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

Probit function technical support document

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substance name	CAS number
Arsine	**7784-42-1**

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.

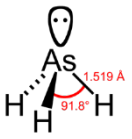
This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have 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 Arsine

1. Substance identification



CAS-number:	7784-42-1
IUPAC name:	arsine
Synonyms:	arsenic hydride, hydrogen arsenide
Molecular formula:	As-H_3
Molecular weight:	77.9 g/mol
Physical state:	gas (at 20°C and 101.3 kPa)
Boiling point:	-62°C (at 101.3 kPa)
Vapour pressure:	1030 kPa (at 20°C)
Saturated vapor conc:	N/A, gas (at 20°C)
Conversion factor:	1 mg/m^3 = 0.309 ppm (at 20°C and 101.3 kPa)
	1 ppm = 3.240 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	Human H330, 373

2. Mechanism of action and toxicological effects following acute exposure

Acute effects: Arsine is a potent haemolytic agent. The main target organs and tissues for inhalation exposure to arsine are erythrocytes and the kidney. Arsine causes intravascular hemolysis, which is usually delayed for several hours or several days. Arsine has poor warning properties, and many case reports are available where humans suffered severe delayed health effects without sensory warning of exposure. Symptoms of high exposure are delayed and include malaise, headache, abdominal pain, chills, nausea, vomiting, oliguria/anuria, hematuria, and bronze skin colour. Lethality results from acute oliguric renal failure.
Long-term effects: There are no definitive data on the effects of chronic (low level) arsine exposure. Most likely, chronic exposure would result in some hemolysis. Some forms of inorganic arsenic are considered to be known human carcinogens; there are no data regarding the carcinogenic potential of arsine.

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. AEGL final TSD, ERPG document and EU RAR and reference database for arsine, 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 5 studies were identified -with 11 datasets for 6 species- with data on lethality following acute inhalation exposure. One dataset was assigned status A for deriving the human probit function, 2 datasets were assigned status B and 8 were assessed to be unfit (status C) for human probit function derivation.

Sensory irritation

No studies were on sensory irritation were found.

