National Institute for Public Health
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Probit function technical support document

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Document id: 20230316-Nitric acid smaller equal to 70%-INHOUDELIJK VASTGESTELD
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substance name	CAS number
Nitric acid ≤70%	**7697-37-2**

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 "inhoudelijk vastgesteld" (approved content).

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 Nitric acid ≤70%

1. Substance identification[1,2]

CAS-number:	7697-37-2
IUPAC name:	Nitric acid
Synonyms:	Aqua fortis, azotic acid
Molecular formula:	HNO_3
Molecular weight:	63 g/mol
Composition:	70% HNO_3, 30% water
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	104-122°C (at 101.3 kPa)
Vapour pressure:	ca 0.95 kPa (at 20°C)
Saturated vapour conc:	9500 ppm = 24900 mg/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.382 ppm (at 20°C and 101.3 kPa)
	1 ppm = 2.621 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H314, H331[3]

2. Mechanism of action and toxicological effects following acute exposure[4]

Acute effects: Nitric acid is a highly corrosive, strongly oxidizing acid. Nitric acid may exist in the air as a vapour, mist, fume, or aerosol. Inhalation of nitric acid involves exposure to nitric acid as well as nitrogen oxides, such a nitrogen dioxide and nitric oxide. [5]

Toxicity after inhalation exposure to nitric acid is similar in humans and animals. Nitric acid may cause immediate irritation of the respiratory tract, pain, and dyspnea, followed by a period of recovery that may last several weeks. A relapse may occur resulting in death caused by bronchopneumonia and pulmonary fibrosis. At nonlethal concentrations, allergic or asthmatic individuals appear to be sensitive to acidic atmospheres.

Long-term effects: It is noted that recovery of irritation of respiratory tract upon inhalation may last several weeks or may result in permanent damage. No further information.

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.

Documentation on accidental exposure to nitric acid solutions are available (ECHA, 2011, 2017), but this does not provide sufficient details on exposure levels. However, documentation on some experimental studies is available and presented below.

Sackner and Ford (1981) presented a study in which five healthy volunteers were exposed to nitric acid fumes at a concentration of 1.6 ppm (4.2 mg/m^3) for 10 min

[1] Based on Dutch Chemiekaarten on Nitric acid, 20-65% in water. It is noted that nitric acid exist in different forms, dependent on the concentration in water, red fuming etc. It is noted that the physical-chemical characteristics such as boiling point and vapour pressure are dependent on the percentage pure HNO_3

[2] AEGL (2013): Commercial formulations of the compound contain approximately 56-68% nitric acid.

[3] it is noted that classification and labelling with Acute Tox. 1 (H330) has been advised by ECHAs Committee for Risk Assessment (RAC) for nitric acid >70%, whereas classification and labelling with Acute Tox. 3 (H331) has been advised for nitric acid ≤ 70% (https://echa.europa.eu/documents/10162/a518fd6c-fc62-b985-fa79-a4bb4e24dde7). This advice is currently not yet included in the CLP-regulation (EC) No. 1272/2008, but is published in Regulation (EC) No. 2020/1182, being the 15th ATP to the CLP-regulation. This classification and labelling shall apply from 1 March 2022.

[4] AEGL (2013); Chemiekaarten (2019)

[5] AEGL (2013) further states: Exposure to light causes the formation of nitrogen dioxide, which gives the liquid a yellow colour. Concentrated nitric acid containing dissolved nitrogen dioxide is termed fuming nitric acid, which evolves suffocating, poisonous fumes of nitrogen dioxide and nitrogen tetroxide. White fuming nitric acid contains 0.5% dissolved nitrogen dioxide while red fuming nitric acid contains 14% dissolved nitrogen dioxide.

and had no changes in pulmonary function (vital capacity, respiratory resistance, and forced expiratory volume [FEV_1]) over a one hour follow-up period.

No changes in pulmonary function, lavage constituents, or bronchial biopsy specimens were found in 10 healthy, athletic subjects exposed to nitric acid gas at 500 $\mu g/m^3$ for 4 h during moderate exercise (Aris et al., 1993).

Some anecdotal data are available. Self-exposure at 12 ppm (31.5 mg/m^3) for 1 hour result a.o. in irritation of the respiratory tract, whereas in a second study even higher concentrations could be tolerated (Diem, 1907; Lehmann and Hasegawa, 1913; as cited in AEGL).

4. Animal acute toxicity data

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

1. AEGL final TSD, ERPG document and EU RAR and reference database for nitric acid, 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 six studies were identified -with seven datasets for three species- with data on lethality following acute inhalation exposure. None of the datasets were assigned status A for deriving the human probit function, one dataset (Nitric acid 70%) was assigned status B and six were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

No studies on sensory irritation were found.

5. Probit functions from individual studies

All available acute lethality data on nitric acid are displayed in Figure 1.

