National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Probit function technical support document

Date: 18 May 2020
Comments before: 13 July 2020
Document id: 20200518- n-butyl acetate -PROPOSED
Status: proposed
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substance name	CAS number
n-butyl acetate	**123-86-4**

This draft document describes the derivation of a probit function for application in a quantitative risk analysis (QRA). The probit function has been derived according to the methodology described in RIVM report 2015-0102.

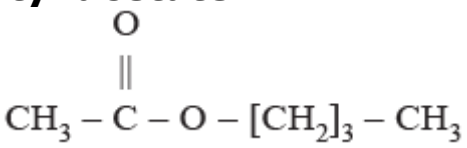
This document has been checked for completeness by the Netherlands' National Institute for Public Health and the Environment (RIVM) and has been assigned the status "proposed". The scientific expert panel on probit functions has approved this document for public discussion and comments. Interested parties are invited to submit comments and suggestions concerning this document within 6 weeks after the issue date to the email address mentioned above.

If the proposed probit function is approved by the expert panel on scientific grounds, after review and revisions following of public comments, the status of the document and probit function will be raised to "interim".

Subsequently, the Ministry of Infrastructure and the Environment will decide whether the probit function will be formally implemented. The decision on actual implementation will primarily be based on the results of a consequence analysis.

Detailed information on the procedures for the derivation, evaluation and formalization of probit functions is available at http://www.rivm.nl/en/Topics/P/Probit_functions.

Technical support document n-butyl acetate



1. Substance identification

CAS-number:	123-86-4
IUPAC name:	n-butyl acetate
Synonyms:	Acetic acid, n-butyl ester; 1-butyl acetate; butyl ethanoate
Molecular formula:	$C_6H_{12}O_2$
Molecular weight:	116.2 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	126°C (at 101.3 kPa)
Vapour pressure:	1.2 kPa (at 20°C)
Saturated vapor conc:	12000 ppm = 58 g/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.207 ppm (at 20°C and 101.3 kPa)
	1 ppm = 4.834 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H336

2. Mechanism of action and toxicological effects following acute exposure[1]

Acute effects: The main target organs and tissues for inhalation exposure to n-butyl acetate are the respiratory tract tissues (primarily throat), conjunctiva and the central nervous system. The health endpoints are irritation to the eyes and respiratory tract (in animals and humans), and decreased motor activity, lethargy, ataxia, narcosis and death in animals. Symptoms of high exposure are lung oedema, unconsciousness and mortality. Lethality likely results from respiratory damage.
Long-term effects: Chronic exposure produces similar effects as described above. No information concerning irreversible effects is available.

3. Human toxicity data

No informative reports on human toxicity following acute inhalation exposure were identified in which details about both health effects and the exposure have been documented in sufficient detail.
In the study by Iregren et al. (1993), three chamber experiments were conducted to study the irritation produced by acute inhalation exposure to analytically determined concentrations of n-butyl acetate in 24 healthy non-smoking male and female subjects without any history of occupational solvent exposure. The highest concentrations tested (i.e., 1426 mg/m^3 for 20 minutes and 711 mg/m^3 for four hours) elicited only minimal irritation to the eyes and respiratory tract. The physical form of exposure (vapour or aerosol) was not stated.
A second human volunteer study on n-butyl acetate has been described (WHO, 2005). Ten volunteers were exposed to approximately 970 mg/m^3 and 1400 mg/m^3 for 3 to 5 minutes. The subjects reported that the lower exposure was irritating to the throat and the higher exposure concentration to be irritating to the nose and eyes and very irritating to the throat (Nelson et al., 1943; as cited in WHO, 2005).
Men exposed to approximately 1.4, 0.7, and 0.33 percent (approximately 68000 mg/m^3, 34000 mg/m^3, 16000 mg/m^3) n-butyl acetate vapour in air even for a "short time" reported the atmosphere extremely disagreeable because of its strong odour and irritation to eyes and nasal passage (Sayers et al., 1936). Details concerning these exposures of humans to n-butyl acetate were not provided.