5. Probit functions from individual studies

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

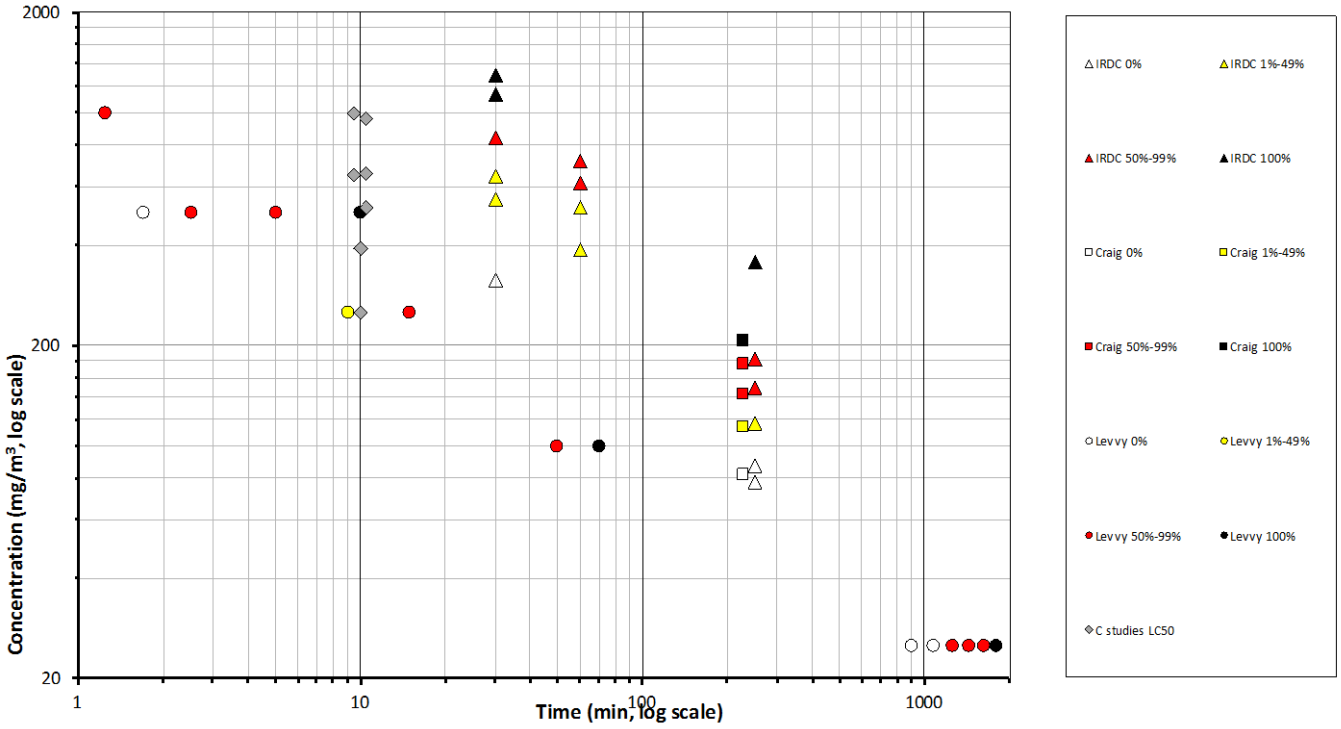


Figure 1 *All available acute lethality data for arsine.*

The data that were selected for primary analysis of the animal probit function are presented in Table 2 and Figure 3.

Both A and B1 studies were selected for derivation of the animal probit function for arsine.

To enable intra-species pooling, LC_{50}-values of study B1.1 was scaled using the rat n-value of 1.24 for arsine with the following formula (section 6):

$$LC_{50,c} = LC_{50,test}\left(\frac{t_{test}}{t_c}\right)^{(1/n)}$$

With $LC_{50,c}$ = scaled LC_{50} value for common exposure duration t_c
$LC_{50,test}$ = observed LC_{50} value for tested exposure duration
t_c = common exposure duration for intra-species pooling
t_{test} = tested exposure duration
n = species specific (for [species]) / overall / default n-value

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 1 *Data selected for initial analysis of the animal probit function of arsine.*

Study ID	Species	Probit (C in mg/m^3, t in min)	LC_{50} at tested exposure duration (mg/m^3) 95% C.I.	LC_{50}, 30 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
A.1	Rat	-24.3 + 3.09×lnC + 2.49×lnt		857 (758 - 958)	1.24 (1.09-1.39)
B1.1	Rat	*240-min LC_{50}*	138 (122 - 152)	*739*	N/A
B2.1	Mouse	-10.4 + 2.21×lnC + 1.29×lnt		148 (81.2 - 197)	1.71 (1.44–1.99)

The data from study B2.1 were excluded from the further analyses because an A-study was available. Further analyses will be based on the data from studies A.1 and B1.1; the data of these studies with rats are presented graphically below.

