of action; toxicokinetics; metabolism; genotoxicity; carcinogenicity; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance



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1) MAK value applies for the sum of the concentrations of 2-butoxyethanol and 2-butoxyethylacetate in the air.

# 2-Butoxyethanol (Ethylene glycol monobutyl ether)[1)]

[111-76-2]

**Supplement 2018**

| | |
|---|---|
| **MAK value (2006)** | **10 ml/m³ (ppm) ≙ 49 mg/m³** |
| **Peak limitation (2006)** | **Category I, excursion factor 2** |
| **Absorption through the skin (1980)** | **H** |
| **Sensitization** | **–** |
| **Carcinogenicity** | **–** |
| **Prenatal toxicity (1985)** | **Pregnancy Risk Group C** |
| **Germ cell mutagenicity** | **–** |
| **BAT value (2015)** | **150 mg butoxyacetic acid (after hydrolysis)/g creatinine** |
| **1 ml/m³ (ppm) ≙ 4.903 mg/m³** | **1 mg/m³ ≙ 0.204 ml/m³ (ppm)** |

2-Butoxyethanol causes phaeochromocytomas in rats, carcinomas and haemangiosarcomas in the liver and forestomach papillomas in mice. Since the supplement of 2007 (supplement "2-Butoxyethanol" 2010), the Commission has re-assessed the relevance for humans of phaeochromocytomas in rats (Greim et al. 2009). 2-Butoxyethanol was one of the example substances in this publication. In the meantime, more recent studies of the mechanism leading to the formation of liver haemangiosarcomas and the significance of forestomach tumours to humans have been published. For this reason, the relevance for humans of the tumours induced by 2-butoxyethanol is re-evaluated here.

1) MAK value applies for the sum of the concentrations of 2-butoxyethanol and 2-butoxyethylacetate in the air.

# 1 Toxic Effects and Mode of Action

2-Butoxyethanol was irritating to the eye in the Draize test with rabbits and caused irritant effects in the eyes and upper airways in a study with volunteers.

The metabolite butoxyacetic acid is responsible for the systemic effects, which in rodents are dominated by haemolysis. In humans, haemolysis is known after oral intoxication; metabolic acidosis is, however, the main effect. Haemolysis in the form of haematuria has likewise been described after inhalation of unknown, prenarcotic concentrations.

After inhalation exposure for 2 years, 2-butoxyethanol caused phaeochromocytomas in the adrenal medulla of female F344 rats, hepatocellular carcinomas and haemangiosarcomas in the liver of male B6C3F1 mice, and squamous papillomas and epithelial hyperplasia in the forestomach of female B6C3F1 mice. The in vitro and in vivo genotoxicity data show that neither 2-butoxyethanol nor butoxyacetic acid are genotoxic. Data for the metabolite butoxyacetaldehyde indicate a mutagenic potential in vitro, but are insufficient for a conclusive assessment to be made. The liver tumours and phaeochromocytomas in mice and rats, respectively, are very probably secondary results caused by sequelae of the haemolytic effects of butoxyacetic acid. Due to toxicokinetic and toxicodynamic differences, the exposure concentrations necessary to induce haemolysis in humans are so high that they would not be permanently tolerable at the workplace because of the expected irritant and central nervous effects of 2-butoxyethanol. The forestomach tumours in mice are not relevant to humans.

# 2 Mechanism of Action

## 2.1 Haemolysis

One of the main effects of 2-butoxyethanol is haemolysis, caused by its metabolite butoxyacetic acid. The mechanism of action is not known exactly. Butoxyacetic acid presumably produces an increased influx of calcium and sodium into the erythrocytes. The increased sodium content leads to an increased uptake of water and to osmolysis. Intracellular calcium initially delays the start of haemolysis by activating the calcium-dependent potassium channel, which causes potassium to be released, although it could then subsequently activate proteases, which additionally impair the membrane function (Udden and Patton 2005). Butoxyacetic acid has also been suggested to cause a disturbance of membrane transport proteins (Udden 2005).

### 2.1.1 Species differences

There are marked species differences in sensitivity to the haemolytic effects of butoxyacetic acid; the erythrocytes of dogs, cats, pigs, guinea pigs and humans being less sensitive than those of rodents and rabbits (supplement "2-Butoxyethanol" 2010). The reasons for the lower sensitivity of human erythrocytes compared with rat erythrocytes are not known. Species differences in transport proteins and the calcium-dependent effects mentioned above have been suggested (Udden and Patton 2005).