[1] ERPG (2014); Norris et al. (1997)

4. Animal acute toxicity data

During the literature search the following technical support documents and databases were consulted:

1. ERPG document and reference database for n-butyl acetate, covering references before and including 1995.
2. An additional search covering publications from 1980 onwards was performed in HSDB, MEDline/PubMed, Toxcenter, IUCLID, ECHA, RTECS, IRIS and ToxNet with the following search terms:
   - Substance name and synonyms
   - CAS number
   - lethal*
   - mortal*
   - fatal*
   - LC_{50}, LC
   - probit
3. Unpublished data were sought through networks of toxicological scientists.

Animal lethal toxicity data focused on acute exposure are described in Appendix 1. A total of 13 studies were identified -with 14 datasets for 4 species- with data on lethality following acute inhalation exposure. None of the datasets were assigned status A for deriving the human probit function, 8 datasets were assigned status B1 and 6 were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

A total of three studies were identified in which sensory irritation was studied. In these studies the following RD_{50} values were observed:

Table 1 *Sensory irritation data for n-butyl acetate*

Species/strain	RD_{50} (mg/m^3)	Exposure duration (min)	Author/year
Mouse / Swiss OF1	$3,529^{NS}$	5	Muller and Greff, 1984 (as cited in ERPG 2014)
Mouse / BALB/c	$8,340^{NS}$	Not stated	Korsak and Rydzynski, 1994 (as cited in WHO, 2005)
Mouse / Swiss-Webster	$3,553^{NS}$	30	Dow Chemical, UCC 1993 (as cited in ERPG 2014)

NS: not specified if a plateau in response was reached.

5. Probit functions from individual studies

All available acute lethality data on n-butyl acetate are displayed in Figure 1.