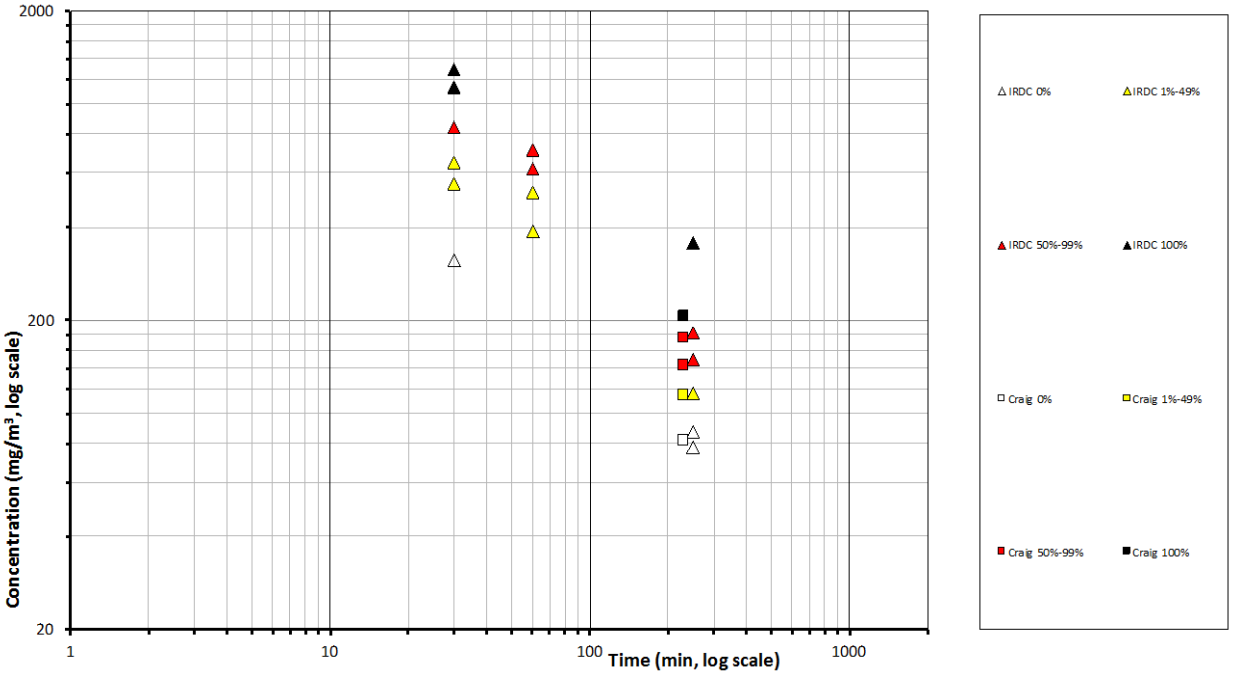


Figure 2 *Data selected for the initial analysis of the animal probit function of arsine.*

Based on visual inspection and the criteria outlined in the guideline, the data from studies IRDC A.1 and Craig B1.1 were selected for the final dataset for the derivation of the animal probit function. Both studies are well conducted and show consistent results. The 240-minute data were believed to be eligible, despite an exposure duration longer than a factor 2 outside the target range of 30-60 minutes, because the exposure range in study A.1 also extended to 240 minutes.

The final data eligible for calculating the animal probit function contains 2 datasets from 2 studies and includes data from 1 animal species.

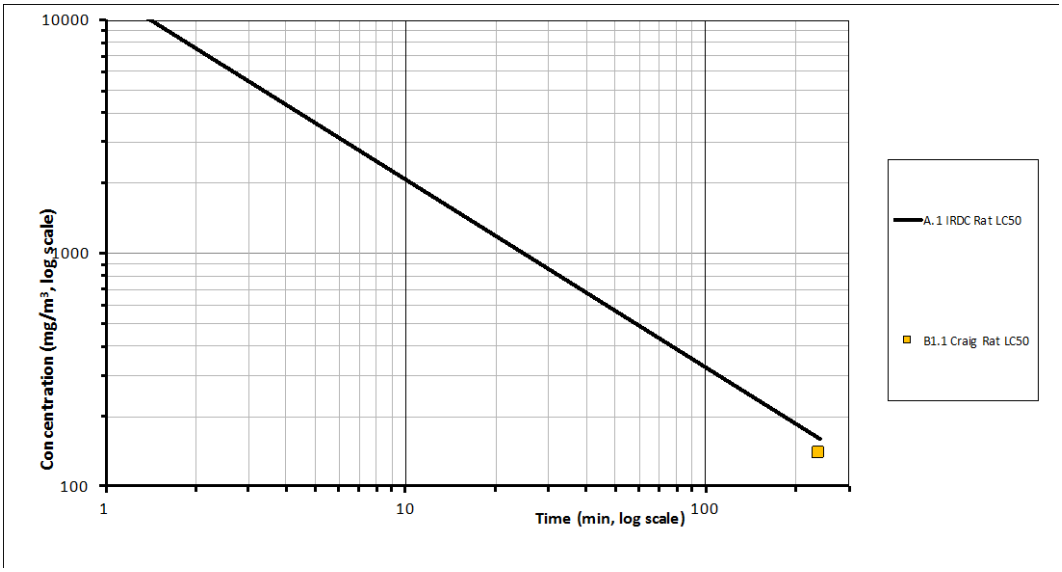


Figure 3 *LC_{50} values of A and B1 datasets for arsine, over time where available.*

Table 2 *Data selected for the derivation of the animal probit function of arsine.*

Study ID	Species	Probit (C in mg/m³, t in min)	LC_{50} at tested exposure duration (mg/m³) 95% C.I.	LC_{50}, 30 minutes (mg/m³) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
A.1	Rat	-24.3 + 3.09×lnC + 2.49×lnt		857 (758 - 958)	1.24 (1.09-1.39)
B1.1	Rat	*240-min LC_{50}*	138 (122 - 152)	*739*	N/A

The data of the selected datasets are presented graphically below.