A minimal decrease in deformability and increasing osmotic fragility are the initial stages of haemolysis and have been described in washed human erythrocytes at butoxyacetic acid concentrations of 7.5 to 10 mmol/l, and in washed rat erythrocytes even at 0.05 mmol/l (Udden 2002). The rat erythrocytes therefore reacted at least 100 times more sensitive than the human erythrocytes.

Increased osmotic fragility of the erythrocytes was not found after exposing a total of 6 volunteers for 4 to 8 hours up to the highest 2-butoxyethanol concentration tested of 195 ml/m$^3$ (8 hours, n = 3). On the other hand, in rats, exposure to 2-butoxyethanol concentrations of 62 ml/m$^3$ for 4 hours led to increased osmotic fragility of the erythrocytes (Carpenter et al. 1956; supplement "2-Butoxyethanol" 2010). The corresponding NOAEC (no observed adverse effect concentration) for rats was 32 ml/m$^3$ (Carpenter et al. 1956). Rats are therefore at least 6 times more sensitive than humans. A limitation of this study is that only a few volunteers were investigated.

Incubation of human blood with 8 mM butoxyacetic acid for 4 hours induced slight haemolysis, whereas in rat blood even a butoxyacetic acid concentration of 0.5 mM caused more pronounced haemolysis than 8 mM in human blood. Rat erythrocytes are therefore 16 times more sensitive than human erythrocytes (Ghanayem 1989). The haemolytic effects increased with the duration of exposure both in rat blood and in human blood, so that a greater effect is to be expected after longer exposure, for example over 8 hours at the workplace. For comparison with an 8-hour exposure under workplace conditions however, the slower influx and the slower achievement of the steady-state compared with in the in vitro investigation must be taken into account; the situation in vitro is similar to that after bolus administration, where the maximum concentration is immediately effective.

In another study with a 3-hour incubation period, the $EC_{50}$ value for haemolysis in washed human erythrocytes was 14.4 mM and that in washed rat erythrocytes 4.8 mM. This means that the rat erythrocytes were only 3 times more sensitive to the haemolytic effect of butoxyacetic acid than human erythrocytes (Starek et al. 2008). The slope of the dose–response curve is, however, not given in this study, so that a species comparison with lower relevant blood concentrations is not possible. The comparison of $EC_{50}$ values is less suitable for extrapolation between the species as it does not take into consideration the slope of the dose–response relationship, unlike when comparing the NOAEL (no observed adverse effect level) or LOAEL (lowest observed adverse effect level).

In summary, it can be concluded from the data obtained that, relative to the concentration of butoxyacetic acid in blood, rats are approximately 16 times as sensitive as humans.

### 2.1.2 Interindividual differences

After oral administration of 2-butoxyethanol, older rats were more sensitive to haemolysis than younger rats. This was attributed to reduced glucuronidation and sulfation of 2-butoxyethanol, reduced degradation to $CO_2$ and the lower renal elimination of butoxyacetic acid in older rats. As a result, they are exposed to higher levels of butoxyacetic acid. Thus, toxicokinetic differences are involved here (Ghanayem et al. 1987). In contrast, with 2 mM butoxyacetic acid in vitro, no toxicodynamic

differences in sensitivity as regards haemolysis were found in human erythrocytes from younger (41.6 years) and older (71.9 years) donors (Udden 1994).

## 2.2 Liver tumours in male mice

The occurrence of liver tumours (significantly increased incidences of haemangiosarcomas and hepatocellular carcinomas at 250 ml/m$^3$) was assumed to be a secondary effect of haemolysis. Haemolysis results in iron storage (haemosiderosis) in the liver and oxidative DNA damage via Fenton or Haber-Weiss reactions or the activation of Kupffer cells. As liver tumours occur only in B6C3F1 mice but not in F344 rats, and the oxidative DNA damage after oral administration of 2-butoxyethanol likewise occurs only in B6C3F1 mice but not in F344 rats, this mechanism is plausible. This species difference was explained by the higher antioxidative capacity of F344 rats compared with that of B6C3F1 mice: the vitamin E content in the liver of untreated male F344 rats is about 2.5 times as high as that of untreated male B6C3F1 mice. The oral administration of 2-butoxyethanol reduced the vitamin E content in the liver both in mice and in rats. Even the lowest vitamin E content in the liver of male F344 rats, reduced by exposure to 2-butoxyethanol, was still higher than that of untreated male B6C3F1 mice. Compared with this, the vitamin E content in the human liver is as much as 100 times as high as that in the mouse liver (supplement "2-Butoxyethanol" 2010).

In B6C3F1 mice, haemosiderosis was found in the Kupffer cells (macrophages) in the liver after oral administration of 2-butoxyethanol. An increased synthesis of endothelial DNA was observed. These effects were reduced after the depletion of Kupffer cells. The authors therefore concluded that activation of the Kupffer cells is involved in the induction of haemangiosarcomas in mice as a result of increased DNA synthesis (Corthals et al. 2006; Kamendulis et al. 2010).

