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
Phosphine	**7803-51-2**

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.

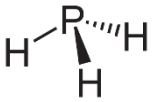
This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have had the opportunity to comment on the derivation of the proposed probit function. The status of this document has now been raised to "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 phosphine

1. Substance identification



CAS-number:	7803-51-2
IUPAC name:	Phosphine
Synonyms:	Hydrogen phosphor, phospine
Molecular formula:	PH_3
Molecular weight:	34 g/mol
Physical state:	gas (at 20°C and 101.3 kPa)
Boiling point:	-88°C (at 101.3 kPa)
Vapour pressure:	3460 kPa (at 20°C)
Saturated vapor conc:	NA (at 20°C)
Conversion factor:	1 mg/m^3 = 0.706 ppm (at 20°C and 101.3 kPa)
	1 ppm = 1.42 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H314, H330

2. Mechanism of action and toxicological effects following acute exposure

Acute effects: The main target organs and tissues for inhalation exposure to phosphine are the respiratory tract and the cardiovascular system. Phospine reacts with cytochrome C and cytochrome C oxydase leading to inhibition of mitochondrial oxygen uptake. Clinicals signs can be caused by this lack of oxygen uptake or from direct effects on the respiratory tract.
Common clinical signs are headache, nausea, vomiting, coughing, shortness of breath, paresthesia, weakness, tremors, and jaundice. Pulmonary congestion, pleural effusion, and congestive heart failure may be observed upon postmortem examination. Damage occurs to epithelial cells in the respiratory tract and high oxygen demanding cells, such as in the myocardium and liver. Lethality results from either lung or heart failure.
Long-term effects: Chronic exposure produces similar effects as seen after acute exposure. Acute effects may be delayed.

3. Human toxicity data

A number of fatal and non-fatal case reports are mentioned in the AEGL document, however data on exposure duration and/or concentration are limited and therefore not considered to be informative. The case reports do suggest that children might be more susceptible to phosphine intoxications.

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 phosphine, covering references before and including 1995.
2. An additional search covering publications from 1980 onwards was performed in HSDB, MEDline/PubMed, Toxcenter, 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 7 studies were identified -with 8 datasets for 2 species- with data on lethality following acute inhalation exposure. Two datasets were assigned status B1 for deriving the human probit function and 6 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 phosphine are displayed in Figure 1.