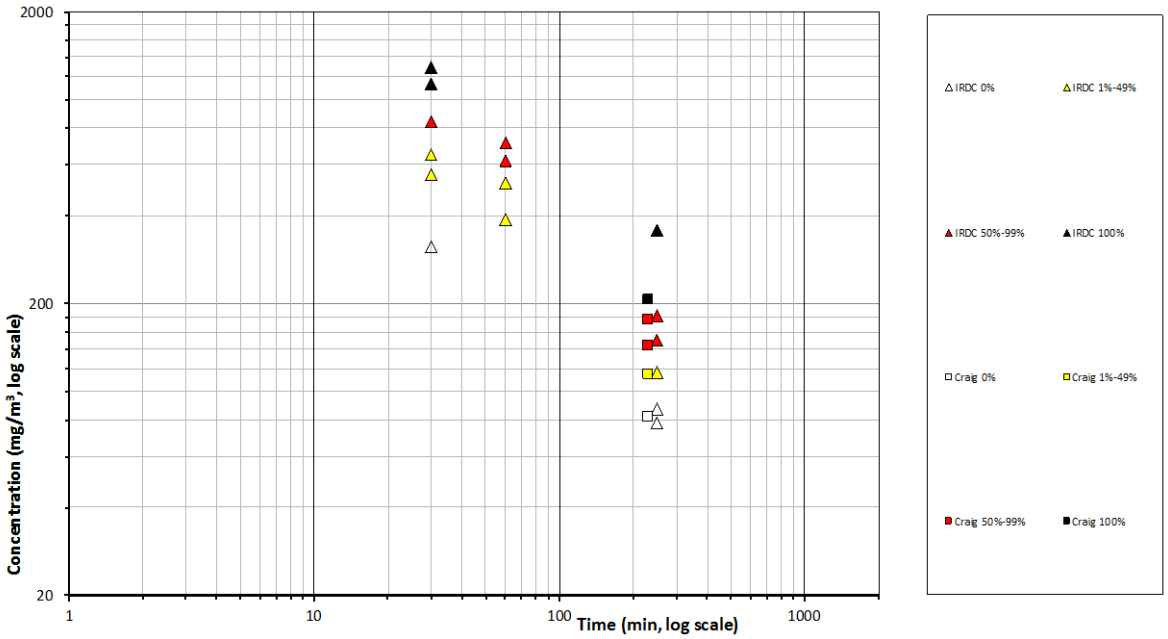


Figure 4 *Final data selected for derivation of the animal probit function of arsine (identical to figure 2).*

6. Derivation of the human probit function

To derive the human probit function the results from IRDC (A.1) and Craig (B1.1) have been used to derive a point of departure. These were the only qualifying studies.

The n-value from the IRDC rat study (A.1) of 1.239 was used as an n-value.

Second, the LC_{50}-values of all applicable A- and B1-studies were calculated for a common exposure duration of 30 minutes. To enable this intra-species pooling, LC_{50}-values of B1-studies were scaled using the rat n-value of 1.239 with the following formula:

$$LC_{50,c} = LC_{50,test}\left(\frac{t_{test}}{t_c}\right)^{(1/n)}$$

With $LC_{50,c}$ = scaled LC_{50} value for common exposure duration t_c
$LC_{50,test}$ = observed LC_{50} value for tested exposure duration
t_c = common exposure duration for intra-species pooling
t_{test} = tested exposure duration
n = species specific (for [species]) / overall / default n-value

Finally, the species-specific geometric mean LC_{50}-value was calculated from the (time-scaled) LC_{50} values of studies A.1 and B1.1. The average rat LC_{50}-value was 795.7 for the rat. The overall formula for the geometric mean of time-scaled LC_{50}-values is as follows:

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

With $\overline{LC_{50}}$ = geometric mean LC_{50}-value across species
$LC_{50,i}$ = LC_{50}-value of study i.
m = number of observations on LC_{50}-values within a species (i=1…m).
s = number of species for which LC_{50}-values are pooled (j= 1…s).

The Point of Departure for the human probit function is a 30-minute geometric mean animal LC_{50} value of 795.7 mg/m^3 and a rat n-value of 1.239.

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	No rationale to deviate from the default value.
Nominal concentration	1	Well conducted studies, no assessment factor required.
Adequacy of database:	1	Well conducted A- and B1-studies, supported by a B2 study.

The estimated human equivalent 240-minute LC_{50} value is 795.7 / 3 = **265.2 mg/m^3**.

The experimentally determined n-value was **1.239** (IRDC, study A.1). Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = **1.614**.