Reactive oxygen species originating either from the activation of Kupffer cells or other biological processes are able, for example, to inhibit the gene expression of MAP kinase/AP-1 and NFkB, and thus to stimulate cell proliferation or inhibit apoptosis (US EPA 2010 b).

A further explanation for the occurrence of haemangiosarcomas is, apart from the inflammation induced by the activation of Kupffer cells, local hypoxia in the liver. This is produced by haemolysis in the liver, and was demonstrated in mice by the increased expression of the hypoxia-inducible transcription factors Hif1-alpha, Epas1 and Arnt (Laifenfeld et al. 2010). These explanations for the formation of angiosarcomas in the mouse agree with a generally applicable mode of action that was developed also for other substances causing these mouse-specific haemangiosarcomas (Cohen et al. 2009).

To summarize, the following mechanisms are found (US EPA 2010 b):

1. 2-Butoxyethanol is metabolized via the aldehyde to butoxyacetic acid.
2. Butoxyacetic acid causes swelling of the erythrocytes, which are sequestered in the spleen by macrophages. When the capacity of these macrophages becomes overwhelmed, the damaged erythrocytes make their way into the liver.
3. Excess haemoglobin from damaged erythrocytes is taken up by phagocytic (Kupffer) cells of the liver and stored as haemosiderin.

4. Oxidative damage and increased synthesis of endothelial and hepatocyte DNA are initiated by one or several of the following events:
   - Generation of reactive oxygen species (ROS) by haemoglobin-derived iron within Kupffer cells and presumably also from within hepatocytes and sinusoidal endothelial cells
   - Activation of Kupffer cells with production of cytokines or growth factors, which suppress apoptosis and promote cell proliferation.
5. ROS result in oxidative DNA damage of hepatocytes and endothelial cells.
6. ROS modulate the gene expression of hepatocytes and endothelial cells.
7. ROS stimulate the cell proliferation of hepatocytes and endothelial cells.
8. ROS promote the initiation of hepatocytes and endothelial cells.
9. ROS promote the formation of tumours.

Except for step 6, these steps have been demonstrated with 2-butoxyethanol. Step 6, however, is plausible in that the induction of oxidative damage changes the gene expression in mammalian cells. Steps 8 and 9 agree with the absent genotoxicity of 2-butoxyethanol and the high incidence of spontaneous endothelial liver neoplasms in male mice (US EPA 2010 b).

### Species differences

Also in humans, haemolysis could lead to the accumulation of iron in the liver. Such an overloading of the liver with iron is, in the case of haemochromatosis, a risk factor for liver cell carcinomas in humans (Fonseca-Nunes et al. 2014).

However, in humans, haemolysis is to be expected at markedly higher 2-butoxyethanol concentrations than in rats, which are less sensitive to oxidative liver damage than mice. In addition, the vitamin E content in the human liver is 100 times as high as that in the liver of mice (supplement "2-Butoxyethanol" 2010).

## 2.3 Forestomach papillomas in female mice

The deposition and retention of 2-butoxyethanol or its metabolites in the forestomach of rats and mice has also been confirmed after inhalation exposure. 2-Butoxyethanol and the resultant metabolites butoxyacetaldehyde and butoxyacetic acid induce chronic irritation of the forestomach tissue, cell proliferation and clonal expansion of spontaneously initiated cells in the forestomach. In male mice, no significantly increased incidences of forestomach tumours were demonstrated. However, the initial stage, forestomach hyperplasia, was found, which indicates that this mechanism is possible also in male animals. It is unclear whether the possible genotoxicity of butoxyacetaldehyde contributes to these effects. However, it is assumed on the basis of structure–activity relationships that the interaction of aldehydes with the DNA decreases with increasing chain length. For this reason butoxyacetaldehyde can be assumed to have a smaller effect than, for example, acetaldehyde (supplement "2-Butoxyethanol" 2010).

### Species differences

The aldehyde dehydrogenase in the forestomach of mice has a far larger capacity to metabolize butoxyacetaldehyde to the acid than that of the rat. At the same dose and exposure duration, mice are therefore able to form more butoxyacetic acid in the forestomach than can rats. This could explain the different sensitivity for the development of forestomach tumours (US EPA 2010 b).

Unlike the rodent forestomach, the human stomach has a shorter time of passage, is protected against irritating substances by a layer of mucus, and the localization of the enzymes necessary for metabolism to butoxyacetic acid in the human stomach is not the same as in the rodent forestomach (US EPA 2010 b).