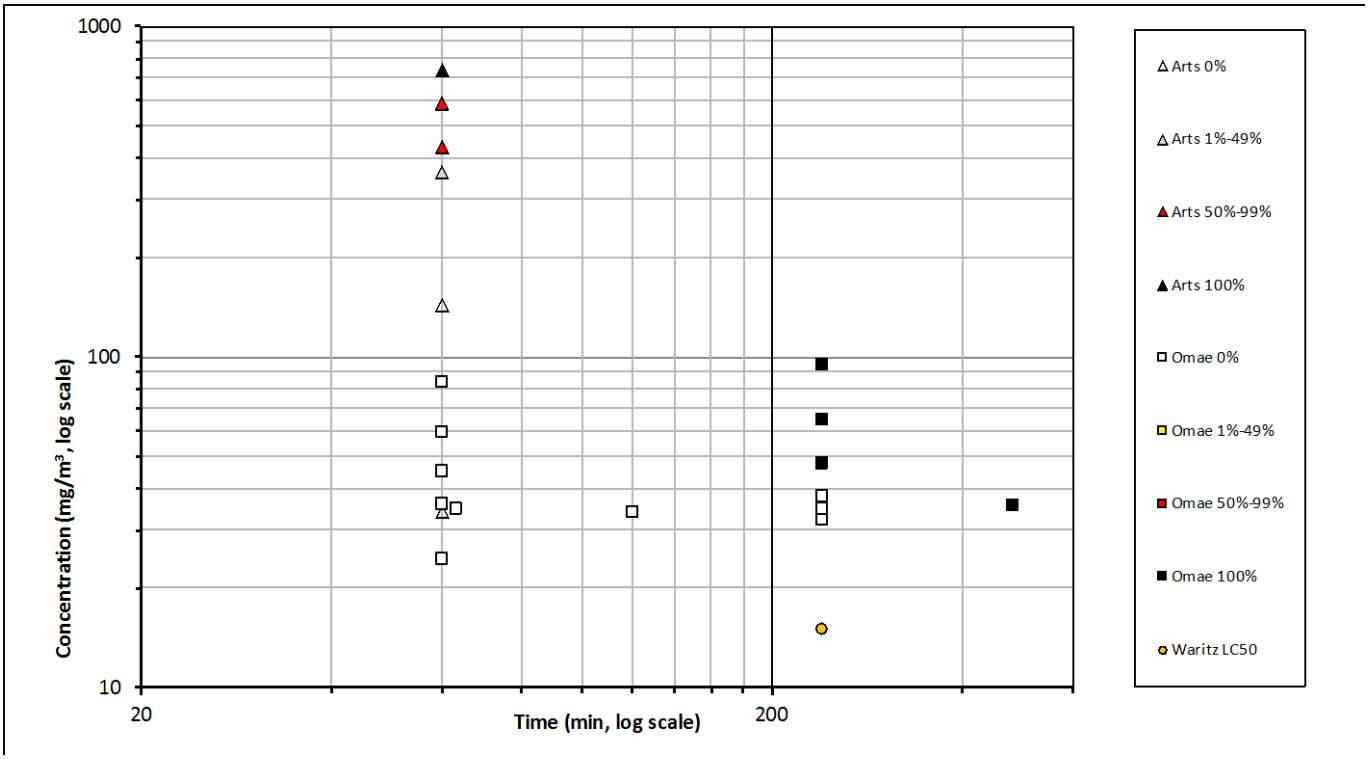


Figure 1 *All available acute lethality data for phosphine.*

The data that were selected for primary analysis of the animal probit function are presented in Table 1 and Figure 2.

Based on both study's quality the data from studies B1.1 and B1.2 were selected for the initial dataset for the derivation of the animal probit function. Three datapoints were left out of the data from study B1.2 as described in appendix 1, because sacrifice may have been premature. The outcome of study B1.2 was hardly influenced by this data selection. Effectively, study B1.2 was reduced to a 240-min LC_{50} study.

To enable pooling, LC_{50}-values of B1-studies were scaled using the default n-value of 2 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 phosphine.

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}, 60 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
B1.1	Rats	*60-min LC_{50}*		361 (224 – 417)	N/A
B1.2	Mice	*240-min LC_{50}*	42.4 (40.1 – 45.0)	*84.8*	N/A

The data of studies B1.1 (rats) and B1.2 (mice) are presented graphically below.