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): $5 = a + 1.614 \times \ln (265.2^{1.239} \times 30)$ resulting in the a-value of **–11.652**.

$Pr = -11.7 + 1.61 \times \ln (C^{1.24} \times t)$ with C in mg/m^3 and t in min.

The derived human probit function has a scientifically sound basis. The probit function is based on 2 studies in the rat with A and B1 quality, where a total of 410 rats were exposed to concentrations ranging from 80-1300 mg/m^3 and durations ranging from 30-240 minutes.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 49 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 34 mg/m^3.

Table 4 *LC-vales 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	59	34
1% lethality, this probit	86	49
AEGL-3 (2000, final)	2.0	1.6
ERPG-3 (2009)		4.9
LBW (2015)	8.7	4.9

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are higher. The AEGL and ERPG values are based on a non-lethal level in a limited mouse study (Peterson and Bhattacharyya 1985) described as a C-study.

Appendix 1 Animal experimental research

Study ID: A.1

Author, year: ***IRDC 1985***
Substance: Arsine
Species, strain, sex: Sprague-Dawley rat, male and female
Number/sex/conc. group: 10 / sex / group
Age and weight: age 47-64 days, weight 162-274 grams
Observation period: 14 days post exposure

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*GLP statement provided*
Study carried out according to OECD 403 guideline(s)	*No statement of compliance with OECD guideline 403 provided. Most criteria appear to be met.*
Stability of test compound in test atmosphere	*Stable*
Use of vehicle (other than air)	*Air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*NA*
Pressure distribution	*Not specified.*
Homogeneity of test atmosphere in breathing zone of animals	*A mixture of 2.1% arsine (99.995% purity) in nitrogen was metered into the chamber.*
Number of air changes per hour	*Flow of 168-257 in ℓ/min through a 160ℓ exposure chamber (> 10 Air Changes/h)*
Equilibration time (t95)	*2-3 minutes*
Start of exposure relative to equilibration	*not specified*
Actual concentration measurement	*Exact location unspecified. Samples were taken every 10 minutes, absorbed on two-stage charcoal tubes and analysed with AAS.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*NA*
Assessment of Reliability	***A*** *Well conducted study with concentration-time-lethality data for 3 exposure durations.*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
Rat	314		30	0/10	0/10
	551		30	0/10	2/10
	648		30	0/10	5/10
	842		30	2/10	7/10
	842		30	5/10	8/10
	1134		30	10/10	10/10
	1296		30	10/10	10/10
	389		60	0/10	2/10
	518		60	1/10	6/10
	616		60	4/10	6/10
	713		60	6/10	10/10
	713		60	7/10	9/10
	78		240	0/10	0/10
	87		240	0/10	0/10
	117		240	0/10	5/10
	149		240	1/10	10/10
	181		240	6/10	9/10
	356		240	10/10	10/10

Probit function

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

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

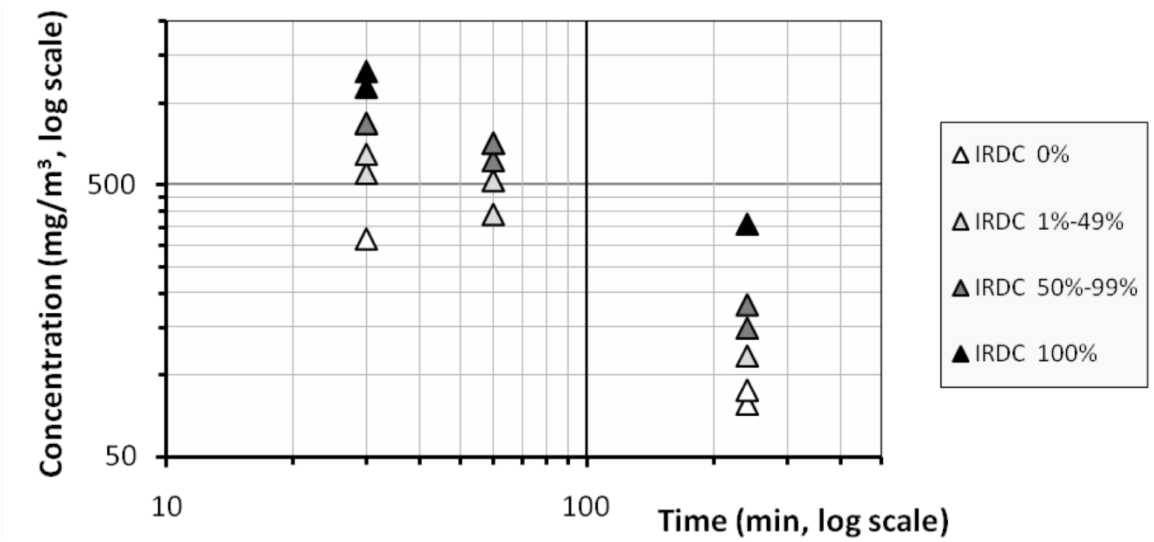
with C for concentration in mg/m^3, t for time in minutes and S for sex (0 = female, 1 = male).

