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Probit function technical support document

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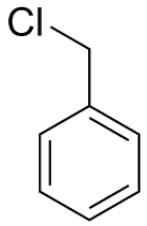
substance name	CAS number
Benzyl Chloride	**100-44-7**

This 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 of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have had the opportunity to comment on the derivation of the proposed probit function. The status of this document has now been raised to "interim", pending a decision on its formal implementation.

The decision on actual implementation depends on the results of a further consequence analysis.

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



Technical support document Benzyl chloride

1. Substance identification

CAS-number:	100-44-7
IUPAC name:	Benzyl chloride
Synonyms:	α-chlorotoluene, chloromethyl benzene, chlorophenyl methane
Molecular formula:	C_6H_5-CH_2Cl
Molecular weight:	126.6 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	179°C (at 101.3 kPa)
Vapour pressure:	0.12 kPa (at 20°C)
Saturated vapor conc:	1200 ppm = 6320 mg/m^3 (calculated, at 20°C)
Conversion factor:	1 mg/m^3 = 0.190 ppm (at 20°C and 101.3 kPa)
	1 ppm = 5.266 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	Human H 302, 315, 318, 331, 335, 350, 373

2. Mechanism of action and toxicological effects following acute exposure

Acute effects: The main target organs and tissues for inhalation exposure to benzyl chloride are the cornea, conjunctiva and respiratory tract. Liver, kidney and heart muscle damage have also been described after inhalation exposure. The health endpoints after acute inhalation to near lethal levels are related to the irritant properties of benzyl chloride.

Damage occurs in the respiratory system, resulting in mucus secretion, upper airway and/or pulmonary oedema and laryngospasm. Symptoms of high exposure are laboured breathing, secretions from nose, mouth and eyes and prostration. The resulting hypoxemia will cause CNS and cardiovascular (myocardial ischemia) effects. Lethality results when the respiratory tissue damage proceeds to inflammation, degeneration and necrosis of affected tissue, atelectasis, emphysema and finally death.

Long-term effects: Benzyl chloride is mutagenic in a number of different in vitro test systems, and is proven to produce DNA adducts in the mouse via alkylation after oral administration. There are no reports of mutagenicity and carcinogenicity following long-term experimental inhalation exposure. There is limited evidence for carcinogenicity in long-term exposed workers. Subacute and subchronic inhalation exposure produces respiratory irritative effects, and increased relative weight of liver, kidney and spleen without a histopathological correlate.

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.

4. Animal acute toxicity data

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

1. ERPG document and EU RAR and reference database for benzyl chloride, covering references before and including 1995.
2. An additional search covering publications from 1980 onwards was performed in HSDB, MEDline/PubMed, Toxcenter, ECHA, IUCLID, 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 4 studies were identified -with 4 datasets for 3 species- with data on lethality following acute inhalation exposure. For deriving the human probit function none of the datasets could be assigned status A, 2 datasets were assigned status B1 and 2 were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

A total of 4 studies was identified in which sensory irritation was studied. In these studies, the following RD_{50} values were observed:

Table 1 *Sensory irritation data for benzyl chloride*

Species/strain	RD_{50} (mg/m^3)	Exposure duration (min)	Author/year
Swiss OF-1 mice	88^{NS}	5	De Ceaurriz 1981
Swiss-Webster mice	138^{NS}	10	Dudek 1995
Sprague-Dawley rat	465^{NS}	10	Dudek 1995
Fischer 344 rat	863^{NS}	10	Dudek 1995

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

5. Probit functions from individual studies

All available acute lethality data on benzyl chloride are displayed in Figure 1.