Unlike rodents, humans have no forestomach and therefore no organ in which 2-butoxyethanol is retained for a longer period and can cause local irritation. As, in addition, only female mice develop forestomach papillomas, but not rats, these tumours are of little relevance to humans.

In a comprehensive assessment of the mechanism of action of 2-butoxyethanol, this type of tumour was not regarded as suitable for risk extrapolation to humans (Gift 2005).

## 2.4 Phaeochromocytomas in female rats

Although the combined incidences of benign and malignant phaeochromocytomas of the adrenal medulla of female rats in the high concentration group of 125 ml/m$^3$ (16%) exceeded the highest incidence of historical controls (13%), there was, however, no statistically significant increase when the incidence was compared with that in the concurrent control group (6%). Also, a concentration-dependent increase is not recognizable. In addition, a non-genotoxic mechanism of action connected with the haemolytic effect seems possible, so that at present 2-butoxyethanol-induced phaeochromocytomas are not assumed to have any notable relevance for humans (supplement "2-Butoxyethanol" 2010).

2-Butoxyethanol is one of the example substances which was used to test the relevance for humans of phaeochromocytomas in the rat (Greim et al. 2009). Toxic effects such as the degeneration of the olfactory epithelium and oxidative stress resulting from haemolysis were seen to be causes for the induction of phaeochromocytomas. As hypoxia was identified in this publication as a mechanism of action for the induction of phaeochromocytomas, and 2-butoxyethanol can induce local hypoxia as a result of haemolysis, although this was not investigated for the adrenal gland (Laifenfeld et al. 2010), also the phaeochromocytomas are to be considered secondary effects of the haemolysis.

In addition, the NTP report on 2-butoxyethanol (NTP 2000) mentions that there were difficulties distinguishing phaeochromocytomas from medullary hyperplasia, and that the tumours in rats treated with 2-butoxyethanol were not substantially larger than the more severe grades of medullary hyperplasia. Furthermore, the incidence of phaeochromocytomas was only slightly above the upper range of the historical controls. Therefore, they were not regarded with certainty as substance-related, and the US EPA did not give significant weight to them in the qual-

itative and quantitative assessment of the carcinogenic potential of 2-butoxyethanol (US EPA 2010 a, b).

### Species differences

As described above, phaeochromocytomas are of minor relevance for humans and, should they be substance-induced, are mechanistically explainable by haemolysis, for which the species differences described in Section 2.1.1 apply.

## 3 Toxicokinetics and Metabolism

In the male rat, both after inhalation and after dermal or oral exposure (via gavage and drinking water), butoxyacetic acid was eliminated with the urine as the main metabolite in amounts of 60% to 75% of the absorbed dose of 2-butoxyethanol (supplement "2-Butoxyethanol" 2010). In humans, during physical exercise (50 watts) on a bicycle ergometer, 57% of the inhaled amount of 2-butoxyethanol was absorbed, of which about 17% to 55% was eliminated with the urine in the form of non-conjugated butoxyacetic acid (Johanson et al. 1986; supplement "2-Butoxyethanol" 2010). The actual amount of butoxyacetic acid formed is, however, higher in humans, who eliminate an additional butoxyacetic acid glutamine conjugate with the urine with high intraindividual and interindividual variance. As a result of conjugation with glutamine, the butoxyacetic acid formed in humans is detoxified. This glutamine conjugate does not occur in the rat; instead, 2-butoxyethanol glucuronide or 2-butoxyethanol sulfate were detected in low quantities in the rat, but not in humans (supplement "2-Butoxyethanol" 2010).

**Volunteer studies**

After whole-body exposure of 7 volunteers to a 2-butoxyethanol concentration of 20 ml/m$^3$ for 2 hours during physical exercise (50 watts) on a bicycle ergometer, a plateau of about 800 µg 2-butoxyethanol/l blood was reached after 2 hours. The half-life of 2-butoxyethanol was 40 minutes, the elimination of butoxyacetic acid with the urine is, on the other hand, markedly slower. The substance was absorbed via the lungs and through the skin (Johanson et al. 1986). In the blood, the initial half-life of butoxyacetic acid was 13 minutes (50 ml 2-butoxyethanol/m$^3$, 2 hours, at rest; Jones and Cocker 2003) and 4 hours for the secondary phase (20 ml 2-butoxyethanol/m$^3$, 2 hours, 50 W physical exercise; Johanson and Johnsson 1991). In 6 volunteers, the renal elimination of free and total butoxyacetic acid was investigated after inhalation through the mouth of 20 ml 2-butoxyethanol/m$^3$ for 30 minutes or after the exposure of 40 cm$^2$ of skin to 50% aqueous 2-butoxyethanol for 4 hours. The half-lives for the elimination of total butoxyacetic acid with the urine were 3.4 hours after inhalation and 5.1 hours after dermal exposure. However, in both cases, butoxyacetic acid was still being eliminated with the urine 16 hours after the exposure, so that with repeated exposure accumulation is to be expected. This applied particularly in the case of dermal exposure. The urinary elimination of total butoxyacetic acid was highest in the period between 0 and 4 hours after terminating dermal exposure, with values of 300 mg/l or about 350 mmol/mol creatinine.

