The MAK Collection for Occupational Health and Safety

Ethyl acetate

MAK Value Documentation, addendum – Translation of the German version from 2017

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Ethyl acetate

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 maximum concentration at the workplace (MAK value) of ethyl acetate [141-78-6] of 400 ml/m^3, considering all toxicological endpoints. Available publications and unpublished study reports are described in detail. The critical effect of ethyl acetate is irritation of eye and nose in humans and of the olfactory mucosa in rats; in addition, neurotoxic effects are observed in animals at high concentrations. In three studies with volunteers exposed to 400 ml ethyl acetate/m^3, slight irritation in the nose, throat and eye were observed, however the physiological parameters blinking frequency and nasal resistance, both indicators of irritation were not affected. Overall, 400 ml ethyl acetate/m^3 is not an unequivocal NOAEC. Accordingly, the MAK value has been lowered to 200 ml/m^3. As local effects are critical, the assignment to Peak Limitation Category I and the excursion factor of 2 are confirmed. Taking into consideration the data for the metabolites acetic acid and ethanol, damage to the embryo and fetus is unlikely when the MAK value for ethyl acetate is observed. Therefore, ethyl acetate remains classified in Pregnancy Risk Group C. Ethyl acetate is not genotoxic and there are no carcinogenicity studies. Skin contact is not expected to contribute significantly to systemic toxicity. Skin sensitization is not expected from the limited data. There are no data concerning the potential for respiratory sensitization.

Keywords

ethyl acetate; acetic acid ethyl ester; acetic ether; mechanism of action; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; irritation; allergenic effects; reproductive toxicity; fertility; developmental toxicity; genotoxicity; carcinogenicity; peak limitation; prenatal toxicity; germ cell mutagenicity; absorption through the skin; sensitization; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

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2028

Ethyl acetate

[141-78-6]

Supplement 2017

MAK value (2016)	**200 ml/m³ (ppm) ≙ 730 mg/m³**
Peak limitation (1996)	**Category I, excursion factor 2**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (1996)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**

1 ml/m³ (ppm) ≙ 3.656 mg/m³ **1 mg/m³ ≙ 0.274 ml/m³ (ppm)**

The previous MAK value for ethyl acetate of 400 ml/m³ was derived from a volunteer study in which groups of 16 male volunteers were exposed once for 4 hours or twice for 4 hours to an ethyl acetate concentration of 400 ml/m³ (documentation "Ethyl acetate" 1999; Seeber et al. 1992 a).

Two other volunteer studies and inhalation studies in rats have been published in the meantime, making a re-evaluation of the MAK value necessary. The data for germ cell mutagenicity are also reviewed.

Mechanism of Action

As is the case for vinyl acetate and methyl acetate, damage to the nasal mucosa is not caused by ethyl acetate itself, but by acetic acid that is formed locally by the cleavage of the ester. The cleavage is induced by carboxylesterases. Therefore, the decisive factor for the local toxicity of ethyl acetate is not its tissue burden, but primarily the carboxylesterase activity in the olfactory epithelium, which leads to the toxic metabolite acetic acid. The carboxylesterase activities in the olfactory epithelium of rats and humans for vinyl acetate, which is hydrolysed to acetaldehyde and acetic acid, are similar (Bogdanffy et al. 1998). The same is assumed for the enzymatic cleavage of ethyl acetate to acetic acid.

A comparison of the RD_{50} values of ethyl acetate and of its metabolites ethanol and acetic acid in mice (580, 27 314 and 163 ml/m³) also demonstrates that hydrolysis to acetic acid plays an important role in the mechanism of irritation caused by ethyl acetate (Riihimäki 1990).

Toxicokinetics and Metabolism

Absorption, distribution, elimination

After inhalation exposure to ethyl acetate, volunteers absorbed 57% of the substance via the lungs. Fractions of 10% to 35% of the external concentration of 90 mg/m³ were deposited in the isolated upper respiratory tract of rats (documentation "Ethyl acetate" 1999).

In a worker who was found dead, lying face down inside a tank containing ethyl acetate, the highest concentration of ethyl acetate was determined in the testes (Coopman et al. 2005). This suggests that absorption probably occurred after death.

In vitro studies of the dermal absorption of ethyl acetate yielded a flux of 0.5 mg/cm² and hour with a lag phase of 24 hours for human skin (Catz and Friend 1990). Thus, 1000 mg of the substance would be absorbed after the exposure for one hour of a 2000 cm² surface area of skin to undiluted liquid ethyl acetate.

The half-lives of ethyl acetate in the blood after intraperitoneal injection of 1.6 ml/kg body weight were between 5 and 10 minutes in 4 male Sprague Dawley rats. Between 1.19 and 5.18 µM ethyl acetate was determined in the blood of untreated rats (Crowell et al. 2015).

