



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

Method for derivation of probit functions for acute inhalation toxicity

RIVM Report 2015-0102

M.M.W.M. Ruijten et al.



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Colophon

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Dutch Expert Panel on Probit Functions

M.M.W.M. Ruijten

J.H.E. Arts

P.J. Boogaard

P.M.J. Bos, RIVM

H. Muijser

A. Wijbenga

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P.O. Box 1 | 3720 BA Bilthoven

The Netherlands

www.rivm.nl/en

Synopsis

Method for the derivation of probit functions to predict acute lethality following inhalation of toxic substances

A method is in place to assess the risks attached to the use, transport and storage of dangerous substances, the so-called quantitative risk analysis (QRA). Part of the QRA method comprises the prediction of the percentage of people that will die after inhaling substances that are acutely toxic. These predictions are calculated using 'probit functions'. A probit function describes the relationship between the concentration of a substance, the duration of exposure and the part of the exposed population that demonstrates a certain effect.

To derive a probit function, animal data are translated to humans. This report describes the methodology used to perform this derivation. The method has been developed by the Dutch Expert Panel on probit functions, by order of the Netherlands' Ministry of Infrastructure and the Environment, and replaces the previous version of the method from 2001. The method has been thoroughly revised and subsequently reviewed internationally.

Keywords: probit function, inhalation toxicity, quantitative risk analysis, QRA, third party risk

Publiekssamenvatting

Methode voor de afleiding van probitrelaties om acute sterfte te voorspellen na inhalatie van giftige stoffen

Er bestaat een methode om risico's van het gebruik, vervoer en de opslag van gevaarlijke stoffen inzichtelijk te maken, de zogeheten kwantitatieve risicoanalyse (QRA). Als onderdeel hiervan wordt voorspeld welk percentage mensen overlijdt na het inademen van stoffen die acuut giftig zijn. Deze voorspellingen worden berekend met behulp van 'probitrelaties'. Een probitrelatie geeft het verband weer tussen de concentratie van een stof, de duur van de blootstelling en het deel van de blootgestelde personen dat een bepaald effect vertoont.

Om de probitrelaties te kunnen afleiden worden onderzoeksgegevens van dieren vertaald naar de mens. In dit rapport staat beschreven hoe deze afleiding moet worden uitgevoerd. De methodiek is in opdracht van het ministerie van Infrastructuur en Milieu opgesteld door de Toetsgroep probitrelaties en vervangt de vorige versie van de methodiek uit 2001. De methodiek is grondig herzien en vervolgens internationaal gereviewd.

Kernwoorden: probitrelatie, inhalatietoxiciteit, kwantitatieve risicoanalyse, QRA, omgevingsveiligheid

Contents

Part 1 Scientific justification of the probit derivation methodology – 11

1 Introduction – 13

- 1.1 Relevant issues outside the scope of this document – 15

2 Philosophy for the derivation of a probit function – 17

- 2.1 Modelling the response to acute inhalation exposure – 17
 2.2 Vulnerability models – 17
 2.3 Quality assessment of animal toxicity data – 18
 2.3.1 Data completeness requirements – 18
 2.3.2 Study quality requirements – 19
 2.3.3 Classification of overall study quality – 20
 2.4 Acute toxicity data: characteristics and sources – 21
 2.4.1 Literature search and selection of experimental data – 21

3 Evaluation of animal data to derive a probit function – 23

- 3.1 Data adjustment, quality assessment and primary data selection – 24
 3.1.1 Overview of available data – 24
 3.1.2 Adjustment of data with short exposure duration – 25
 3.1.3 Datasets with only 0% and 100% response – 26
 3.1.4 Test atmosphere generation and characterization of the concentration – 28
 3.1.5 Quality evaluation of each available study – 31
 3.2 Stepwise approach to evaluate the animal data – 32
 3.2.1 Probit calculation for individual eligible studies – 32
 3.2.2 Exploring the analysis of pooled data – 34
 3.2.3 Derivation of a point of departure for the human probit function – 35
 3.3 Interspecies extrapolation – 36
 3.4 Assessment of sensory irritation in animals – 40
 3.5 Assessment and adjustment for overall quality of the study – 43
 3.6 Intra-species extrapolation – 43
 3.7 Verification with primate data – 45

4 Discussion and conclusions – 47

Part 2 Completion of the probit TSD format – 49

5 Stepwise explanation of the probit TSD format – 51

- 5.1 Title page – 51
 5.2 Substance identification and physical-chemical characteristics – 51
 5.3 Mechanism of action in humans – 51
 5.4 Human toxicity data – 52
 5.5 Literature search strategy – 52
 5.5.1 Sensory irritation – 53
 5.6 Probit functions derived from individual studies – 53
 5.7 Evaluation – 54

6 Description and interpretation of studies in the probit TSD – 57

- 6.1 Study identification – 57
 6.2 Evaluation of study quality – 57

- 6.3 Presentation of the data — 58
- 6.4 Probit function — 58
- 6.5 Calculation of LC-values with the model — 58
- 6.6 Plot of underlying data — 58
- 6.7 References — 58

7 Annexes — 59

- 7.1 Annex to head/nose only versus whole body exposure — 59
 - 7.1.1 Selection of an inhalation chamber — 59
 - 7.1.2 Nose-Only Exposure Technique — 61
 - 7.1.3 Whole-Body Exposure Technique — 61
- 7.2 Annex to interspecies extrapolation — 62
- 7.3 Annex to intraspecies extrapolation — 62

References — 65

List of abbreviations — 69

Internet literature resources — 71

Summary

A probit function for the acute inhalation toxicity of a chemical describes the lethality rate in an exposed population as a function of any combination of the exposure concentration and exposure duration. Probit functions are used in Quantitative Risk Analysis (QRA) to estimate the proportion of fatalities following exposure to toxic chemicals. This document describes the methodology used for the derivation of probit functions. The methodology, that was formerly described in the 'Green Book' (part 4 of PGS1), has been updated and thoroughly revised. This revised version of the methodology is a product of the Netherlands' Expert Panel on probit functions, that operates under authority of the National Institute of Public Health and the Environment (RIVM).

After introducing the basic philosophy of deriving a probit function, the methodology describes in detail the interpretation and use of animal data and the derivation of the probit function. Stepwise guidance is provided for drafting a probit technical support document.

Major revisions to the methodology compared with the previous version include the following:

- The revised procedure puts higher demands on the quality of inhalation toxicological data.
- Many datasets do not meet the quality criteria set by the Expert Panel on probit functions. The Panel has defined quality criteria for the inclusion or exclusion of studies in the probit derivation.
- The derivation of probit functions based on 'life threatening values' (LBW) is no longer allowed.
- The derivation of probit functions via LC_{50} values and the old flow chart based on allometric scaling is not allowed.
- The procedure to raise the LC_{50} value as a point of departure when data from two or more animal species is available is no longer considered to be valid.

Part 1

Scientific justification of the probit derivation methodology

1 Introduction

A probit function for acute inhalation toxicity of a chemical describes the lethality rate in an exposed population as a function of any combination of the exposure concentration and exposure duration. Probit functions for the acute toxicity of chemicals are important instruments in the Netherlands' external safety policy. They are used in Quantitative Risk Assessments (QRAs) to estimate the proportion of fatalities following (accidental) exposure to toxic chemicals. The primarily responsible ministry, the Ministry of Infrastructure and the Environment, has initiated the unification of methods, procedures and information for external safety QRAs. As a part of this unification process, the probit functions and the scientific and administrative procedures used to derive probit functions have been reviewed and revised.

This methodology describes the current, standing technical operating procedure used to develop a probit function for human lethality due to an acute airborne (mainly inhalation) exposure. This procedure, that was formerly described in Chapter 4 of PGS1 or the 'Green Book' (VROM 2005), has been approved by the Dutch Expert Panel on probit functions. The revised procedure places high demands on the quality of inhalation toxicological data before acceptance as a Point of Departure (PoD). This is a major shift away from the previous situation, in which an LC₅₀ value from RTECS was considered to be an acceptable PoD. Even if an LC₅₀ value for a single exposure duration is used as a PoD, the revised procedure requires a review of the primary report and data, and a verification of the calculated value. Other significant methodological changes include:

- The derivation of probit functions based on LBW values is no longer allowed.
- The derivation of probit functions via LC₅₀ values and the old flow chart based on allometric scaling is not allowed.
- The procedure whereby the LC₅₀ value as a PoD is multiplied by a factor 2 when data from two or more animal species is available is no longer considered to be valid.

The objective of this document is to outline what information and scientific risk assessment procedures are required to prepare a Technical Support Document (TSD), including the development of a draft (procedural status: proposed) probit function for human lethality following an acute airborne exposure to a hazardous substance.

A number of general assumptions and basic principles underlie the development and application of probit functions:

1. For the Dutch implementation of the Seveso III directive, a choice has been made to include only lethality as an endpoint for external safety assessment. The endpoint for all probit functions, therefore, is lethality. Non-lethal health effects are not considered in the external safety assessment. Guidelines and models for an external safety assessment of transport of dangerous goods follow the same philosophy.

2. Probit functions are designed to predict the lethality following acute inhalation exposure. The probits are explicitly not designed to guarantee the prevention of all levels of toxicity. For this reason, probit functions have been developed without the safety factors usually applied for protective exposure guidelines (such as air quality guidelines).
3. The probit functions assume an 'average' population, including susceptible subjects. Depending on the demographic characteristics of the population actually exposed, the calculated risk zones may be a more or less accurate reflection of the site and scenario specific, expected human lethality.
4. The probit functions assume that the exposed persons are not protected by personal protective equipment or shelter in place, nor that they receive medical treatment following exposure.
5. Possible lethality from delayed effects, as in the case of carcinogenicity or reproductive toxicity, is not taken into account.
6. Possible lethality following secondary exposure is not taken into account.
7. Possible secondary lethality from causes other than acute toxicity (e.g. from mechanical trauma due to falling caused by toxic incapacitation) is not taken into account.

In March 2009, the then Ministry of Housing, Spatial Planning and the Environment appointed the members of the Dutch Expert Panel on probit functions (the Panel). The mission of the Panel is:

1. To advise the Ministry's director of risk policy about the toxic properties of chemicals and, particularly, to provide the best possible support for a probit function based on current scientific understanding.
2. To develop, maintain and publish the robust protocols and criteria necessary to produce the advice mentioned under 1 in a transparent and reproducible manner.

Since 2014, the Panel operates under authority of RIVM.

Secretarial support for the Panel is provided by RIVM. The membership register of the expert Panel can be found on the RIVM website (www.rivm.nl, cf 'internet resources'). The guidelines and procedures contained in this document have been developed and approved by the Panel. The current version of this document can be found on the RIVM website. While reviewing draft probit TSD documents, the Panel may identify toxicological or procedural issues that require an update of the standing operating procedures. Changes in the toxicological risk assessment approach will result in an update of this document, which will be posted on the RIVM website. Therefore the actual guidelines to develop probit functions consist of:

- the most recent version of this document;
- the procedural guidelines posted on the RIVM website.

Both documents indicated above can be found on the RIVM website.

All guidelines presented in this document are preferred standard procedures. The Panel recognizes that the availability and quality of the data may sometimes seriously limit the ability to strictly follow these guidelines. The preferred procedures outlined in this document do not exclude the TSD author's or Panel's option to take case-by-case

decisions based on expert judgment of specific information for a given substance. Such decisions should be justified in the TSDs.

After the derivation of about 40 probit functions, it was deemed appropriate to organize a peer review of the methodology and the derived probits. As a result, an international invitational expert meeting was convened in September 2013 with the objective of improving the scientific basis, validity, appropriateness and acceptance of the methodology used (at that time) to derive probit functions for acute lethality in a normal human population following a single airborne exposure. This objective was met by bringing together renowned international experts in the field of risk assessment for acute airborne exposures in order to discuss its strengths, weaknesses and opportunities to improve the methodology.

The participants of the workshop were invited to recommend improvements to the methodology. The probit Panel asked that the recommendations should ideally be:

- practical and feasible with reasonable data requirements (minimizing the need to generate new data);
- a clear improvement over the existing methodology, both from a scientific and societal point of view;
- philosophically and practically compatible with other risk assessment methodologies (so far as these are applicable to the risk assessment of incident scenarios) as far as possible, particularly those generally applied in Europe.

The current version of this document incorporates the recommendations made by this meeting (Ter Burg et al., 2013).

Even after the expert meeting, this document is considered to be a living document and the presented procedures are subject to periodic review and revision. The Panel will evaluate new scientific insights that are relevant for the derivation of human concentration-time-lethality functions for acute inhalation exposure and the procedures will be adjusted accordingly, if necessary. In addition, all interested parties with suggestions for changes of the methodology are invited to submit sufficiently justified and supported recommendations to the Panel's secretariat (via the website).

1.1 Relevant issues outside the scope of this document

This document serves as a technical guideline for the development of probit functions. The following relevant and related information will not be described, and can be found on the RIVM website:

- A list of substances scheduled for the development or revision of a probit function. The selection of substances for the derivation of probits is the responsibility of the Ministry of Infrastructure and the Environment. Among other things, the selection is based on the inclusion of substances in external safety reports submitted to the RIVM.
- All proposed, interim and final probits TSDs; the documents database can be searched by name or CAS number on the RIVM website. The site also provides access to the regularly updated 'Reference Manual Bevi Risk Assessments', which includes a list of existing probit functions.

- The administrative procedure and guidelines for drafting and submitting a probit Technical Support Document (TSD) to the Panel, as well as a format of the probit TSD.
- TNO publication (Arts and Muijser 1999) on quality criteria for animal inhalation experiments (see Section 2.3.2).

Due to size limitations, this document is not intended as an exhaustive scientific justification of the applied approach. The document is designed as a 'how-to' guidance with an explanation of the assumptions, principles and justification of the often pragmatic choices that need to be made.

2 Philosophy for the derivation of a probit function

This chapter provides the basic philosophy for modelling the lethal response to acute inhalation exposure, the data demands to meet the modelling needs and the identification of data sources.

2.1 Modelling the response to acute inhalation exposure

The toxic response of a human or animal population to a chemical exposure is determined by:

- the chemical substance;
- the exposure route (inhalation, dermal, oral or parenteral);
- the exposure concentration of the chemical in the contact medium (air, water, food, etc.);
- the duration of exposure;
- the species (test animals or human);
- physiological characteristics of the individuals in the exposed population.

This document deals with the lethality, immediately or shortly after the exposure, of a single (up to eight hours) airborne exposure in an average population. Risk evaluations for chemical exposures by other routes, contact media and exposure durations require different data, procedures and assessments from those presented in this document. The procedures described here may not be suitable for those situations and scenarios.

For obvious reasons, experimental test data on humans resulting in lethality are not available. In some cases, information is available from accidental poisoning, but in such cases the levels of exposure are usually poorly characterized. Therefore almost all information for the derivation of probit functions originates from the results of animal experiments. Any data on health effects in humans or other primates can provide supportive evidence (cf. Section 3.7).

2.2 Vulnerability models

The modelling for external safety requires that the percentage of lethality in the exposed population can be assessed for each combination of exposure concentration and duration. The concentration-time-lethality relationship can be described using a number of statistical models including (log) probit, (log) logit and Weibull models. All mentioned models make assumptions about the underlying statistical distribution of the concentration-time-response ($C \times t$) data, and usually describe the $C \times t$ relationship of acute lethality data about equally well in the actual experimental exposure range (interpolation and limited extrapolation); for risk assessments that require predictions of toxicity well outside the actual experimental exposure range, the models may produce widely different health outcomes for identical exposure-response scenarios. The log probit model has been selected as the most simple and straightforward model to describe the human vulnerability distribution for use in modelling acute lethality for external safety.

The probit model for concentration-time-lethality data is described as:

$$\text{Pr} = a + b_1 \times \ln(C) + b_2 \times \ln(t)$$

Equation 1 Standard bivariate probit model. *C* is concentration in mg/m³ and *t* is exposure duration in minutes.

A frequently used alternative presentation is:

$$\text{Pr} = a + b \times \ln(C^n \times t)$$

Equation 2 Alternative bivariate probit model; $b = b_2$ and $n = b_1/b_2$. The dose metric ($C^n \times t$) is often referred to as 'toxic load'.

In some cases, the probit function can be extended with the use of a covariate for e.g. sex. In that case, the basic version of the probit model is described as:

$$\text{Pr} = a + b_1 \times \ln(C) + b_2 \times \ln(t) + b_3 \times (X)$$

Equation 3 Probit model with interaction term. *X* is the covariate (log-transformed, if appropriate, or 0/1 in case of sex)

In some cases, the model fit of the data can be improved by adding cross-terms for interactive effects between the model parameters (usually concentration and time) or a threshold response level (concentration or time). Before the probit model is complicated by such an addition, strong biological and statistical rationales are required (Section 3.1).