Probit function	Species	a	b	c	d	n-value
Sex as variable	Rat	-29.2	3.66	2.96	-1.25	1.24 (1.12 - 1.36)
Sexes combined	Rat	-24.3	3.09	2.49		1.24 (1.09 - 1.39)

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/m^3) 95%-C.I. Male	LC_{50} (mg/m^3) 95%-C.I. Female	LC_{50} (mg/m^3) 95%-C.I. Combined
10	2483 (2079 - 2948)	1763 (1443 - 2105)	2080 (1663 - 2542)
30	1022 (916 - 1143)	726 (638 - 813)	857 (758 - 958)
60	584 (533 - 644)	415 (374 - 455)	490 (449 - 532)

A graphical overview of the data is presented below. Each concentration-time combination (with 10 male and 10 female animals) represents one point in the plot.


Concentration (mg/m³, log scale)
500
50
10
100
Time (min, log scale)
IRDC 0%
IRDC 1%-49%
IRDC 50%-99%
IRDC 100%

1
2
3
4

Study ID: B1.1

Author, year: ***Craig 1988***
Substance: Arsine
Species, strain, sex: Sprague-Dawley rat, male and female
Number/sex/conc. group: 5 / sex / group
Age and weight: 56(M) or 61(F) days, weight 140 - 206 grams
Observation period: 14 days post exposure

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*EPA GLP statement provided*
Study carried out according to OECD 403 guideline(s)	*No statement of compliance with OECD guideline 403 provided. Most criteria seem to be met.*
Stability of test compound in test atmosphere	*Stable*
Use of vehicle (other than air)	*Air (HEPA and charcoal filtered)*
Whole body / nose-only (incl. head/nose-only) exposure	*Nose only*
Type of restrainer	*Cannon-type nose-only tubes*
Pressure distribution	*0.5 inch negative water pressure*
Homogeneity of test atmosphere in breathing zone of animals	*Test atmosphere was generated by metering arsine gas from a cylinder of compressed gas into the airstream leading to the exposure system*
Number of air changes per hour	*6 ℓ/min for 10 animals*
Equilibration time (t95)	*Not specified nor able to calculate. Cannon (1983) claims that 30 sec are required 'for the animals to receive the full concentration' in this type of chamber.*
Start of exposure relative to equilibration	*Not explicitly stated, probably at start of concentration build-up.*
Actual concentration measurement	*Test atmosphere was drawn at from an unspecified location in the exposure system 5 times per exposure and analysed with a calibrated IR spectrophotometer.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*Not applicable - arsine is a gas*
Assessment of Reliability	***B1*** *Well-conducted study with only 1 exposure duration*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
Rat	Measured	Adjusted		Male	Female
	82		240	0/5	0/5
	114		240	0/5	2/5
	143		240	2/5	3/5
	176		240	4/5	5/5
	206		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 = female, 1 = male).

Probit function	Species	a	b	d	n-value
Sex as variable	Rat	-24.7	6.15	-1.10	N/A
Sexes combined	Rat	-21.1	5.30		N/A

The LC_{50} values (240 min) 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/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	149 (129 - 170)	125 (106 - 143)	138 (122 - 152)

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

Study ID: B2.1

Author, year: ***Levvy 1947***
Substance: Arsine
Species, strain, sex: mice ('inbred stock'), male and female
Number/sex/conc. group: 15 / sex / group
Age and weight: age unspecified, weight 25-30 grams
Observation period: unspecified, but at least 4 days post exposure[1]