Free butoxyacetic acid was eliminated more rapidly. The proportion of conjugated butoxyacetic acid was, during the first 4 hours after the start of the exposure, about 45% and increased to about 92% after 48 hours. The dermal flux amounted to 3.5 mg/cm$^2$ and hour (Kezic et al. 2004).

In 4 volunteers exposed to 2-butoxyethanol concentrations of 50 ml/m$^3$ by inhalation only or via skin only, the uptake of the substance after two hours was investigated. The dermal absorption of 2-butoxyethanol from the gaseous phase was 3 times as high as the absorption after inhalation exposure. In this study, the concentration of 2-butoxyethanol in the capillary blood was used as a measure of systemic exposure (Johanson and Boman 1991). The concentration of 2-butoxyethanol in the capillary blood during dermal exposure, however, does not reflect the systemic exposure, but above all the local concentration under the exposed skin. The butoxyacetic acid concentration in venous blood is a better measure of systemic exposure. The actual contribution of dermal absorption to the total uptake during whole-body exposure is therefore about 15% to 27%, depending on air humidity. During physical activity, this proportion decreases to between 5% and 9% (Corley et al. 1997). This lower dermal uptake was confirmed in another study, in which dermal absorption from the gaseous phase under resting conditions while wearing shorts and T-shirts at 25 °C with 40% air humidity was 11% of the total exposure. The wearing of overalls at 30 °C with 60% air humidity increased this amount to 39% (Jones et al. 2003). In the toxicokinetic model of Corley et al. (1994), the contribution of absorption through the skin from the gaseous phase was assumed to be 20%.

**Workplace studies**

In 31 workers, who were exposed via their unprotected hands to a 10% aqueous solution of 2-butoxyethanol during the adhesion of stickers, the urinary elimination of total butoxyacetic acid was determined. The arithmetic mean values for the butoxyacetic acid concentrations during one working week were 446 mg/g creatinine at the end of the Monday shift and 619 mg/g creatinine on Friday. The concentrations of 2-butoxyethanol in the air determined via personal air sampling were 1.9 ml/m$^3$ on Monday and 1.5 ml/m$^3$ on Friday. The concentrations of 2-butoxyethanol in the air and of butoxyacetic acid in the urine were not correlated. As, according to a toxicokinetic model of Franks et al. (2006), 8-hour inhalation exposure to 1.9 ml/m$^3$ corresponds to the urinary elimination of about 20 mg total butoxyacetic acid/g creatinine, it is plausible that the high levels of butoxyacetic acid eliminated in this study can almost exclusively be attributed to the direct skin contact with 2-butoxyethanol, which the authors also assume (Hung et al. 2011). This study revealed that 40% more butoxyacetic acid was eliminated at the end of the working week compared with the Monday value, thus reflecting the accumulation of butoxyacetic acid.

In 17 workers who were exposed to average 2-butoxyethanol concentrations of 1.1 (< 0.1–8.1) ml/m$^3$ during the formulation of varnishes, the post-shift concentration of 2-butoxyethanol in blood was 121.3 (< 5–570) µg/l and that of free butoxyacetic acid in the urine 10.5 (0.6–30) mg/l. Direct skin contact with 2-butoxyethanol occurred. The 2-butoxyethanol concentration in the air correlated neither with that of 2-butoxyethanol in the blood nor with that of butoxyacetic acid in the urine. For

this reason, it was not possible to calculate the urinary excretion of butoxyacetic acid in relation to the concentration of 2-butoxyethanol in air (Angerer et al. 1990).

**PBPK models**

According to a PBPK (physiologically based pharmacokinetic) model (Corley et al. 1994), whole-body exposure of humans to a vapour atmosphere saturated with 2-butoxyethanol of 1160 ml/m$^3$ for 6 hours leads to a concentration of 1.5 mM butoxyacetic acid in the blood. Consequently, the concentration of 8 mM butoxyacetic acid necessary to induce haemolysis of human erythrocytes (Ghanayem 1989) is not attained. Under these conditions, about 4 mM is to be expected following whole-body exposure of rats, whereby 0.5 mM already leads to slight haemolysis of rat erythrocytes (Corley et al. 1994; Gift 2005).