2.3 Quality assessment of animal toxicity data

The Panel evaluates the quality of data from animal experiments on the basis of two criteria:

1. The ability to derive a probit function from the dataset (data completeness requirements).
2. The technical conduct of the study and the quality of the report (study quality requirements).

These two quality aspects will be discussed below, followed by the Panel's rules for the classification of studies.

2.3.1 Data completeness requirements

A dataset will enable the assessment of all parameters of a probit function as a basis for a human probit function if the following information is available and verifiable from the literature source:

1. A sufficient number of exposure concentration-time combinations, with at least three exposure durations and three concentrations per exposure duration. Studies with two qualifying exposure durations are considered unfit to assess the n-value for the particular chemical without supporting data.
2. The number of animals and the number of fatalities per concentration-time combination. Preferably also the time of death is provided.

3. The dataset must produce a model outcome with the DoseResp or BMDS software packages. In practice, the statistical models require a number of exposure conditions with a partial response to produce a model output. Studies carried out according to the OECD guideline 403 C×t protocol (OECD, 2009) only produce responses of 0%, 50% or 100% (and sometimes 25% and 75%, when 2 animals per sex per concentration-time combination are exposed). These studies are eligible because many concentration-time combinations for longer and shorter durations and lower and higher concentrations are available. A C×t dataset can produce estimates of the relevant parameters LC_{50} and 'n' in itself, as opposed to single duration LC_{50} studies.
For studies with 1 exposure duration, at least one partial response is required (in practice, at least two) to produce a model output. The assumptions that need to be made to derive a probit function will be discussed in Section 3.1.
4. In addition to the animal experimental data described above, every effort should be made to collect data on the response in humans and other primates, even if such data only concern non-lethal effects. Experience indicates that such data can be crucial to supporting the validation of animal data for human risk assessment.

2.3.2 Study quality requirements

The first quality aspect concerns compliance with international testing guidelines, particularly OECD TG 403 and GLP. Studies that are used as a point of departure for the derivation of a probit function should be performed according to OECD guideline 403 or equivalent (first version introduced in 1981). For the present purpose, the C×t protocol of the OECD guideline 403 (2009 revision) is the preferred study protocol. The C×t protocol is therefore recommended when new or additional acute inhalation toxicity studies are needed. Studies performed according to good laboratory practice (GLP) and OECD guidelines are preferred (EPA GLP introduced in 1976, OECD GLP introduced in 1981). Non-GLP studies will be evaluated for their reliability and suitability for probit derivation.

Acute inhalation toxicity studies are technically a relatively complex undertaking. Arts and Muijser (1999) described in detail which aspects of inhalation toxicity studies need to be reviewed to assess a study's technical quality (in Dutch). The following critical study characteristics should be described adequately and these quality requirements should be met to be eligible to qualify for 'A' status of the study:

- Purity and stability of the test substance.
- Head/nose only or whole body exposure.
- Dynamic test atmosphere and airflow (for head/nose only) or air exchange rate (for whole body).
- Use of vehicle, if other than air.
- Pressure distribution in the test system.
- Homogeneity of the test atmosphere in the test system.
- Actual concentration measurement (frequency, location, analytical procedure).
- Presence of aerosol. Measurement of aerodynamic particle size distribution if aerosol was or may have been present.

- Exposure duration, equilibration time of the chamber/test system.
- Test species, strain, sex and age.
- Number of animals per concentration, exposure duration or concentration-time combination.
- Post-exposure observation period and criteria for sacrifice of moribund animals.
- Calculated LC₅₀ value for the exposure duration(s).
- The appropriate use of suitable restraining tubes.

For most of the characteristics mentioned, detailed information needs to be provided in the probit TSD (Chapter 6). Arts and Muijser (1999) provide a list of additional, less critical characteristics contributing to study quality.

Most of the modern acute inhalation toxicity studies are conducted with a head/nose-only exposure setup. OECD GD 39 provides an extensive justification for the preference of head/nose only studies over whole-body studies in general, if technically well performed (cf. Annex 7.1). The main caveats mentioned in OECD GD 39 that may disqualify nose-only lethality studies include the restraining tube design, pressure distribution in the inhalation chamber and the airflow. All these factors are therefore carefully assessed to ensure that the study qualifies for probit development. Most of the more recent acute mortality studies used by the Panel are designed to adequately control most of the critical factors.

2.3.3 *Classification of overall study quality*

The Panel distinguishes three quality levels of animal data based on the quality of the study and the ability to derive a probit function from the data:

'A' quality studies

'A' quality studies provide sufficient data to assess all the parameters of a probit function: a , b ($=b^2$) and n . While the n -value can be estimated mathematically from a study with two exposure durations, the Panel will accept n -values derived from at least 3 eligible exposure durations.

Another data applicability criterion requires that the response rates, as determined in the study, cover the whole response range. In practice, at least two partial responses should be available.

In addition, the study should sufficiently meet the 'study requirements' listed above.

Finally, all study quality requirements listed above must be met for a study to qualify as an 'A' quality study. 'A' quality studies can be used to derive a PoD for probits without restriction.

'B' quality studies

Studies are qualified as 'B' quality in two cases:

1. Not all the parameters of the concentration-time-lethality function can be assessed, but the quality of the study is adequate and an LC₅₀ or LT₅₀ value can be estimated from the data using DoseResp or BMDS software (B1 studies);
2. All parameters of the concentration-time-lethality function can be assessed, but the study fails on quality issues such as test

atmosphere generation, concentration assessment, etc. (B2 studies).

'B' quality studies can be used, with restrictions, to derive a PoD for probits when no 'A' quality studies are available. When the pooling of data is appropriate, B1 studies can be included in the pooled data. B2 studies can be used, with restrictions, if there are no suitable A or B1 studies.

'C' quality studies

Studies should not be used for development of a probit function ('C' quality status) if the criteria for classification as an 'A' quality or 'B' quality study have not been sufficiently met. 'C' quality studies cannot be used to derive a PoD for a probit, but can serve as supporting evidence.

2.4 Acute toxicity data: characteristics and sources

The derivation of a probit function for lethality requires much more information than the derivation of a threshold value for lethality. The derivation of a threshold only requires that the lower end of the exposure-response curve can be estimated from the data and, in some cases, a high non-lethal exposure can serve as a PoD. For the derivation of a probit function, information must be available over the full concentration-time-response range from a single study. In some cases, data from different studies can be combined to cover the concentration-time-response range, but such a procedure requires a detailed explanation and rationale and is not preferred by the Panel.

For many substances, these high data demands cannot be met. In such cases, the absolute minimum data requirement for the derivation of a probit function is a high quality animal LC₅₀ value from a primary literature source for an exposure duration of between 10 and 240 minutes. With these data and some default assumptions outlined and justified in Section 3.2, a probit function can be derived. The Panel does not accept LC₅₀ values from secondary sources as a point of departure for a probit.

2.4.1 Literature search and selection of experimental data

The nature of the probit derivation places very high demands on the quality and completeness of the data. Primary literature has the highest likelihood that the information as specified above is available and has a lower likelihood of containing copying and interpretation errors. This is why primary literature is always preferred over secondary literature. Primary sources include, in order of preference:

1. Original toxicity study reports.
Reports of toxicological experiments containing all the raw data offer the most complete and reliable information to assess the chemical's toxicity and the study's validity, even though these reports do not undergo formal peer review outside the test laboratory. This situation requires that the TSD author perform the peer review, which requires a thorough understanding of inhalation toxicology.
2. Peer reviewed journals.

Publications in peer-reviewed journals often provide much less detailed information than the original study reports. The cautious approach, therefore, is to request the original study report. This may be problematic if the study is old or proprietary. The advantage of this type of publication is the peer review, if well conducted.

3. Non-peer reviewed journals.

Even though a peer review was not performed, the quality and usefulness of such publications can be adequate. The absence of peer review makes great demands with respect to the knowledge and experience of the TSD author.

Secondary literature sources include:

1. AEGL, EU RAR, ERPG, REACH and SMAC documentation or other documents of risk assessments from authoritative agencies (US EPA, ATSDR, occupational guidelines, etc.). These sources contain summaries of the relevant data and are essentially unsuitable as a surrogate for data from primary references. These documents can be a valuable source to identify available data.
2. Databases such as RTECS, IUCLID, HSDB and others contain summarized information on the acute inhalation toxicity of chemicals. The citations are known to contain errors and should, under no circumstances, be used as a PoD to derive a probit. These databases can be a valuable source to identify available data.

In addition to the aforementioned databases containing summaries of relevant data, other databases without such information can be used to identify primary data. Examples are ESIS, HPV, ToxNet, Medline and NTIS. This will be explained further under search strategy in Chapter 5. A list of Internet resources for literature research is provided in the appendix 'Internet literature resources'.

3 Evaluation of animal data to derive a probit function

Chapter 2 described data requirements and data sources for probit function development. This chapter justifies the approach prescribed by the Panel and describes a number of critical, underlying methodological (extrapolation) issues that play a role in the process of probit derivation from the available data. Figure 1 outlines the methodological process used to derive probit functions and outlines the relationship between the methodological steps.

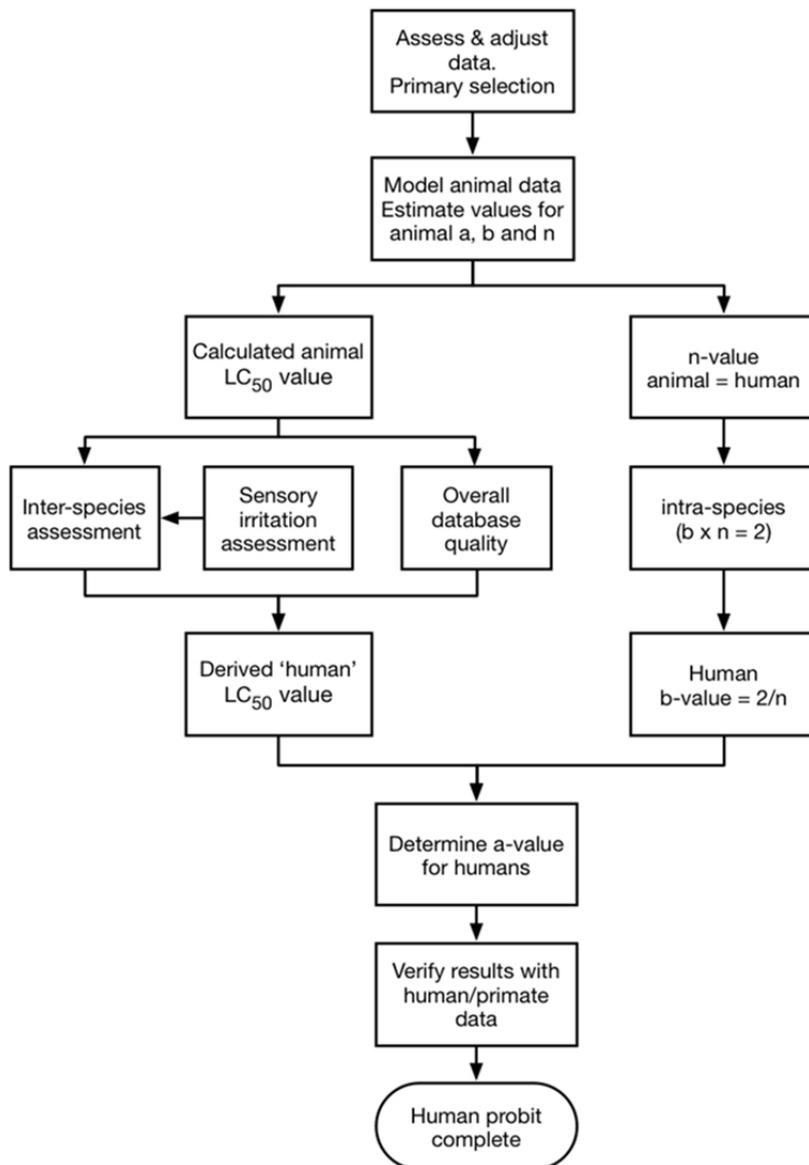


Figure 1 Flowchart for developing a human probit function from animal lethality data.

The derivation of a probit function that is believed to be valid for a heterogeneous human population requires an LC₅₀ value and an n-value from lethality data in animals as a starting point. The derivation of a valid animal LC₅₀ and n-value, therefore, are the first step in the process of human probit derivation. The following brief outline of the necessary steps to derive a human probit function from animal data (assuming an ideal C×t dataset) is provided to promote an understanding of the issues and the prescribed risk assessment approach:

1. Determine which information from which studies should be included in the modelling of animal data, adjusting for data from short exposure durations, test atmosphere characterization and datasets with only 0% and 100% responses.
2. The selected (and if necessary: adjusted) animal data are used to model values for the parameters a, b and n for the test species.
3. From the animal probit function, an animal LC₅₀ value (preferably for 30 minutes) and an n-value are calculated.
4. The animal n-value is assumed to be valid for humans as well.
5. The value of (b×n) as a metric for variability within a human population is usually assumed to be 2 (see Section 3.6). The b-value is usually calculated as 2/n.
6. To derive a human LC₅₀ value, AFs for inter-species extrapolation and overall study quality are applied.
7. The a-value is calculated with the derived human LC₅₀ value, b-value and n-value.

Each of the assessment steps is described and justified below.

3.1 Data adjustment, quality assessment and primary data selection

3.1.1 Overview of available data

The TSD presents a graph summarizing all the data reviewed by the Panel. The format previously applied for overview plots (as presented below) will be applied. Each animal species is represented by a different colour. A different marker represents each study within an animal species. Different response percentages are represented by shades of the primary colour.

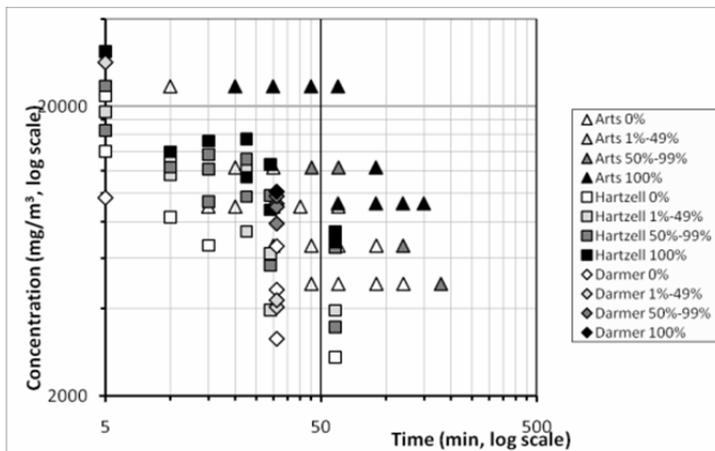
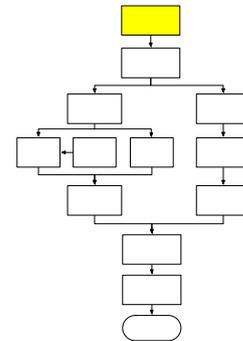


Figure 2 Example of a scatterplot with available animal data from three studies.

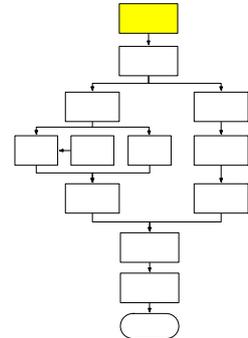
- other analytically valid information about the actual exposure concentration is available.
4. If animals are placed in the system *prior to equilibration*, an adjusted exposure concentration is calculated as follows:
 - a. The adjusted concentration is calculated as the average concentration $\langle C \rangle$ of the test atmosphere as follows:

$$\langle C \rangle = C_{\text{equilibrium}} \times (1 - t_{95}/3t) \times (1 - \text{EXP}(-3t/t_{95}))$$
 with
 - t_{95} = equilibration time (in min),
 - t = exposure duration (in min) and
 - $C_{\text{equilibrium}}$ = the equilibrium concentration.
 A new tab has been added to the probit worksheet to make this calculation.
 - b. The probit Panel has the option of performing probit calculations with the adjusted exposure concentration.
 5. For all data points for which the animals were placed in the system after equilibration and for data points for which the concentration was adjusted as proposed above, the goodness of fit of the short term data in the overall dataset is evaluated as follows:
 - a. Visual inspection of the data and/or the LC_{50} values for all time periods.
 - b. Calculation of the probit function with and without the short-term data and an assessment of the 30-minute LC_{50} from both calculations.
 - c. Statistical goodness-of-fit tests with and without the short-term data.
 Any substantial differences in the model outcome or statistics may indicate issues with the assumption that the short-term data are part of the same statistical distribution as the longer duration data, or that the response of the animal species to the exposure follows an atypical pattern. The issues underlying the deviation may include a different response mechanism or the inability of the probit model to describe short exposures.
 6. Expert judgment is exercised to determine whether or not data from animals that were placed in the inhalation chamber after equilibration and data for which the concentration was adjusted as proposed above can be combined in one statistical model (if applicable). In either case, the judgement provides a clear reason for the choice made.
 7. Based on the evaluation of the data, an expert judgment is made as to which of the data should be included in the probit calculation.
 8. In cases in which the only option to calculate a probit function from the data is to include questionable data, the option of reverting to the default model parameters remains open. This can be an option particularly in cases in which the default parameters produce a more conservative probit.

