



ACUTEX
Acute Exposure
PROJECT



ACUTEX

METHODOLOGY TO DEVELOP AETLs

January 2006



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1 INTRODUCTION

The ACUTEX (ACUTE EXposure) project is an EU-funded research project in support of the practical application of the European Council Directive 96/82/EC (the Seveso II Directive)¹ for the control of major-accident hazards involving dangerous substances. The project aims at the development of a European methodology for producing Acute Exposure Threshold Levels (AETLs) for toxic substances to be applied in major hazard control, and in particular emergency response and land-use planning. Creating this methodology is viewed as part of an overall effort to facilitate greater consistency in information used across Europe to support risk management decisions. Moreover, the project seeks to address concerns about increasing demand for acute exposure values in Europe to meet an expanding range of applications, while reducing the overall cost of creating these values by making available an accepted European methodology.

Nine scientific partners from government, industry and academia in Europe² have collaborated on this project and results have also been critiqued by a Critical Review Panel of stakeholder representatives. This collaboration of European scientists and the process of deliberation among stakeholders was specifically established to achieve the following key results: (1) facilitate wide acceptance of the methodology in Europe by both the scientific community and communities of different end-users; (2) to provide greater equivalence and transparency in implementation of the Seveso II Directive across the Member States, specifically through the development of common scientific bases for assessing risks and making risk management decisions related to toxic releases; and (3) to produce a methodology that remains open to future collaboration on derivation of acute exposure levels on a global basis.

The AETL methodology builds on existing approaches and also incorporates newly developed techniques in chemical risk assessment. The range of values produced through the methodology has been designed to accommodate typical needs of European end-users for control of major-accident hazards from industrial installations, primarily associated with the Seveso II Directive, i.e. for emergency and land-use planning. The AETL values are offered as an alternative to other types of acute exposure values for use in implementation of the Seveso Directive, or other related types of activities, and competent authorities may choose to accept them for compliance with various legal requirements, as they deem appropriate.

The methodology also takes into account existing exposure level methodologies currently in use within the Member States, in particular Member State's own methodologies where they exist, and that of the US AEGL programme. To the extent that it has been feasible, the project has sought to retain compatible methods for deriving values as well as threshold structures that are somewhat complimentary to these existing systems. Moreover, it is hoped that transparency in the development of the AETL methodology will enable scientists and end-users, both in Europe and abroad, to understand and overcome the differences between the AETL and other methodologies.

¹ Council Directive 96/82/EC of 9 December 1996 on the control of major-accident hazards involving dangerous substances. Official Journal of the European Communities. Vol. L 10, 14.1.97, pp. 13-33. Luxembourg.

² Funded in late 2002 under the European 5th Framework Programme for Research and Technology Development (RTD), the project is co-ordinated by INERIS (France) and includes partners from five different EU Member States, and also partners from European industry and from the European Commission's Joint Research Centre.

Particular innovative elements of the AETL methodology include:

- a priority-setting procedure, which makes use of modern decision-making algorithms to develop a procedure by which stakeholders can prioritise chemicals for the AETL process;
- a definition of a set of toxicity levels that exploits the full range of the dose-effect relationship;
- a highly innovative approach for modelling the dose-effect relationship for toxic effects. It is expected that applying this methodology will reduce uncertainty and that smaller confidence intervals would allow adoption of smaller safety factors. Moreover, including different species in the data analysis may allow estimation of interspecies variation with higher precision, resulting in smaller scaling factors;
- the matrix approach developed in this project uses both kinetic and dynamic properties of toxic substances, thus enabling more precise definition of the degree of susceptibility that is to be expected in certain subpopulations.

The ACUTEX project aims to support the methodology through the publication of this Technical Guidance Document (TGD) and making available a complimentary software tool (via the Acutex website, <http://www.acutex.info>) for applying some aspects of the methodology. In addition to the creation of these support materials, the project also performed a rigorous validation exercise to test and improve the methodology. Following a case-study approach, the draft methodology was applied to 22 substances and modified according to the results achieved.