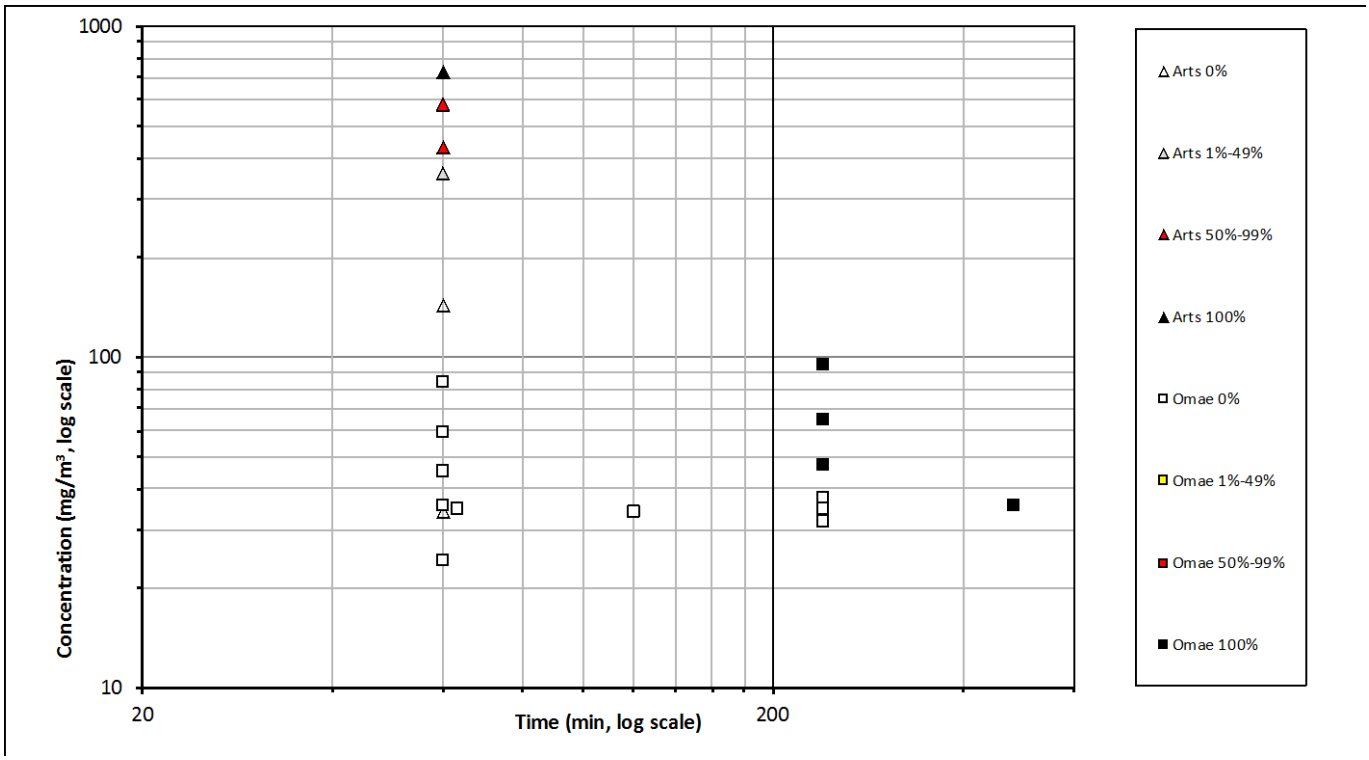


Figure 2 *Data selected for the initial analysis for the derivation of the animal probit function of phosphine.*

The data from the two remaining B1 datasets will not be pooled, since the uncertainty involved with time-scaling the 240-min data from study B1.2 is considered too high. The final data eligible for calculating the animal probit function contains 1 dataset from 1 study (B1.1) and includes data from 1 animal species.

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

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}, 60 minutes (mg/m^3) 95% C.I. *(underline italic for scaled values)*	n-value 95% C.I.
B1.1	Rats	*60-min LC_{50}*		361 (224 – 417)	N/A

The data of the selected datasets are presented graphically below.