3.1.3 *Datasets with only 0% and 100% response*

The statistical models used by the Panel to calculate probit functions require that the datasets include response rates different from 0% and 100%. Sometimes, the steepness of the dose-response curve appears relatively small with respect to the spacing of exposure durations and/or

the concentrations chosen in a study. For steep dose-response curves, in particular, a 30-40% increase in exposure concentration or duration can result in an increase of the response from 0% to 100%. When the study is ongoing, an attempt can be made to test intermittent durations or concentrations, but more often the Panel is confronted with a dataset that includes only 0% and 100% responses without the ability to perform additional testing. This situation may occur for a range of exposure durations at a fixed exposure concentration in a C × t study, or a range of concentrations at a fixed exposure duration (as in the more common fixed duration LC₅₀ studies). Without data adjustment, such studies would not contribute to the chemical’s database and the Panel would be unable to calculate an LC₅₀ value from such a study.



The following data adjustment will be used to overcome a situation in which the plotted data suggest a well-fitting probit relation that the software is unable to calculate. Half of the animals tested at the highest 0% level and half of the animals tested at the lowest 100% level are assigned to a new fictitious exposure duration which equals the assumed LT₅₀ value (estimated as the GM of the two durations).

The same procedure would work for exposure concentrations in studies in which the exposure duration is fixed, which applies to many classical OECD 403 tests. In this case, the GM exposure concentration is calculated and some of the observations are assigned to that GM concentration.

The procedure is explained using the example below for a fictitious chemical with a dataset of lethal responses following an exposure to a fixed concentration for 5 different exposure durations as part of a C × t study in which a number concentrations were tested. The spacing of the exposure durations is by a factor of SQRT(2). For each concentration/ duration combination, 2 males and 2 females were tested.

Table 1 Hypothetical C × t data with only 0% and 100% response at a fixed concentration level.

	Actual exposure duration (min)									
	30		42		60		85		120	
Mortality males	s	s	s	s	†	†	†	†	†	†
Mortality females	s	s	s	s	†	†	†	†	†	†

s: survived, †: fatal

The observed data will be adjusted as follows:

1. Addition of an exposure duration that was not actually tested (50: the GM of 42 and 60);
2. Assign half of the animals from the 42 and 60 minute groups to the 50 min group;
3. Assume 50% lethality in the 50-minute group.

This adjustment would produce the following adjusted exposure-response data table:

- Target concentrations, the concentrations that the investigator plans to produce and for which all instrument settings are chosen. There is no verification of the extent to which this concentration has been actually achieved in the test system.

This section provides guidance to conclude whether studies only reporting nominal concentrations could still be used for evaluation and inclusion in setting probit relationships. Note: *target concentrations*, i.e. intended concentrations, can never be used for this purpose unless solid evidence is available that the target concentrations are likely to reflect the actual concentrations accurately.

Gases

For gases, the efficiency for dynamic test atmosphere generation is expected to be near 100%, hence in cases in which only nominal concentrations have been reported in a study, it is assumed that the actual concentrations will be close to the nominal concentrations reported if the gas and the dilution airflow have been measured correctly.

Dusts

In cases of dry aerosols, the generation efficiency can vary to a large extent, depending on the method used, the nature of the substance and the size of the particles in the substance to be tested. Because efficiencies can be very low (<5%), the Panel will not accept the use of a dust generation study for evaluation in cases in which only the nominal concentration has been reported.

Vapours

A vapour test atmosphere can be generated in two ways, viz. by:

1. Evaporation of the liquid, e.g. by passing (heated) air through the liquid substance,
2. Aerosolizing the liquid (nebulization), e.g. small droplets are being generated that will (partly) evaporate on their way to the test animals.

With regard to the evaporation method, the nominal concentration will be close to the actual concentration unless condensation has occurred. This can happen if the vaporization was performed at a higher than ambient temperature and the concentration generated was close to the saturation concentration at the temperature in the exposure chamber. This could result in loss of material.

With regard to liquid aerosolization, depending on the vapour pressure and the concentration tested, it can be estimated whether the test atmosphere would consist mainly of vapour or mist. In case of vapour, the nominal concentration is expected to be close to the actual concentration; in case of mist, the actual concentration can be much lower than the nominal concentration.

Differentiation between vapour and mist is therefore made on the basis of the saturated vapour concentration (SVC) for a volatile substance, and can be calculated as follows:

$$SVC \left(\frac{mg}{L} \right) = 0.412 \times MW \times Vapour \ Pressure$$

Equation 5 Calculation of the Saturated Vapour Concentration (SVC) in mg/L. MW = molecular weight in Dalton, Vapour pressure in kPa.

A substance generated at a concentration well below the SVC will mainly consist of vapour, whereas a substance generated at a concentration close to or above the SVC will consist of a mixture of vapour and mist.

Suppose we have a substance with a molecular weight of 96 and a vapour pressure of 1 kPa; this would mean a saturated vapour concentration of: $0.412 \times 96 \times 1 = 40$ mg/L.

If the concentration tested is 10 mg/L, it could reasonably be assumed that most of the test atmosphere would consist of vapour and, as such, the nominal concentration would be close to the actual concentration.

Although in principle a vapour concentration close to 40 mg/L for the substance mentioned above could be generated, this requires a well-designed dynamic generation system that avoids any condensation. In addition, vapour generation at the saturation concentration may not be feasible for all substances, particularly for those having a low rate of vaporization. Therefore, such studies must be evaluated with care and the actual concentration cannot be considered to be close to the nominal concentration without detailed information on the method of generation. For volatility, the scheme in Table 3 is used (COSHH essentials):

Table 3 COSHH scheme to assess volatility based on vapour pressure.

Vapour pressure	kPa
Very/extremely high	> 13.3
Moderate	2.7-13.3
Slightly	0.13-2.7
Very low	< 0.13

If only boiling temperatures are available, the graph in Figure 3 is used:

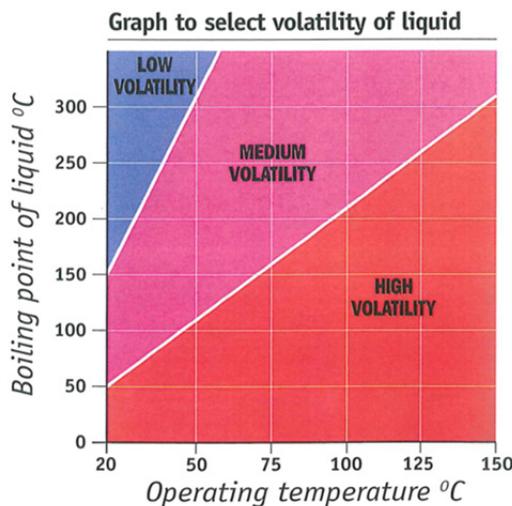


Figure 3 Assessment of volatility of liquids by boiling point and operating temperature.

In some cases, therefore, particularly for data-poor chemicals, B2 studies may be included in the data selection for further analysis. For chemicals with a larger database of studies meeting A or B1 criteria, B2 studies will usually not be considered in the further analysis. The presence or absence of an OECD/GLP certificate for a particular study is not a reason in itself to include or exclude a study from further consideration. The Panel will always make its own quality assessment based on the available information.

The studies selected to carry over to the next step in the procedure are selected on the basis of the criteria outlined above and the inclusion or exclusion from further analysis is clearly justified in the study description.

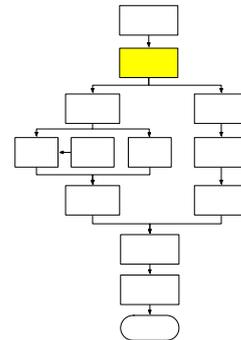
3.2 Stepwise approach to evaluate the animal data

The following procedure is applied to select the data for the final analysis of the animal concentration-time-lethality curve, which will preferably produce 30-minute LC₅₀ values and an n-value as a POD for the human probit function.

3.2.1 *Probit calculation for individual eligible studies*

For each of the studies that passed the steps outlined in Section 3.1, the concentration-time-response (C×t) data in the animal experiment are analysed with a multivariate probit model. Single exposure studies with exposure durations between 10 and 240 minutes are preferred. Concentration and exposure duration are transformed logarithmically (based on the natural logarithm). Deviations from this principle must be motivated. Studies with a range of exposure concentrations and durations can be designed as:

1. A series of LC₅₀ experiments, in which the concentration-lethality curve was assessed for a number of exposure durations.
2. (One or) a number of fixed concentration levels with varying exposure durations (old LT₅₀ studies or recent C×t studies respectively).



Both types of datasets can be analysed identically. For exposure durations of less than 3 times t₉₅, the calculations are performed with the adjusted exposure concentrations.

If only concentration-response data (for one exposure duration) or time-response data (for one exposure concentration) are available, these can be analysed following the same strategy and using the same software. Obviously, if the exposure duration is not equal to 30 minutes, the LC₅₀ and comparison of sex differences must be calculated for the tested exposure duration.

Sometimes information on a covariate (such as sex) is available for analysis. Depending on the circumstances, the covariate can be included in the analysis. The analysis of sex differences in the response is mandatory and can follow two approaches:

1. An analysis with male and female data and sex as a (dummy) covariate. This approach enables easy testing of the statistical significance of any sex difference. Based on this analysis, an LC₅₀ for each of the sexes can be calculated.
2. An analysis of the data of each sex separately and the calculation of an LC₅₀ for each sex.

Sex differences are disregarded and data are pooled, unless the following conditions are met:

- The 30 minute LC₅₀ differs more than a factor 2 between the sexes;
- The sex difference is statistically significant.

If a sex difference is found and there is no clear explanation for gender-related differences, then data from the most susceptible sex are used to derive the human probit function. No gender-specific probit functions are derived.

Sometimes the inclusion of cross-terms to assess statistical interaction between parameters or a threshold response level (concentration or time) can improve overall model fit. The Panel only accepts the results of such complex models with a very strong, mechanistically based rationale (i.e. it should not be used merely for model fit improvement).

Software programs available for calculating the model parameters of the presented multidimensional probit function are Doseresp (Ten Berge, 2015) and the Benchmark Dose Software BMDS (US EPA, 2015). These software packages can be downloaded and installed on a local computer, and produce almost identical parameter estimates. These software packages are the preferred statistical modelling tools to derive probit functions. Besides the software mentioned above, these probit models can be built using most statistical software packages.

The objective of modelling the concentration-time-response function is to assess the values of the parameters a , b and n . Using the derived substance-specific model, the animal LC₅₀ value is determined. Such a calculation must be made for all 'A' and 'B' quality studies (see Section 2.3).

If applicable, separate probit functions are calculated for different sexes and animal species. In a study with multiple species, this step treats the data from each species as a separate study. Each study description in the TSD will present:

1. A table with study characteristics to aid in deciding about the study quality and a clear rationale for the assigned study quality.
2. A table presenting the raw data, specified by observed (and adjusted, if applicable) exposure concentration and duration, the group size and number of fatalities, and the animals' sex and species.
3. The probit function per sex, with all data pooled (using a dummy to identify the groups and an indication of the statistical significance of the dummy variable).
4. A graph including:
 - a. all responses for the smallest possible subgroups;

- b. the Maximum Likelihood Estimate (MLE) of the LC₅₀ curve over the tested time range, if possible.
5. LC₅₀ values for 10, 30 and 60 minutes for each of the sexes and for the sexes or species combined.
6. An evaluation of the significance of sex differences and a rationale for combined or separate analysis of the data.

3.2.2 *Exploring the analysis of pooled data*

Sometimes acute inhalation exposure data are available from more than one study or more than one animal species. In such cases, the procedure below describes whether or not multiple studies can be pooled in the analysis.

As a next step to evaluate the total body of data, lethality data from single species are presented as follows per species:

- For each species, a single table listing the probit functions and the LC₅₀ values for 10, 30 and 60 minutes in a way that allows identification of individual studies.
- For each species, a single figure with data points and an assessment of the LC₅₀ values over time in a way that allows identification of individual studies.

If data from two or more species are available, the species-specific tables and figures mentioned above are then combined into a single table and figure to represent all data that passed the quality criteria from step 2. Since allometric scaling between species is believed to be implicitly included when comparing exposure concentrations of inhalation studies with different species, no further adjustment is needed.

Analysis of pooled data

Based on the numerical and visual information, an expert-based assessment is made to determine whether the body of data is likely to converge. Convergence is believed to be likely if all available LC₅₀ values for the same exposure duration fall within one order of magnitude from each other and the slopes of the LC₅₀-time curves are roughly parallel.

Three situations may occur:

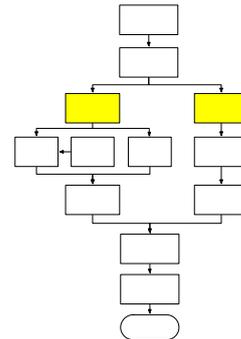
1. Data from different studies and different species (including species other than rodents) show convergence. In this case, the differences between species are probably limited and animal data are generally thought to reflect the LC₅₀ value and n-value in humans well. In these cases, the LC₅₀ values and n-values of all qualifying studies are used to assess a PoD for the human probit calculations.
2. Clear differences are apparent in susceptibility or C x t relationships between species. In this case, discussion should focus on which species and/or data are the most relevant for derivation of the human probit function. The study selection criteria may include anatomical or physiological resemblance and (dis)similarities in metabolism of the animal species in relation to humans, the number of animals in the study or other aspects of study robustness, relevant exposure duration, etc. In general, the response levels themselves (e.g. LC₅₀ values) do not qualify as a selection criterion.

3. If the assessment above does not produce a logical species or study that should be used for human probit derivation, no information is available to define which data are the most relevant for the derivation of a human probit function. In this case, the most susceptible species is used for deriving a probit for humans as a default method. A sufficiently justified deviation from this procedure is possible and will be made on the basis of expert judgment.

For all three possible outcomes, one or more studies may qualify to serve as a PoD for the human probit calculations. If two or more studies qualify equally, the LC₅₀ value and n-value can be estimated as a weighted average of the eligible LC₅₀ values and n-values. The weight is inversely proportional to the confidence interval of the LC₅₀ value or the n-value from the study, as outlined in the probit excel worksheet. The data selected to calculate the LC₅₀ values and n-values as a PoD for human probit assessment are selected on the basis of the criteria outlined above and the inclusion or exclusion from further analysis is clearly justified.

3.2.3 Derivation of a point of departure for the human probit function

A human probit function will be derived with 2 points of departure (PoD): an LC₅₀ value for a specified exposure period and an n-value. The studies and data that were selected in Section 3.2.2 are used to derive these parameters. Data from rat studies are used frequently because of the large database and because rats are the preferred species in the OECD 403 test guideline. The TSD format provides standard text for the identification of the key study (studies), the LC₅₀ value and a rationale for this choice. The DoseResp worksheet template (available from the RIVM website) can be used to calculate the (weighted) LC₅₀ value.



LC₅₀ values from secondary sources or databases without raw exposure-time-lethality data are not acceptable as a PoD for probit derivation.

In the absence of inhalation toxicity data, probit derivation from oral toxicity data will only be considered if:

- Human data establish that the mode of action from inhalation exposure is driven by systemic toxicity;
- It can reasonably be excluded that serious respiratory system toxicity precedes death in humans;
- A validated PBPK (or PK) model is available to support the route-to-route extrapolation.

The n-value calculated from the animal data is assumed to be a chemical-specific characteristic of the exposure-time-response relationship and is assumed to be valid for humans, in part because the lack of a credible alternative. All of the data selected for the final analysis using the criteria outlined in Section 3.2.2 are used in the final calculation of the probit parameters n and b.

For the selection of the n-value, three options are open:

1. If more than one eligible C×t or other studies are selected using the criteria outlined in Section 3.2.2, a justified choice is made to either derive the n-value from a single study or as a weighted average n-value of multiple studies. The weight is inversely proportional to the 95% confidence interval of the n-values. A calculation tool is included in the calculation template that is available from the RIVM website.
2. If only one eligible C×t study is available (i.e. with ≥ 3 exposure durations and ≥ 3 exposure concentrations/duration and sufficient variation in lethal responses), the n-value is selected from this C × t study.
3. If no C×t study or set of other eligible studies is available to derive a probit function, an n-value derived for a structurally similar and, mechanistically, similarly acting chemical can be used. Substitution of the n-value with an n-value of another compound will only be accepted if the substitute n-value itself is robust and if the validity of the substitution is sufficiently justified.