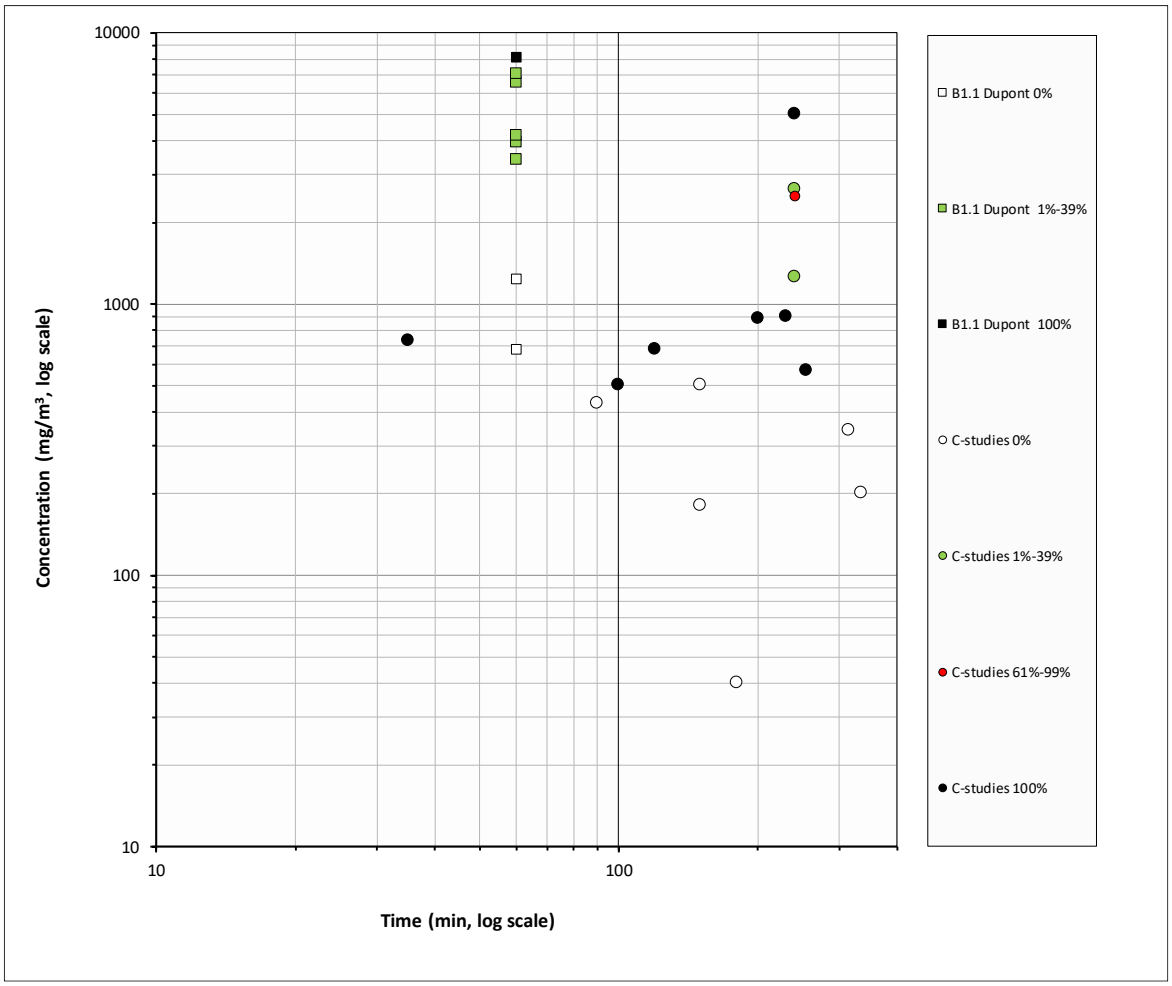

Figure 1 *All available acute lethality data for nitric acid (note: the majority of the presented datapoints of the C-studies concern n=1 animal of the study of Diem (1907)).*

The data that were selected for initial analysis of the animal probit function based on criteria outlined in the guideline, are presented in Table 1 and Figure 2.

It was possible to derive a probit function for nitric acid ≤70% based on the available study with B1 quality (with nitric acid 70% as test item). Therefore, the probit function was derived using these data. Because the data encompassed only one exposure duration, the default value for " n" had to be used to derive a concentration-time-lethality relationship.

Probit functions have been calculated and reported in Appendix 1 and the results are presented in Table 1.

Table 1 *Data selected for initial analysis of the animal probit function of nitric acid.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} at tested exposure duration (mg/m^3) 95% C.I.	n-value 95% C.I.
B1.1	rat	*60-min LC_{50}*	6682 (5696 - 8720)	N/A

The data of study B1.1 with rats are presented graphically below.