Rats were given intravenous bolus doses (4 animals/group; 10 or 100 mg ethyl acetate/kg body weight) or were treated via infusion (10 or 50 mg ethyl acetate/kg body weight; 3–4 animals/group; 15 minutes), and the blood concentrations of ethyl acetate and ethanol were determined. Following bolus injection, the ethyl acetate concentrations in the blood decreased very rapidly with a clearance of more than 90% of the substance during the first minute after administration. The ethanol concentrations in the blood were highest 20 to 40 seconds after administration, which was the first sampling time. They then decreased logarithmically over the next 200 to 400 seconds. The blood concentrations were linear to the administered ethyl acetate dose with the highest concentration of 1 mM ethanol in the group treated with 100 mg/kg and 0.1 to 0.3 mM ethanol in the group treated with 10 mg/kg. During the infusion, the ethyl acetate concentrations in the blood reached a plateau within the first 2 to 5 minutes, and ethyl acetate was no longer detected in either concentration group 3 minutes after the end of the infusion. The ethanol concentrations increased steadily without reaching a plateau; at the end of the infusion, they decreased more slowly compared with the ethyl acetate concentrations (Crowell et al. 2015).

A group of 3 anaesthetized rats was exposed to an ethyl acetate concentration of 2000 ml/m³ in a closed system for 2 hours. The blood concentrations of ethyl acetate and ethanol were determined at regular intervals. The ethyl acetate concentrations increased rapidly, remained high during the first 30 minutes of exposure (C_{MAX} = 40 – 80 µM) and decreased slowly over the further course of the exposure

period. The ethanol concentrations in the blood were between 20 and 140 μM; they reached a maximum after 40 minutes and then decreased again (Crowell et al. 2015).

After the exposure of rats to 50 000 ml/m³ for 15 minutes, only low concentrations of ethyl acetate were found in the blood and brain, but not in the liver, as a result of rapid hydrolysis, degradation and the metabolites acetic acid and ethanol entering intermediary metabolism (documentation "Ethyl acetate" 1999).

Metabolism

Inhaled ethyl acetate is hydrolysed by carboxylesterases in the epithelial cells of the respiratory tract to yield ethanol and acetic acid or acetate. In the isolated upper respiratory tract of hamsters and rats, 63% to 90% and 40% to 65%, respectively, of the amounts deposited there were hydrolysed; therefore, only small amounts were systemically available in unchanged form (IRK 2014). In the blood, ethyl acetate is also rapidly hydrolysed by plasma esterases; the metabolites that are formed are degraded mainly in the body and used as acetyl-CoA in the intermediary metabolism.

Effects in Humans

Single exposure

Oedema was found in the brain and lungs of the worker who died while cleaning the inside of a tank containing ethyl acetate (see above). In addition, haemorrhage in the lungs was detected in the histopathological examination. Oxygen deficiency was assumed to be the cause of death (Coopman et al. 2005).

A volunteer study was described in the 1996 documentation (documentation "Ethyl acetate" 1999) that was used to derive the MAK value of 400 ml/m³ in 1996. Exposure of 16 male volunteers once for 4 hours or twice for 4 hours to an ethyl acetate concentration of 400 ml/m³ led to "irritation" (irritation of the eyes, throat and nose, and unpleasant odour). It is assumed that the odour of ethyl acetate was the primary cause of the increased "irritation". For the end point "complaints" (physical well-being and malaise with and without physical symptoms), self-evaluation by the test persons did not yield significant differences between the two exposure conditions and the control condition (see above). Likewise, the reaction times and performance in choice reaction or short-term memory tests did not reveal any essential effects of the exposure (documentation "Ethyl acetate" 1999; Seeber et al. 1992 b).

In another study, volunteers were exposed for 15 minutes or 4 hours. The 9 volunteers in the short-term exposure group were exposed to ethyl acetate concentrations of 600 to 1000 ml/m³, and 5 and 6 participants in the 4-hour exposure groups were exposed to 200 and 400 ml/m³, respectively. Questionnaires were used to record the irritation and odour perceived by the participants; in addition, the blinking frequency and eye redness were determined. At the low concentration, the number of symptoms was not increased, but an increase was observed after exposure to 400 ml/m³, particularly as the length of exposure increased. In addition, increases in headaches (n = 2; before the beginning of exposure: n = 0), distraction (n = 3 compared with n = 1) and sleepiness (n = 4 compared with n = 0) were reported;

these complaints were all assessed as "weak". Even after 15-minute exposure to 600 to 1000 ml/m^3, subjective symptoms in the mouth, nose, eyes and throat were reported, but they were not rated as "severe" in any of the persons. The blinking frequency was not increased under any of the exposure conditions, and objective eye redness was not observed (HSE 1997). In 2000, the Commission did not consider the findings after 15-minute exposure to be sufficient reason to change the excursion factor of 2 because the effects were slight, there was no concentration–effect relationship and the localizations were inconsistent in some cases (supplement "Ethylacetat" 2000, available in German only).