The data from the workplace studies do not contradict – as a result of the great influence of dermal exposure to liquid 2-butoxyethanol – the relationships between the concentration of 2-butoxyethanol in the air and butoxyacetic acid in the blood derived using the PBPK model, as these were determined in volunteers under the exclusion of direct skin contact.

As dermal absorption of liquid 2-butoxyethanol can be high, a PBPK model for this exposure route was developed. The exposure of 10% of the body surface in humans for 6 hours was assumed (about 1900 cm$^2$), and the skin permeability coefficient for guinea pigs was applied. It is known that 2-butoxyethanol is absorbed better from aqueous solutions than in its undiluted form. For this reason, the maximum concentration of 1.266 mM butoxyacetic acid in blood was calculated for solutions of 20% to 80%. In the case of undiluted 2-butoxyethanol, the corresponding concentration was 0.367 mM (Corley et al. 1994).

**Worst-case scenario for inhalation and dermal exposure**

In the following, using the concentrations of butoxyacetic acid in blood calculated by Corley et al. (1994) after 6-hour exposure, a worst-case estimation has been carried out. For inhalation, the increased respiratory volume at the workplace must be taken into account. Inhalation exposure alone to the saturated vapour concentration of 1160 ml 2-butoxyethanol/m$^3$ for 6 hours leads to a butoxyacetic acid concentration in the blood of 1.2 mM. With the increased respiratory volume, a doubling of this value is assumed, therefore 2.4 mM. Additionally contributing is the dermal uptake from the gaseous phase with about 0.3 mM (values from the graph in Corley et al. 1994) and the uptake from direct skin contact with diluted 2-butoxyethanol with about 1.3 mM (values from the graph in Corley et al. 1994), resulting in a total of 4 mM. This concentration is extrapolated in a linear fashion from 6 to 8 hours, and a 40% accumulation during the working week is taken into account according to the data from Hung et al. (2011). The butoxyacetic acid concentration in blood is thus 7.4 mM and within the range of the critical concentration of 8 mM. Longer-term exposure to 2-butoxyethanol concentrations of 1160 ml/m$^3$ is, however, hypothetical, as even concentrations of as little as 100 ml/m$^3$ cause irritation of the eyes and the respiratory tract (Section 4). Likewise, permanent wetting of the hands and forearms (about 1900 cm$^2$) with a 2-butoxyethanol solution for 8 hours is clearly a worst-case assumption. If the urinary elimination of about 350 mmol total butoxyacetic acid/mol creatinine (about 360 mg/g creatinine) after the exposure for

4 hours of 40 $cm^2$ of skin reported by Kezic et al. (2004) is extrapolated in a linear fashion to the exposure of 1900 $cm^2$ of skin for 8 hours, the amount of butoxyacetic acid eliminated with the urine is 34 200 mg/g creatinine. This is about 75 times the amount measured by Hung et al. (2011) of about 450 mg/g creatinine determined after the first working day in workers exposed to 2-butoxyethanol mainly via the skin.

Butoxyacetic acid has a calculated pKa value (acid constant) of 3.65 (Starek et al. 2008). At the physiological pH value it is therefore present in dissociated form. According to the Henderson-Hasselbalch equation (concentration of $CO_2$ in the blood 1.2 mmol/l, concentration of $HCO_3^-$ 24 mmol/l, pKa value 6.1), a concentration of 7.4 mM in the blood would lead to the reduction of the blood pH to 7.24, which corresponds to metabolic acidosis, as often found in cases of oral intoxication (Section 4). Also for these reasons, longer-term exposure under the assumed worst-case conditions is not likely to occur.

**Ingestion**

In a case of poisoning, a maximum concentration of 4.86 mmol butoxyacetic acid/l blood was found 16 hours after the ingestion of a window cleaning agent. No earlier determinations were carried out. The absorbed amount corresponded to between 1100 and 1500 mg 2-butoxyethanol/kg body weight (US EPA 2010 b).

## 4 Effects in Humans

The critical effect in humans is the sensory irritation found in volunteers at 100 ml/$m^3$ and above. At this concentration, nasal and ocular irritation occurred in all 5 volunteers. At 195 ml/$m^3$, irritation in the throat was observed in all 3 volunteers. As reported by the volunteers, the concentration of 195 ml/$m^3$ was too high to be tolerated continuously (Carpenter et al. 1956). In a toxicokinetic study, exposure to 20 ml/$m^3$ for 2 hours under light physical exercise produced neither clinical signs of adverse effects nor subjective complaints in 7 volunteers (Johanson et al. 1986). After the exposure of volunteers to 50 ml/$m^3$ at rest, no subjective symptoms were described; it must be noted, however, that they were not questioned about this by the testing personnel (Jones and Cocker 2003).