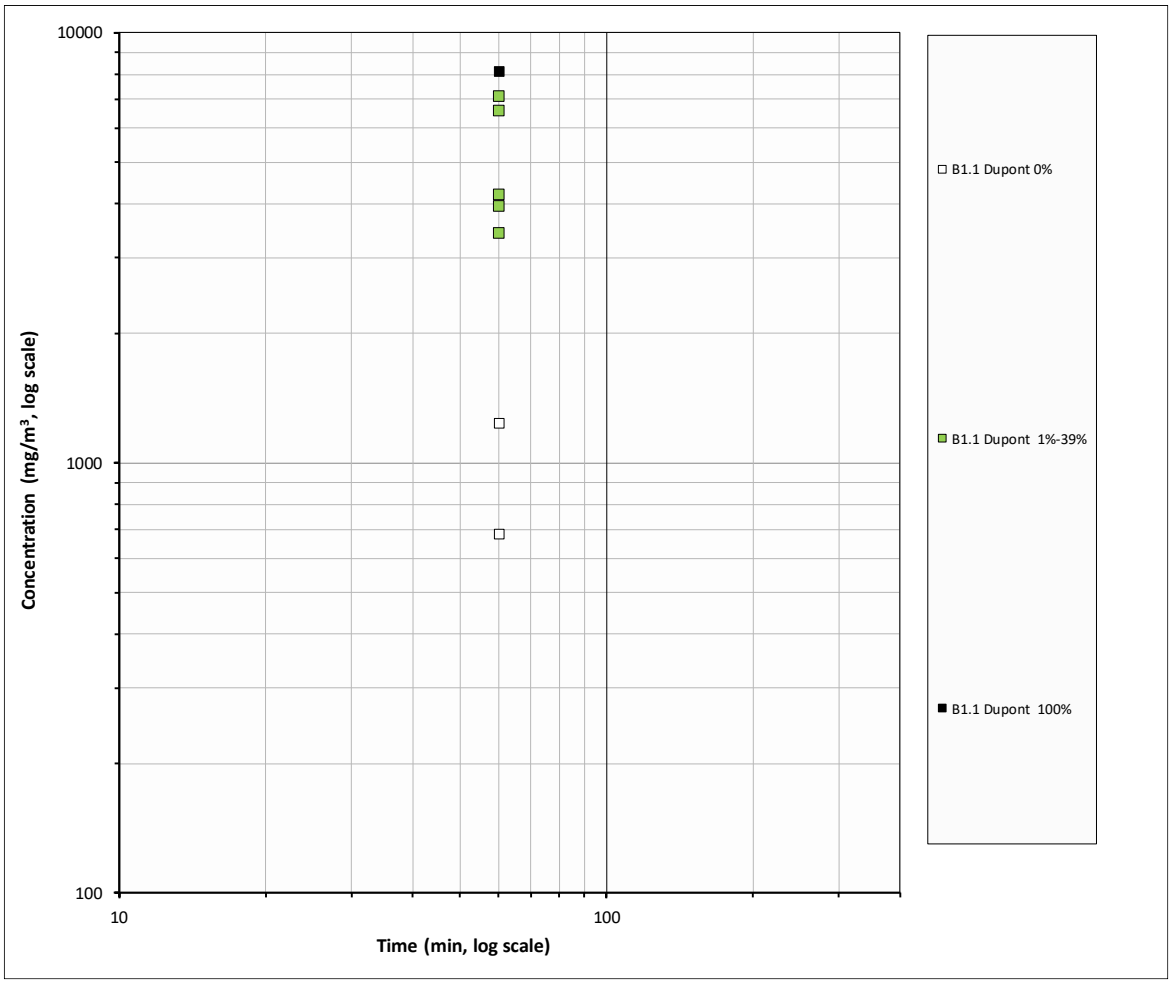

Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of nitric acid.*

6. Derivation of the human probit function

The current probit TSD is focussed on nitric acid with a concentration **≤70%**.
It is noted that a discussion has been raised within the context of the CLP-regulation (classification, labelling and packaging) concerning the acute inhalation toxicity of nitric acid.
It has been considered that there is a non-linear relationship between the nitric acid concentration and acute inhalation toxicity (ECHA 2017, 2018). At a concentration of 69.2 wt%, nitric acid forms a maximum-boiling azeotrope with water (Thiemann et al., 2005). At this concentration, the gas phase has the same composition as the liquid phase. Because of the azeotropic properties of nitric acid, it was argued that there would be a non-linear relationship between the nitric acid content in the gas phase and liquid phase. Nitric acid in a concentration below the azeotropic point at approximately 70% would be less acutely toxic by inhalation than derived by the additivity formula of CLP (ECHA, 2017).
Concentrated fuming nitric acid is characterised by the release of NOx (nitrous fumes). However, results of technical trials performed by industry suggested that diluted nitric acid up to concentrations of about 70% has a comparably low vapour pressure and does not release nitrous fumes by itself (only in contact with metals) (BASF, 2015ab; ECHA, 2020).
This discussion within the context of the CLP-regulation resulted in a distinction in classification for acute inhalation toxicity between highly concentrated nitric acid

>70% (Acute Tox. 1; H330) and diluted nitric acid ≤70% (Acute Tox. 3; H331). Substances either having a H330 or a H331 labelling are considered relevant within the context of external safety and thus relevant for derivation of human probit function.[6] Nevertheless, evaluation of the current probit TSD is limited to nitric acid ≤70%.
It is noted that the acute inhalation toxicity study used as basis for the category 3 classification for acute inhalation toxicity of the ≤70% nitric acid is the study of BASF (2015a) with 70% nitric acid as test item. This study is given the C-status in the current evaluation, due to including solely a single exposure duration - concentration combination with no probit derivation or LC_{50} derivation possible.

The evaluation of the individual animal studies resulted in one dataset being eligible for calculating the animal probit function for nitric acid ≤70% (see section 5). This study (B1.1; Dupont, 1987) used 70% nitric acid as test item.
Though the study of BASF (2015a; using also 70% nitric acid as test item and an exposure duration of 240 minutes) was given the C-status, it is noted that the results of this study are not in conflict with the results of the study of Dupont (1987) when the default n-value of 2 is used to derive a 240-min LC_{50}. This time-scaled 240-min LC_{50} would be 3341 mg/m^3, whereas the 240 min LC_{50} of the study of BASF (2015a) is >2650 mg/m^3.