Another volunteer study investigated 3 different 4-hour exposure patterns with different concentrations in the air. The 24 men and women were exposed to either constant ethyl acetate concentrations of 2 ml/m^3 (odour control group) or 400 ml/m^3 or alternating concentrations of 5 to 800 ml/m^3 (mean time-weighted concentration of 400 ml/m^3; 4 peaks lasting 15 minutes at 1-hour intervals). The adverse chemosensory effects of ethyl acetate were investigated; they were recorded with physiological parameters, subjective symptoms and behavioural tests. Chemosensory perceptions (olfactory and trigeminal: odour intensity, annoyance, eye and nose irritation, tickling, sneeze, burning, pungent and sharp) and acute complaints (for example, dizziness, shortness of breath and eye redness) were assessed by means of a 6-point severity scale. The blinking frequency and changes in nasal resistance or narrowing of the main nasal cavity were recorded as physiological parameters. The Mackworth clock test (vigilance) and divided attention tasks were selected as the behavioural test procedures.

Subjectively, odour intensity was rated as "severe", whereas trigeminal perceptions (for example, eye irritation) were rated as "slight" to "moderate". No significant differences in chemosensory perception were observed between constant and variable exposures; only odour intensity was perceived as stronger after constant exposure. The absence of substantial trigeminal ratings was supported by the recorded physiological parameters. There was thus no significant increase in the blinking frequency or in nasal resistance; both parameters are indicators of irritation. Likewise, performance in the behavioural tests was not affected (Kleinbeck et al. 2008).

Local effects on skin and mucous membranes

The 48-hour occlusive application of 10% ethyl acetate in petrolatum did not produce skin reactions in 25 test persons, whereas daily application (concentration not specified) for 60 minutes over a period of 6 days caused defatting of the skin and damage to the stratum corneum in 3 volunteers (documentation "Ethyl acetate" 1999; Riihimäki 1990). A cumulative occlusive patch test with an unspecified number of volunteers was carried out with 5 applications a week for a total period of 21 days. At weekends, the test persons did not undergo treatment; however, the test patches were not removed from the application site, which remained the same over the entire test period. Undiluted ethyl acetate did not cause any irritation of the skin (Friend et al. 1991).

Allergenic effects

No valid or sufficiently documented findings have been published to date. One marked reaction (2+) and one severe reaction (3+) to undiluted ethyl acetate were observed in patch tests in 6 workers with contact eczema who worked in a plant that manufactured flavourings. A marked positive reaction was observed also in 1 of 102 control persons (Hegyi 1971).

Animal Experiments and in vitro Studies

Acute toxicity

RD_{50} values for ethyl acetate of 580 ml/m³ and 614 ml/m³ were obtained in mice (documentation "Ethyl acetate" 1999). The corresponding values for acetic acid and ethanol were 163 and 27 314 ml/m³, respectively (Riihimäki 1990).

The relationship between the RD_{50} for ethyl acetate in mice and the concentrations used in the volunteer studies is unusual because long-term exposure to RD_{50}-concentrations usually induces irritation in animals that can be detected histopathologically and severe irritation is expected to occur in humans (Riihimäki 1990). However, only moderate irritant effects were found in the volunteer studies even at peak levels of 800 ml/m³ (see above; Kleinbeck et al. 2008). However, the relationship between the RD_{50} value for acetic acid and its MAK value almost corresponds to the empirically derived relationship "TLV for irritants = 0.03 × RD_{50}" (Schaper 1993). This difference might indicate that ethyl acetate is metabolized to acetic acid in humans to a far lesser extent than in mice; this may be due to differences between the two species as regards carboxylesterase activity.

All rats survived 6-hour exposure to ethyl acetate concentrations of up to 6000 ml/m³. Concentration-dependent body weight losses on the day after exposure and depression of the central nervous system were the primary effects (see below) (CMA 1995 a).

A group of 3 anaesthetized rats was exposed to an ethyl acetate concentration of 2000 ml/m³ in a whole-body plethysmograph for 2 hours. At the beginning of exposure, respiratory depression (reduced respiratory frequency and reduced tidal volume) was initially observed, followed by an increase in the respiratory minute volume that persisted throughout the exposure period (Crowell et al. 2015).

Neurotoxicity

Neurobehavioural effects were investigated in groups of 8 mice after 20-minute exposure to ethyl acetate concentrations of 0, 250, 500, 1000 or 2000 ml/m³. The motor activity was tested and neurobehavioral effects were investigated using the functional observational battery (FOB). At the high concentration, ethyl acetate led to a significant decrease in motor activity, decreased arousal and delayed righting reflexes, and to convulsions, reduced sensorimotor reactivity and increased eyelid closure. Clonic convulsions and sudden jumps with all four feet in the air were observed at 500 ml/m³ and above (no other details). The animals recovered rapidly within a few minutes after the end of exposure (Bowen and Balster 1997).