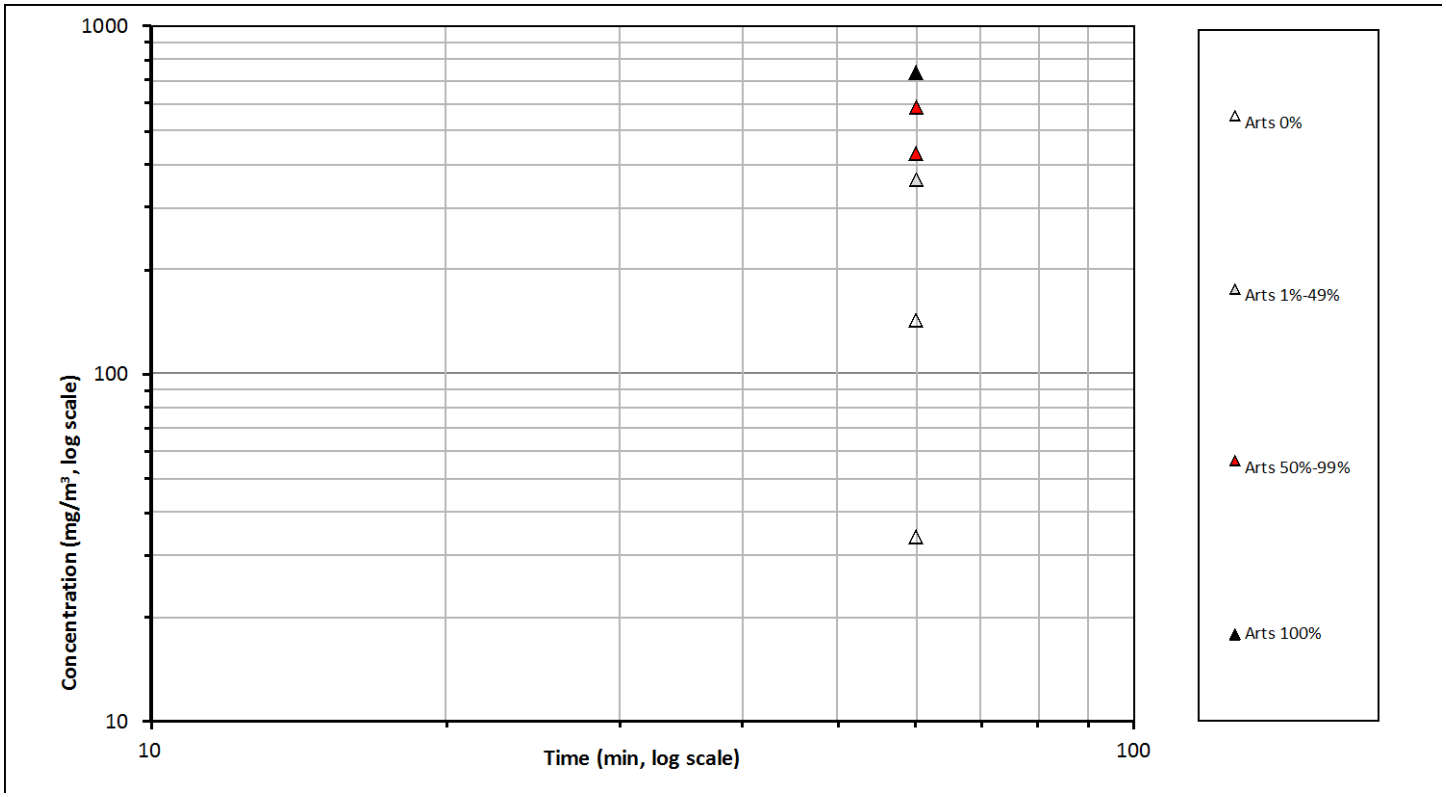


Figure 3 *Final data selected for derivation of the animal probit function of phosphine.*

6. Derivation of the human probit function

The data from the two remaining B1 datasets will not be pooled, since the uncertainty involved with time-scaling the 240-min data from study B1.2 is considered too high. To derive the human probit function the rat data from study B1.1 were preferred over the mouse data from study B1.2 because of the shorter exposure duration, the observation of partial lethality in the dataset and the general preference of rat over mouse data.

As a point of departure for deriving the human probit function, the 60 min 361 mg/m^3 LC_{50} value for the rat from study B1.1 was taken. Since no c×t study was available, the default n-value of 2 was used.

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 reason to deviate from the standard factor of 3.
Nominal concentration	1	The study reported analytically determined concentrations.
Adequacy of database:	1	One well conducted B1 study supported by information from one B1 study and several C studies.

The estimated human equivalent 60-minute LC_{50} value is 361 / 3 = **120 mg/m^3**.

The default n-value was **2**. 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 60 min LC_{50} using the above parameters to solve the following equation to obtain the a-value (the intercept): $5 = a + 1 \times \ln (120^2 \times 60)$ resulting in the a-value of **-8.67**.

$Pr = -8.67 + 1 \times \ln (C^2 \times t)$ 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 one study in the rat with B1 quality, where 60 animals have been exposed to concentrations ranging from 34 – 733 mg/m^3 for 60 minutes.

The human 60 min LC_1 (Pr = 2.67) calculated with this probit equation is 37 mg/m^3 and the calculated human 60 min $LC_{0.1}$ (Pr = 1.91) is 26 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	36	26
1% lethality, this probit	53	37
AEGL-3 (2007, final)	10	5.1
ERPG-3 (2016)		9.0
LBW (2015)	10	5.1

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are higher, probably because of the higher assessment factors used in the derivation of AEGLs and ERPGs to account for susceptible subpopulations.

Appendix 1 Animal experimental research

Study ID: B1.1

Author, year:	***Arts 1987***
Substance:	phosphine
Species, strain, sex:	SPF-reared (Bor:WISW) rats, both sexes
Number/sex/conc. group:	5 animals/sex/concentration group
Age and weight:	age not specified, mean body weights are 281 and 180 g for males and females, respectively.
Observation period:	two weeks

Evaluation of study quality

Criteria	***Comment***
Study carried out according to GLP	*Yes*
Study carried out according to OECD 403 guideline(s)	*According to OECD guideline 403*
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, individually housed in a horizontally placed glass tube.*
Type of restrainer	*N/A*
Pressure distribution	*No information*
Homogeneity of test atmosphere in breathing zone of animals	*A phosphine test atmosphere was generated by mixing an adjustable flow of test material with the airflow before entering the exposure chamber.*
Number of air changes per hour	*Air flow was 1.1-1.3 m^3/hr with a chamber volume of 0.015 m^3, which produced 73.3 to 86.7 air changes/hr.*
Equilibration time (t95)	*42-49 seconds*
Start of exposure relative to equilibration	*At start of concentration build-up*
Actual concentration measurement	*Concentrations were determined by vapour phase infrared spectrometry (Miran 1A).*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *Well conducted and reported study, only 1 exposure duration*

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
Rat	34		60	0/5	0/5
	143		60	0/5	0/5
	361		60	1/5	3/5
	431		60	5/5	4/5
	583		60	4/5	5/5
	733		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