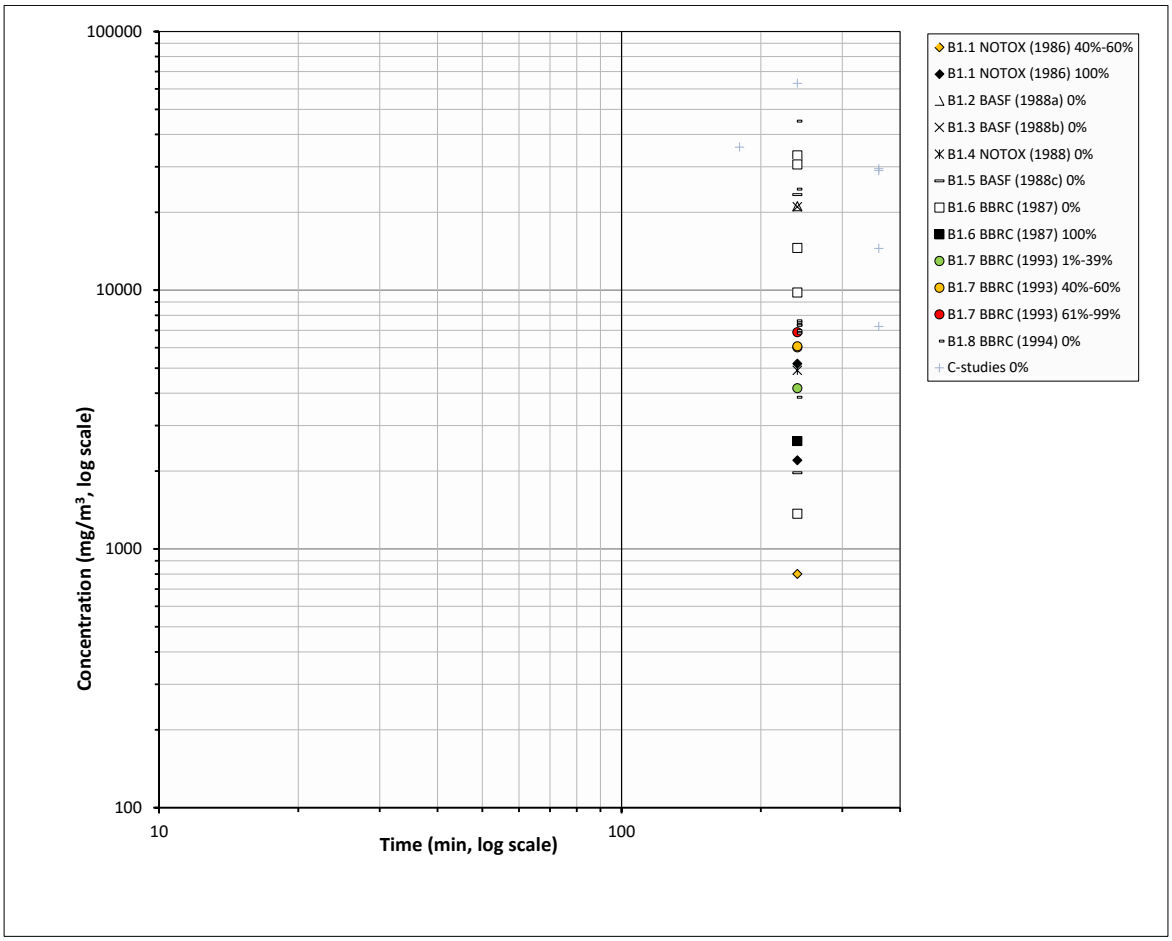


Figure 1 *All available acute lethality data for n-butyl acetate.*

6. Derivation of the human probit function

It is not possible to derive a human probit function for n-butyl acetate with sufficient reliability. The database of the acute inhalation toxicity studies for n-butyl acetate, described under Appendix 1 and presented in Figure 1, shows inconsistent results. The database of n-butylacetate consists of 8 B1-studies and several C-studies. All B1-studies were well performed and documented. Since only one duration was considered these studies were deprived the A-status.

In three out of eight B1 studies mortality was found, of which two could be used to derive an 240 min LC_{50} value (i.e. 799 mg/m^3 based on study B1.1 (Notox 1986) and 5,298 mg/m^3 based on study B1.7 (Bushy Run Research Center (1993)). One other study (Bushy Run Research Center (1987; study ID B1.6) also found mortalities, the data however did not allow probit derivation.
In several other studies, at exposure concentrations far exceeding the concentrations of the abovementioned studies where mortalities were observed, all animals survived.

No clear explanations could be found for this inconsistency in the results, although attempts were made (ERPG, 2014; Norris et al., 1997) to rule out possible exposure factors such as vapour generation methods and whole body versus head/nose-only exposure. A possible explanation for the inconsistencies might be the presence of impurities in the old production batches. That might explain the mortality observed in some of the studies. However, based on the available data, no definite conclusions can be drawn on this point.

If we were to ignore the inconsistency of the data and use the studies B1.1 (Notox, 1986) and B1.7 (Bushy Run Research Center, 1993) as point of departure for the human probit function (see appendix 2), lethal concentrations will be derived that are approximately at the same level and even below the concentrations used in the two human volunteer studies (see section 3). In the volunteer studies only irritation of the nose, eyes and respiratory tract were noted.

Additionally, a repeated dose toxicity study is available with rats exposed at 3000 ppm (14,501 mg/m^3) n-butyl acetate for 6h/d 5d/week for 14 weeks. No mortalities were observed during the study (David et al., 1998).

For the abovementioned reasons the studies that reported mortality are not suitable for derivation of a human probit function since such a function would conflict with other animal and human data. Since mortality data is lacking in other studies, it is concluded that no human probit function for n-butyl acetate can be derived with sufficient reliability.