2 COMPARISON OF EXISTING METHODOLOGIES FOR SETTING THRESHOLD LEVELS FOR ACCIDENTAL AIRBORNE ACUTE EXPOSURE AND THEIR AREA OF APPLICABILITY

2.1 Introduction

Existing systems of acute exposure values were reviewed so as to understand the range of definitions and parameters in current use and to identify the various differences between them (see Tables 2-1 and 2-2). This information served as an important input to the development of the AETL methodology. In particular, the findings helped to define which aspects of these systems the project would choose to imitate, or imitate with slight improvement, within the AETL methodology. The review also pointed out areas where the AETL methodology, by adopting a new approach, could provide significant value to the derivation of acute exposure levels and to the European end-user.

This review targeted the types of values most commonly applied in the European Union for implementing requirements of the Seveso II Directive (primarily in emergency planning and land-use planning applications) that is AEGL (EPA-USA), ERPG (AIHA-USA), TEEL (DOE-USA) and EEI (ECETOC)³. A less detailed review of some other approaches used in

³ The list represents values that have been specifically identified by Member State competent authorities (in Austria, Belgium, France, Germany, Italy, the Netherlands, Spain, and the United Kingdom) as values that they have applied within certain Seveso-related programme areas. Although IDLH values by NIOSH (McGinnis *et al.*, 2003) were also identified, they have not been included as they primarily serve a purpose which is outside the scope of this project (worker protection). The IDLH values were established to allow workers to escape from contaminated environments without loss of life or irreversible health effects in the event of failure of respiratory protection equipment.

the EU, namely SEL/SEI (F), SLOD/SLOT (UK) and Intervention Values (NL) was also conducted (References: see Table 2-1).

The findings from this review, presented in the following paragraphs, describe and compare the different methodologies, focusing specifically on the intended use of the values produced by the methodology, and the definition of threshold levels and the reference periods.

2.2 Intended use of existing acute exposure levels

The intended uses of existing acute exposure levels vary considerably, but in general can be summarised as belonging to either of the following two categories of use:

- emergency response;
- land-use planning.

The official definitions of the AEGL (Acute Exposure Guideline Levels), ERPG (Emergency Response Planning Guidelines), TEEL (Temporary Emergency Exposure Levels) and EEI (Emergency Exposure Indices) values each indicate that these values have been explicitly designed for emergency response applications. They can be used to guide decision-making during an emergency, but also can play an important role in emergency planning and preparedness.

The SEL (Seuil des Effets Létaux) and DTL (Dangerous Toxic Load) values are principally intended for use in land-use planning.

2.3 Definition of threshold levels

The following levels have been identified in most of the systems evaluated:

Threshold level 3: indicating potential or certain fatalities.

Threshold level 2: denoting irreversible (but not fatal) effects, possibly defined to include serious (e.g. 'slowly reversible') toxic effects. May also include escape impairment and need for medical attention.

Threshold level 1: denoting reversible effects and discomfort/irritation.

Sensory awareness level: denoting detectability (e.g. odour).

Table 2-1: Acute Exposure Values/Methodologies Reviewed by Acutex

Acute Exposure Guideline Levels (AEGL), developed by the US National Advisory Committee on AEGLs (NAC/AEGLs) managed by the US EPA. According to the Standard Operating Procedures for Developing Acute Exposure Guideline Levels (AEGLs) for Hazardous Chemicals, AEGLs are guideline levels for once-in-a-lifetime, short-term exposures to airborne concentrations of acutely toxic, high-priority chemicals (NRC, 2001).

Dangerous Toxic Load (DTL), developed by the UK Health and Safety Executive. The Dangerous Toxic Load (DTL) describes the exposure conditions, in terms of airborne concentrations and duration of exposure, which would produce a particular level of toxicity in the general population. HSE has defined SLOT (Specified Level of Toxicity) DTLs and SLOD (Significant Likelihood of Death) DTLs. No exposure period is given, but the DTL is expressed as an equation, which allows the calculation of the concentrations, which relate to any chosen short time period (Fairhurst and Turner, 1993).

Emergency Exposure Indices (EEI), developed by ECETOC. The EEI (t₁)-1 is defined as “that airborne concentration for exposures lasting up to a specified exposure time (t₁)-1 below which direct toxic effects are unlikely to lead to discomfort in the exposed population (including susceptible but excluding hypersusceptible groups) and above which, as the concentration increases, discomfort would become increasingly more common”. EEI (t₂)-2 and EEI (t₃)-3 for *disability* and *death/permanent incapacity* respectively are defined similarly (ECETOC, 1991).