Likewise, neurobehavioural effects were investigated in groups of 14 Sprague Dawley rats after single 6-hour exposures to ethyl acetate concentrations of 0, 600, 3000 or 6000 ml/m^3 (analysed concentrations: 612, 3037 and 6060 ml/m^3). Transient body weight losses were observed; necropsy yielded no unusual findings. At the middle concentration and above, the first FOB investigation immediately following the exposure revealed signs of an impairment of the nervous system and motor activity. In the animals of the high concentration group, reduced motor activity was still observed on the day following exposure (CMA 1995 a). Thus, a NOAEC (no observed adverse effect concentration) of 600 ml/m^3 was determined in this study for acute neurotoxicity in rats.

Subacute, subchronic and chronic toxicity

Groups of male and female Sprague Dawley rats were exposed to ethyl acetate concentrations of 0, 350, 700 or 1500 ml/m^3 for 94 days, for 6 hours a day, on 5 days a week (a total of 68 exposures). The group size was 10 animals per sex and concentration. The purity of the test substance was > 99.9%. This study was carried out at the same time as two 90-day neurotoxicity studies (see Table 1). Clinical signs, body weights and feed consumption were recorded regularly during the study. Blood and urine samples were taken from all surviving animals after 43 to 44 and 85 to 86 days, respectively. The eyes were examined immediately before the beginning of the study and after 73 days. It was not possible to determine a local NOAEC because minimal degeneration of the olfactory nasal epithelium was observed in 8 of 20 animals of the low concentration group. The systemic NOAEC was 350 ml/m^3 and was derived on the basis of reduced body weight gains, reduced feed consumption and acute sedation in the next-higher concentration group (CMA 1998).

It was not possible to derive a systemic NOAEC in the parallel neurotoxicity study that included the FOB and examined the motor activity of the animals (Christoph et al. 2003; CMA 1997 a) because reduced body weight gains were observed in this study even in the animals exposed to 350 ml/m^3. In addition, there was a transient decrease in the motor activity of the female rats of the high exposure group (see below), and neurobehavioural effects (reduced response to an acoustic signal) were transiently observed in the animals of both sexes at 750 ml/m^3 and above (CMA 1997 a).

Neurotoxicity

Numerous studies with acute (see above), subacute and subchronic exposure examined the neurotoxic effects of ethyl acetate in rats (see Table 1). In a 90-day inhalation study, motor activity was reduced in the female animals exposed to 1500 ml/m^3, but this effect was no longer observed at the end of the 4-week recovery period. The authors interpreted this finding as a secondary systemic effect of reduced body weight gains and reduced feed consumption. A diminished response to an acoustic signal was found at an ethyl acetate concentration of 750 ml/m^3. This acute effect was no longer observed 30 minutes after the end of exposure. At 350 ml/m^3 and above, the body weight gains in the male animals were reduced in a concentration-dependent manner compared with those of the control group. These effects were completely or partially reversible during the 4-week recovery period. The neuropathologi-

Table 1 Effects of ethyl acetate after repeated inhalation exposure

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 10 ♂, 10 ♀	94 days, 68 exposures, 6 hours/day, 5 days/week, 0, 350, 750, 1500 ml/m³	**350 ml/m³**: ♂, ♀: **local LOAEC**: minimal degeneration of the olfactory mucosa (8/20), **systemic NOAEC** **750 ml/m³**: ♂, ♀: minimal to moderate degeneration of the olfactory mucosa (20/20) ≥ **750 ml/m³**: ♂, ♀: reduced response to an acoustic signal (acute sedative effect, transient, returned to normal within 30 minutes after the end of exposure); ♀: body weight gains ↓, feed consumption and feed efficiency ↓, relative kidney weights ↑; ♂: triglycerides in serum ↓ **1500 ml/m³**: ♂, ♀: minimal to severe degeneration of the olfactory mucosa (20/20), absolute spleen weights ↓, relative adrenal gland weights ↑; ♂: body weight gains (n. s.; –14% compared with controls) ↓, feed consumption ↓, number of erythrocytes, haemoglobin concentration and haematocrit ↓; ♀: absolute liver weights ↓, relative liver, lung and spleen weights ↑, triglycerides, albumin and total protein levels in serum ↓	CMA 1998
neurotoxicity studies			
rat, Sprague Dawley, 10 ♂ (5 with reduced diet at body weights of 300 g; 5 ad libitum), 5 ♀ (ad libitum)	14 days (range finding), 6 hours/day, 5 days/week, 0, 1500, 3000, 6000 ml/m³ (analysed concentrations: 1491, 3066, 6024 ml/m³) ad libitum: FOB and motor activity (week before beginning of exposure, at the end of first and second weeks of exposure)	≥ **1500 ml/m³**: ♂ (ad libitum), ♀: body weight gains ↓, feed consumption ↓; ♂ (ad libitum): water consumption ↑, absolute and relative spleen weights ↓, changes in motor activity ≥ **3000 ml/m³**: ♂, ♀: changes in FOB parameters, hypoactivity, blepharospasm, absence of startle reflex; ♀: motor activity ↓ **6000 ml/m³**: ♂, ♀: absolute (reduced diet: ♂) and relative lung weights ↑; ♀: water consumption ↑, absolute and relative ovary weights ↓	CMA 1995 b; OECD 2008