Appendix 1 Animal experimental research

Study ID: B1.1

Author, year: ***Notox (1986; sponsored by 3M)***
Substance: n-butyl acetate
Species, strain, sex: rat, Wistar, male and female
Number/sex/conc. group: 5
Age and weight: 8-12 weeks; 196-294 g (m) and 209-250 g (f)
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes*
Study carried out according to OECD 403 guideline(s)	*Yes*
Stability of test compound in test atmosphere	*No information*
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	*Head-only*
Type of restrainer	*Animals are placed in Perspex animal confinement cages. The inner compartment of the cages can be adjusted to animals of different sizes in such a way that the animals can only breathe the aerosol-containing air through a perforated Perspex plate at the front of their cages. The animal's tail rests outside the cage through a small opening allowing dissipation of body heat.*
Pressure distribution	*Positive pressure at the nose of the animals (central cylinder), negative pressure in surrounding hood.*
Homogeneity of test atmosphere in breathing zone of animals	*A dynamic spraying nozzle was used to generate the test substance. The substance is delivered by an infusion syringe pump.*
Number of air changes per hour	*Air flow of 10 l/min*
Equilibration time (t95)	*Insufficient information to calculate t95*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Chamber atmosphere was sampled at the same level as the breathing orifices. Samples were taken hourly and analyzed by gas chromatography.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Sampling of test atmosphere with a low pressure cascade impactor close to the breathing zone of the animals.* *The test substance completely vaporized at the lower concentrations.*

Assessment of Reliability	***B1*** *Well-performed study, limited to one exposure duration.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	0.8×10^3		240	3/5	3/5
Rat	2.2×10^3		240	5/5	5/5
Rat	5.2×10^3		240	5/5	5/5

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, 2016) as

$Pr = a + b\times lnC + d\times S$

with C for concentration in mg/m^3 and S for sex (0 = male, 1 = female).

Probit function	Species	a	b	d	n-value
Sex as variable*	Rat	-	-	-	-
Sexes combined	Rat	-1942	291	-	-

* due to identical lethality data for male and female animals, analysis with sex as variable was not possible

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Combined
240	799 (CI could not be estimated due to large variances)

It is noted that the calculated 4-hour LC_{50} values (as calculated by the Probit TSD author) is approximately at the lowest value of the range of tested concentrations. The study author calculated a 4-hour LC_{50} of 740 mg/m^3.

No C × t probit function could be calculated from these data alone.

Study ID: B1.2

Author, year: ***BASF (1988a)***
Substance: n-butyl acetate
Species, strain, sex: rat, Wistar, male and female
Number/sex/conc. group: 5
Age and weight: 8-9 weeks; 258 g (m) and 189 g (f)
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*yes*
Study carried out according to OECD 403 guideline(s)	*yes*
Stability of test compound in test atmosphere	*No information*
Use of vehicle (other than air)	*No*
Whole body / nose-only (incl. head/nose-only) exposure	*Head/nose-only*
Type of restrainer	*Animals were restrained in tubes and their snouts projected into the inhalation chamber; not further specified*
Pressure distribution	*By means of an exhaust system the pressure ratios were adjusted in such way that the amount of exhaust air was about 33.3% lower (excess pressure). This prohibits dilution of the test substance with laboratory air.*
Homogeneity of test atmosphere in breathing zone of animals	*A liquid aerosol was generated by means of a continuous infusion pump and a 2-component atomizer.*
Number of air changes per hour	*Air flow of 600 l/h (compressed air)*
Equilibration time (t95)	*Insufficient information to calculate t95*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Gas chromatography (GC) was used to analyze the atmosphere. Location was immediately adjacent to the animals' noses. One sample about hourly.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Measured by means of 1) collecting disks followed by GC and 2) light scattering photometer. No particles detected.*
Assessment of Reliability	***B1*** *Well-performed study, limited to one exposure duration.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	$21{,}1 \times 10^3$		240	0/5	0/5

Probit function

A probit function could not be derived based on the data by BASF (1988a).

Study ID: B1.3

Author, year: ***BASF (1988b)***
Substance: n-butyl acetate
Species, strain, sex: rat, Wistar, male and female
Number/sex/conc. group: 5
Age and weight: 8-9 weeks; 271 g (m) and 197 g (f)
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes*
Study carried out according to OECD 403 guideline(s)	*Yes*
Stability of test compound in test atmosphere	*No information*
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	*Head/nose-only*
Type of restrainer	*Animals were restrained in tubes and their snouts projected into the inhalation chamber; not further specified*
Pressure distribution	*By means of an exhaust system the pressure ratios were adjusted in such way that the amount of exhaust air was about 10% lower (excess pressure). This prohibits dilution of the test substance with laboratory air.*
Homogeneity of test atmosphere in breathing zone of animals	*A vapour/air mixture was generated by means of a continuous infusion pump and a glass vaporizer with thermostat (evaporation by heating).*
Number of air changes per hour	*Air flow of 1500 l/h*
Equilibration time (t95)	*Insufficient information to calculate t95*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Gas chromatography (GC) was used to analyze the atmosphere. Location was immediately adjacent to the animals' noses. One sample about hourly.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*A quartz wool plug was used as aerosol barrier. Particle detection was not performed.*
Assessment of Reliability	***B1*** *Well-performed study, limited to one exposure duration.*