Emergency Response Planning Guidelines (ERPG), developed by the American Industrial Hygiene Association (AIHA). The Emergency Response Planning Guideline (ERPG) values are intended to provide estimates of concentration ranges above which one could reasonably expect to observe adverse effects as defined according to the different threshold levels (ERPG-1, -2, and -3) (AIHA, 1987).

Intervention Values for Dangerous Substances, developed by the Dutch Ministry of Housing, Spatial Planning and Environment, are tiered thresholds representing concentrations of substances above which health effects can occur as defined in each tier, as follow the ‘instruction guidance values’ (VRW), ‘alarm boundary values’ (AGW) and ‘life threatening values’ (LBW) (Arts, 2002; Ruijten and van Doorn, 2004; Ruijten *et al*, 2004).

SEI and SEL (Threshold of Lethal Effects and Threshold of Irreversible Effects), developed by the French Ministry of Environment, INERIS, INRS, IPSN, University Hospitals, and Industry. The ‘irreversible effects threshold’ (SEI) and the ‘lethal effects threshold’ (SEL) were developed to represent acute effect thresholds in the event of an accidental release into the atmosphere from an industrial site. These thresholds are used to calculate the distance over which effects occur. These distances are taken into account in controlling urban development around Seveso installations (Ministère de l’Ecologie et du Développement Durable, 2004; Pichard and Tissot, 2003).

Temporary Emergency Exposure Levels (TEEL), developed by the US Department of Energy (DOE). Temporary Emergency Exposure Limits (TEELs) were developed by the US DOE to help with emergency planning at DOE sites when ERPGs are not available. Once an ERPG is assigned to a chemical, the ERPG replaces the TEEL. The TEEL programme uses occupational exposure limits to derive TEELs (Craig and Lux, 1998; Craig *et al*, 2000).

Table 2-2: Health Effects and Exposure Durations Targeted by Threshold Levels⁴ of Acute Exposure Levels Used in the EU

| Health Effect | Duration of Exposure | | | | | | | | |
|--|----------------------|---------------|-----------------|--------|-----------------|----------------------------------|--------|--------|---------------------|
| | 1 min | 10 min | 15 min | 20 min | 30 min | 1 hr | 4 hrs | 8 hrs | Probit ⁵ |
| No appreciable risk of health effects, not likely to suffer discomfort | | | EEI-1 TEEL-0 | | EEI-1 | EEI-1 | | | |
| Objectionable odour | | | TEEL-1 | | | EPRG-1 VRW | | | |
| Mild effects, discomfort, irritation | | AEGL-1 | EEI-1 TEEL-1 | | AEGL-1 EEI-1 | AEGL-1 EPRG-1 EEI-1 VRW | AEGL-1 | AEGL-1 | |
| Likely to suffer severe distress | | | | | | | | | SLOT |
| Medical attention required | | | EEI-2 | | EEI-2 | EEI-2 | | | SLOT |
| Impairment of an individual's ability to take protective action or escape | | AEGL-2 | TEEL-2 EEI-2 | | AEGL-2 EEI-2 | AEGL-2 EPRG-2 EEI-2 AGW | AEGL-2 | AEGL-2 | |
| Serious health effects, serious injury requiring prolonged treatment | | AEGL-2 | TEEL-2 EEI-2 | | AEGL-2 EEI-2 | AEGL-2 EPRG-2 EEI-2 AGW | AEGL-2 | AEGL-2 | SLOT |
| Immediate or delayed permanent adverse health effects, irreversible health effects | SEI | SEI AEGL-2 | TEEL-2 | SEI | SEI AEGL-2 | SEI AEGL-2 EPRG-2 AGW | AEGL-2 | AEGL-2 | |
| Permanent incapacity | | EEI-3 | | | EEI-3 | EEI-3 | | | |
| Life-threatening effects | | AEGL-3 | TEEL-3 | | AEGL-3 | AEGL-3 EPRG-3 LBW | AEGL-3 | AEGL-3 | |
| Likely to cause death, lethal effects | SEL | SEL EEI-3 | | SEL | SEL EEI-3 | SEL EEI-3 LBW | | | SLOD |

⁴ Threshold levels represent the maximum concentration at which the health effect is not expected to occur.