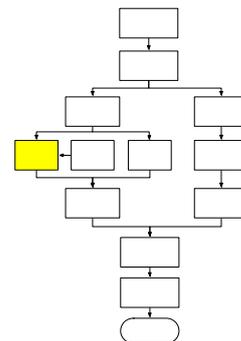
If none of the three conditions above apply, an n-value of 2 is assumed for humans over the whole range of exposure durations (10 minutes to 8 hours).

Based on the assumption that $(b \times n) = 2$, the value of b can be calculated as $2/n$. See also the explanation given in Section 3.6 about the product $(b \times n) = 2$ based on assumed intra-species variability.

For each of the available acute toxicity studies, an LC_{50} value is calculated, being statistically the most reliable response estimate (preferably for a 30 minute exposure). If the studies are not equally suitable, the LC_{50} value of the most appropriate study is used.

3.3 Interspecies extrapolation

For obvious reasons, human probit functions will generally be based on lethality data in animal species. Species will vary in susceptibility and therefore show differences in response. If no substance-specific data are available on differences in susceptibility between experimental animals and humans, which is usually the case, the application of an interspecies extrapolation factor is required. The point of departure (PoD) for the derivation of a human probit function will be an experimental animal LC_{50} value. To this value, an interspecies extrapolation (assessment) factor will be applied to derive an estimated human LC_{50} value.



Differences between species can be subdivided into two different aspects: differences in body size and differences in biokinetics and biodynamics. Experience reveals that equal doses of a chemical (expressed as mg/kg of body weight) generally result in more severe toxic effects in larger animals, an observation that cannot be explained by only assuming a greater susceptibility. It appears that a biological response following exposure to a xenobiotic substance depends on the

rate of physiological processes, e.g. the metabolic rate (US EPA, 1992; Kalberlah and Schneider, 1998). The rate of these processes is inversely related to body size. Differences in body size can be accounted for by allometric scaling, which refers to the empirical observation that several anatomical body compartments and physiological processes scale according to the allometric equation:

$$y = a \times BW^p$$

Equation 6 Allometric equation

in which y is some biological response (here the LC₅₀), a is a constant of proportionality, BW is body weight and p is the allometric scaling power (Adolph, 1949). A certain dose in animals can be extrapolated to a (toxicologically) equivalent dose in humans by dividing this dose by the allometric scaling factor (ASF) that can be calculated by:

$$ASF = \left(\frac{HumanBW}{AnimalBW} \right)^{1-p}$$

Equation 7 Determination of the Allometric Scaling Factor

The allometric scaling factor should not be regarded as a 'safety factor' and an additional factor may be needed to account for interspecies differences in biokinetics and dynamics.

Several studies on interspecies differences in response following oral exposure have been published, in which NOAELs for a chemical observed in different animal species were compared (Dourson et al., 1992; Rennen et al., 2001; Schneider et al., 2004; Travis and White, 1988; Vermeire et al., 1999; Watanabe et al., 1992). These studies show that, after application of an ASF, the animal species were on average equally susceptible.

Rhomberg and Wolff (1998) have a different point of view, suggesting that single and repeated dosing regimens may have different scaling properties for severe toxic effects. They reported a stronger support for cross-species extrapolation on a body weight basis (p=1) for single exposures. But they compared LD₅₀ values obtained from the RTECS database that – as also acknowledged by Rhomberg and Wolff – only reports the lowest reported LD50 in literature for any species. Schneider et al. (2004) showed that this bias results in a shift towards a higher value for p (closer to 1). Using geometric mean LD₅₀ values, they concluded that cross-species extrapolation in single exposure regimens is also in line with allometric scaling (p=0.75).

A study in which rats and mice were compared for differences in susceptibility based on benchmark dose (BMD) values confirmed these findings (Bokkers and Slob, 2007). In addition, a study on a comparison of different kinetic parameters in different animal species (e.g. C_{max}, t_{1/2}) showed similar results (Kalberlah and Schneider, 1998). Overall, the evidence leads to the conclusion that, after application of an allometric

scaling factor, the ratios of NOAELs approximately follow a lognormal distribution of around one. Although it can thus be stated that, on average, animal species will be equally susceptible, without appropriate data it cannot be determined whether this holds for a specific chemical or that either of two species is the most susceptible.

Most human risk assessment frameworks focus on lifetime oral exposure. In these frameworks, such as the derivation of the acceptable daily intake (ADI), a default interspecies factor of 10 is typical. In these frameworks, scaling from an animal species to humans is based on body weight and no separate step for allometric scaling is applied. Differences related to differences in metabolic rate can therefore be considered to be an implicit part of the default factor of 10. The metabolic rate allometric scaling factor (with $p = 0.7$) is approximately 5 for extrapolation from rats to humans (RatBW = 0.3 kg; HumanBW = 70 kg), leaving a remaining factor of 2 for differences in biokinetics and biodynamics (Bokkers and Slob, 2007).

In several frameworks, similar views exist as to interspecies extrapolation, whereby the ASF is set at 4 for the rat-to-human extrapolation and a factor of 2.5 is taken to account for remaining differences related to kinetics and dynamics – together resulting in a factor of 10 (e.g. REACH guidance). If smaller species are concerned, a higher test-species-to-human ASF is applied (e.g. for mice an ASF of 7).

Interspecies extrapolation for single inhalation exposures in the lethal dose range

The point of departure (PoD) for the derivation of a human probit function will be an experimental animal LC_{50} value. Allometric scaling is not necessary for inhalation exposure, since the respiratory volume already scales between species according to metabolic rate, when air concentrations expressed as mg/m^3 or ppm are extrapolated between species, as is the case for the extrapolation of a rat LC_{50} to a human LC_{50} . A phenomenon that occurs in rodents is the elicitation of the nervus trigeminus, which in rodents (as opposed to humans) produces a reflex post-inspiratory apnoea, resulting in a decreased breathing rate and thus minute volume, often expressed in terms of the RD_{50} value. Although this phenomenon is part of the interspecies discussion, it will be dealt with in Section 3.4.

Although adequate human data are not available for lethality endpoints, it is assumed that the principle of equal susceptibility also holds true, on average, for the susceptibility of humans compared to animals on the condition that animals and humans are, on average, biologically the same. Therefore, if adequate data on insight into interspecies differences (including humans) are not available for a specific substance, then there is uncertainty as to whether humans will be more or less susceptible than the animal species that provides the point of departure. From a precautionary point of view, therefore, it is assumed that the average human, by default, may be more susceptible than the animal species producing the PoD (generally the rat), unless substance-specific data indicate otherwise.

The question then remains of what the default value should be for the additional factor required to account for differences in biokinetics and biodynamics for single exposure via inhalation.

In Table 5 below, the default values used in several frameworks are given, such that the default values differ for systemically and locally acting agents. In general, for systemic effects a default value of 3 is applied to cover remaining interspecies differences and therefore uncertainty in the extrapolation from the average animal to the average human. For local effects (also referred to point of entry effects), a lower default value is often considered. Since the remaining differences for kinetics and dynamics are not likely to play a role for locally acting agents, a factor of 1 is generally proposed.

Table 5 Overview of default interspecies and intraspecies assessment factors in setting limits for acute inhalation exposure^a

	Interspecies assessment factor	Intraspecies assessment factor
AEGL SOP (2007)	10	
Acutex (AETL, 2008)	systemic effects: 3 local effects: 1	Systemic effects: 3 ² Local effects: 3 ³
Dutch Intervention values	3	3
ECETOC (Emergency Exposure Indices)	1: Humans are assumed to be as susceptible as animals	1: Humans are assumed to be as susceptible as animals

^a ECHA and OECD presented methodologies for setting limits for acute exposures, however these do not concern lethality.

The U.S. Department of Homeland Security (DHS) proposed mode-of-action-specific interspecies factors. The classification of chemicals based on toxicodromes (e.g. (anti)-cholinergic, coagulants, opioids, lower and upper pulmonary) for medical response purposes was evaluated for its usefulness in developing substance-specific interspecies assessment factors based on mode of action (US DHS 2012). However, data to support a specific assessment factor for the listed mode of actions were not found in the documentation.

For modes of action related to straightforward toxicological endpoints, such as tissue destruction caused by corrosive chemicals (e.g. HCl, HF, acrolein) or inhibition of vital physiological functions such as oxygen utilization (CO, HCN), the differences in toxicodynamics across species may be limited. Since toxicokinetics may have already (to a large extent) been covered by allometric considerations, a default interspecies factor of 1 may suffice. Toxicity in primates compared to rodents with a limited number of volatile corrosive substances (monomethylhydrazine, HCl, HF) appear to indicate that primates can be less susceptible to such

² Factor of 1 used for AETL-3a

³ Factor of 1 used for AETL-3a

corrosive substances, which would support an interspecies factor of 1 (Ter Burg et al., 2013).

Conclusion

Overall, there is a consensus that the average human is equally susceptible as the average animal, but it is also acknowledged that there may be differences between animal species and humans. From a precautionary point of view, therefore, it is assumed that the average human, by default, may be more susceptible than the animal species producing the PoD (generally the rat), unless substance-specific data indicate otherwise. A default assessment factor of 3 is used for the animal LC₅₀ value to obtain the human equivalent thereof, covering the uncertainty related to possible remaining differences in biokinetics and dynamics between animals and humans.

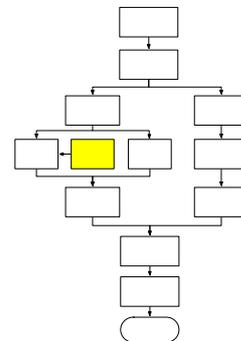
Table 6 Default interspecies and intraspecies assessment factors for Dutch Probit Panel

	Interspecies assessment factor	Intraspecies assessment factor
Dutch probits (RIVM, 2015)	3 ⁴	3 ⁵

The default factor of 3 is also in line with other frameworks focused on acute inhalation exposures such as in the derivation of the Dutch Intervention Values, AETLs and the AEGLs. ECETOC suggests not applying an assessment factor for interspecies differences, based on the consideration that animals and humans, on average, are equal. In the AETL framework, for local effects a default factor of 1 is proposed. It is concluded that, if the local effects are portal of entry effects, an assessment factor of 1 can be used if data supports this value. In all other cases, modes of action or types of effect, a deviation from the default factor of 3 must be supported by sufficient substance-specific evidence.

3.4 Assessment of sensory irritation in animals

Sensory irritants are defined as substances that stimulate free trigeminal nerve endings in the corneal, buccal, laryngeal and nasal mucosa and produce a stinging or burning sensation in humans. This perception can be accompanied by irritation of the eyes and throat and coughing from stimulation of laryngeal nerve endings (Bos et al., 1992). Within the context of sensory irritation, the trigeminal, glossopharyngeal and vagal nerves are the most important. The boundary of sensory innervations between the nose, nasopharynx and larynx are rather diffuse and the areas innervated by the three respective nerves overlap. These nerves respond to airborne chemicals and mediate irritant responses. In addition, these and other nerves are involved in the effects of sensory irritants on heart rate (vagal nerves) and blood



⁴ Sometimes 1 for locally acting substances.

⁵ $(b \times n) = 2$ in a normal population implies that $LC_{50} / LC_{01} = 3$

pressure (splanchnic nerves). But the trigeminal nerve appears to be the only nerve involved in a reduction in respiratory rate. Since this effect is directly important for the derivation of a human probit function from animal data, as will be discussed below, the present section focuses on sensory irritation resulting from stimulation of the trigeminal nerve.

In rodents sensory irritants induce, among other effects, a reflex post-inspiratory apnoea resulting in a decreased breathing rate and thus minute volume. Through body plethysmograph recordings, it can be verified whether a chemical stimulates only the trigeminal nerve or also other nerves that affect respiration via other mechanisms. In mice, a decrease in respiratory rate of 80-90% can be provoked for a significant period of time. Alarie (1973) developed a test in mice and proposed that the RD_{50} , the concentration that provokes a 50% decrease in respiratory rate, be used as a parameter to compare the sensory irritating properties of substances. Exposure durations for such a test should be (at least) 30 minutes (ASTM 2004). In the literature, the exposure duration has been found to range from 5 minutes up to 4 hours.

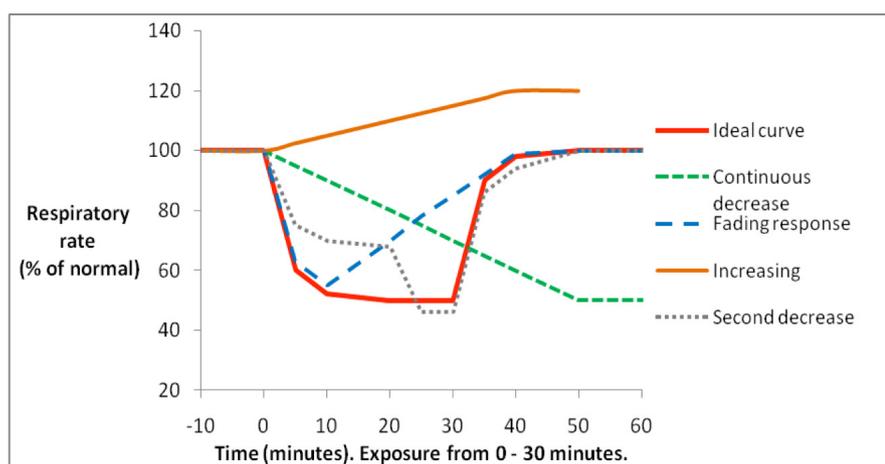


Figure 4 Sensory irritation response curves

The typical response pattern of an RD_{50} test is displayed as the red-coloured curve in Figure 4: a rapid decrease in respiratory rate followed by a plateau (during exposure), followed by a rapid increase after cessation of exposure. Yet in many cases, non-typical response patterns have been observed (Figure 4), which may indicate that response mechanisms other than trigeminal nerve stimulation drive the reduction of respiratory rate. The plethysmograph recordings may confirm this. In many publications, only the RD_{50} value is reported, which seriously limits the ability to assess if a reduction in respiratory rate was due to sensory irritation.

Stimulation of the nervus trigeminus generally does not induce major changes in the tidal volume, meaning that the inhaled breath, and thus the chemical, will reach the alveoli. Expiration is delayed and the inhaled air remains in the lungs for a longer duration. The inhaled chemical is thus available for absorption or induction of local effects in the lower regions of the respiratory tract. The duration of the post-inspiratory apnoea appears to be concentration-related. The result of this effect is

that the respiratory rate decreases and the total inhaled amount of a chemical decreases with increasing concentrations.

Sensory irritation is unrelated to the toxicity profile of a chemical and may start at a concentration at which no other effects are observed or at (near-) lethal concentrations (Bos et al., 1992, 2002). If sensory irritation occurs at a concentration below lethal concentrations, the interpretation of a rodent LC₅₀ that is calculated from external air concentrations is not straightforward. The animal LC₅₀ value is, in this case, biased upwards and may underestimate human toxicity since humans are much less capable of reducing their minute volume similarly for an extended duration of exposure (Arts et al., 2006). Hence, the derivation of the appropriate point of departure to extrapolate to humans requires consideration.

It is recognized that the anatomical and physiological differences between the rodent and human respiratory tracts are substantial. Nevertheless, despite differences in defence mechanisms, lethality data obtained with rodents in acute toxicity studies under normal conditions are generally considered to be predictive for the response in humans. From that point of view, a decrease in respiratory rate can be regarded as an additional defence mechanism in rodents, which presumably is less present in humans. Although structure activity relationships are reported within homologous series of chemicals, no generic rule can be derived to predict sensory irritation and to verify how humans may react to these chemicals. Simultaneously with the respiratory depression, other changes will occur in the rodent, e.g. a lowering of the body temperature and a lowering the basal metabolic rate. The effect of these changes on the internal exposure to a chemical and on the toxicity is unknown and difficult to predict without knowledge of the mechanism of action underlying the mortality.

Another issue that needs consideration is the exposure duration. The recommended standard exposure duration for the sensory irritation test is 30 minutes (ASTM, 2004), but many RD₅₀ values are obtained with shorter exposure duration. Yet, in acute toxicity studies, exposure durations may vary from 10 minutes up to 8 hours. Since mortality will occur at lower concentrations with increasing exposure duration, the effect of respiratory depression on the study outcome may depend on the exposure duration of the acute toxicity study. For instance, for a specific chemical, a 30-minute RD₅₀ for a chemical may be lower than a 15-minute LC₅₀, but higher than a 4-hour LC₅₀. Therefore, sensory irritation might have affected the 15-minute LC₅₀, yet have affected the 4-hour LC₅₀ less or not at all. But then, the value for *n* derived from such a study may also be affected and steered towards a lower value (bias towards higher LC₅₀ values for shorter exposure durations). Therefore the effect of sensory irritation should be evaluated both for LC₅₀-values as well as for a value for *n* obtained from animal studies, taking into account the exposure duration at which an RD₅₀ is derived compared with the exposure durations in the acute toxicity study (or studies).