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

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	d	n-value
Sex as variable	Rat	-17.6	3.78	-0.55	N/A
Sexes combined	Rat	-16.1	3.59		N/A

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 and LC_{50} value.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. Female	LC_{50} (mg/m^3) 95%-C.I. Male	LC_{50} (mg/m^3) 95%-C.I. Combined
60	337 (180 – 416)	389 (253 – 475)	361 (224 – 417)

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

Study ID: B1.2

Author, year: ***Omae 1996***
Substance: phosphine
Species, strain, sex: male ICR mice
Number/sex/conc. group: 10 per concentration group
Age and weight: four weeks old, weights not reported, but were measured
Observation period: 2 weeks or 3 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)	*No statement of compliance with OECD guideline 403 provided*
Stability of test compound in test atmosphere	*Stable*
Use of vehicle (other than air)	*highly purified nitrogen*
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	*The phosphine-nitrogen mixture was mixed with HEPA filtered room air prior to introduction into the 550 L air tight exposure chamber at a constant flow rate.*
Number of air changes per hour	*No information*
Equilibration time (t95)	*No information*
Start of exposure relative to equilibration	*No information*
Actual concentration measurement	*The exposure concentration was measured with gas chromatography every 12 minutes. Oxygen levels were also determined with a oximeter.*
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure	*N/A*
Assessment of Reliability	***B1*** *Study with 3 exposure durations, but details missing about technical aspects of exposure generation, only 0% and 100% response and uncertainty about the validity of the* c×t probit function.

This study consisted of two experiments: an LC_{50} study conducted at 1 and 4 hour exposure, and an acute study aimed to clarify the pathology of acute toxicity in a 1, 2, 4 and 8 hour exposure group. All fatalities in the LC_{50} study died within 3 days. The animals exposed in the acute study were sacrificed for histopathological examination after 3 days.

Results

Species	Concentration (mg/m^3)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Exposed	Lethal
Mice	24.4		60	10	0
	35.6		60	10	0
	45.0		60	10	0
	59.1		60	10	0
	84.1		60	10	0
	32.0		240	10	0
	37.6		240	10	0
	47.4		240	10	10
	64.6		240	10	10
	95.0		240	10	10
	34.8		60	10	0
	33.9		120	10	0
	34.8		240	10	0
	35.4		480	10	10

The greyed area represents the data from the acute study, where animals were sacrificed after 3 days. These were not considered for the lethality calculations.

Probit function

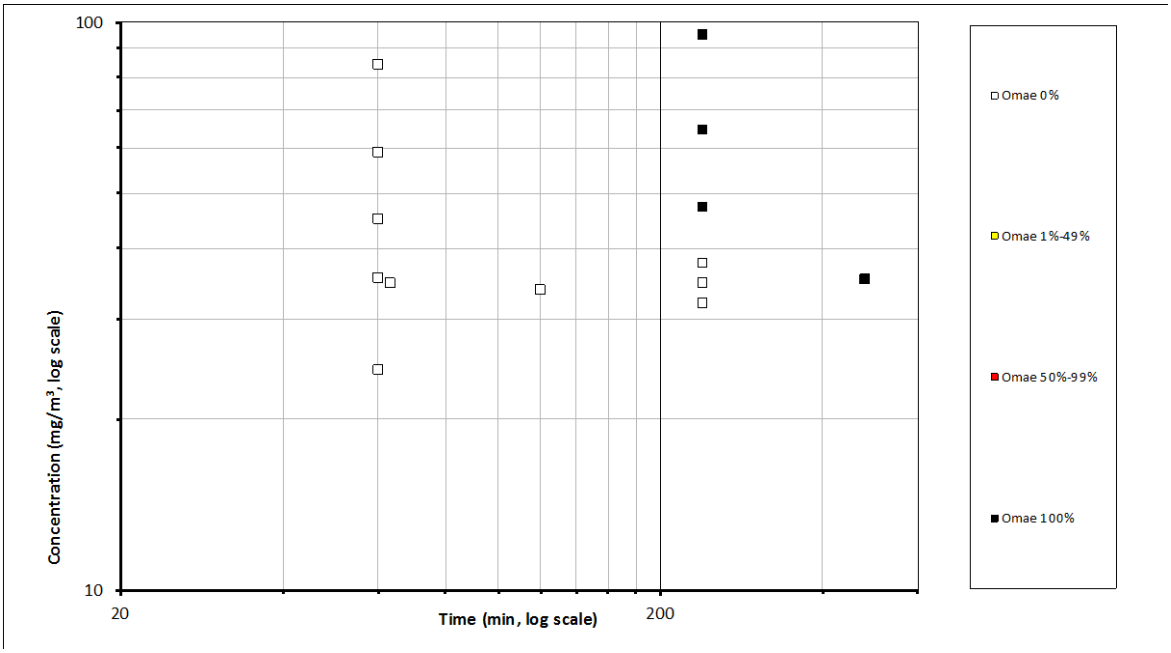
While the model produced a c×t probit function, it was decided that this function may be biased. At 480 minutes the only exposure produces 100% lethality and at 60 minutes all exposures produce 0% lethality. The data are therefore inconclusive: it is possible that lower exposure concentrations at 480 minutes would also produce (high) lethality, and higher concentrations at 60 minutes would also produce no lethality. In that case, the slope of the dose-effect curve would be different from the calculated slope.