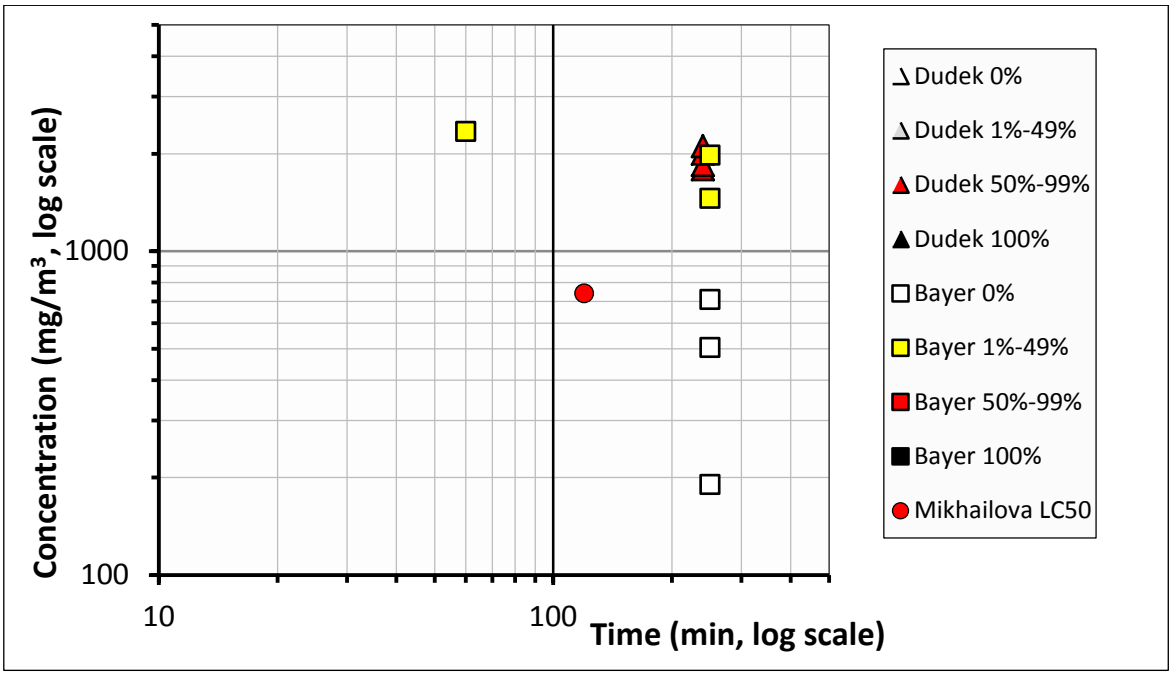


Figure 1 All acute lethality data for benzyl chloride

The data that were selected for primary analysis of the animal probit function are presented in Table 2 and Figure 2. The probit function was derived by pooling the data from the two studies with B1 quality.

Probit functions have been calculated and reported in Appendix 1 for each of the reported studies. The results of the calculations are presented in the table below.

Table 2 *Data selected for initial analysis of the animal probit function of benzyl chloride.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} **240 minutes** (mg/m^3), 95% C.I.	n-value (95% cfd-i)
B1.1	Rat	*240-min LC_{50}*	1795 (852 – 1873)	N/A
B1.2	Rat	*240-min LC_{50}*	1989 (1670 - 3098)	N/A

The data of the two B1 studies with rats are presented graphically below.