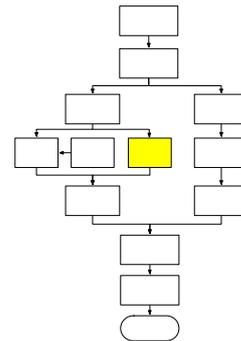
As discussed, several issues must be considered and a generic rule on how to account for respiratory depression in an animal experiment is difficult to define. Nonetheless, the phenomenon is considered to

potentially affect the outcome of an acute toxicity study in such a way that it might hamper the derivation of the most appropriate point of departure and of the value of n from an animal study – two key parameters in the derivation of a human probit function. Considering all uncertainties involved, the Panel will not derive a separate default assessment factor for sensory irritants, but will evaluate the impact of an available RD_{50} value within the context of interspecies extrapolation, regarding both the most appropriate point of departure and the value for n . The Panel favours RD_{50} values derived from studies with exposure durations of at least 10 minutes in which a plateau response clearly has been reached. On the condition that the respiration is affected only via trigeminal nerve stimulation (i.e., 'pure' sensory irritation, which should be verifiable from the available data), the decrease in respiratory rate (as depicted by an RD_{50}) will further be weighted as one of the possible interspecies differences to be considered in the final choice of an interspecies extrapolation factor.

3.5 Assessment and adjustment for overall quality of the study

The Assessment Factors (AFs) introduced above are designed to adjust the derived animal PoDs for frequently occurring or methodologically foreseeable issues. Since animal inhalation experiments are complex and sometimes the only option is to use a key study with significant shortcomings, a general assessment factor for overall study quality has been introduced. Examples of situations in which this AF has been applied are:

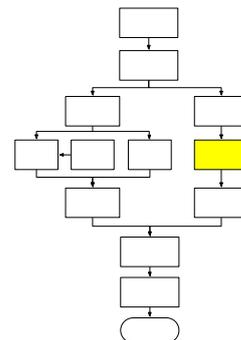
- The value for n and/or the LC_{50} cannot be determined with sufficient certainty (e.g. if data are available for just 1 duration of exposure).
- Database shortcomings.
- The probits are based on the probit function of a hydrolysis product, but it is uncertain whether the particular hydrolysis product's toxicity suffices to predict the parent compound's toxicity.
- The key study has other significant shortcomings, such as low air exchange rate.



In such cases, an additional AF of 2 (or, in rare cases, 3) can be applied to the LC_{50} . Every case in which an AF is applied for the total study quality needs a specific and clear justification. This AF should not be used for issues that have been covered by the previous AFs.

3.6 Intra-species extrapolation

Differences in response between humans will be reflected by the slope of the dose-response curve; the steeper the slope the less variability in susceptibility between individuals. Since an inbred strain of one or more species is tested in animal experiments, the animal dose-response curve is usually rather steep. However, the slope in the animal experiment provides no information about the inter-individual variability in humans. A reasonable



assumption is that the slope of the human probit function will be less steep than in the animal experiment. No data are available to estimate the inter-individual variability in humans regarding lethality.

The probit function can be expressed as:

$$Pr = a + b \ln (C^n \times t) \text{ (Eq. 2)}$$

The steepness of the slope of the probit function can be estimated from the ratio of the concentrations producing responses of, for instance, 99% and 1%. For a certain exposure duration, this ratio can be calculated as:

$$\frac{C_{99}}{C_{01}} = \exp \left[\frac{(Pr_{99} - Pr_{01})}{b \times n} \right]$$

Equation 8 *The ratio of concentrations with a 99% and 1% response.*

such that C_{99}/C_{01} equals the ratio of the concentrations producing 99% and 1% lethality, respectively, and Pr_{99} and Pr_{01} are the probit values associated with 99% and 1% lethality, respectively ($Pr_{01} = 2.6737$ and $Pr_{99} = 7.3263$).

From Equation 8, it can be deduced that the ratio C_{99}/C_{01} depends on the product ($b \times n$). In the 2005 version of the 'Green Book' (VROM 2005), a fixed value of 2 for the product ($b \times n$) was considered to yield acceptable and appropriate results. If the product ($b \times n$) equals 2, it can be calculated from Equation 8 that the C_{99}/C_{01} ratio will be approximately a factor of 10, which corresponds with a 10-fold difference in susceptibility between the 99 and 1 percentile points in the human susceptibility distribution. Lower values of ($b \times n$) (i.e. a less steep slope) will correspond with a larger range in the inter-individual variability; for instance a value of 1 corresponds with an approximate 100-fold difference between the 99 and 1 percentile points in the human susceptibility distribution.

As previously mentioned, there are no data available to provide a scientific basis for any value for the product ($b \times n$) in a human probit function. As a pragmatic choice, therefore, the use of a default value of 2 for the product ($b \times n$) in the human probit function will be continued, unless appropriate data are available which clearly justify a deviation from this principle.

For comparison, an intraspecies extrapolation factor is introduced to cover differences in susceptibility between the average human and the susceptible human. Hence, a ten-fold difference in response (i.e. $C_{99}/C_{01} = 10$) would correspond with an intraspecies extrapolation factor of 3 ($\approx \sqrt{10}$) (LC_{50}/LC_{01}) in the human target population. An intraspecies extrapolation factor of 3 is often used in other frameworks dealing with acute inhalation exposures, such as in the derivation of the Dutch Intervention Values, AETLs and the AEGs. The REACH guidance and OECD document on the derivation of the Acute Reference Dose use a default of 10. In the AETL framework, an intraspecies factor of 1 is

suggested for AETL-3a, but the scope of AETL-3a is the average healthy man, not taking into account susceptibility variations in the general population (see Table 6 for an overview of the default values in several frameworks). A default value of 2 for the product ($b \times n$), therefore, is consistent with the defaults used in other frameworks. The difference, compared with these other frameworks, is that the intraspecies AF for the derivation of probits is applied by defining that: $(b \times n) = 2$, rather than applying a factor of 3 to a certain PoD.

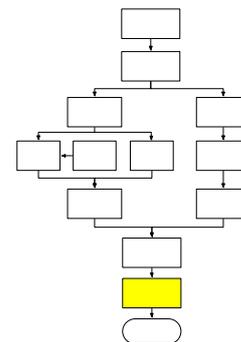
In rare cases, it may occur that the animal $b \times n$ product is smaller than 2, implying a greater variability in lethality response in the animal than is assumed for the general human population. Furthermore, it can reasonably be assumed that the variability in susceptibility in the human population will be larger than it is in an inbred experimental animal strain. The variability in lethal response, however, results not only from the intrinsic variation in susceptibility in the test animal, but also includes other factors that influence the response, such as experimental conditions, measurement errors etc. Correcting for these factors in a quantitative way is not possible in practice. Moreover, the baseline principle is that the animal probit relation (i.e. $b \times n$) does not contain information on the human product of $b \times n$. As a result, the default approach of $b \times n = 2$ will be maintained even in cases where the animal $b \times n < 2$ (see Annex 7.3 to this chapter).

Conclusion

The intraspecies variability in humans is assessed by assuming that $(b \times n) = 2$ and that the n -value in the animal model is valid for humans. Based on these assumptions, the b -value is calculated as $b = 2/n$. The human variability is assumed to be equal for all substances, which in reality may be an overestimation or an underestimation of the human lethality response.

3.7 Verification with primate data

The standard procedure to derive a probit function for human lethality following a single acute inhalation exposure requires a number of assumptions and extrapolations. As a part of the derivation process, the Panel actively searches for and evaluates acute inhalation toxicity data from humans and primate species for referencing and benchmarking purposes. Such data may include, but are not limited to, controlled laboratory exposures, regular and accidental workplace exposures and experiments with primates.



There is a theoretical possibility that human or primate data may suffice for the derivation of a probit function for lethality following a single inhalation exposure. In most cases, the study size and/or characterization of the exposure (concentration and duration) preclude the application of such data for purposes other than referencing and benchmarking.

It is possible, and has been shown to happen on a few occasions, that rigorous application of the guidance may result in a probit function that is not supported by data on lethal and non-lethal responses in humans or primates. Such data can include, but are not restricted to, human experimental studies on irritancy or discomfort produced by airborne exposure, data on accidents with well-documented human exposure, the therapeutic use of chemicals (or a precursor) and experimental studies in primates. In rare cases, data on repeated exposures in rodents may also provide evidence against the reasonableness of the probit function.

If lethality assessments produced with the probit based on rodent data are incompatible with experimental or observational data from humans or primates, a modification of the probit function based on rodent data will be considered.

4 Discussion and conclusions

The methodology used to derive a probit function for human lethality following a single acute inhalation exposure has been drastically revised, as compared with the previous version (VROM 2005). The results of the discussion at the international meeting to review the draft methodology have helped to further improve the methodology (Ter Burg et al., 2013). The toxicological evaluation of data and the application of quality criteria to toxicological data have become more prominent elements of the derivation process. Draft probit functions, included in a standardized probit TSD presenting all the prominent data, are evaluated and technically adopted by the Dutch Expert Panel on probit functions. This Panel will not accept draft probit functions based on the previous version of the methodology. The science and assumptions underlying the current approach are described in detail in Chapters 2 and 3 and in Figure 1.

Probit TSD authors are encouraged to follow the formats provided for the TSD and the calculations, and to use the exposure-response assessment approach presented in this document. The Panel will consider a sufficiently justified deviation from this approach. The Panel will reject deviation from the presented risk assessment approach if no clear justification is given.

Many datasets do not meet the quality criteria set by the Panel. Rather than not deriving a probit function for those substances, the Panel has chosen to use the existing data to its limits and to appropriately compensate for the introduced uncertainty.

The Panel is aware that many scientific issues remain unresolved. But it is also aware that when more experience is developed, new scientific insights are obtained. The Dutch Expert Panel has the right to amend the methodology at any time based on these insights. If such a change in methodology is made, this document will be updated and posted on the RIVM website with an incremental version number. TSD authors are encouraged to verify that they use the most recent version of this document.

Methodological changes may also be applicable to probit relationships established in the past. The Panel has decided that it will not continuously update all existing probit functions. Instead, the Panel has suggested that the existing probit functions be reviewed periodically. In such reviews, both the development of additional substance-specific data and new scientific insights can be taken into account.

The 2005 version of this methodology discusses non-lethal health outcomes. Evaluation of such endpoints could be an interesting and valuable addition to the instruments available for QRA in the Dutch external safety policy. The information and methodology underlying such development could be developed further from existing methodology to derive acute exposure thresholds (such as AEGL, ERPG, etc.). The current Dutch external safety policy applies QRA with lethality as the

only endpoint. Since this document aims to describe the current methodology, the possible extension of the lethality probits with probits for non-lethal levels will not be discussed further.

Part 2

Completion of the probit TSD format

5 Stepwise explanation of the probit TSD format

Draft Technical Support Documents (TSD) and probit functions must be submitted to the Panel's secretariat at the RIVM in the prescribed format (available on the RIVM website). This chapter presents a stepwise guide for toxicologists that are drafting a probit TSD to complete the TSD's main text. The TSD format contains standard text, where appropriate, an indication of the type of information required in individual sections and other guidelines. Standard text, tables and other formats must be followed, unless this would hamper the readability and consistency of the document. The TSD format includes red text to indicate alternative formulations or to provide information on expected input. Text that is not applicable can be deleted and all text transformed to black before submission. Chapter 6 will provide details on how to complete the description of individual studies in the probit TSD appendix. The most recent format of the probit TSD can be found on the RIVM website. The administrative procedure to develop a probit TSD and propose a probit function can be found on the RIVM website.

5.1 Title page

The explanatory text on the title page of draft documents is prescribed. The name of the document and the Word file that should be submitted to RIVM always takes the form: YYYYMMDD <substance name>-proposed, where YYYYMMDD stands for the year, month and day of submission to RIVM (also to be completed in the page headers on pages 1 and 2). The TSD author and the organization that commissioned the draft TSD and probit must be explicitly mentioned. RIVM will set the date for comments. The contact details can be found on the RIVM website.

5.2 Substance identification and physical-chemical characteristics

The standard source for physical-chemical data is the 'Chemiekaartenboek' (most recent or online version); use of another source must be specified. Standard temperature and pressure for all Physical and Chemical data are 20 °C and 101.3 kPa. The saturated vapour concentration is calculated by assuming that 1 kPa vapour pressure equals 10,000 ppm. The concentration conversion factor (at 20 °C) is:

$$\text{Conc [mg/m}^3\text{]} = \text{Conc [ppm]} \times (\text{M}/24.05), \text{ with M} = \text{molecular weight.}$$

5.3 Mechanism of action in humans-

This concise qualitative section provides some mechanistic information about the inhalation toxicity of the substance, with a focus on the mechanism and cause of lethality following an acute inhalation. Long-term effects can be the residual effects of a single exposure to a high concentration (such as RADS) or relevant hazards associated with repeated exposures (e.g. carcinogenicity or reprotoxicity). If appropriate, a third section on 'special considerations' can precede the 'acute effects' section. Among other things, this section has been used to justify a chemical class approach, the derivation of a probit on the

basis of a hydrolysis product and to demonstrate the inappropriateness of certain animal species for predicting the human response to a particular substance.

5.4 Human toxicity data

The section on human toxicity data can serve two purposes. Firstly, human data might be available to derive a probit function. Yet this is hardly ever the case. Secondly, human data on lethality and non-lethal endpoints may serve to verify the lethal concentrations as predicted by the derived human probit function. This is a very important function, since experience has shown that rigorous application of the procedure and AFs as presented in Chapter 3 may lead to estimated human 1% lethal levels that have been used for human experimental studies without serious health effects.

5.5 Literature search strategy

A literature search should at least include the consultation of the following publications and databases:

1. AEGL final/interim/proposed TSD, ERPG, OECD HPV, EU RAR, SMAC documents and reference database, REACH registration for [substance]. For these documents, the latest version (to be specified) is preferred. Other possible sources to identify data are criteria documents for occupational guidelines, ATSDR, US EPA, Dutch Health Council, etc. This search should cover references dated before and including 1995.
2. An additional search covering publications dated from 1980 onwards, all animal species included, must be performed in HSDB, MEDline/PubMed, Toxcenter, IUCLID, RTECS, IRIS and ToxNet with the following search terms:
 - substance name and synonyms, hydrolysis products, if appropriate;
 - CAS number;
 - inhalation;
 - 'lethal*' or 'mortal*' or 'fatal*' or 'lethal*' or 'killing' or 'dead' or 'oecd403';
 - LC₅₀, LC;
 - acute effect;
 - probit;
 - (sensory) irr.*, irritation, irri.*;
 - 'sublethal*' or 'intoxication' or 'case report' or 'serious effect' AND 'human*' or 'subject' or 'primate'.
3. A well-documented and thorough attempt should be made to obtain unpublished data identified in any of the sources presented above. Usually such documents are from the chemical industry and/or contract laboratories that are performing studies on their behalf.

This section of the TSD describes the number of studies and datasets that have been identified, their quality ('A','B' or 'C') and how many animal species they cover. A study can contain more than one dataset, e.g. for different species or (as for HCl) for vapour or aerosol exposure.

5.5.1 Sensory irritation

This section summarizes the available information on sensory irritation. The table includes the test species, exposure duration, the RD_{50} value, the reference and the type of response (see Figure 3). Compilations of available RD_{50} values can be found in Schaper (1993), Bos et al. (1992 and 2002), <http://www.yvesalarie.com/> and the publications indicated above. These references do not always provide information on the type of responses (see Chapter 3.4), so they serve more as a bibliographic source rather than as a source of data. Every effort must be made to obtain primary references to evaluate the substance's sensory irritation properties. This table can be deleted if no eligible sensory irritation studies are available, but the text should be retained.

5.6 Probit functions derived from individual studies

This section lists $C \times t$ probit functions from A-studies or B-studies described in the appendices of the probit TSD (see Chapter 6). While probit functions can also be calculated for datasets with one exposure duration or one concentration, such probit functions do not need to be listed in this section. Probit functions must be listed with concentration in mg/m^3 , exposure duration in minutes and concentration and time logarithmically transformed.

All data from all studies are presented in a first 'overall' scatter plot containing raw, unadjusted data.