Seven workers who were occupied for 0.5 to 4 hours in a room in which a floor cleaning agent containing 2-butoxyethanol had been used suffered from eye and respiratory irritation, marked dyspnoea, nausea, and faintness, suggesting an exposure concentration in the air of 200 to 300 ml/$m^3$. The symptoms persisted for 3 days. Analyses of the air in the room carried out about one week later did not reveal any 2-butoxyethanol, however, but formaldehyde concentrations of 0.1 to 0.2 ml/$m^3$. The cleaning agent had a pH of 11.5. No haematological examinations were made until 8 months later and the results were normal except for an increased erythrocyte sedimentation rate (Raymond et al. 1998; IARC 2006). It is possible that the irritation was partly caused by the alkaline ingredients in the cleaning agent used.

This publication also reported the case of a man who suffered from drowsiness, anaemia and haematuria after cleaning with an agent containing 2-butoxyethanol

in an unventilated car for between 4 and 6 hours. Two other exposed persons reported headaches and dizziness after using a floor cleaner containing 2-butoxyethanol in an unventilated room (Raymond et al. 1998).

In 31 male workers, the erythrocyte count, haemoglobin, haematocrit, MCV (mean corpuscular volume of the erythrocytes), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration), haptoglobin, reticulocyte count and osmotic resistance (osmotic fragility) as well as the concentrations of free 2-butoxyacetic acid, retinol binding proteins and creatinine in the urine were investigated. The workers had been exposed for 1 to 6 years to a concentration (geometric mean) of 0.6 ml 2-butoxyethanol/$m^3$, that is 20 workers were exposed to an average of 0.75 ml 2-butoxyethanol/$m^3$ and 11 to 0.46 ml/$m^3$. Another 21 workers from the same company who were not exposed, and were matched for age, sex and smoking habits were used as controls; their inhaled air, however, was not analysed. There was a significant correlation ($r = 0.55$; $p = 0.0012$) between the concentration of free butoxyacetic acid in the urine after the end of the shift (14–20 mg/l, 9–12 mg/g creatinine) and the 2-butoxyethanol concentration in the inhaled air. Compared with the values for the control group, there was a statistically significant ($p = 0.03$) decrease in the haematocrit (43.9% compared with 45.5%) and a statistically significant ($p = 0.02$) increase in the MCHC (33.6 g/dl compared with 32.9 g/dl) in the exposed workers (Haufroid et al. 1997; supplement "2-Butoxyethanol" 2010). The haematocrit values and the MCHC were, however, within the normal biological variability and were not dependent on the exposure concentration (ATSDR 1998). These marginal findings were therefore not taken into account for the derivation of the MAK value (supplement "2-Butoxyethanol" 2010).

In 31 workers, who excreted 446 to 619 mg total butoxyacetic acid/g creatinine with the urine after inhalation and especially dermal exposure to 2-butoxyethanol, no statistically significant decrease in the haemoglobin concentration in blood was found (Hung et al. 2011). Therefore, at an exposure level which was 3 to 4 times as high as the BAT (biological tolerance) value and around 20 times as high as that obtained in the study by Haufroid et al. (1997), there was no evidence of haemolysis.

In a case of oral poisoning with 2-butoxyethanol doses of 1100 to 1500 mg/kg body weight, no signs of haemolysis were reported. The main effect was acidosis from the butoxyacetic acid. Further cases of poisoning showed that, in humans, ingestion of 400 to 1500 mg 2-butoxyethanol/kg body weight causes only mild haemolytic effects (US EPA 2010 b). When extrapolated to 70 kg body weight, 400 mg/kg body weight corresponds to 28 g 2-butoxyethanol.

## 5 Animal Experiments and in vitro Studies

**Genotoxicity**

The available data for in vitro and in vivo genotoxicity indicate that neither 2-butoxyethanol nor its metabolite butoxyacetic acid have genotoxic effects. The data for butoxyacetaldehyde indicate a mutagenic potential in vitro, but are not sufficient for a conclusive evaluation to be made (supplement "2-Butoxyethanol" 2010).

To investigate whether compensatory erythropoiesis affects the results of the Pig-a assay, with which gene mutations are demonstrated in vivo in erythrocytes or

reticulocytes, 2-butoxyethanol, as a non-genotoxic haemolytic substance, was used in doses of 10 to 450 mg/kg body weight and day. In Wistar rats it was found that haemolysis and pronounced compensatory erythropoiesis are induced by single gavage doses of 2-butoxyethanol or doses administered over 28 days of 250 mg/kg body weight and above. This, however, did not lead to positive results in the Pig-a assay. 2-Butoxyethanol was therefore not found to be mutagenic in this test (Kenyon et al. 2015).