To derive the human probit function the results from Dupont (1987; study B1.1) have been used to derive a point of departure as outlined above.

First, the default n-value of 2 was selected as no experimentally derived value for n was available.
Second, the LC_{50}-value of B1.1 was calculated to be 6682 mg/m^3 for 60 minutes.

The Point of Departure for the human probit function is a 60-minute animal LC_{50} value of 6682 mg/m^3 and the default n-value of 2.

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

Table 3 *Rationale for the applied assessment factors.*

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3	Default
Nominal concentration	1	The concentrations were measured analytically in the B1.1 study.
Adequacy of database:	2	Only one B1 study available (70% nitric acid test item). The study was performed using only one exposure duration. One C-study available (70% nitric acid test item) that does seem to support the B1 study.

The estimated human equivalent 60-minute LC_{50} value is 6682 / 6 = **1114 mg/m^3**.

No reliable 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**.

[6] https://www.rivm.nl/documenten/selectiemethodiek-toxische-en-ontvlambare-stoffen

The human probit function is then calculated on the human equivalent 60 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1 \times \ln(1114^2 \times 60)$ resulting in the a-value of **-13.1**.

$Pr = -13.1 + 1 \times \ln(C^2 \times t)$ with C in mg/m³ and t in min.

The derived human probit function has a scientifically acceptable basis. The probit function is based on 1 study in the rat with B1 quality, with 5 animals per sex per experimental group, one exposure duration (60 minute) and eight exposure concentrations included and response rates between 0 and 100%.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 235 mg/m³ and the calculated human 60 min LC_1 (Pr = 2.67) is 343 mg/m³.

Table 4 *LC-values calculated with the derived probit function compared with existing acute inhalation exposure guidelines.*

Estimated level	30 min (mg/m³)	60 min (mg/m³)
0.1% lethality, this probit	332	235
1% lethality, this probit	485	343
AEGL-3[7] (2013, final)	315	241
ERPG-3[7] (2010)	-	197
LBW[8] (2021)	300	240

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

[7] AEGL and ERPG values were converted from ppm to mg/m³ with the conversion factor calculated in section 1. Therefore, the AEGL and ERPG values in mg/m³ can deviate slightly from those reported in the AEGL and ERPG TSDs.
[8] The Dutch Intervention Values are applicable to 70% nitric acid

Appendix 1 Animal experimental research

Study ID: B1.1

Author, year: ***Dupont, 1987***
Substance: Nitric acid 70%
Species, strain, sex: Rat, Crl:CD®BR, male and female
Number/sex/conc. group: 5
Age and weight: males: 8 weeks old, 233-285 g; females: 9-11 weeks old, 188-258 g
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 statement of compliance with OECD guideline 403 provided*
Stability of test compound in test atmosphere	*The test atmospheres were generated without heat to prevent oxidation or reduction reactions from occurring in the generation system. To verify stability, the atmospheric concentration of nitrogen dioxide was monitored during each exposure at the same time that the athmospheric concentration of nitric acid was measured. Under these conditions, the test material was assumed to be stable throughout the exposure phase of the test.** *Glass and Teflon® equipment was used whenever possible due to the corrosive nature of the test material.*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Nose-only*
Type of restrainer	*Perforated, stainless steel cylinder with a conical nose piece.*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*In general, atmospheres of nitric acid were generated by aerosolizing the aqueous nitric acid solution with various types of nebulization equipment.** *Airborne test material was dispersed with a baffle as it entered the 38 L cylindrical glass exposure chamber.*
Number of air changes per hour	*Flow rate was 20-28 L/min for highest exposure group. For the lower concentrations air flow was approximately 26 L/min.* *38 L glass exposure chamber*
Equilibration time (t95)	*t95 varied between 4.1 and 5.7 min*
Start of exposure relative to equilibration	*Not specified*

Actual concentration measurement	*Atmospheric concentration of nitric acid was determined, from the animals' breathing zone at 20 minute intervals during exposure, by quantitating the nitrate ion concentrations in aqueous solutions collected from the test atmosphere. The nitrate ion concentration in solution was assumed to be directly proportional (1:1) to the nitric acid concentration in the test atmosphere. In addition, the nitrite ion concentrations in the same solutions were monitored. The solutions were analysed with an ion chromatograph. With this equipment, the nitrate and nitrite ions are separated on a strong base anion exchange resin, converted back to the acid form on a suppressor column, and quantitated with a conductivity detector.* *During most exposures, at least 1 gravimetric filter sample was collected to monitor the amount of nitric acid present as an aerosol.* *For those exposures for which an appreciable aerosol component was determined either by gravimetric sampling or visual observation, particle size (MMAD en percent respirable) was determined with a cascade impactor.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*See table below as copied from Dupont study report ***:* *Aerosol content was assumed (not analytically verified) to be 100% at the three highest concentrations and ranged from 15-73% at the lower concentrations (as measured on a gravimetric filter sample).* *<3407 mg/m^3: particle size not measured* *≥3407 mg/m^3: 59-74% respirable, 2.0-6.6 µm MMAD* *See also BASF expert statement below***
Assessment of Reliability	***B1*** *Well-performed study, limited to one exposure duration.*