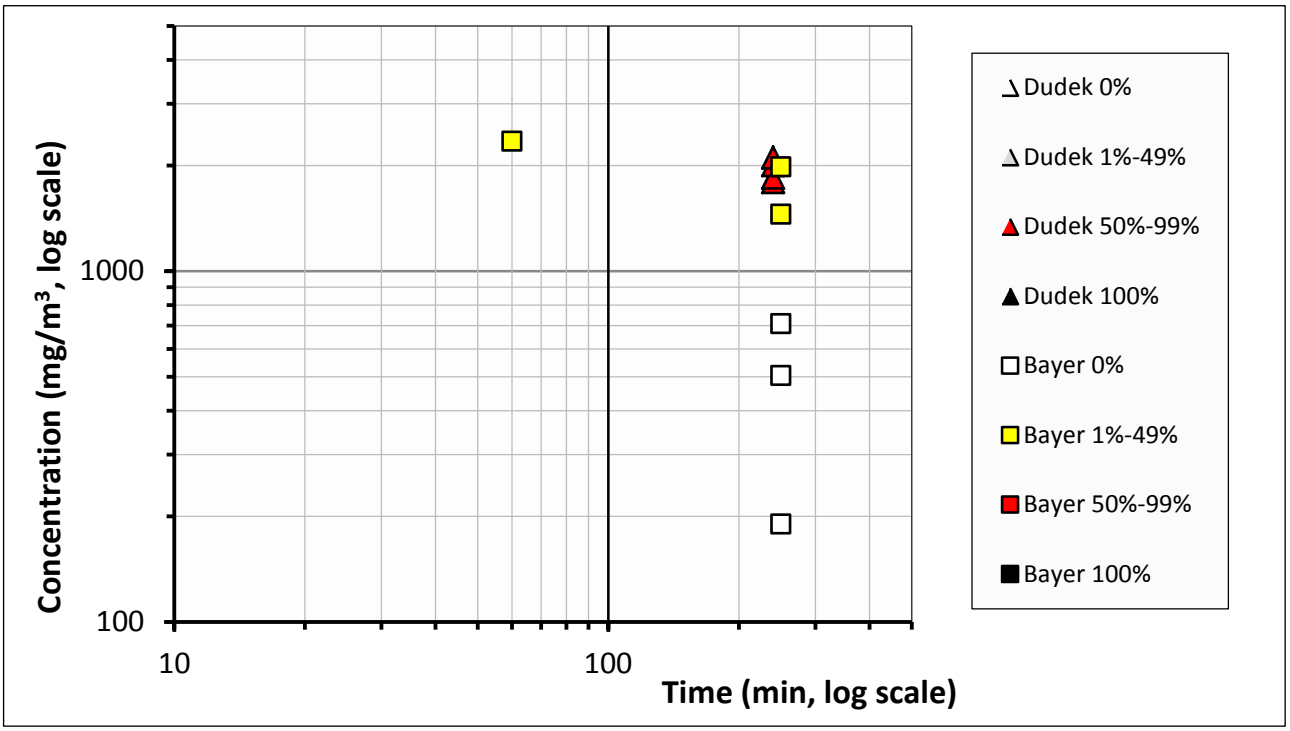


Figure 2 *Data selected for the initial analysis of the animal probit function of benzyl chloride.*

Based on study quality characteristics the data from studies B1.1 (Dudek 1994) and B1.2 (Bayer 1979, only 240-minute data) were selected for the final dataset for the derivation of the animal probit function. All response rates were ≥ 50% in study B1.1, and <50% in study B1.2. The point estimates of the two 240 minute LC_{50} values were almost equal (B1.1: 1795 mg/m3 and B1.2: 1989 mg/m^3) and confidence intervals could be assessed for both LC_{50} values.

The final data eligible for calculating the animal probit function contain two datasets from two studies and include data from one animal species (rat).

Table 3 *Data selected for the derivation of the animal probit function of benzyl chloride (identical to table 2).*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} **240 minutes** (mg/m^3) 95% C.I.
B1.1	Rat	*240-min LC_{50}*	1795 (852 – 1873)
B1.2	Rat	*240-min LC_{50}*	1989 (1670 - 3098)

The data of the selected datasets are presented graphically below.