Because of this uncertainty the panel decided to use this study as a 240-min LC_{50} study, based on the data from animals with a 14-day observation period.

No data were available to assess sex differences in the response to phosphine inhalation.

Duration (min.)	LC_{50} (mg/m^3) 95%-C.I. All date
240	42.4 (40.1 – 45.0)

A graphical overview of the data is presented below. Each concentration-time combination (with 10 male animals) represents one point in the plot. The presented data include the groups with 0/10 lethality in the acute lethality study.



Study ID: C studies

Groups of four male F344 rats were exposed to 0, 1, 5, or 10 ppm (0, 1.4, 7.1, 14.2 mg/m^3) of phosphine for 6 h/day for up to 4 days. All rats died by the end of the third exposure to 10 ppm (Morgan et al. 1995). Deaths were not observed in rats exposed to 5 ppm (7.1 mg/m^3), suggesting a steep concentration-response curve. Morgan et al. (1995) exposed 18 F344 rats/sex/group to 0, 1.25, 2.5, or 5 ppm of phosphine 6 h/day, 5 days/week, for 2 weeks. None of the rats died as a result of phosphine exposure.
Groups of four male B6C3F1 mice were exposed to 0, 1, 5, or 10 ppm (0, 1.4, 7.1, 14.2 mg/m^3) of phosphine for 6 h/day for 4 days (Morgan et al. 1995). All mice were killed moribund after the fourth exposure to 10 ppm (14.2 mg/m^3). Pathology data were collected, and there were no treatment-related effects observed in mice exposed to 1 or 5 ppm. Anemia, decreased leukocyte counts, increased serum ALT and SDH activities, increased urine nitrogen, degeneration and necrosis of renal tubule epithelium, myocardial degeneration, and liver foci and degeneration were observed in the 10-ppm group.
In a follow-up study, Morgan et al. (as cited in AEGL) exposed 18 male and female rats and mice per group to 0, 1.25, 2.5, or 5 ppm of phosphine for 6 h/day, 5 days/week, for 2 weeks. Six male animals/group were sacrificed after 1, 5 and 10 exposures. All females were sacrificed on the morning after the final exposure. None of the rats or mice died as a result of the phosphine exposure.

Muthu *et al* (1980) studied the inhalation toxicity of 2 brands of aluminium phosphide products in groups of 6 adult female albino CFT-Wistar rats. The calculated mean concentrations (probably of phosphine) ranged from 30-90 mg/m^3 and exposure durations ranged from 120-240 minutes. The authors reported a 2-fold difference between the LC_{50} values of the two products. The probit function was not calculated, because the data are considered unreliable.

Newton (1991) exposed Sprague-Dawly rats (5/sex/concentration group or 10 males per group) for 6 hours to phosphine concentrations ranging from 1.8-39.8 mg/m^3. Five out of 6 exposure concentrations did not produce lethalities, the highest exposure concentration of 39.8 mg/m^3 cause lethality of 2/5 female and 3/5 male rats. This study did not allow to calculate LC-values, but was otherwise well conducted.

Newton et al (1993) exposed Fischer 344 rats (15/sex/group) 6 hours to phosphine concentrations of 3.4, 7.0 and 14.2 mg/m^3. None of the exposure concentrations produced lethalities. This study did not allow to calculate LC-values, but was otherwise well conducted.

Waritz and Brown (1975) exposed male Charles-River CD rats (6/group) for 4 hours to unspecified concentrations of phosphine. The authors reported a 4-hour LC_{50} value of 15 mg/m^3 (95% confidence interval 11 - 21 mg/m^3).

Appendix 2 Reference list

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