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*GLP did not exist at the time (OECD GLP 1981 is point of reference, EPA GLP 1978, FDA GLP 1976)*
Study carried out according to OECD 403 guideline(s)	*OECD guideline 403 did not exist at the time.*
Stability of test compound in test atmosphere	*Stable*
Use of vehicle (other than air)	*Ambient air*
Whole body / nose-only (incl. head/nose-only) exposure	*Whole body*
Type of restrainer	*NA*
Pressure distribution	*Negative pressure of 5 mm H_2O.*
Homogeneity of test atmosphere in breathing zone of animals	*An arsine-hydrogen mixture, produced by a chemical reaction of sodium arsenite, was produced and stored in a sealed bottle before introduction into the exposure chamber.*
Number of air changes per hour	*Flow of 1.5-2.0 ℓ/min through a 142 ℓ exposure chamber (< 1 Air Changes/h). For the 'very short' exposure durations the chamber atmosphere was static. For the 25 mg/m^3 exposures a 3.5 ℓ chamber was used with an airflow of approx 1.5 ℓ/min (26 Air Changes/h)*
Equilibration time (t95)	*213-284 minutes for the high concentrations, 7 minutes for the 25 mg/m^3 exposures*
Start of exposure relative to equilibration	*After complete equilibration and after 'it was found by sampling that the concentration of arsine was steady at the figure desired'. Animals were moved quickly into and out of the chamber on a slide. For the 25 mg/m^3 exposure a 3.5 ℓ chamber was used and equilibrated to the desired concentration before introduction of the animals.*

[1] The average period before death was stated to be 4 days

Actual concentration measurement	*Samples were taken at unspecified intervals in an apparently appropriate location, absorbed in an impinger and analysed by titration.* *For the 25 mg/m^3 exposures, 6 samples were taken over the exposure duration.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*NA*
Assessment of Reliability	***B2*** *Reasonable C×t study, but unspecified brief observation and actual exposure uncertain due to low air exchange rate.*

Representation of the hinged door mechanism to quickly move mice into and out of the exposure chamber.

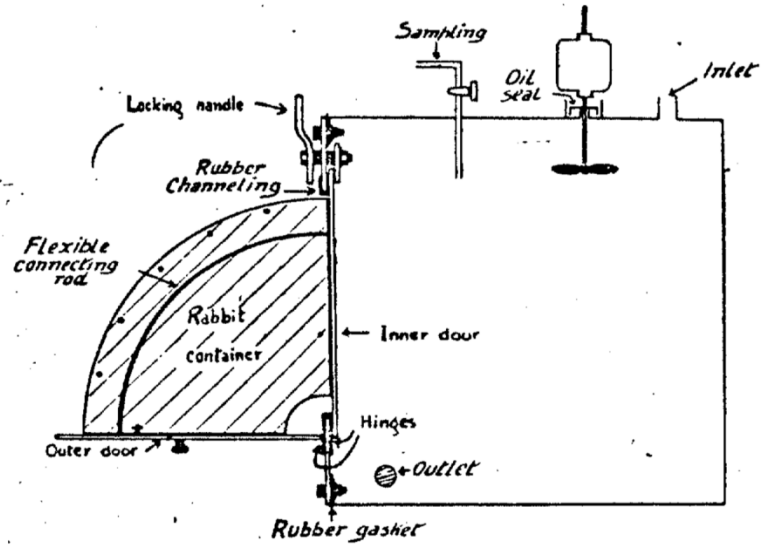


FIG. 2.—Diagram showing construction of chamber for accurately timed exposures of animals to predetermined concentrations of arsine.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality
	Measured	Adjusted		Sexes combined
Mouse	2500		0.5	28/30
	2500		0.33	6/30
	1000		1.25	17/30
	1000		0.83	5/30
	500		10	30/30
	500		5	28/30
	500		2.5	17/30
	500		1.7	0/30
	250		15	21/30
	250		9	10/30
	100		70	30/30
	100		50	15/30

	25		900	0/6
	25		1080	0/6
	25		1260	3/6
	25		1440	3/6
	25		1620	3/6
	25		1800	6/6