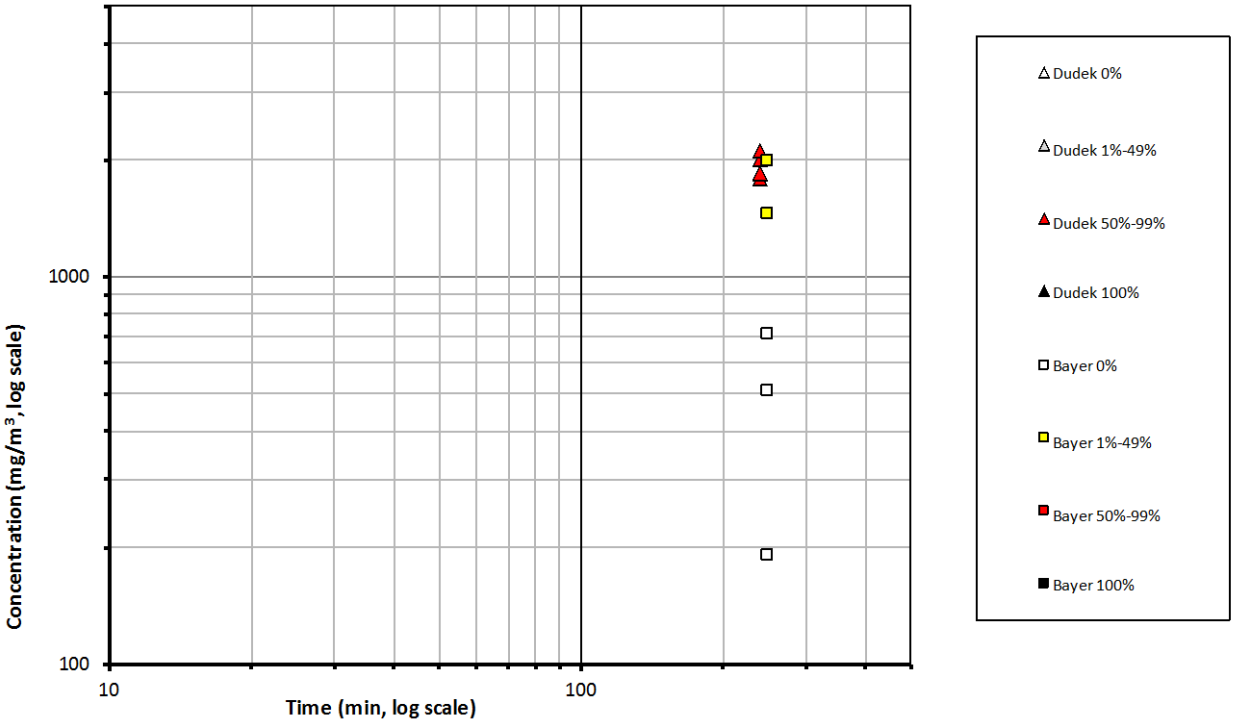


Figure 3 *Final data selected for derivation of the animal probit function of benzyl chloride.*

6. Derivation of the human probit function

To derive the human probit function the results from study B1.1 (Dudek 1994) and B1.2 (Bayer 1979, excluding the 60-minute data[1]) were used to derive a point of departure. A geometric mean LC_{50}-value for studies B1.1 and B1.2 was 1890 mg/m^3 was calculated.

In absence of information about the n-value the default n-value of 2 was used for the probit function.

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

[1] For 60 minutes only 1 exposure concentration was tested.

Table 4 *Rationale for the applied assessment factors.*

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	No rationale to deviate from the default assessment factor. In addition, sensory irritation as defined by the RD_{50} value is well below calculated LC_{50} values, indicating an additional protection mechanism in the test species in comparison to humans.
Nominal concentration	1	Actual concentrations reported
Adequacy of database:	1	No A-study available. Two reasonably well performed B1-studies with very similar outcomes, however with only 240-min data.

The estimated human equivalent 240-minute LC_{50} value is 1890 / 3 = **630 mg/m^3**.

No experimentally determined n-value was available, so the default n-value of **2** 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**.

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): $5 = a + 1 \times \ln(630^2 \times 240)$ resulting in the a-value of **-13.372**.

$Pr = -13.4 + 1 \times \ln(C^2 \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically acceptable basis. The probit function is based on 2 studies in the rat with B quality.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 340 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 233 mg/m^3.