A second scatter plot contains all selected datasets from A-studies and B-studies that are actually used in the derivation of the probit functions. The format of this scatter plot is prescribed and can be found in the DoseResp worksheet template that is available from the RIVM website. All data, including data from short-term exposure durations, should be included in the scatter plot after appropriate adjustment (for nominal concentration, the start of exposure before chamber equilibrium was reached and 0/100% response). This plot represents the data as they will be evaluated for eligibility in the final probit equation.

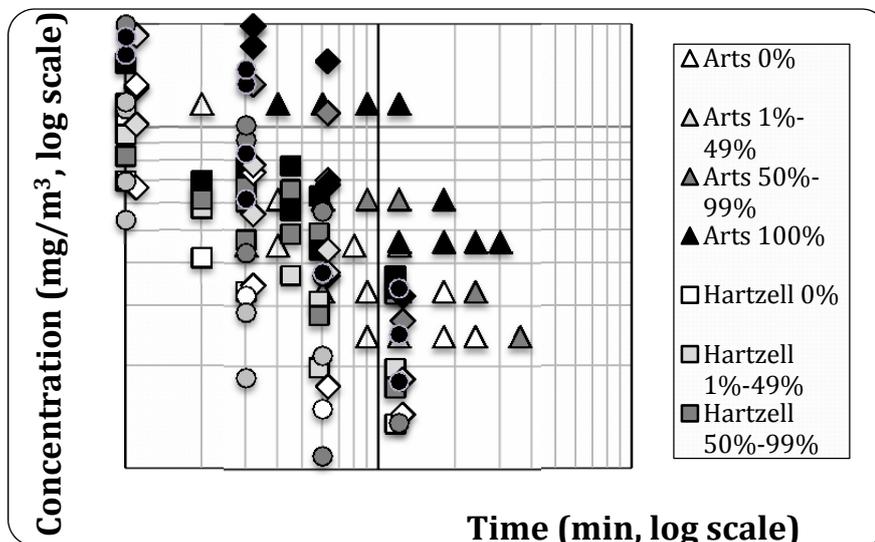
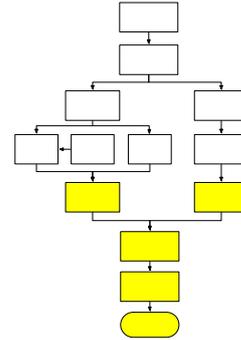


Figure 5 Example of a scatter plot. Data from studies by Hartzell et al., Darmer et al. and Arts et al. on HCl.

This table and scatter plot will also be presented if no C×t probit functions can be calculated from available individual datasets. The TSD format provides a standard text, which can be used to indicate the absence of C×t probit functions from single studies.

5.7 Evaluation

The animal LC₅₀ to be used as a PoD for the human LC₅₀ is taken from the most appropriate study or combination of studies, as outlined in Section 3.2.3. The TSD format provides a standard text for the identification of the key study (studies), the LC₅₀ value and a rationale for this choice. If two or more studies are equally suited as a PoD, usually a weighted geometric mean of those LC₅₀ values is used. The weight of each LC₅₀ is inversely proportional to the LC₅₀'s 95% confidence interval. The DoseResp worksheet template (available from the RIVM website) can be used to calculate such a weighted LC₅₀ value.



The AFs, as presented in Chapter 3, are applied to the animal LC₅₀ value⁶ as appropriate to derive a human LC₅₀ value. For each AF, the chosen value is justified in a table.

The n-value calculated from the animal data is assumed to be a chemical-specific characteristic of the exposure-time-response relationship and is assumed to be valid for humans, in part because the lack of a credible alternative. All of the data selected for the final analysis using the criteria outlined in Section 3.2.2 are used in the final calculation of the probit parameters n and b.

For the selection of the n-value, three options are open:

1. If more than one eligible C×t or other studies are selected using the criteria outlined in Section 3.2.2, a justified choice is made to either derive the n-value from a single study or as a weighted average n-value of multiple studies. The weight is inversely proportional to the 95% confidence interval of the n-values. A calculation tool is included in the calculation template that is available from the RIVM website.
2. If only one eligible C×t study is available (i.e. with ≥3 exposure durations and ≥ 3 exposure concentrations/duration and sufficient variation in lethal responses), the n-value is selected from this C × t study.
3. If no C×t study or set of other eligible studies is available to derive a probit function, an n-value derived for a structurally similar and, mechanistically, similarly acting chemical can be used. Substitution of the n-value with an n-value of another compound will only be accepted if the substitute n-value itself is robust and if the validity of the substitution is sufficiently justified.

⁶ A discussion about the application of assessment factors to the toxic load (Cⁿ × t) or the concentration was concluded by the decision to apply the AFs to the concentration.

If none of the three conditions above apply, an n-value of 2 is assumed for humans over the whole range of exposure durations (10 minutes to 8 hours).

The a-value is then calculated with the known human LC₅₀ value, b-value and n-value. This completes the derivation of values for all model parameters and the model is presented:

- All model parameters are presented with three significant figures.
- If default values of b=1 and n=2 are used, these values are presented with 1 significant figure.
- C should always be expressed in mg/m³, t in minutes.

The scientific credibility of the derived probit function is described with one of the four prescribed descriptors (unacceptable, weak, acceptable, sound) based on the quality of the underlying data. The rationale for this qualification is provided. If the data do not allow the derivation of a human probit function with sufficient certainty, no probit function will be recommended.

Any available relevant data on toxicity following an acute exposure of humans or other primates to the chemical or a precursor are used for verification. If the human lethality as predicted by the derived probit function does not match other relevant data, it may be appropriate to adjust the derived probit function on a case-by-case basis to provide a more realistic probit function.

To verify the outcome of the probit function, the 0.1% and 1% lethality levels for 30 and 60 minutes, as calculated with the probit function, are compared with lethal and non-lethal human (as presented in Section 3 of the TSD) and animal data and acute exposure guidelines. The concentrations should be calculated using the exact values of the model parameters, as presented in the final model, to avoid rounding errors (e.g. in Excel). The DoseResp worksheet template, available from the RIVM website, offers an accepted calculation method of the model parameters and lethality estimates. Any deviations between the model results and existing data and guideline values should be mentioned and justified.

If lethality assessments produced with the probit are incompatible with experimental or observational data from humans or non-human primates, a modification of the probit function based on rodent data should be considered.

6 Description and interpretation of studies in the probit TSD

Every acute inhalation toxicity study on the substance, as well as any supporting data, needs to be described in the appendices. This chapter serves as a guideline to complete these appendices of the probit TSD. A study-reporting format has been developed to ensure that the reporting of the available studies is transparent and reproducible.

'A' quality and 'B' quality studies must be described in detail in the study-reporting format provided. 'C' quality studies can be described in the study-reporting format, but can also be reported in 1-2 paragraphs under a common heading 'C quality studies'. The study description should justify the assignment of a 'C' quality status to the study. Supporting studies on lethal and non-lethal (inhalation) toxicity in humans and other primates should be described in sufficient detail to allow justification of the deviation from the standard derivation procedure.

The TSD format includes red text to indicate alternative formulations or to provide information on expected input. Texts that are not applicable can be deleted and all text should be transformed to black before submission.

6.1 Study identification

Every study receives a code consisting of the qualification ('A', 'B1', 'B2' or 'C') and a consecutive number. 'A' quality studies are reported first, followed by 'B1' and 'B2' quality and finally 'C' quality studies. The numbering of studies restarts for 'B' quality and 'C' quality studies. The absence of any of the requested data under study identification must be explicitly mentioned (not just left blank).

If one study includes several datasets (e.g. different species), one or more study descriptions can be made. For large datasets and particularly for key studies, a separate description for each dataset is highly recommended. The description of the datasets can be combined for smaller datasets, non-critical studies and when a combined description is still unambiguous.

6.2 Evaluation of study quality

The evaluation criteria in the table determine – to a large extent - the assignment of a quality indicator A/B/C. The red coloured text in the table indicates which information should be provided for the particular study. The evaluation criteria concern:

- compliance with GLP and OECD TG 403;
- stability of the test substance;
- the use of a vehicle other than air;
- whole body or head/nose only exposure, restrainer type;
- the pressure distribution in the test system;
- homogeneity of the test atmosphere in the animals' breathing zone;
- the air exchange in the test system;
- actual concentration measurement;
- particle size distribution.

Studies without GLP status are labelled as follows: 'GLP did not exist at the time' (for studies conducted before 1981, the introduction year of

OECD GLP) or 'No GLP statement provided' (for studies conducted from 1981 onward). Similarly, studies not performed according to OECD guideline 403 are labelled as follows: 'OECD guideline 403 did not exist at the time' (for studies conducted before 1981) or 'No OECD guideline 403 statement provided' (for studies conducted from 1981 onward).

Additional comments can be added under the table. The evaluation of these study quality criteria leads to the assignment of the 'A', 'B1', 'B2' or 'C' quality status.

6.3 Presentation of the data

All raw data are presented without any pooling or summarizing. The concentration unit is mg/m³, the unit for time is minutes. The description itself should include the species, sex, exposure duration and concentration, and the number of exposed animals and the number of fatalities for each concentration-time combination.

6.4 Probit function

The parameters of the probit model area derived as described in Section 3.1. Probit function calculation is mandatory for all 'A' quality studies and 'B1' quality studies. For 'B2' quality studies with a Cxt dataset, the probit calculation is only mandatory if no 'A' quality or 'B1' quality studies are available and optional if 'A' or 'B1' studies are available. For studies without a Cxt dataset, a statement should be included to indicate that no Cxt probit could be derived from the particular study.

If a covariate was considered in the analysis, the probit function parameters should be presented from the analysis including and excluding the covariate. A rationale is provided for including or excluding the covariate(s) in the probit function.

6.5 Calculation of LC-values with the model

The LC₅₀ values for each sex and for sexes combined should be presented in the table for 10, 30 and 60 minute exposure durations. It is crucial to compare the calculated LC-values with those calculated by the authors. Deviations that are > 5-10% should raise suspicion of calculation errors and should be explained.

Obviously, for studies with a single exposure duration (or concentration), only the LC₅₀ (or LT₅₀) for the tested exposure duration can be presented.

6.6 Plot of underlying data

For all studies with a Cxt dataset, a scatter plot should be provided in the format provided in the TSD template (available from the RIVM website).

6.7 References

All references used to complete the study evaluation form should be included. If data from a secondary source are used, the secondary source should be listed instead of the primary source listed in the secondary source.

7 Annexes

7.1 Annex to head/nose only versus whole body exposure

A possible problem with head/nose-only studies is that the restrainers (tubes) may promote hyperthermia and stress in the exposed animals. In general, few problems are expected with the Battelle tubes (current standard). In older studies and when other restrainers have been used, it is advised that tube training, the pressure distribution in the inhalation chamber and the risk of hyperthermia should all be evaluated. For all head/nose-only inhalation studies, the type of restrainer should be described in the study description. When carrying out a study adhering to the guideline (OECD 403) or guidance document (GD39) and when using atypical restraining-tube designs, test laboratories should demonstrate that the tubes do not cause undue stress to exposed animals in a sham-exposed population of the same species and strain as judged by:

1. body temperature;
2. stress hormone production;
3. feeding behaviour following exposure.

If such information is not available, the system is suspect and should be evaluated by a Panel member with substantial experience with the design and conduct of inhalation toxicity testing. This evaluation should provide a sufficiently justified conclusion to accept or reject the test system, and thereby for the acceptance or rejection of the toxicity data produced with it. The outcome of studies with other chemicals under the same testing conditions, compared with other studies conducted with a more standard test system, may contribute to the evaluation.

The following text on issues related to head/nose-only and whole body exposure for acute inhalation studies is taken from OECD (2009).

7.1.1 *Selection of an inhalation chamber*

A dynamic, validated inhalation system with suitable control of all inhalation chamber parameters is required for acute inhalation toxicity studies. Dynamic inhalation systems include nose-only chambers and whole-body chambers. The preferred mode of exposure is nose-only (which term includes head-only, nose-only, or snout-only) for the following reasons:

1. Exposure and/or uptake by any other route than inhalation (oral route via preening or dermal route) are minimized, especially when testing aerosols.
2. Technician exposure from handling exposed animals is minimized.
3. A minimum of test article is needed due to low chamber volume.
4. High concentrations (e.g., limit concentrations) are readily achieved.
5. The instability of test articles (e.g., reactivity with excreta or humidity) and test atmosphere in-homogeneity are of minimal concern.

6. The time required to attain inhalation chamber equilibration (t₉₅) is negligible relative to the duration of exposure and therefore not an issue.
7. Adding or removing animal restraining tubes during exposure to a fixed steady state chamber concentration allows for multiple exposure durations in one single test (the C x t protocol, utilizing the same exposure concentrations for multiple exposure durations).
8. The exposure of individual animals can be interrupted at any time during the course of exposure to avoid undue suffering of animals.
9. Animals are readily accessible for specific physiological measurements (e.g., respiratory function, body temperature) or the collection of blood, if applicable.
10. The pre-conditioning of air prior to entering the inhalation chamber (e.g., in order to eliminate ubiquitous environmental constituents such as ozone, nitrogen oxides, hydrocarbons, and particulates, or to allow testing under defined humidity or gas conditions) is technically less demanding with nose-only chambers than with larger whole-body inhalation chambers.

The principal advantages and disadvantages of nose-only vs. whole body exposure have been detailed elsewhere (Phalen 2009). The nose-only mode of exposure-specific mild immobilization stress, following repeated inhalation exposure has been examined (Pauluhn et al., 1997). Apart from differences in food and water intake, it was concluded that mode of exposure-associated differences in cardiovascular endpoints and respiration did not occur.

The design of animal restrainers may differ from one laboratory to another. When atypical restraining-tube designs are used, test laboratories should demonstrate that they do not cause undue stress to exposed animals (see also paragraph 58⁷). While nose-only is the preferred mode of exposure, special objectives of the study may give preference to the whole-body mode of exposure. The use of other modes of exposure should be based on the focus of the study and should be justified in the study report.

In directed-flow (flow-past) nose-only inhalation chambers, the inhalation exposure air flow and the exhalation flow are separated so the exhaled air from one rat cannot be inhaled by another. Directed-flow chambers are preferable to chambers of small volume using a mixed-flow operation principle (Cannon et al., 1983, Moss et al., 2006) in which the inhalation exposure airflow and the exhalation flow can mix and be re-breathed. When an animal is confined to a restraining tube the observation of its behaviour and physical condition is somewhat⁸ restricted. Subtle clinical signs may be⁹ obscured due to impaired locomotion and limited capability to evoke specific neurobehavioral responses. If the focus of a study is on neurobehavioral changes over the course of an exposure, this is sufficient justification for using an alternative exposure mode such as whole-body exposure. A detailed

⁷ Paragraph 58 of the OECD GD 39

⁸ The probit Panel believes that this qualification should read 'highly' instead of 'somewhat'.

⁹ The probit Panel believes that 'may be' should rather read as 'are'.

analysis and recording of clinical signs should be made, but not limited to, the time when maximal systemic toxicity is expected, which is usually on the exposure day. Details have been published elsewhere (Lipnick et al., 1995, Cannon et al., 1983, Moss et al., 2006, Pauluhn et al., 2007). Because of the study design of the C x t protocol, a nose-only chamber should always be used when performing the study.

7.1.2 *Nose-Only Exposure Technique*

During nose-only exposure, animals are exposed to the test article while in restraining tubes. The restraining tubes should not impose undue physical, thermal, or immobilization stress on the animals. Restraint may affect physiological endpoints such as body temperature (hyperthermia) and/or respiratory minute volume. If generic data are available to show that no such changes occur to any appreciable extent, then pre-adaptation to the restraining tubes is not necessary. When precise dosimetry is the objective of the study, however, pre-adaptation may decrease inter-animal variability. Urine and faeces should escape from the restraining tubes during the course of exposure.

To provide optimal exposure of animals, a slight positive balance of air volumes supplied to and extracted from the exposure system should be ensured to prevent dilution of the test article at the animals' breathing zone. The design of the restraining tube and the pressure difference should make it impossible for animals to avoid inhalation exposure. If leakages from the inhalation equipment cannot be excluded by design, the inhalation equipment should be operated in a well-ventilated chemical hood to avoid harming laboratory personnel. Maintenance of slight negative pressure inside the hood will prevent leakage of the test article into the surrounding area.

Animals exposed in flow-past inhalation equipment designed to sustain a dynamic airflow that ensures an adequate air exchange of at least 2-3 times the respiratory minute volume of animals exposed (*i.e.*, at least 0.5 L/min per exposure port for rats). Each exposure port should have similar exposure conditions with an oxygen concentration of at least 19% and a carbon dioxide concentration not exceeding 1%. The design and operating conditions of the chamber should minimize the re-breathing of exhaled atmosphere. A significant disturbance of airflow dynamics during the collection of test atmosphere should be avoided (Moss et al., 2006, Pauluhn et al., 2007).