Table 1 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 18 ♂, 18 ♀ (0, 1500 ml/m³), 12 ♂, 12 ♀ (350, 750 ml/m³) beginning of exposure at 54 days of age	99–100 days, 68–69 exposures, 6 hours/day, 5 days/week, 0, 350, 750, 1500 ml/m³ FOB, motor activity (before the beginning of exposure and after 4, 8 and 13 weeks; 12 ♂, 12 ♀: 4-week recovery period (0 and 1500 ml/m³)) and neuropathology	**≥ 350 ml/m³**: ♂: body weight gains ↓, feed efficiency ↓ **≥ 750 ml/m³**: ♂, ♀: body weights compared with controls ↓, reversible reduced response to an acoustic signal (see above); ♂: feed consumption ↓; ♀: body weight gains ↓, feed efficiency ↓ **1500 ml/m³**: ♀: transient decrease in motor activity, feed consumption ↓; ♂: stained chin (as a result of skin irritation) **1500 ml/m³, recovery**: ♂, ♀: feed consumption and feed efficiency returned to normal; ♀: body weight gains returned to normal; no unusual findings in the neuropathological examination	Christoph et al. 2003; CMA 1997 a
rat, Sprague Dawley, 10 ♂, ad libitum up to body weights of 280–313 g, then reduced diet, constant body weights, beginning of exposure at 146 days of age	89 days, 65 exposures, 6 hours/day, 5 days/week, 0, 350, 750, 1500 ml/m³ 2-week recovery period (0 and 1500 ml/m³) investigation of operant behaviour	**350 ml/m³: NOAEC** **≥ 750 ml/m³**: reversible reduced response to an acoustic signal (see above), feed consumption and feed efficiency ↓ **1500 ml/m³**: body weights compared with control animals (≤ 2.5%) ↓ no effects on operant behaviour	Christoph et al. 2003; CMA 1997 b

FOB: functional observational battery; n. s.: not statistically significant

cal examination did not reveal any unusual findings (Christoph et al. 2003; CMA 1997 a, b). These studies, which examined motor activity and operant behaviour and investigated neurobehavioral effects using a FOB, did not reveal any cumulative or persistent effects on the nervous system of rats after 90-day inhalation exposure to concentrations of up to 1500 ml/m³.

A PBPK model was used to determine human equivalent concentrations (HEC) under workplace conditions (8 hours a day for 5 days a week) for the systemic LOAEC (lowest observed adverse effect concentration) of ethyl acetate of 350 ml/m³ (reduced body weight gains) and for the LOAEC for the neurotoxicity of ethyl acetate of 1500 ml/m³. Concentrations of 495 and 2120 ml/m³ were calculated from the AUC (area under the curve) for the ethyl acetate concentrations in the blood of rats, and ethyl acetate concentrations of 257 and 747 ml/m³ were calculated from the AUC for the ethanol concentrations in the blood (Crowell et al. 2015).

Summary: After 90-day exposure, non-specific effects on feed efficiency and body weight gains and local damage to the olfactory epithelium were observed in rats at the lowest concentration tested of 350 ml/m³ and above. Acute sedative effects were observed at 750 ml/m³ and above.

Local effects on skin and mucous membranes

Skin

The 4-hour semi-occlusive application of 0.5 ml undiluted ethyl acetate to the skin of 3 New Zealand White rabbits (sex not specified) did not cause irritation (ECHA 2016); likewise, no irritation was observed after 24-hour open application of 10 µl undiluted ethyl acetate to the skin of 5 rabbits (strain not specified) (Smyth et al. 1962). A test evaluated the irritant effects on the skin of ethyl acetate, which was used as a permeation enhancer in a hydroxypropyl cellulose gel containing a contraceptive (levonorgestrel). A chamber containing a 50 µm or 100 µm-thick permeable membrane was filled with the gel (500 µl) and attached to the shaved skin of 4 female New Zealand White rabbits with non-occlusive cloth. The 24-hour application for up to 7 days induced mild erythema on the shaved skin (Friend et al. 1991).

Eyes

In a study carried out with 4 New Zealand White rabbits according to OECD Test Guideline 405, 0.1 ml undiluted ethyl acetate induced redness of the conjunctivae and corneal opacity; in addition, effects on the iris were observed in 1 animal on day 1 after treatment. After 24, 48 and 72 hours, the scores per animal were 1 × 0.6 and 3 × 0.3 for corneal opacity, 1 × 1 and 3 × 1.3 for redness of the conjunctiva and 1 × 0.3 and 3 × 0.6 for swelling of the conjunctiva. All reactions were reversible at the end of the observation period of 7 days (ECETOC 1998). Therefore, ethyl acetate caused mild irritation in the rabbit eye.