* The study report states the following: "*No nitrite ion was detected in the water samples collected from most exposures, indicating that nitric acid was stable under the generation conditions used. However, during exposure to 260 and 1300 ppm* [681 and 3407 mg/m^3]*, an unknown ion, believed to be nitrite ion was detected by liquid chromatography. These exposures correspond chronologically to the 4th and 5th exposures conducted; samples from these exposures were analysed by drawing the*

test atmosphere through glass fiber filters inserted in front of the impingers. Because of the differences observed between these exposure and the other 6 exposures, we believe the presence of nitrite ion was an artefact of the sampling method used (possibly due to the reduction of nitrate ion by trace metals known to be present in the glass fiber filters). Furthermore, the amount of nitrite ion observed during the 1300 ppm exposure was below the limit of detection (approximately 3 mg/L in solution), while the concentration observed in the 260 ppm exposure was approximately 14% of the total detected test material."

** For the 5 lowest exposure concentrations tested, the test atmospheres were generated by pumping liquid nitric acid with a Harvard Model 975 compact infusion pump into a Spraying Systems nebulizer. Air introduced at the nebulizer (approximately 26 L/min) aerosolized and evaporated the liquid, and swept the aerosol/vapour mixture through a 1-liter glass cyclonic elutriator. The cyclone removed large aerosol particles by inertial impaction, while it also provided additional residence time for evaporation. For the next 2 highest exposure concentrations (2500 and 2700 ppm [6553 and 7077 mg/m^3]), the cyclonic elutriator was removed in an attempt to achieve higher concentrations. For the highest concentration tested, the test atmosphere was generated using both a Devilbiss and a Thomas-John nebulizer operating simultaneously. With both of these nebulizers, an aerosol was produced by drawing liquid nitric acid from a reservoir and aerosolizing it with a high pressure air stream (~20-28 L/min for each nebulizer). The reservoirs were replenished regularly by pumping liquid nitric acid into the systems within the infusion pump.

*** Table copied from Dupont (1987)

Aerosol content and particle size				
Nitric acid concentration #		**Percent aerosol[a]**	**Particle size**	
ppm	**mg/m^3**		**% respirable[b]**	**MMAD[c]**
260	681	15%	NM	NM
470	1232	NM	NM	NM
1300	3407	73%	70%	4.0 µm
1500	3932	13%	72%	3.2 µm
1600	4194	32%	71%	3.3 µm
2500	6553	NM	59%	6.5 µm
2700	7077	NM	61%	6.6 µm
3100	8125	NM	74%	2.0 µm

NM = not measured

[a] percent of test material present as an aerosol as determined by gravimetric analysis of filter samples and liquid chromatographic analysis of impinger samples; assumed to be approximately 100% for the 3 highest exposure concentrations.

[b] percent by weight of particles with aerodynamic diameter less than 10 µm (considered respirable by Dupont).

[c] mass median aerodynamic diameter.

the test atmosphere concentrations were presented by Dupont (1987) in ppm as (rounded) mean values of multiple measurements (i.e. 20-minute intervals). It is assumed that Dupont converted the measured values (mg/m^3) to values expressed as ppm (conversion factor not specified by Dupont). The TSD author converted these ppm-values to mg/m^3, using the conversion factor as presented in section 1 of the current TSD.

The Probit Expert Panel noted that the above presented MMADs were highly variable and not in line with the general observation that the higher the concentration, the larger the particle size (viz. at the highest concentration, the smallest MMAD was observed). At the concentrations of 2500 and 2700 ppm (i.e. 6553 and 7077 mg/m^3), the MMAD was 6.5 and 6.6 µm, respectively, which exceeded the OECD TG 403 testing guideline value of 1-4 µm. However, based on the test results of BASF

(2015b) as presented below (see ****), the test atmospheres were assumed to consist mainly of vapour with a minor aerosol fraction.

**** Within the CLH-framework, a discussion was raised concerning the DuPont (1987) study based on certain analytical limitations as it was considered that the study did not contain sufficient evidence whether the rats were exposed to a test atmosphere mainly consisting of a vapour or an aerosol[9]. Therefore, BASF (2013) performed a technical trial involving generation of a HNO_3 atmosphere under the conditions of the DuPont study to more clearly elucidate the acute toxicity potential of 70% and lower concentrations of nitric acid. BASF (2013) concluded at that time that "*results showed that aerosol was the main fraction of the atmosphere (with only very low amounts of NO_2) which must be considered for classification*".