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
0.1% lethality, this probit	338	233
1% lethality, this probit	495	340
AEGL-3	not available	not available
ERPG-3 (2017)		260
LBW (2015)	140	110

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are slightly higher. ERPG and LBW values are set with higher assessment factors to allow a wider margin of safety for susceptible individuals.

Appendix 1 Animal experimental research

Study ID: B1.1

Author, year: ***Dudek 1994[2]***
Substance: Benzyl chloride
Species, strain, sex: Male and female Sprague-Dawley rats
Number/sex/conc. group: 5 / concentration / sex, total number of animals was 40.
Age and weight: Age and weight: approx 7 weeks, 247-304 grams (males) and 156-193 grams (females)
Observation period: 14 days

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*No GLP statement provided*
Study carried out according to OECD 403 guideline(s)	*Equivalent to OECD guideline 403*
Stability of test compound in test atmosphere	*Stable*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*N/A*
Pressure distribution	*No information provided*
Homogeneity of test atmosphere in breathing zone of animals	*Air flow rate was high, the compound was mixed into the airstream before entering the chamber, and the in- and outlets positioned such that the air had to pass the animals*
Number of air changes per hour	*71-72 ℓ/min for 10 rats in a 300 ℓ exposure chamber, which equals 14 air changes / h.*
Equilibration time (t95)	*3 × 300 / 71 = 12.7 min.*
Start of exposure relative to equilibration	*Not specified, but probably at start of concentration build-up*
Actual concentration measurement	*Four samples of 10 ℓ chamber atmosphere were passed through an impinger with isooctane over the 4-hr exposure period. Gas chromatographic analysis. Appears adequate.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *B1-study because only one exposure duration was tested and all response rates were ≥ 50%, otherwise well performed.*

[2] In other reviews referenced as Monsanto 1994

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Nominal	Analytical		Male	Female
Rat	2317	1790	240	2/5	3/5
Rat	2264	1843	240	2/5	4/5
Rat	2633	2001	240	4/5	5/5
Rat	2896	2106	240	3/5	2/5

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, December 2015) as

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

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

An exploratory analysis with sex as variable did not indicate sex differences.

Probit function	Species	a	B
Sexes combined, <u>including</u> 2106 mg/m^3 data	*Rat*	*-5.89*	*1.48*
Sexes combined, <u>excluding</u> 2106 mg/m^3 data	*Rat*	*-82.2*	*11.6*

The analysis including all the data produced an LC_{50} value of 1556 mg/m^3 and a very poor model fit. This was probably due to the unexpected lethality outcome at the 2106 mg/m^3 exposure level.

The study author (Dudek) calculated the LC_{50} values on the basis of the lower three exposure concentrations, because:

- the lethality from the 2106 mg/m^3 exposure did not follow the response pattern
- if the responses to the 2016 mg/m^3 exposure were included, the LC-curve for the females had a negative slope.

In the present TSD the LC_{50} value was also calculated on the basis of the 1790, 1843 and 2001 mg/m^3 data, producing an LC_{50} of 1795 mg/m^3.

Duration (minutes)	LC_{50} (mg/m^3) 95%-C.I. Male	LC_{50} (mg/m^3) 95%-C.I. Female	LC_{50} (mg/m^3) 95%-C.I. Combined
240			1795 (852 – 1873)

Study ID: B1.2

Author, year: ***Bayer 1979***
Substance: Benzyl chloride
Species, strain, sex: Rat, male and female Wistar
Number/sex/conc. group: 10 / sex / group, 120 animals in total
Age and weight: young adult, weight unspecified
Observation period: 21 days (extended from 14 days due to delayed mortality)