Amounts of 0.1 ml of ethyl acetate solutions of 3%, 10%, 30% in propylene glycol or 100% were instilled into the conjunctival sac of the eyes of 4 to 6 New Zealand White rabbits. Swelling of the cornea was determined as a parameter and compared with the corneal thickness before treatment. In addition, the eyes were evaluated accord-

ing to Draize, but only the first readings were reported (Draize scores of 2, 3, 5 and 18 of a maximum of 110 for 3%, 10%, 30% and 100% ethyl acetate). The authors considered undiluted ethyl acetate to cause mild irritation of the eyes (Kennah et al. 1989).

When one drop of ethyl acetate was instilled into the conjunctival sac of rabbits (number not specified), it caused redness and mild swelling of the conjunctiva; the effects were about the same as those caused by 2% acetic acid. The irritation subsided after 1 to 2 days (Flury and Wirth 1933).

Allergenic effects

Sensitizing effects on the skin

A maximization test with female Dunkin Hartley guinea pigs yielded negative results for ethyl acetate. Intradermal induction was carried out with 10% ethyl acetate in corn oil, and topical induction and challenge treatments were carried out with the undiluted substance. Sodium dodecyl sulfate was not applied before the topical induction. The readings after 24 and 48 hours did not reveal reactions in any of the 20 guinea pigs (ECHA 2016; OECD 2008).

Sensitizing effects on the airways

There are no data available.

Reproductive and developmental toxicity

Fertility

In the 90-day inhalation study described above in the Section "Subacute, subchronic and chronic toxicity", Sprague Dawley rats were exposed to ethyl acetate concentrations of 0, 350, 750 or 1500 ml/m^3; the sperm parameters examined (number or concentration of spermatids in the testes or of sperm in the epididymis, sperm motility and morphology) did not yield any unusual findings (CMA 1998). No histopathological changes were found in the testes or epididymis, which were investigated in the neurotoxicity study described above. There was a statistically significant increase in the relative weights of both organs, which was associated with the lower terminal body weights compared with those of the control animals (CMA 1997 a).

Five male Wistar rats were exposed twice daily for 7 days to ethyl acetate concentrations, which led to the loss of the righting reflex (no other details). At the end of exposure, the weights of the testes, epididymis, vas deferens, prostate gland and seminal vesicles, and the testosterone levels in the plasma, the acid phosphatase activity in the prostate gland and the spermatozoa counts in the epididymis were determined. Ethyl acetate led to a statistically significant decrease in the testis and prostate weights and reduced spermatozoa numbers in the epididymis compared with the values in the control animals. In addition, the acid phosphatase activity and the testosterone level were decreased. Also the body weights were lower than those of the control animals (Yamada 1993).

Developmental toxicity

There are no developmental toxicity studies available in rodents. Teratogenic effects were not observed when up to 25 mg ethyl acetate was injected into the yolk sac or air pocket of hens' eggs at the beginning of incubation or 95 hours after the beginning of incubation (IRK 2014).

Genotoxicity

In vitro

Ethyl acetate was not mutagenic in bacteria in the strains TA97, TA98, TA100, TA1535 or TA1537 in either the presence or absence of a metabolic activation system up to the highest concentration tested of 10 000 µg/plate (Zeiger et al. 1992). Other studies, for example with the strains TA1538 or TA92 and TA94, likewise yielded negative results (Ishidate et al. 1984; OECD 2008).

At high concentrations (1.96% or 2.44%) and under specific culture conditions (ice cooling), ethyl acetate induced mitotic aneuploidy in Saccharomyces cerevisiae, but no mitotic recombinations or point mutations. The aneuploidogenic effects were explained by a disturbance in the spindle apparatus. Concentrations of 1.23% to 2.44% in the medium with ice cooling caused a concentration-dependent loss of chromosomes in diploid, triploid and tetraploid strains. It was not reported whether lower concentrations were tested (documentation "Ethyl acetate" 1999).

A test for sister chromatid exchange in Chinese hamster ovary (CHO) cells yielded positive results in the presence of a metabolic activation system in one of two tests, but only at very high concentrations (4020 and 5020 µg/ml). In the other test, the incidence of sister chromatid exchanges was increased at the highest concentration tested of 6020 µg/ml, but the increase was not yet statistically significant (Loveday et al. 1990).

The same laboratory carried out tests for chromosomal aberrations with CHO cells, which yielded negative results both with and without the addition of a metabolic system up to the highest concentration tested of 5010 µg/ml (Loveday et al. 1990). Aberrations including gaps were induced in Chinese hamster lung (CHL) cells at very much higher concentrations (up to 9000 µg/ml). A questionable result was obtained after 24-hour incubation, and the result was only just positive after 48 hours (11% of metaphases with aberrations; result is regarded as positive at 10% and above). A metabolic activation system was not used (Ishidate et al. 1984).