7.1.3 *Whole-Body Exposure Technique*

Animals should be tested with inhalation equipment designed to sustain a dynamic airflow of at least 10 air changes per hour. Higher airflow rates may be useful to meet specific requirements imposed by the test article. An oxygen concentration of at least 19%, a carbon dioxide concentration not exceeding 1%, and an evenly distributed exposure atmosphere should be ensured. Where concerns might apply, these gas levels should be measured in the vicinity of the animals' breathing zone. All animals should be individually housed to preclude them from breathing through the fur of their cage mates, thus reducing their aerosol exposure. To ensure stability of a chamber atmosphere, the total "volume" of the test animals should not exceed 5% of the chamber volume. Maintenance of slight negative pressure inside the chamber will

prevent leakage of test article into the surrounding area. Food and drinking water should be accessible for exposures exceeding 8 hours. In a dynamic whole-body chamber, the test article concentration initially rises rapidly, and then slowly approaches a theoretical equilibrium provided:

1. the output of the test article is constant; and,
2. the test article is instantaneous and thoroughly mixed throughout the chamber.

Under these conditions, an exponential built-up of concentration is seen throughout the chamber. The time to 95% atmosphere equilibrium (t_{95}) in minutes is calculated using the following simplified formula:

$$t_{95}(min) = 3 \times \left(\frac{\text{chamber volume}}{\text{chamber flow}} \right)$$

More details are presented in Phalen (2009).

7.2 Annex to inter-species extrapolation

In Technical Report 86 and 110 by (2003; 2011), a further discrimination is made for local irritants: i.e. water soluble vapours and gases, low water soluble vapours and gases, and particulates and aerosols (likely referring to non-soluble liquids and solids). According to ECETOC, rats are a factor 2 to 4 more susceptible to water soluble irritants that elicit effects in the nasal cavity compared to humans. This is based on back calculation of the exposure, where metabolism in nasal epithelial cells are thought to be indicative for exposure, which was higher in rats compared to humans. The main criticism for this approach is that other parts of the respiratory tract were not considered (exposure determined only in nasal regions), and not showing whether at what external concentrations effects occurred in rats vs. humans. In case of low soluble irritants, amounts reaching the lower tract may be lower in rodents, because rodents extract larger amount in the nasal cavity and may reduce the respiratory volume. On the other hand the surface area is linear compared to body mass, while alveolar ventilation to body mass $\wedge 0.75$. ECETOC therefore concludes that exposure in the deeper lungs is likely to be lower. An AF of 1 or even below 1 is appropriate. ECETOC further states that determining a generic guidance is not possible for particulates. In a previous report by ECETOC (ACUTEX 2006), an interspecies factor of 1 is suggested for local effects and a factor of 3 for systemic effects, in case toxicodynamics and ADME differences exist between rodents and humans.

7.3 Annex to intra-species extrapolation

The adoption of the default approach of $b \times n = 2$ for the human probit function regardless of the animal $b \times n$ product has been subject to debate. In light of the default approach, the two main assumptions that have been taken into consideration can result in a conflict when the animal $b \times n < 2$. The first assumption is that the animal data, i.e. the concentration \times time and lethality response, do not provide information on the variability in susceptibility in human lethality response. The second assumption is that it is reasonable to consider that the variability in the general human population is greater than the variability observed in the animal species, usually an in-bred strain of rodents.

In the rare case that the animal probit function resulted in an animal $b \times n < 2$ and the default approach of $b \times n = 2$ for humans is pursued, the second assumption above is neglected. Alternative approaches were considered to address the smaller human variability than in the in-bred strain animal. The alternative approaches discussed were to adopt the animal $b \times n$ product, or to apply an additional 'safety' margin where the human $b \times n$ product is at least 0.5 point lower than the animal $b \times n$ product or is arbitrarily set at 1. The suggested alternative approaches, though adhering to the second assumption above; do not comply with the first assumption that the animal data do not provide reliable information on human variability on human lethality response, since the alternatives rely on the animal $b \times n$ product as starting point.

The reason for the first assumption is that the animal test population is principally/by default more homogeneous and experimental errors having a major influence on the variability in the results. If the experimental error can be decreased to a low level, the estimate of the true variability in response in the test animal becomes more accurate. In practice, the information on the experimental error in animal studies is too limited to correct for in a quantitative way. Hence, it cannot be estimated what the actual influence of the experimental error is in respect to the true variability in the response. From experience with the analyses of acute lethality studies, variability in lethality response are generally rather small, unless there are apparent contrasting results in specific dose groups with respect to other dose groups. Therefore, it is thought that in case an animal $b \times n < 2$ is found, experimental errors are general the cause. Taking the abovementioned into consideration, the only parameters that are used in human probit function derivation are the LC_{50} value and n -value, as they are least affected by experimental error.

These contemplations would suggest refraining from taking the animal $b \times n$ product as starting point for intraspecies extrapolation. The default approach of $b \times n = 2$ for human lethality response will be applied as it considered the most appropriate approach, accepting that the true variability and human lethal response may be underestimated in cases where the animal $b \times n$ product suggests a larger variability.

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List of abbreviations

ACUTEX	Acute Exposure project under EC FP5, aimed at developing methodology to derive acute exposure guidelines.
ADI	Acceptable Daily Intake
AEGL	Acute Emergency Guideline Level (National Research Council and US EPA)
AETL	Acute Exposure Threshold Level
AF	Assessment Factor, also called Uncertainty Factor (UF, e.g. in AEGL)
ASF	Allometric Scaling Factor
ATSDR	Agency for Toxic Substances and Disease Registry
BMDS	Benchmark Dose Software, available from US EPA
BW	Body Weight
C×t protocol	efficient protocol for acute inhalation toxicity experiments designed to produce data for derivation of a concentration-time-response relationship, as presented in the 2009 revision of OECD TG 403.
C×t study	acute inhalation toxicity study in which lethality was studied for more than one exposure duration and more than one exposure concentration per exposure duration, either conducted in conformity with the C×t protocol included in the OECD TG 403 (2009) or from another study design.
CAS number	Chemical Abstract Service number; unique identifier for a substance or mixture
COSHH	Control of Substances Hazardous to Health
DHS	Department of Homeland Security
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EPA	US Environmental Protection Agency
ERPG	Emergency Response Planning Guideline
ESIS	European chemical Substances Information System
EU RAR	European Union Risk Assessment Report
GLP	Good Laboratory Practice
GM	Geometric Mean
HSDB	Hazardous Substances Databank
HPV	High Production Volume chemicals (OECD program)
IRIS	Integrated Risk Information System (US EPA)
IUCLID	International Uniform Chemical Information Database, a software application to capture, store, maintain and exchange data on intrinsic and hazardous properties of chemical substances.
LBW	Levensbedreigende waarde (Life-threatening level), Dutch acute inhalation exposure guideline representing the threshold for lethality or life-threatening health effects.
LC ₅₀	Lethal concentration 50%, a concentration that is calculated to produce 50% lethality in a population during exposure or in the specified post-exposure observation period.

LD ₅₀	Lethal dose 50%, a dose calculated to produce 50% lethality in a population.
LT ₅₀	Median lethal time, a time interval calculated to produce 50% lethality in a population at a given concentration.
Medline	Database system with references to medical articles; available on-line and on CD-ROM.
MLE	Maximum likelihood estimate
MW	Molecular weight
NOAEL	No observed adverse effect level
NTIS	National Technical Information Service. Online searchable database including toxicological information submitted to the US government.
OECD	Organisation for the Economic Cooperation and Development
Panel	Dutch Expert Panel on Probit functions
PBPK	Physiologically-based pharmacokinetic model
PGS	Publicatiereeks gevaarlijke stoffen
PoD	Point of Departure: derived toxicological endpoint used as the starting point for the derivation of a probit function.
ppm	parts per million
QRA	Quantitative Risk Assessment
RADS	Reactive Airways Dysfunction Syndrome
RD ₅₀	Respiratory decrease 50%, concentration producing a 50% reduction in the respiratory rate of test animals.
RIVM	National Institute for Public Health and the Environment
RTECS	Registry of Toxic Effects of Chemical Substances; database with summarized toxicological information.
SQRT	Square root
SVC	Saturated vapour concentration
Toxcenter	Database system with references to toxicological articles; available online.
ToxNet	Online search system for toxicological information
TSD	Technical Support Document
US EPA	United States Environmental Protection Agency

Internet literature resources

AEGL:

<http://www.epa.gov/opptintr/aeql/pubs/chemlist.htm>

ERPG

<http://www.aiha.org/INSIDEAIHA/GUIDELINEDEVELOPMENT/ERPG/Pages/default.aspx>

EU Risk Assessment Reports (EU RAR)

<http://esis.jrc.ec.europa.eu/>

Toxnet (NLM) providing access to HSDB, IRIS, ITER, Toxline, inter alia.

<http://toxnet.nlm.nih.gov/>

IUCLID

<http://iuclid.eu/>

PubMed, Medline:

<http://www.ncbi.nlm.nih.gov/pubmed/>

REACH registered chemicals

<http://apps.echa.europa.eu/registered/registered-sub.aspx>

RIVM

<http://www.rivm.nl/probitrelaties>

RTECS

<http://ccinfoweb.ccohs.ca/rtecs/search.html>

SMAC:

www.nasa.gov/centers/johnson/pdf/485930main_SMACsGuidelines.pdf

Probit TSD format

Draft Technical Support Documents (TSD) and probit functions must be submitted to the Panel's secretariat at the RIVM in the prescribed format, which is contained in this annex. The most recent format of the probit TSD can be found on the RIVM website.

Probit function technical support document

Date: Day, Month, 2015
 Comments before: Day, Month, 2015
 Document id: 2015MMDD-<substance>-proposed
 Status: proposed
 Author: <name and affiliation of author>
 <disclosure of organization that commissioned the draft>

substance name	CAS number
Substance	XXX-XX-X

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

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

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

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

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

1 **Technical support document Substance^{10,11}**

2

3 **1. Substance identification**

4 CAS-number:

5 IUPAC name:

6 Synonyms:

7 Molecular formula:

8 Molecular weight: XX g/mol

9 Physical state: gas/liquid/solid (at 20°C and 101.3 kPa)

10 Boiling point: XX°C (at 101.3 kPa)

11 Vapour pressure: XX kPa (at 20°C)

12 Saturated vapour conc: XX ppm = YY mg/m³ (at 20°C and 101.3 kPa)

13 Conversion factor: 1 mg/m³ = XX ppm (at 20°C and 101.3 kPa)

14 1 ppm = XX mg/m³ (at 20°C and 101.3 kPa)

15 Labelling: H-sentences

16

17

18 **2. Mechanism of action and toxicological effects following acute**
 19 **exposure¹²**

20

21 **Acute effects:** [Length of description roughly 5-10 lines. Aim for (close
 22 to) standard sentences for frequently encountered endpoints, including
 23 cause of acute mortality.]. The main target organs and tissues for
 24 inhalation exposure to substance are .

25 The health endpoints are [describe mechanism of action].

26 Symptoms of high exposure are [describe symptoms].

27 Damage occurs [describe tissues and organs affected by toxicity].

28 Lethality results [describe cause of lethality].

29 **Long-term effects:** [includes irreversible / residual effects of acute
 30 exposure and health consequences of chronic exposure - 5-10 lines
 31 maximum.]. Chronic exposure produces.

32 ** information on susceptible individuals only required in special cases

33 **

34

35

36 **3. Human toxicity data**

37 **In most cases, include this text:** No informative reports on the health
 38 effects in humans following acute inhalation exposure were identified.
 39 Such reports are considered informative if both health effects as well as
 40 the exposure have been documented in sufficient detail.

41 ** Provide description of studies on human fatalities following acute
 42 inhalation exposures, with references. Pay particular attention to the
 43 determination of the level and duration of exposure. Also include data
 44 on controlled human exposures that can serve as a validity/reality check
 45 for the lethal concentrations calculated with the derived probit. **

46

47

48 **4. Animal acute toxicity data**

49 Animal lethal toxicity data focused on acute exposure are described in
 50 Appendix 1. A total of XX studies were identified -with YY datasets for

¹⁰ Consistently use the most common substance name.

¹¹ Insert figure with molecular structure, aligned to the right margin.

¹² References for mechanism of action and toxicological effects following acute exposure.

1 **ZZ** species- with data on lethality following acute inhalation exposure.
 2 **XX** datasets **was/were** assigned status A for deriving the human probit
 3 function, **YY** datasets **was/were** assigned status B and **ZZ** were assessed
 4 to be unfit (status C) for human probit function derivation.

5

6 During a literature search, the following technical support documents
 7 and databases were consulted:

- 8 1. AEGL **final/interim/proposed** TSD, ERPG document and EU RAR and
 9 reference database for **substance**, covering references before and
 10 including **1995**¹³.
- 11 2. An additional search covering publications from **1980** onwards was
 12 performed in HSDB, MEDline/PubMed, Toxcenter, IUCLID, RTECS,
 13 IRIS and ToxNet with the following search terms:
 14 • Substance name and synonyms
 15 • CAS number
 16 • lethal*
 17 • mortal*
 18 • fatal*
 19 • LC₅₀, LC
 20 • probit
- 21 3. Unpublished data were sought through networks of toxicological
 22 scientists.

23

24 **Sensory irritation**¹⁴

25

26 A total of **XX** studies **were/was** identified in which sensory irritation was
 27 studied. In these studies, the following RD50 values were observed:

28

Table 7 *Sensory irritation data for <substance>*

Species/strain	RD50 (mg/m ³)	Exposure duration (min)	Author/year
	XXP		
	YYND		

29

30 P: a plateau was reached, C: continuously decreasing response, F:
 31 fading of response during exposure, S: second decrease during
 32 exposure, I: increase in respiratory rate, NS: not specified if a plateau in
 33 response was reached. **** Retain applicable notations only. ****

34

35

36 **5. Probit functions from individual studies**

37

38 All available acute lethality data on ***substance*** are provided in Figure
 39 1.

¹³ Usually a safe assumption.

¹⁴ Delete table if no applicable studies are identified. In that case, include text: No studies on sensory irritation were found.

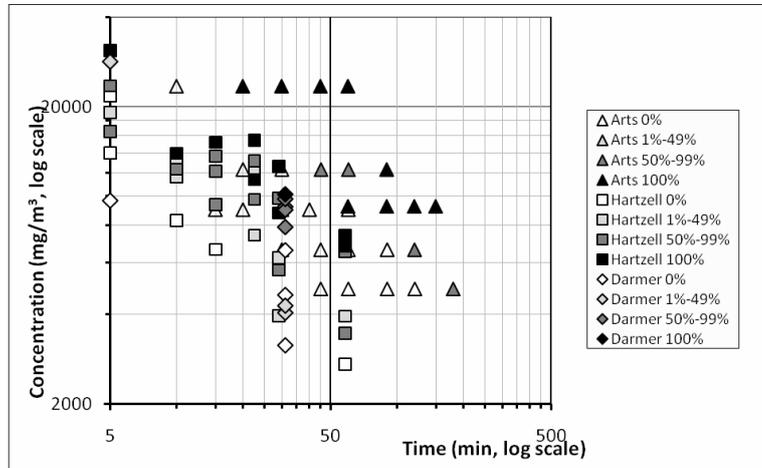


Figure 6 All available acute lethality data for <substance name>

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

All A and B1 studies were selected for derivation of the animal probit function for <substance name>.

OR, if NO studies with a Cxt dataset are available:

It was possible to derive a probit function for <substance name> based on the available studies with B1 quality by pooling data. Therefore, the probit function was derived using data from the studies with B1 quality, none of which enabled us to produce a concentration-time-lethality relationship.