1 **Results**

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	$21{,}0 \times 10^3$		240	0/5	0/5

2

3 **Probit function**

4 A probit function could not be derived based on the data by BASF (1988b).

Study ID: B1.4

Author, year: ***NOTOX (1988; sponsored by BASF)***
Substance: n-butyl acetate
Species, strain, sex: rat, Wistar, male and female
Number/sex/conc. group: 5
Age and weight: 8 weeks; 302 g (m) and 215 g (f)
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	Yes
Study carried out according to OECD 403 guideline(s)	*OECD guideline 403; EU method B.2*
Stability of test compound in test atmosphere	*No information*
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	*Head-only*
Type of restrainer	*Radially mounted cylindrical Perspex animal confinement cages*
Pressure distribution	*Positive pressure at the nose of the animals (central cylinder), negative pressure in surrounding hood.*
Homogeneity of test atmosphere in breathing zone of animals	*A dynamic spraying nozzle was used to generate the test substance. The substance is delivered by an infusion pump.*
Number of air changes per hour	*Air flow of 10 l/min*
Equilibration time (t95)	*Insufficient information to calculate t95*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Chamber atmosphere was sampled at the same level as the breathing orifices. Samples were taken hourly and analyzed by gas chromatography.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Sampling of test atmosphere with a low pressure cascade impactor close to the breathing zone of the animals. Main part was present as vapour rather than as an aerosol.*
Assessment of Reliability	***B1*** *Well-performed study, limited to one exposure duration.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	$4{,}9 \times 10^3$		240	0/5	0/5

Probit function

A probit function could not be derived based on the data by NOTOX (1988).

Study ID: B1.5

Author, year: ***BASF (1988c)***
Substance: n-butyl acetate
Species, strain, sex: rat, Wistar, male and female
Number/sex/conc. group: 5
Age and weight: 8-9 weeks; 299 g (m) and 196 g (f)
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes*
Study carried out according to OECD 403 guideline(s)	*Yes*
Stability of test compound in test atmosphere	*No information*
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	*Head/nose-only*
Type of restrainer	*Animals were restrained in tubes and their snouts projected into the inhalation chamber; not further specified*
Pressure distribution	*By means of an exhaust system the pressure ratios were adjusted in such way that the amount of exhaust air was about 10% lower (excess pressure). This prohibits dilution of the test substance with laboratory air.*
Homogeneity of test atmosphere in breathing zone of animals	*An aerosol was generated by means of a continuous infusion pump and a 2-component atomizer.*
Number of air changes per hour	*Air flow of 1500 l/h (compressed air)*
Equilibration time (t95)	*Insufficient information to calculate t95*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Gas chromatography (GC) was used to analyze the atmosphere. Location was immediately adjacent to the animals' noses. One sample about hourly.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Measured by means of metal collecting disks in the impactor followed by GC. No aerosol particles were detectable.*
Assessment of Reliability	***B1*** *Well-performed study, limited to one exposure duration.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	1.97×10^3		240	0/5	0/5

Rat	23.4 $\times 10^3$		240	0/5	0/5

Probit function

A probit function could not be derived based on the data by BASF (1988c).