**Carcinogenicity**

In the 2-year inhalation study of the NTP (2000), F344 rats were exposed to 2-butoxyethanol concentrations of 0, 31, 63 or 125 ml/m³ and B6C3F1 mice to concentrations of 0, 63, 125 or 250 ml/m³. There was an increase in benign and malignant phaeochromocytomas in the adrenal medulla (0 ml/m³: 6%, 31 ml/m³: 8%, 63 ml/m³: 2%, 125 ml/m³: 16%, not significant) of female rats. In male B6C3F1 mice, the incidences of hepatocellular carcinomas (0 ml/m³: 20%, 63 ml/m³: 22%, 125 ml/m³: 33%, 250 ml/m³: 43%, $p \leq 0.01$) and haemangiosarcomas in the liver (0 ml/m³: 0%, 63 ml/m³: 2%, 125 ml/m³: 4%, 250 ml/m³: 8%, $p \leq 0.01$) were increased. In the forestomach of female B6C3F1 mice, the incidences of squamous cell papillomas (0 ml/m³: 0%, 63 ml/m³: 2%, 125 ml/m³: 4%, 250 ml/m³: 10%, $p \leq 0.05$) were increased, combined with a concentration-dependent increase in ulceration and epithelial hyperplasia (supplement "2-Butoxyethanol" 2010).

## 6 Manifesto (carcinogenicity)

The critical effects are the local irritation of the olfactory epithelium of the rat and the sensory irritation in humans.

**Carcinogenicity.** After 2-year inhalation exposure to 2-butoxyethanol, the incidences of benign or malignant phaeochromocytomas in the adrenal medulla of female F344 rats, hepatocellular carcinomas and haemangiosarcomas in the liver of male B6C3F1 mice, and squamous cell papillomas in the forestomach of female B6C3F1 mice were increased. The mechanism by which these tumours are formed is assumed to be non-genotoxic. The liver tumours in mice and the phaeochromocytomas in rats are very probably sequelae of haemolysis, provided that the latter are indeed substance-induced. In humans, haemolysis is possible after high oral doses of 2-butoxyethanol of about 30 g, but also after inhalation of 2-butoxyethanol at concentrations that produced marked CNS effects. Haemolysis can result in the overloading of the liver with iron. Such an overloading of the liver, as can be found in haemochromatosis, can increase the risk of liver cancer. However, for this to occur, the haemolysis would have to be so severe that the spleen is overwhelmed with the degradation of erythrocytes. In the case of haemolysis, and therefore for the resultant haemosiderosis in the liver, humans are considerably less sensitive than rats and mice. This species difference is so great that even after whole-body exposure to a vapour atmosphere saturated with 2-butoxyethanol (1160 ml/m³) for 8 hours, and taking into consideration the increased respiratory volume and simultaneous 8-hour dermal absorption by direct skin contact (1900 cm²), the butoxy-

acetic acid concentrations attained in human blood are only minimally haemolytic. Additionally, it is to be expected that, at this concentration of butoxyacetic acid in the blood, metabolic acidosis would occur. As, however, irritation was observed in volunteers at concentrations of as little as 100 and 200 ml/m$^3$, even more severe irritation and presumably additional prenarcotic effects are to be expected at the concentration of 1160 ml/m$^3$. This means that continuous inhalation exposure at a level at which haemolysis and haemosiderosis – as precursors of carcinogenic effects in humans – can occur, is not possible. Furthermore, due to the higher antioxidative capacity (vitamin E content) of the human liver compared with that of the mouse, it is better protected against the sequelae of haemosiderosis (the release of ROS, oxidative DNA damage, cell proliferation, tumour initiation and promotion). In this respect, the situation in humans is closer to that in rats, in which no liver tumours are formed – although, at concentrations similar to those found with mice, rats do develop haemosiderosis – as the vitamin E content in the rat liver is higher than that in the mouse. In addition, male B6C3F1 mice are particularly susceptible for the initiation and promotion of hepatocellular tumours due to the high spontaneous incidence of liver tumours. The forestomach tumours in mice are not relevant for humans, as the human stomach has a shorter time of passage, is protected against irritants by a layer of mucus, and the localization of the enzymes necessary for the metabolism of the substance to butoxyacetic acid is not the same as in the forestomach of rodents. Consequently, longer-term irritation and subsequent tumour formation in the stomach do not occur. 2-Butoxyethanol has therefore been withdrawn from Carcinogen Category 4.

## 7 References

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