OR, if B2 (and B1) studies but no A studies with a Cxt dataset are available:

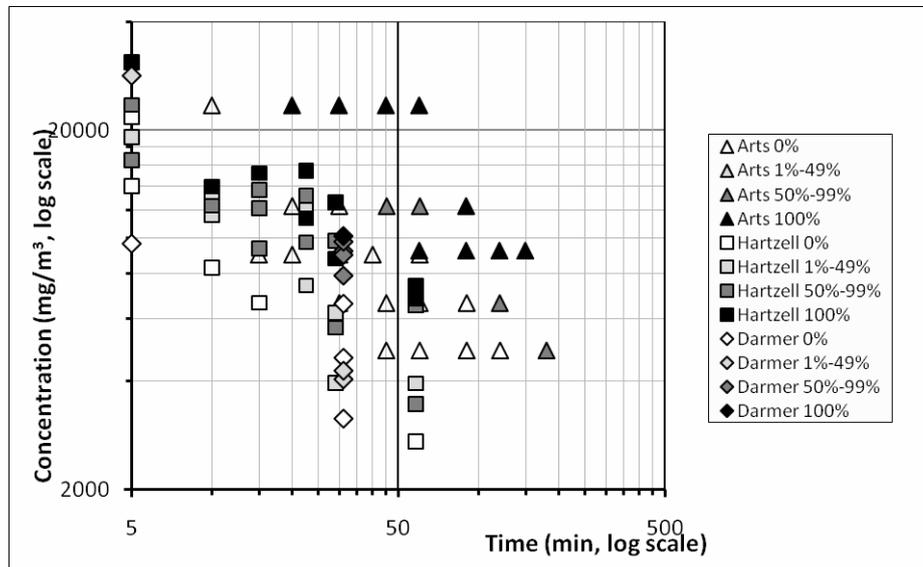
It was not possible to derive a probit function for <substance name> based on studies with A quality. Therefore, the probit function was derived using data from the study/studies with B2 <and B1> quality listed in the table below.

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 8 Data selected for derivation of the animal probit function of <substance name>

Study ID	Species	Probit (C in mg/m ³ , t in min)	LC ₅₀ , 30 minutes (mg/m ³) 95% C.I.
A.1	Rat	-a + b×lnC + c×Int	
B1.1		-a + b×lnC or -a + b×Int	
B2.1		-a + b×lnC + c×Int	

The data of the XX A studies <and study B.1-X> with rats are presented graphically below.



1
2 **Figure 7** Data selected for the initial analysis for the derivation of
3 the animal probit function of *<substance name>*
4

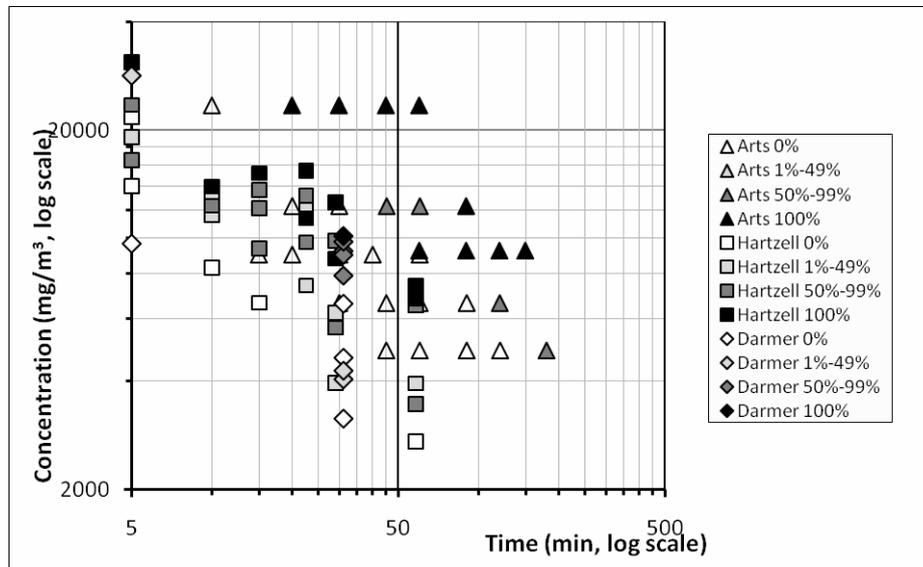
5 Based on *<visual inspection and statistical analysis>*, the data from
6 studies *<study identifiers>* were selected for the final dataset for the
7 derivation of the animal probit function. *<Rationale for the selection of
8 studies, datasets within studies, animal species>*.

9 The final data for calculating the animal probit function contains
10 *<number>* datasets from *<number>* studies and includes data from
11 *<number>* animal species.
12

13 **Table 3** Data selected for the initial analysis for the derivation of the
14 animal probit function of *<substance name>*

Study ID	Species	Probit (C in mg/m ³ , t in min)	LC ₅₀ , 30 minutes (mg/m ³) 95% C.I.
A.1	Rat	-a + b×lnC + c×Int	
B1.1		-a + b×lnC or -a + b×Int	
B2.1		-a + b×lnC + c×Int	

15
16 The data of the selected datasets are presented graphically below.



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Figure 8 Final data selected for the derivation of the animal probit function of <substance name>

6. Derivation of the human probit function

To derive the human probit function, the results from *study identifier(s) including study ID* have been used to derive a point of departure. The reason was .

As a point of departure for deriving the human probit function, the XX min LC₅₀ value of XX mg/m³ for the rat from the study name/ID study was taken <if applicable: after adjustment of concentrations for the <XX minute data due to issues with chamber equilibration>¹⁵. The human equivalent LC₅₀ was calculated by applying the following assessment factors:

Assessment factor for:	Factor	Rationale
Animal to human extrapolation:	3 (1)	AF can be 1 in datasets when there is strong support that the local effects are portal of entry effects, and support AF=1.
Nominal concentration	1-4	Rationale
Adequacy of database:	1-3	Rationale

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The estimated human equivalent QQ-minute LC₅₀ value is XX / YY = ZZ mg/m³.

The experimentally determined n-value was N.NN (reference). Assuming a regression coefficient (b×n) of 2 for the slope of the curve, the b-value can be calculated as 2 / n = B.BB.

¹⁵ If applicable. As a general rule, data from exposure durations of less than 10 minutes are excluded because uncertainties in chamber conditions and the ability of animals to temporarily reduce their minute volume, unless concentrations can be adjusted based on the provided information in the respective studies on chamber equilibrations.

1 OR

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

6
 7 The human probit function is then calculated on the human equivalent
 8 **QQ** min LC₅₀ using the above parameters to solve the following equation
 9 to obtain the a-value (the intercept): $5 = a + Y.YY \times \ln(ZZZ^{N.NN} \times QQ)$
 10 resulting in the a-value of **-AA.AA**¹⁶.

11
 12 $Pr = -AA.AA + B.BB \times \ln(C^{N.NN} \times t)$ with C in mg/m³ and t in min¹⁷.

13
 14 The derived human probit function has a scientifically **unacceptable /**
 15 **weak / acceptable / sound** basis. The probit function is based on **# #**
 16 studies in the **rat** with **A/B** quality, **** mention numbers of animals,**
 17 **exposure durations and response rate ranges****.

18
 19 The human 60 min LC₁ (Pr = 2.67) calculated with this probit equation is
 20 **VVV** mg/m³ and the calculated human 60 min LC_{0.1} (Pr = 1.91) is **VVV**
 21 mg/m³.

Estimated level	30 min (mg/m ³)	60 min (mg/m ³)
1% lethality, this probit		
0.1% lethality, this probit		
AEGL-3 (year, status)		
ERPG-3 (year)		
LBW (year)		

23
 24 Compared with equivalent (inter)national guideline levels as presented
 25 in the table above, the lethal levels derived with this probit function are
 26 **lower / approximately identical / higher**.

27 **** No value judgment on the level of agreement, but factual description**
 28 **and possible rationales for any agreement or difference. ****

29

¹⁶ Present with 2 decimal digits.

¹⁷ Present a, b and n with 3 significant numbers

1 **Appendix 1 Animal experimental research**

2

3 **Study ID: A.1**

4 Author, year:

5 Substance:

6 Species, strain, sex:

7 Number/sex/concentration group:

8 Age and weight:

9 Observation period:

10

11 Evaluation of study quality

Criteria	Comment
Study carried out according to GLP	No GLP statement provided / 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 guideline(s)	No statement of compliance with OECD guideline 403 provided / OECD guideline 403 did not exist at the time (prior to 1981)
Stability of test compound in test atmosphere	aerosol formation / condensation, hydrolysis, decomposition, etc.
Use of vehicle (other than air)	
Whole body / nose-only (incl. head/nose-only) exposure	
Type of restrainer	[add evaluation criteria]
Pressure distribution.	Adequate testing conditions require: Positive pressure at the nose of the animals (central cylinder), negative pressure in the surrounding hood for nose-only exposures. Negative pressure for whole-body exposures.
Homogeneity of test atmosphere in breathing zone of animals	Test atmosphere generation, particularly for liquids (spraying, evaporation, other) and solids. Mixing of test atmosphere in the exposure system.
Number of air changes per hour	Flow in l/min/animal for head/nose only studies (at least XX l/min/rat), air changes/hour for whole body studies (at least YY ACH)
Equilibration time (t95)	t95 in minutes
Start of exposure relative to equilibration	At start of concentration build-up # min into equilibration time (# min) after complete equilibration
Actual concentration measurement	Where (breathing zone?), how often, sampling and analytical methods.
Particle size distribution measurement in breathing zone of the animals in case of aerosol exposure;	If appropriate

Assessment of Reliability	A Rationale for B1/B2/C studies, why the study is NOT A-level
---------------------------	--

1
2 <additional comments if applicable>

3
4 ** For studies with > 1 animal species, several presentation options are
5 possible. All data and calculations can be pooled in one study
6 description, but not for the key study used to derive the probit function.
7 For studies with many datapoints per species, separate datasheets per
8 species are recommended for a better overview of the data **
9

10 **Short-term exposure data**

11 In this study, animals have been placed in the exposure chamber before
12 equilibrium of the test atmosphere has been reached. Therefore, the
13 concentrations of all exposure durations less than $3 \times T95$ have been
14 adjusted¹⁸. All calculations have been performed with the adjusted
15 concentrations.

17 **Zero and 100% responses**

18 In this study, one or more data series had only 0% or 100% response
19 rates. To enable the use of such data by the software package, the data
20 have been manipulated as described in the methodology document. The
21 manipulated data can (in the green section of the table below) be used
22 for the analysis.

24 **Results**

Species	Concentration (mg/m ³)		Exposure duration (min)	Lethality	
	Measured	Adjusted		Male	Female
				Dead/tested	
			42	0/1	0/1
			50	1/2	1/2
			60	1/1	1/1

26 **Probit function**

27 The probit function and associated LC-values have been calculated using
28 the DoseResp program (Wil ten Berge, 2015) *or other software* as

$$29 \text{Pr} = a + b \times \ln C + c \times \ln t + d \times S$$

30 with C for concentration in mg/m³, t for time in minutes and S for sex
31 (0 = female, 1 = male).

32

Probit function	Species	a	b	C	d	n-value
Sex as covariate	Rat	-73.9	6.67	4.19	0.53	1.59 (1.26 - 1.92)
Sexes combined	Rat	-73.3	6.64	4.17		1.59 (1.26 - 1.93)

¹⁸ The required calculations can be performed with the worksheet 'Average concentration' in the Probit panel's version of DoseResp.

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The LC₅₀ 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.

OR

Results per sex showed a difference between the LC-values for the males and females with a factor of more than 2. However, the sex difference observed in the study cannot be explained by physiological differences in male and female animals. The data from both sexes, therefore, were pooled and analysed to derive the animal probit function.

OR

Results per sex showed a difference between the LC-values for the males and females with a factor of more than 2. The sex difference observed in the study could be explained by physiological differences in male and female animals. **<Provide explanation.>** Therefore, the data from the **<male/female>** animals, which are considered to be the most sensitive animals, were used to derive the animal probit function.

Duration (minutes)	LC50 (mg/m3) 95%-C.I. Male	LC50 (mg/m3) 95%-C.I. Female	LC50 (mg/m3) 95%-C.I. Combined
10	XX (YY - ZZ)		
30			
60			

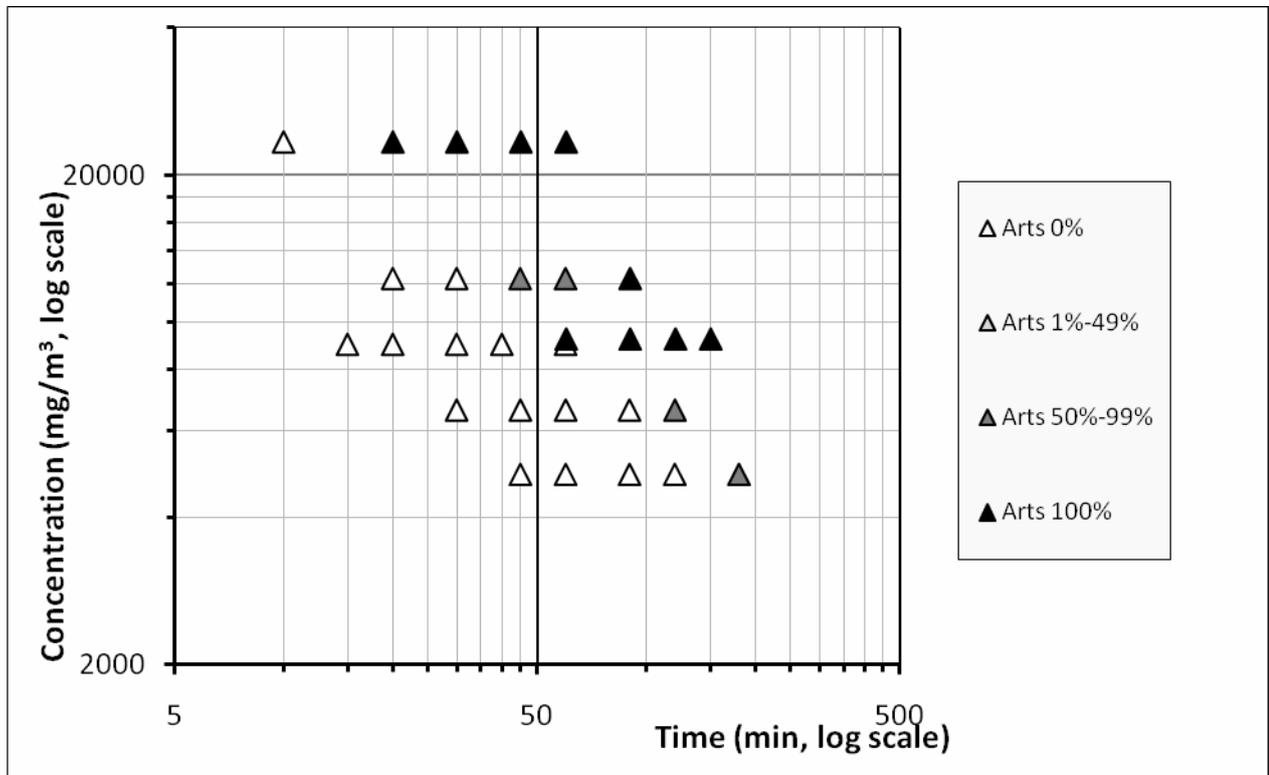
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** Comment on sex differences and choice to use 1 sex only or to combine sexes> **
<The results for males and females were derived from the analysis with sex as a covariate or from analysis with 1 sex only>.

< For B1-studies: No C × t probit function could be calculated from these data alone.>

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

** Dataplots are only mandatory for A studies and B studies with a 'C×t' dataset. **



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1 **Study ID: C studies**

2

3 ** C studies can be summarized without the need to complete a table as
4 required for A and B studies. The description should clarify why the C
5 study criteria apply. **

6

7 ** Also include as C-studies relevant data (e.g. toxicological endpoints
8 in a few primates) even if no lethality occurred. **

9

10 ** Include descriptions of studies that were identified (e.g. in IUCLID)
11 but not used due to inadequate / incomplete data. **

12

13 ** Refer to secondary references as 'Author primary reference' as cited
14 in 'secondary reference'. List the secondary reference in the reference
15 list. **

16

1 **Appendix 2 Reference list**

2

3 ** For all A studies, primary references are mandatory. Every effort
4 should be made to obtain the original study reports with all study
5 details, rather than the publication in peer reviewed journals derived
6 from such reports. For all B and C studies from OECD member states
7 published in or after than 1970, primary references are required. **

8

9 Arts, J.H.E., C. Mommers, and H. Muijser: Toxic Effects from Accidental
10 Releases of Hazardous Substances (TEARHS) – Lethal and non-lethal
11 effects in rats upon exposure during short periods of time. TNO Nutrition
12 and Food Research, report V99.1136. Zeist (2000).

13

14 Hartzell, G.E., A.F. Grand, and W.G. Switzer. Modelling of toxicological
15 effects of fire gases: VI. Further studies on the toxicity of smoke
16 containing hydrogen chloride. J. Fire Sci. 1985;5:368-391.

17

18 NAC/AEGL. Acute Exposure Guideline Levels for Selected Airborne
19 Chemicals. Interim TSD for XXX. Washington, US EPA, 200X.

20

21 National Research Council. Acute Exposure Guideline Levels for Selected
22 Airborne Chemicals. Volume XX. Washington, DC. The National
23 Academies Press, 200X.

24

25 University of Arizona Emergency Medicine Research Center. Advanced
26 Hazmat Life Support (AHLS). Provider Manual, 3rd ed. Tucson, AZ,
27 2003.

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