However, in 2015 an amendment was provided by BASF (2015b) and their previous conclusion was revised as follows:
The results of new technical pre-tests conducted as preparation for the in vivo acute inhalation study with nitric acid 70% (BASF, 2015, project no. 13I0234/13I464) strongly indicate that the vapour fraction in the nitric acid atmospheres was underestimated in the previously performed technical trial (BASF, 2013, project no. 12I0234/13I023). Comparison of concentrations measured with or without quartz wool plug showed that this aerosol blocker did not only trap aerosols but also vapour, suggesting the large cyclone filter unit used as aerosol blocker in the technical trial did the same. As the atmosphere fraction determined after the aerosol filter was considered vapour, the low concentration after the filter was misinterpreted as a very low vapour fraction (and thus high aerosol fraction) of nitric acid in the atmosphere. The quartz wool plug, which was used to determine the aerosol fraction in the new experiment captured a large fraction of aerosol, though not quantitatively. Measurement by an aerosol spectrometer showed significantly lower particle counts than without quartz wool plug, which were still higher than in the control air. Moreover, due to vacuum applied while sampling, trapped aerosol may have been evaporated. Due to these uncertainties the aerosol fraction in the new experiments may be slightly underestimated. However, as the content of nitric acid of both the quartz wool plug and in tandem impingers were determined quantitatively, the determined total atmospheric concentration of nitric acid is considered reliable. The majority of the determined nitric acid was in the absorption solvent of the tandem impingers and therefore considered vapour.
Based on the data of the new technical pre-tests, BASF concluded – in contrast to the technical trial from 2013 – that the main fraction of an atmosphere generated from 70% nitric acid by nebulization consisted of vapour and that aerosol is a small fraction only.

Upon evaluation, the Probit Expert Panel agrees with these considerations in the amended expert statement of BASF (2015b). It is expected that upon nebulization the nitric acid droplets will evaporate rather quickly (probably almost instantly), unless reactions occur with, for example, parts of the technical equipment. Nitric acid is a very reactive chemical and these reactions may also occur in cyclones, quartz wool plugs or in a humid atmosphere. Therefore, tandem impingers can reliably measure nitric acid concentrations.

[9] https://echa.europa.eu/documents/10162/2a9d7f75-dd2a-6af8-765f-6d7bd8e3706a
Note that within the CLH-framework, different classification criteria (i.e. concentration borders) for acute inhalation toxicity are applied for a liquid aerosol vs. vapour.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
rat	681	N/A	60	0/5	0/5
rat	1232	N/A	60	0/5	0/5
rat	3407	N/A	60	1/5	0/5
rat	3932	N/A	60	1/5	0/5
rat	4194	N/A	60	2/5	0/5
rat	6553	N/A	60	2/5	1/5
rat	7077	N/A	60	2/5	1/5
rat	8125	N/A	60	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

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

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

Probit function	Species	a	b	d	n-value
Sex as variable	Rat	-17.6	2.52	0.82	N/A
Sexes combined	Rat	-15.1	2.28	-	N/A

Duration (min.)	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
60	5706 (4573 - 7337)	7897 (6304 - 11560)	6682 (5696 - 8720)

The study authors of Dupont (1987) calculated 60-min LC_{50} values of 5766 mg/m^3 (95%-C.I.: 4194 – 11010 mg/m^3) for males, 7339 mg/m^3 (95%-C.I.: 6553 – 8125 mg/m^3) for females, and 6553 mg/m^3 (95%-C.I.: 5766 – 8649 mg/m^3) for sexes combined.

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.

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

Study ID: C.1

Author, year: ***BASF, 2015a***
Substance: Nitric acid 70%
Species, strain, sex: Rat / Wistar / RccHan:WIST, male and female
Number/sex/conc. group: 5
Age and weight: males: 8 weeks old, 230.0-236.8 g; females: 10 weeks old, 204.0-216.8 g
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)	*In this study report it is stated that the study is carried out according OECD 403. However, it is noted that this study only includes a single exposure duration-concentration combination.*
Stability of test compound in test atmosphere	*Aerosol formation (0.8%)*
Use of vehicle (other than air)	*N/A*
Whole body / nose-only (incl. head/nose-only) exposure	*Nose-only*
Type of restrainer	*Nose-only inhalation system INA 20 (glass-steel construction, BASF SE, volume V 55 L): the animals were restrained in glass tubes and their snouts projected into the inhalation system.*
Pressure distribution	*Positive pressure inside the exposure systems.*
Homogeneity of test atmosphere in breathing zone of animals	*Various modifications of vapour generation procedures were tested in a technical trial prior to the main study (i.e. vapour generator, bubbling air through the test substance, nebulizing the test substance)** *For the main experiment the vapour was produced by continuously pumping amounts of the test substance to a two-component atomizer. Using compressed air, the aerosol was produced first with the atomizer inside the aerosol-mixing vessel. Due to the high surface area of the aerosol droplet, the test substance evaporated immediately within the glass vessel and was passed into the exposure system.*
Number of air changes per hour	*55 L volume, 1.5 m^3/h air flow resulting in 27 air changes per hour*
Equilibration time (t95)	*0.11 h ~ 6.6 min*
Start of exposure relative to equilibration	*The animals were exposed to the inhalation atmosphere for 4 hours plus equilibration time of the inhalation systems (t99 about 10 min).*