Study ID: B1.6

Author, year: ***Bushy Run Research Center (1987; sponsored by Union Carbide Coorporation)***
Substance: n-butyl acetate
Species, strain, sex: rat, Sprague-Dawley albino, male and female
Number/sex/conc. group: 5
Age and weight: 50-62 days; 199-290 g (m) and 157-214 g (f)
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes*
Study carried out according to OECD 403 guideline(s)	*No information*
Stability of test compound in test atmosphere	*Using the atomizer for vapour generation resulted in aerosol formation*
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*n-Butyl acetate test atmosphere was generated in three different ways: statically, dynamically by using a evaporator and dynamically by using an atomizer.* 1. *Static exposure: test material was placed in an open tray at the top of a sealed 120 l chamber. Vapours were allowed to achieve equilibrium after 17 hours after which the animals were placed into the chamber.* 2. *Dynamically by evaporation: test material was metered with a pump into a heated evaporator. The vapour mixture was carried to the chamber using a countercurrent air stream that entered the bottom of the evaporator.* 3. *A metered amount of test material was introduced to a atomizer with a liquid and air nozzle. The atomizer was placed at the top of the chamber. Liquid aerosol/vapour was diluted to the desired vapour concentration and dispersed throughout the chamber by filtered supply air.*

Number of air changes per hour	1. *static* 2. *air flow of 200 l/min gives 13.3 air changes per hour (900 l chamber)* 3. *Air flow of 250 to 300 l/min gives 11.5 to 13.8 air changes per hour (1300 l chamber)*
Equilibration time (t95)	1. *N/A* 2. *13.5 min* 3. *13-15.6 min*
Start of exposure relative to equilibration	*Static: after equilibrium* *Dynamic: no information*
Actual concentration measurement	*Concentrations were measured by gas chromatography at least 10 times during the 4h exposure period. The analytical/nominal ratios ranged from 0.88 to 0.92.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Not determined.*
Assessment of Reliability	***B1*** *Well-performed study, limited to one exposure duration.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	33,192 (static)		240	0/5	0/5
Rat	30,621 (evaporator)		240	0/5	0/5
Rat	14,564 (evaporator)		240	0/5	0/5
Rat	9,798 (evaporator)		240	0/5	0/5
Rat	2,610 (atomizer)		240	5/5	5/5
Rat	1,368 (atomizer)		240	0/5	0/5

Probit function

Based on the data it was not possible to derive a probit function. Under the exposure conditions where mortality occurred, only two exposure concentrations were tested. The author stated a 4h LC_{50} value of 1,890 mg/m^3 for male and female rats with a confidence interval (95%) of 1,426 to 2,499 mg/m^3.

Study ID: B1.7

Author, year: ***Bushy Run Research Center (1993; sponsored by Union Carbide Coorporation)***
Substance: n-butyl acetate
Species, strain, sex: rat, Sprague-Dawley albino, male and female
Number/sex/conc. group: 5
Age and weight: age not specified; 218-290 g (m) and 172-213 g (f)
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes*
Study carried out according to OECD 403 guideline(s)	*No information*
Stability of test compound in test atmosphere	*Author indicates that aerosol formation occured during exposure.*
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution	*Test substance was metered from a pump into an atomizer fitted with a liquid and air nozzle. The atomizer was positioned at the top of the chamber where the aerosol was generated. Either filtered air or dry compressed cylinder air (at the 6,008 mg/m^3 exposure group) was passed through the atomizer.*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere generation, particularly for liquids (spraying, evaporation, other) and solids. Mixing of test atmosphere in the exposure system.*
Number of air changes per hour	*12 to 17 air changes per hour (air flow of 250 l/min and a chamber volume of 900 or 1300 l)*
Equilibration time (t95)	*10.8-15.6 min*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Concentrations were measured using gas chromatography, equipped with a flame ionization detector, 9 to 16 times*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*The particle size distribution was measured using a TSI aerodynamic Particle Sizer Model (for two exposure groups).*
Assessment of Reliability	***B1*** *Well-performed study, limited to one exposure duration.*

Results

Species	Concentration (mg/m³)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	4,181		240	1/5	2/5
Rat	6,008		240	2/5	3/5
Rat	6,071		240	4/5	2/5
Rat	6,878		240	5/5	4/5

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, 2016) as

$Pr = a + b \times \ln C + d \times S$

with C for concentration in mg/m³ and S for sex (0 = male, 1 = female).

Probit function	Species	a	b	d	n-value
Sex as variable	Rat	-19.1	2.82	-0.15	-
Sexes combined	Rat	-19.2	2.82	-	-

The LC_{50} values for both sexes did not differ by more than a factor of 2. This does not support the proposition that sex differences exist in the lethal response. For this reason the data from both sexes were pooled and analysed to derive the animal probit function.