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*GLP did not exist at the time*
Study carried out according to OECD 403 guideline(s)	*OECD guideline 403 did not exist at the time; unable to verify compliance.*
Stability of test compound in test atmosphere	*The company toxicologist believed that products of hydrolysis were negligible.*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Nose-only*
Type of restrainer	*No information available*
Pressure distribution.	*Positive pressure (unspecified) in the test unit.*
Homogeneity of test atmosphere in breathing zone of animals	*Not specified*
Number of air changes per hour	*Air flow was 500 mℓ/min per rat*
Equilibration time (t95)	*No information available*
Start of exposure relative to equilibration	*No information available*
Actual concentration measurement	*Sampling in hexane, analysis by spectrophotometry (@ 240 nm); sampling frequency and location not specified.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	*Unlikely that particles were present as the bubbler was operated at room temperature with subsequent air dilution.*
Assessment of Reliability	***B1*** *B1 because all but 1 exposures were tested at the same duration, and all response rates were < 50%. Otherwise well performed, as far as can be assessed from the limited available information.*

The TSD author did not have access to the complete study report, because this was considered confidential business information. Only the results table and information required to complete the most relevant sections of the table above were provided.

Mortality was delayed and associated with lung oedema & hydrothorax. In all groups evidence of respiratory distress/irritation existed. No abnormalities were observed at necropsy up to 708 mg/m^3. Due to the late onset of mortality the post-exposure period was extended to 21 days.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
Rat	190		240	0/10	0/10
Rat	504		240	0/10	0/10
Rat	708		240	0/10	0/10
Rat	1453		240	5/10*	1/10
Rat	1980		240	6/10	3/10
Rat	2343		60	0/10	2/10

* last lethality on day 19

Higher concentrations were not tested for the 1 hour exposure, because of difficulties to reach and maintain a stable test atmosphere (personal communication).

Probit function

The probit function and associated LC-values have been calculated using the DoseResp program (Wil ten Berge, December 2015) as:

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

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

Probit function	Species	a	b	C	d	n-value
Sex as variable	*Rat*	*-19.4*	*2.32*	*1.18*	*0.49*	*1.97 (0.80 - 3.14)*
Sexes combined	*Rat*	*-18.7*	*2.26*	*1.19*		*1.89 (0.76 - 3.03)*

The LC_{50} values for both sexes did not differ by more than a factor of 2 (see table below). 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.

In both statistical analytical approaches the model fit. In addition, the mortality was below 50% in all of the tested concentration-time combinations.

Because just one data point was available for 60 minutes, only the 240 minute data were used in the calculation. The LC_{50} values and their confidence intervals are presented below.

Duration (minutes)	LC_{50} (mg/m^3) 95%-C.I. Male	LC_{50} (mg/m^3) 95%-C.I. Female	LC_{50} (mg/m^3) 95%-C.I. Combined
240	1637 (1269 - 2358)	2416 (no cfd-i)	1989 (1670 - 3098)

Due to the limited data a graphical overview of the data is not informative.

Study ID: C studies

Unspecified numbers of rats and mice were exposed for 2 hours to analytically determined static concentrations of benzyl chloride and observed for 1 month and 2 weeks, respectively (Mikhailova 1964). The reported LC_{50} values was 740 mg/m^3. The acute lethality is inconsistent with that reported by other investigators.

Cats (one per concentration) were exposed to various concentrations of benzyl chloride ranging from 160 to 17,700 mg/m^3 for 3-8 hr (Wolf 1912). The signs reported were indicative of respiratory and ocular irritation (eye blinking, tearing, and at higher concentrations also irregular respiration, nasal/oral secretions and prostration). Pathological findings in animals that died include eye, lung, kidney and liver damage.
A study that apparently followed-up the previous one, also reported on the acute effects of high concentrations of benzyl chloride in cats, rabbits, and a dog and confirmed the findings of Wolf (Schutte 1915). Part of the tested animals were exposed multiple times.

Appendix 2 Reference list

Bayer 1979. Untersuchungen Bayer AG, Bericht Nr. 8419. Leverkusen: Bayer AG, 1979. Partial information obtained from company toxicologist.

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