Actual concentration measurement	*For the quantitative determination of the aerosol and vapour concentration, an ion chromatographic method was used. Vapour and aerosol were analysed separately. 4 samples at about hourly intervals, sampled immediately adjacent to the animals' noses at a separate spare port. A sampling probe (glass, 7 mm diameter) containing a quartz wool plug was used to trap the aerosol fraction. This was connected with 4 impingers in series which were filled with aqueous solution of 0.5 M NaOH as absorption solvent to collect the vapour fraction.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Particle size distribution was determined by an aerodynamic particle sizer. Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were obtained directly by APS. During exposure, 3 measurements with 3 repeats each were performed (sample volume: 5 L).* *Majority of the test atmosphere was nitric acid vapour. Only 0.8 % of the total concentration was liquid aerosol. Particle size measurement showed mass median aerodynamic diameters between 1.66 and 2.33 µm with a GSD 2.35-3.13.*
Assessment of Reliability	***C*** *Well-performed study. Limited to one exposure duration. Only a single exposure duration - concentration combination included; no probit derivation or LC_{50} derivation possible.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
rat	2650	N/A	240	1/5	0/5

***Technical trial (BASF 2015a – appendix 9)**

Preceding the in-life phase of the acute inhalation toxicity study, BASF 2015a performed a technical trial to establish the condition for generating an atmosphere containing (target) 2 mg/L nitric acid using the 70% nitric acid solution as test item. Moreover, the atmosphere was characterized regarding vapour and aerosol fraction. During the current technical trial, mainly three technical setups were tested:

- Vaporizer
- Air bubbling through test substance column
- Nebulization with or without aerosol blocker

In this technical trial, nitrite ion concentration in different samples was below the detection limit of the method (0.5 mg/L solvent). Nitric acid concentration was calculated based on measured concentration of nitrate ion.
In a vaporizer the test substance did not evaporate up to 30°C. As discoloration, indicating heat-induced dissociation of the test compound, occurred shortly after the start of vaporization, this method was not evaluated in detail.
Bubbling air through the substance produced a mixture of vapour and aerosol atmosphere. Initially, a high concentration of 10 mg/L was measured, which is likely due to highly volatile fraction in the test substance. In the subsequent examination, the measured concentrations ranged from 5 to 7 mg/L. This concentration range indicated the vapour saturation concentration under the given test condition. Aerosol fraction was between 2 and 5% under the current condition. This fraction is likely underestimated, because negative pressure during the sampling procedure, trapped aerosol droplets may have evaporated.
Nebulizing the test substance directly in the exposure chamber was an effective way to generate vapour. An aerosol fraction of about 1% was detected at a dosing rate between 2.3 and 7 mL/h. The vapour generation efficiency ranged from 39 to 47%. Increase of the dosing rate to 13 mL/h increased the aerosol fraction to 11%, while the vapour generation efficiency decreased to 31%. Using an aerosol mixing stage and quartz wool plugs before entrance into the exposure chamber reduced the generation efficiency and increased the aerosol fraction. In a further step, the quartz wool plug was removed. The atmospheric concentration was comparable to that in the direct nebulizing system. As the aerosol fraction was slightly lower in the system with aerosol mixing stage than in the direct nebulizing system, the in vivo study was performed with a nebulizer within the aerosol mixing stage. A quartz wool plug installed before entrance into the exposure chamber was not used as it seemed to promote the formation of condensation aerosols.

Clinical data
One in five male animals and none of the five female animals died. It is noted that a large number of severe clinical signs were noted in the remaining nine rats. Within the framework of the CLP-regulation (ECHA, 2018), a discussion was raised whether some of the severe clinical signs (for example, loss of nose tips) should be considered as obvious signs of severe pain and enduring distress and suffering, providing clear evidence of acute inhalation toxicity. For example, OECD TG 403 (paragraph 8) states that "*moribound animals or animals obviously in pain or showing signs of severe and enduring distress should be killed and are considered in the interpretation of the test result in the same way as animals that died on the test*".

Upon contact with the study director of this study, it was stated that the loss of nose tips was considered a local effect, i.e. encrusted red nose, red nasal discharge, and, in some cases, swelling of the nose tissue damage, were observed. This subsequently resulted in formation of a crust which fell off at the end of the observation period, revealing young healthy skin underneath.
See ECHA (2018) for a more detailed response of the study director related to this issue.

Therefore, it seems likely (in line with the conclusions made within the framework of the CLP-regulation) that most animals suffered from the exposure, but according to the additional information of the study director, suffering was concluded not to be sufficiently severe to warrant pre-term humane killing of the animals. The reported mortality data are therefore considered reliable.

Probit function
The study included a single exposure duration – concentration combination.
A probit function or LC_{50} cannot be derived.
The 4h-LC_{50} is estimated to be >2650 mg/m^3.

Study ID: other C studies

Nitric acid ≤70%

Diem (1907) exposed single cats and rabbits to various exposure time combinations (whole body). Exposure to for example 880 mg/m^3 for 200 min, 731 mg/m^3 for 35 min, 500 mg/m^3 for 100 min, 680 mg/m^3 for 120 min resulted in death of the exposed animals (dead animals refer to single cats (n=1) per exposure-time combination). Exposure to 40 mg/m^3 for 180 min, 180 mg/m^3 for 150 min, 200 mg/m^3 for 335 min, 340 mg/m^3 for 315 min, 430 mg/m^3 for 90 min, 500 mg/m^3 for 150 min did not result in death of the exposed animals (refer mostly to single cats (n=1) per exposure-time combination). The author noted that the test atmosphere generation in the chamber resulted in visible differences in concentration within the exposure chamber at several locations. One cat introduced into the chamber was already indicated as 'weak'. Observation periods differ dependent on the visible state of the animals. So animals were re-introduced for a next experiment.