Duration (min.)	LC_{50} (mg/m³) 95%-C.I. Male	LC_{50} (mg/m³) 95%-C.I. Female	LC_{50} (mg/m³) 95%-C.I. Combined
240	5159 (2868 - 6536)	5432 (3412 - 7105)	5298 (3436 - 6233)

The study authors calculated a 4-hour LC_{50} values of 5360 (4495-6395) mg/m³ (males), 5254 (3451-7995) mg/m³ (females), 5298 (4500-6245) mg/m³ (sexes combined).

No C × t probit function could be calculated from these data alone.

Study ID: B1.8

Author, year: ***Bushy Run Research Center (1994; sponsored by Union Carbide Coorporation)***
Substance: n-butyl acetate
Species, strain, sex: rat, Sprague-Dawley, male and female
Number/sex/conc. group: 5
Age and weight: age not specified; 180-296 g (m) and 140-210 g (f)
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes*
Study carried out according to OECD 403 guideline(s)	*No information*
Stability of test compound in test atmosphere	*Author indicates that aerosol formation occured during exposure.*
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*Test substance was metered from a pump into an atomizer (three different types were used) fitted with a liquid and air nozzle. The atomizer was positioned at the top of the chamber where the aerosol was generated. In two exposure groups the filtered air was conditioned to 100% relative humidity.*
Number of air changes per hour	*14 air changes per hour (air flow of 300 l/min and a chamber volume of 1300 l))*
Equilibration time (t95)	*13 min*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*Concentrations were measured using gas chromatography, equipped with a flame ionization detector, 14 to 21 times*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*The particle size distribution was measured using a TSI aerodynamic Particle Sizer Model. Mass Median Aerodynamic Diameters ranged from 0.79 to 2.68 microns. In addition, submicron particles were measured using a TSI Condensation Particle Counter. Submicron particles amounts ranged from 1.56 x 105 to 22.5 x 105 particles/cc.*
Assessment of Reliability	***B1*** *Well-performed study, limited to one exposure duration.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
Rat	3,857		240	0/5	0/5
Rat	6,820		240	0/5	0/5
Rat	6,970[a]		240	0/5	0/5
Rat	7,318[b]		240	0/5	0/5
Rat	7,326[c]		240	0/5	0/5
Rat	7,487[d]		240	0/5	0/5
Rat	7,613[e]		240	0/5	0/5
Rat	24,564		240	0/5	0/5
Rat	45,011		240	0/5	0/5

30 psi operation pressure of atomizer
[a]: exposure with relative humidity of 52% and 20 psi operation pressure of atomizer.
[b]: exposure with relative humidity of 38% and 30 psi operation pressure of atomizer, using an old batch of n-butyl acetate.
[c]: exposure with relative humidity of 100% and 20 psi operation pressure of atomizer.
[d]: exposure with relative humidity of 100% and 20 psi operation pressure of atomizer, using an old batch of n-butyl acetate.
[e]: exposure with relative humidity of 46% and 20 psi operation pressure of atomizer.

Probit function

A probit function could not be derived based on the data by Bushy Run Research Center (1994; sponsored by Union Carbide Coorporation).

Study ID: C studies

Eastman Kodak (1994) performed an acute inhalation neurotoxicity study in Sprague-Dawley rats (10/sex/conc). Animals were exposed to 0, 1500, 3000 or 6000 ppm (0, 7245, 14490 or 28980 mg/m^3) for a single 6 hour period. Beginning immediately after onset of the exposure period and continuing until the end of the exposure period, treated groups had minimal reduced activity (hypoactivity) and minimal reduced responses to extrachamber stimulation (tapping on the outside wall of the inhalation chamber). At 28980 mg/m^3, the severity of hypoactivity was minor to moderate. At 14490 mg/m^3, the severity of hypoactivity in female rats was minor, while male 14490 mg/m^3 rats were characterized as having minimal hypoactivity. Only minimal hypoactivity was observed at 7245 mg/m^3. Sialorrhea was also observed in treated male-rats, but only occasionally in treated female rats. Tearing was also noted occasionally in treated female rats. No deaths were noted during exposure and no clinical conditions were noted at any time post-exposure.