Probit function

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

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

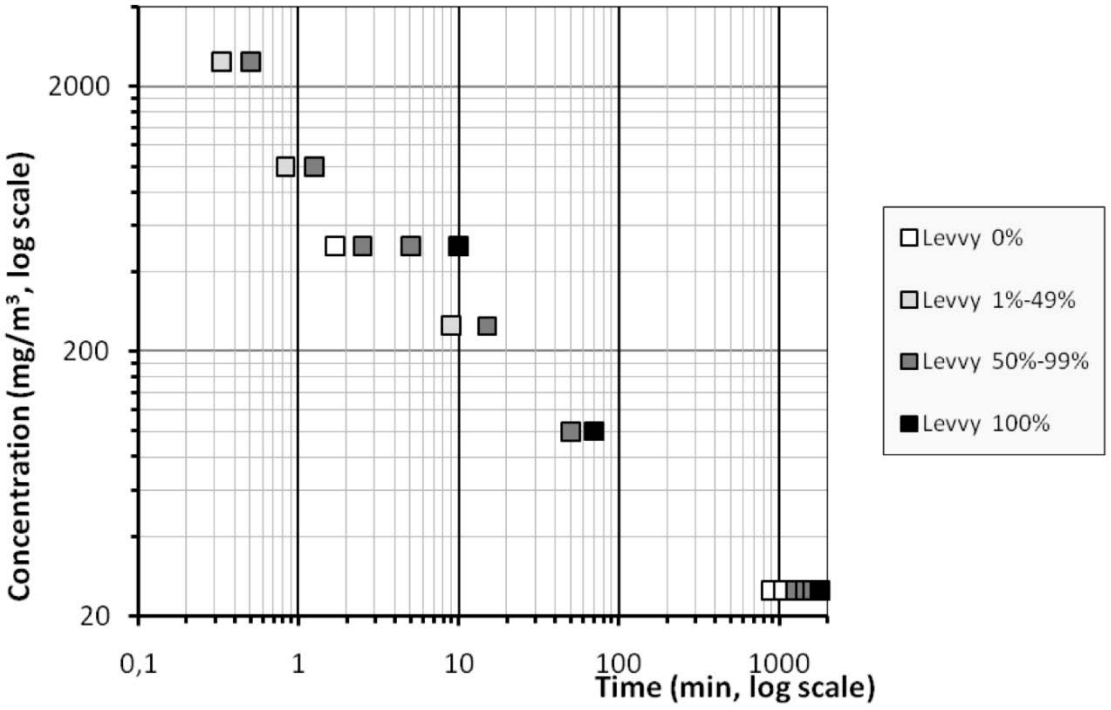
with C for concentration in mg/m^3 and t for time in minutes.

Probit function	Species	a	b	c	d	n-value
Sexes combined	Mouse	-10.4	2.21	1.29		1.71 (1.44 – 1.99)

While both male and female animals were tested, the lethality was only presented for combined sexes. Therefore sex differences could not be assessed.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Combined
10	281 (176 - 360)
30	148 (81-197)
60	98.6 (48.7 - 137)

A graphical overview of the data is presented below. Each concentration-time combination (with 15 male and 15 female animals, 3 male and 3 female animals for the 25 mg/m^3 exposures) represents one point in the plot.



Study ID: C studies

Peterson and Bhattacharyya (1985) exposed female B6C3F$_1$/Anl mice (8/group) to arsine concentrations of 16, 29, 35, 48 and 84 mg/^{3} for 1 hour. The purpose of the study was to characterize the haemolytic response of arsine. All mice in the 84 mg/m^3 group died, all other mice survived the 5 or 11 day observation period.

NDRC (1946) reports on the acute inhalation toxicity of arsine, summarising military research from 1918 onwards. The report states that for concentrations below 500 mg/m^3 $C^2 \times t$ was found to be constant, and for concentrations above 500 mg/m^3 $C \times t$ appeared to be constant. The report presents the following LC_{50} values in a range of species:

Species	minutes	LC_{50} (mg/m^3)	LC_{50} (mg/m^3)
Rat	10	250	520
Mouse	10	390	660
Rabbit	10	650	960
Goat	10	1000	2200
Species	minutes	mg/m^3	lethality
Cat	10	800	3/4 lethal
Cat	1	4100	no lethality
Monkey	15	450	4/5 lethal

Several other studies with have been located. These studies either originate from the early 20th century, are secondary sources or have a limited number of animals (e.g. 3 monkeys). These studies will not be described further.
*

Appendix 2 Reference list

AHLS. University of Arizona Emergency Medicine Research Center. Advanced Hazmat Life Support (AHLS). Provider Manual, 4th ed. Tucson, AZ, 2014.

Cannon WC, EF Blanton, KE McDonald. The Flow-Past Chamber: an Improved Nose-Only Exposure System for Rodents. AM Ind Hyg Assoc J 1983;44:923-928.

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