The REACH registration dossier on nitric acid present an additional acute inhalation study (2012) in rats with 26.1% nitric acid as test item (ECHA, 2020). Female albino Holtzman strain rats (10/group) were exposed for four hours by inhalation (not specified whether nose-only or whole body inhalation was applied) to target concentrations of 1.25, 2.5 and 5.0 ×10^3 mg/m^3. A 14-day post-exposure observation period was applied. According to the registration dossier, no analytical verification of the test atmosphere concentration was done. The method of vapour generation was not specified. Also, it is not clear what the vapour/aerosol fractions of the test atmosphere were.
The table below presents the lethality ratios. The study authors calculated a 4h-LC_{50} of 1.7 ×10^3 mg/m^3.

Concentration mg/L (4h)	No. of rats	Animal death		
		1st week	2nd week	Total
0.0 (control)	5	0	0	0
1.25	10	3	0	3
2.5	10	7	0	7
5.0	10	10	0	10

Lehmann and Hasegawa (1913; as cited in AEGL) conducted a series of experiments using cats (n=1 per conc-time combination) exposed to nitric acid gases. According to AEGL, the generated atmospheres were likely a mixture of nitrogen oxides.
In general, as concentration or duration of exposure to nitric acid increased, death resulted from severe pulmonary edema. At concentrations less than about 388 ppm (1,017 mg/m^3), examination of the concentration and time relationship indicated that Ct products greater than about 900 ppm-h (2359 mg/m^3-h) resulted in death whereas Ct products up to 760 ppm-h (1992 mg/m^3 -h) resulted in only a slight increase in respiration for several hours after exposure. Further, exposure at 287 ppm (752 mg/m^3) for 1.83 h (Ct = 526 ppm-h; 1379 mg/m^3-h) caused no effects, whereas exposure at either 341 ppm (894 mg/m^3) for 3.83 h (Ct = 1,309 ppm-h; 3431 mg/m^3-h) or 217 ppm (569 mg/m^3) for 4.25 h (Ct = 922 ppm-h; 2417 mg/m^3-h) resulted in death. In contrast, at concentrations of 388 ppm (1,017 mg/m^3) or greater, severe clinical signs or death occurred at a Ct product as low as 277 ppm-h (726 mg/m^3-h). Response probably depended on whether either the concentration of the acid or the duration of exposure was great enough to induce corrosive effects leading to edema. Descriptions of this study lack information on the test durations and observation periods, and there is uncertainty if the test concentrations are a mixture of nitrogen oxides and nitric acid rather than nitric acid.

Nitric acid >70%

Gray et al. (1954) compared the toxicities of nitrogen dioxide (98.0% purity), red fuming nitric acid (RFNA) (containing 8-17% nitrogen dioxide), and white fuming nitric acid (WFNA) (containing 0.1-0.4% nitrogen dioxide) by whole body inhalation in albino rats. A 30-minute exposure period with a 3-day post-exposure observation period was applied. For the nitrogen dioxide and RFNA exposure, a 400 L exposure chamber was used. This exposure chamber could however not be used for the WFNA exposures as it was difficult to maintain the desired vapour concentration in this 400 L chamber. This difficulty, due to the low concentration of nitrogen dioxide of the WFNA, made it necessary to use a small glass 20 L chamber. A specification of the applied test concentrations was not presented.

Outcomes related to exposure to RFNA (containing 8-17% nitrogen dioxide) and nitrogen dioxide are reported here to provide a complete description of the study; however, the chemicals are not directly relevant to nitric acid fumes. Exposure concentrations for RFNA and WFNA were measured and reported as nitrogen dioxide. Thirty-minute LC_{50} values were reported to be 174 ppm for nitrogen dioxide, 138 ppm for RFNA as nitrogen dioxide, and 244 ppm for WFNA as nitrogen dioxide. A four hour LC_{50} value of 67 ppm was reported for RFNA as nitrogen dioxide. Deaths were from pulmonary edema.

The dose-response curves for nitrogen dioxide and RFNA for 30-min exposures were parallel statistically, indicating a possible similar mode of action for the two gases. But the curves were somewhat different at lower concentrations for an exposure duration of 240 min. For WFNA, the investigators reported that deaths were not as "predictable" as with the other gases. The approximate 30-min LC_{50} indicates that WFNA is much less toxic (has a higher LC_{50}) than either RFNA or nitrogen dioxide. Therefore, the investigators concluded that the main toxic component of these oxides of nitrogen is nitrogen dioxide.

Based on the results of this study, NIOSH (1976) calculated 30-min LC_{50}s for RFNA and WFNA of 310 ppm and 334 ppm, respectively, on the basis of total nitric acid concentration (*corresponding to 812.51 mg/m^3 and 875 mg/m^3 nitric acid using the conversion factors of page 2 of this document*). The calculations were based on molecular weights and the percentage of nitrogen dioxide in RFNA and WFNA. NIOSH (1976) suggested the possibility of a synergistic toxic effect between nitric acid vapour and nitrogen dioxide since RFNA has a higher nitrogen dioxide content by weight. NIOSH (1976) further stated that this is supported by the results that showed the 30-min LC_{50} for RFNA was at a lower concentration than for nitrogen dioxide when the concentration of each as expressed in ppm nitrogen dioxide.

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