Flury and Wirth (1933) exposed mice and cats (strain, sex and numbers not described) to various (nominal) concentrations n-butylacetate for different exposure durations (10-360 min). Animals showed CNS depression. No deaths were reported. Details on the performance of the study were not presented. A 3-hour exposure to 7400 ppm (35742 mg/m^3) resulted in CNS depression in mice (with the animal recovering). A 6-hour exposure of cats to 6100 ppm (29463 mg/m^3) resulted in irritation of the eyes with no CNS depression.

Sayers et al. (1936) exposed guinea pigs (6/conc) to 3300, 7000 and 14000 ppm (15939, 33810 and 67620 mg/m^3) for multiple exposure durations. A 4 hour exposure to 67620 mg/m^3 resulted in deaths, though the number of animals killed was not presented. No further details presented.

Smyth et al. (1954) described that a 4-hour exposure to a saturated vapour concentration (~63000 mg/m^3) did not result in mortality in rats. No further details presented.

Smyth (1956) described that an 8-hour exposure to a saturated vapour concentration (~63000 mg/m^3) resulted in mortality in rats, though the number of animals killed was not presented.

Appendix 2 Human probit function based on studies B1.1 and B1.7.

To derive the human probit function the results from studies B1.1 (Notox 1986) and B1.7 (Bushy Run Research Center (1993; sponsored by Union Carbide Coorporation)) have been used to derive a point of departure.

The rat geometric mean LC_{50}-value was calculated from the 240 min LC_{50} values of studies B1.1 (799 mg/m^3) and B1.7 (5298 mg/m^3). The rat geometric mean 240 min LC_{50}-value was 2058 mg/m^3. The formula for the geometric mean of time-scaled LC_{50}-values from 1 species is as follows:

$$\overline{LC_{50}} = \left[\prod_{i=1}^{m} LC_{50,i}\right]^{(1/m)}$$

With $\overline{LC_{50}}$ = geometric mean LC_{50}-value
$LC_{50,i}$ = LC_{50}-value of study i.
m = number of observations on LC_{50}-values (i=1…m).

The Point of Departure for the human probit function is a 240-minute geometric mean animal LC_{50} value of 2058 mg/m^3 and the default value for n of 2.

The human equivalent LC_{50} was calculated by applying the following assessment factors:

Table a *Rationale for the applied assessment factors.*

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	default
Nominal concentration	1	Analytical concentrations were used
Adequacy of database:	1	Large dataset including well conducted studies. A conservative approach was applied since other studies observed no mortality at higher concentrations than the LC_{50} values used as point of departure.

The estimated human equivalent 240-minute LC_{50} value is 2058/ 3 = **686 mg/m^3**.

No reliable experimentally determined n-value was available, so the default n-value of **2.0** was used. Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1.0**.

The human probit function is then calculated on the human equivalent 240 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1.0 \times \ln(686^{2.0} \times 240)$ resulting in the a-value of **–13.5**.

Pr = -13.5 + 1 × ln (C ^{2} × t) with C in mg/m^3 and t in min.

The derived human probit function has a scientifically weak basis. The probit function is based on two studies in the rat with B1 quality, where 5 animals/sex/group were

exposed to seven different concentrations for 4 hours. Because there are major inconsistencies in the total dataset for n-butyl acetate the basis for this probit function is considered weak.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 287 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 419 mg/m^3.

Table b *LC-values calculated with the derived probit function compared with existing acute inhalation exposure guidelines.*

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
0.1% lethality, this probit	405	287
1% lethality, this probit	593	419
AEGL-3	-	-
ERPG-3[2] (2014)	-	14500
LBW (2017)	9600	7600

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are lower.

[2] AEGL and ERPG values were converted from ppm to mg/m^3 with the conversion factor calculated in section 1. Therefore, the AEGL and ERPG values in mg/m^3 can deviate slightly from those reported in the AEGL and ERPG TSDs.

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