In vivo

In three valid studies, no micronuclei were induced in the bone marrow of treated animals.

In a study, groups of 10 male and 10 female Chinese hamsters were given single intraperitoneal injections of 473 mg/kg body weight in corn oil or were treated once orally with 2500 mg/kg body weight (2/3 of the LD_{50} in each case) (Basler 1986). It is not clear whether the bone marrow was reached.

Groups of 6 male ddY mice were given single intraperitoneal injections of up to 800 mg/kg body weight or up to 4 doses of 200 mg/kg body weight in a 0.5% carboxymethyl cellulose sodium salt solution. The ratio between polychromatic erythrocytes and the total number of erythrocytes remained unchanged (Hayashi et al. 1988).

The metabolite ethanol caused dominant lethal mutations at systemically toxic doses of more than 1000 mg/kg body weight, but not at lower doses (documentation "Ethanol" 1999).

Carcinogenicity

In a study that does not comply with current test guidelines, groups of 15 male and 15 female A/He mice, which is a strain with a high spontaneous incidence of lung tumours, were given intraperitoneal injections of ethyl acetate of 72 or 360 mg/kg body weight and day, 3 times a week, for a period of 8 weeks. The animals were examined 24 weeks after the first treatment. No increased tumour incidences were found in the examined lungs. Other organs were not investigated (Stoner et al. 1973). The carcinogenic potential of ethyl acetate cannot be evaluated because of the small number of animals and the short duration of treatment.

Manifesto (MAK value/classification)

The critical effect of ethyl acetate is local irritation of the eyes and nose in humans and of the olfactory epithelium in rats. In animal studies, central nervous effects were observed at high concentrations.

MAK value. The previous MAK value for ethyl acetate of 400 ml/m^3 was derived from a volunteer study in which groups of 16 male volunteers reported "irritation" (irritation of the eyes, throat and nose, and unpleasant odour) after they had been exposed once for 4 hours or twice for 4 hours to an ethyl acetate concentration of 400 ml/m^3. It is assumed that the increased "irritation" was primarily caused by the odour of ethyl acetate because odour perception was included under "irritation" and was not recorded separately. For the end point "complaints" (physical well-being and malaise with and without physical symptoms), self-evaluation by the test persons did not yield significant differences between the two exposure conditions and the control condition (documentation "Ethyl acetate" 1999; Seeber et al. 1992 a, b).

A study from 1997 did not reveal any changes in the blinking frequency or eye redness after the exposure of 6 volunteers to an ethyl acetate concentration of 400 ml/m^3 for 4 hours. There was a slight increase in reports of subjective symptoms in the eyes, nose and throat and acute complaints, but a final evaluation is difficult because of the small number of test persons (HSE 1997). In another volunteer study, several 4-hour exposure patterns were tested in 24 test persons who were continuously exposed to 400 ml/m^3 or were exposed to varying concentrations that averaged 400 ml/m^3 with peak concentrations that were twice as high. While odour intensity and annoyance were rated as "strong", eye irritation and other trigeminal perceptions were rated as

"slight" to "moderate". There were no changes in the physiological parameters blinking frequency and nasal resistance, and no effects were observed in the behavioural tests (Kleinbeck et al. 2008).

Studies carried out in rats for 90 days revealed degeneration of the olfactory epithelium and reduced body weight gains at the lowest concentration tested of 350 ml/m³. However, the effects on body weight were observed in only one study and may be interpreted as secondary effects of irritation. The authors suggested that the effects observed in the animals of the neurotoxicity study were possibly related to the more frequent handling of the animals (CMA 1998). Acetic acid was the actual irritant that caused the irritation of the olfactory epithelium of rats; this metabolite is formed by hydrolysis mediated by nasal carboxylesterases. Tests carried out with tissue samples from the nasal cavity demonstrated that the carboxylesterase activity in the olfactory epithelium was about the same in both rats and humans after exposure to vinyl acetate, which is hydrolysed to acetaldehyde and acetic acid (Bogdanffy et al. 1998). This is assumed also for the enzymatic cleavage of ethyl acetate to acetic acid. Therefore, an additional margin between the NOAEC from the study in rats and the MAK value for ethyl acetate is not necessary.

According to the proposals of Brüning et al. (2014), a NAEC (no adverse effect concentration) of 117 ml/m³ would be derived on the basis of the LOAEC of 350 ml/m³ from the 90-day inhalation study in rats, and a MAK value of 50 ml/m³ would be obtained taking an intensification of the effects over time into account. As a NOAEC of 10 ml/m³ was determined for acetic acid in test persons and exposure to an ethyl acetate concentration of 400 ml/m³ caused only mild to moderate effects without any impact on physiological parameters, the suggested MAK value of 50 ml/m³, which was derived for ethyl acetate from the study in rats after taking into account a possible intensification of the effects after chronic exposure, is too low.

Although the physiological parameters did not yield any clear evidence of sensory irritation at 400 ml/m³ and above (Kleinbeck et al. 2008), the odour annoyance (Kleinbeck et al. 2008; Seeber et al. 1992 a, b) and the somewhat increased incidence of subjective symptoms in the eyes, nose and throat as well as the acute complaints described in the study of HSE (1997) indicate that mild irritation may occur at the previous MAK value. As these effects were only slight, the MAK value for ethyl acetate has been lowered to 200 ml/m³.

The MAK values for methyl acetate, *n*-butyl acetate and *n*-propyl acetate of 100 ml/m³ were derived, with the exception of that for methyl acetate, from human data. In view of these values, a MAK value of 200 ml/m³ for ethyl acetate is plausible. In rats, 350 ml/m³ did not cause acute neurotoxic effects and 1500 ml/m³ did not induce persistent neurotoxic effects.

At 400 ml/m³, no effects were observed in behavioural tests in humans (Kleinbeck et al. 2008). Therefore, it is assumed that the MAK value also provides protection from systemic toxicity.

Peak limitation. In view of the local irritation, classification in Peak Limitation Category I has been retained. Physiological parameters (blinking frequency and nasal resistance) did not reveal irritation of the eyes or nose in volunteers after continuous 4-hour exposure to 400 ml/m³ or exposure to a mean value of 400 ml/m³ with

peak concentrations that were twice as high. An excursion factor of 2 has therefore been established.

Prenatal toxicity. No studies of developmental toxicity are available for ethyl acetate. It is assumed that any effects would be caused by acetic acid as a result of rapid hydrolysis and further metabolism. As described in detail in the 1996 documentation (documentation "Ethyl acetate" 1999), acidosis is not expected to occur after exposure at the MAK value. As ethanol, the second metabolite, is classified in Pregnancy Risk Group C at a higher MAK value of 500 ml/m^3, ethyl acetate is not expected to cause prenatal toxicity if the MAK value of 200 ml/m^3 is not exceeded. Ethyl acetate therefore remains in Pregnancy Risk Group C.

Carcinogenicity. None of the studies are suitable for the evaluation of the carcinogenic potential of ethyl acetate. Therefore, ethyl acetate has not been classified in any of the categories for carcinogens.

Germ cell mutagenicity. There are no data for effects on the germ cells. Ethyl acetate did not induce mutations in bacteria. At high concentrations and under specific culture conditions, ethyl acetate caused aneuploidy in Saccharomyces cerevisiae, but no mitotic recombinations or point mutations. Both negative and positive results were obtained for clastogenicity in vitro, but only at high concentrations. In vivo, these questionable results were not confirmed in tests for induction of micronuclei in mouse and hamster bone marrow. Therefore, the studies in somatic cells do not provide an indication for germ cell mutagenicity.

It is not possible to evaluate the metabolite acetic acid conclusively because of the small amount of data available for genotoxicity; the only positive result obtained from a chromosomal aberration test in vitro was the indirect consequence of a change in pH (documentation "Acetic acid" 2010). The metabolite ethanol is classified in Germ Cell Mutagen Category 5 on the basis of positive results from dominant lethal tests after exposure to oral doses of more than 1000 mg/kg body weight. Although ethyl acetate is very rapidly hydrolysed to ethanol and acetic acid, an accumulation of ethanol was not observed in rats after 4-hour exposure to an ethyl acetate concentration of 2000 ml/m^3 (Gallagher and Loomis 1975). At a MAK value for ethyl acetate of 200 ml/m^3, assuming a respiratory volume of 10 m^3 and complete hydrolysis, 3.8 g ethanol is taken up, which is about 50 mg/kg body weight. No positive results are available from in vivo studies with ethyl acetate itself. However, the body burden of ethanol after exposure to ethyl acetate at the MAK value of 200 ml/m^3 is markedly below the ethanol doses that induce germ cell mutagenicity, and ethanol does not accumulate in the blood after inhalation. Therefore, ethyl acetate is not expected to induce germ cell mutagenicity and is not classified in any of the categories for germ cell mutagens.

Absorption through the skin. Data from an in vitro study are available for the absorption of ethyl acetate through the skin. The absorption of 1000 mg after exposure to undiluted ethyl acetate has been estimated for humans under standard conditions (exposure of a 2000 cm^2 surface area of skin for 1 hour). An amount of 14 600 mg would be absorbed after inhalation exposure to ethyl acetate for 8 hours

at the systemic NOAEC for humans (400 ml/m³). Absorption through the skin is thus lower than 25% of the systemically tolerable amount. Therefore, ethyl acetate is not designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. There are still no valid clinical findings available for the sensitizing effects on the skin, and only negative results were obtained in an animal study that used an adjuvant. Likewise, no findings are available for sensitizing effects on the respiratory tract. Ethyl acetate is therefore not designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

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