⁵ Probit as a function of concentration or a given time period.

2.3.1 Threshold level 3

Definitions for this tier, as described in Appendix I, can be categorised as follows:

- For the AEGL, ERPG and TEEL methodologies, this threshold concentration is defined as the point at which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.
- The EEI-3 refers not only to death but also to permanent incapacity.
- The French SEL value is based on the maximum concentration of pollutant in the air. For a given exposure duration, this corresponds to a concentration above which mortality can be observed in the exposed population. The lethal concentration (LC₀₁) is used as the lethal effect threshold.
- The Dutch life threatening value (LBW) refers specifically to death and life threatening situations that may develop within a few days. This is comparable with the EEI approach which considers effects that occur immediately or soon after exposure.
- The UK's SLOD - DTL corresponds to the exposure conditions which are predicted to cause mortality of 50% of an exposed population and which is equivalent to the Dutch approach.

2.3.2 Threshold level 2

Definitions for this tier can be categorised as follows:

- The definitions for AEGL-2, EPRG-2, and TEEL-2 explicitly encompass both irreversible effects and impaired ability to escape in one definition. The definitions also cover severe health effects, without any other descriptor, which implies coverage of severe effects that are reversible as well as irreversible.
- The UK SLOD - DTL takes a similar approach to that of the AEGLs etc. above, in that it encompasses a number of types of serious effects in one definition. However, the definition does not specifically refer to irreversibility, reversibility or incapacity (although to some extent incapacity is implied).
- The EEI-2 is a threshold denoting disability. Disability is being defined as:
 - External assistance is needed because persons are disabled by exposure and cannot take actions necessary to protect themselves or escape.
 - Exposed persons acquire an illness or condition:
 - The outcome or duration of which can be significantly modified by treatment or nursing care.
 - With permanent or long-lasting residual effects including effects on the outcome of an existing or subsequent pregnancy.

Implicitly it can be read that the EEI approach also includes a distinction between reversible and irreversible effects.

- The SEI solely refers to irreversible effects and the concepts of incapacity or impairment to escape are not part of the definition of any tiers within the French system. Irreversible corresponds to health effects that are persistent in time and take the shape of a lesion or functional impairment directly following the exposure.

- The definition of the Dutch alarming threshold (AGW) refers to both irreversible as well as other severe health effects. However, in the methodology so-called effect categories have been defined and those are similar to the ones being used by the EEI. These have also been adapted to be in line with the AEGL and ERPG approach. At this level, the effect category disability includes impairment to escape. Thus, similar to the French approach, the Dutch system does not deal with incapacity or impairment to escape.

2.3.3 Threshold level 1

For the definition of threshold level 1 there appears to be considerable agreement between the methodologies. The aim is, more or less, to prevent discomfort. Discomfort is generally described as the occurrence of irritation and/or mild and transient health effects. Also, effects in this category are, by and large, expected to be reversible. Most uncertainty in the current approaches is in the role of odour; although there appears to be some consensus that objectionable odour (ERPG and TEEL) and annoying odour leading to complaints (VRW) should be included in this level. Odour not leading to complaints or not annoying should be included under the level of sensory awareness.

More specifically, the definitions for the different methodologies include the following:

- The AEGL definition makes specific reference to reversibility.
- ERPG-1 and TEEL-1 specifically refer to objectionable odour at this level.
- EEI-1 makes reference to discomfort, which even in the absence of medical attention will not evolve into more serious effects.
- The French SER (reversible effects threshold) focuses solely on the reversibility of the effect, while the Dutch VRW value focuses at this level on the occurrence of discomfort, or minor and quickly reversible health effects.
- The DTL (UK) approach does not define a value for these severity levels.

2.3.4 Sensory awareness level

Until recent only the TEEL system considered a level 0, which is defined as the level below which most people will experience no appreciable risk of health effects. It is unclear what is meant by no appreciable risk of health effects compared to mild transient health effects as defined at level 1. The French approach most recently added a tier to incorporate this concept, that is, a perception threshold (SP). This threshold level is defined as sensory detection of the substance by the exposed population.

In recent years, the US AEGL committee has been considering to add this level to their system. Within the AEGL methodology the odour level plays a key role. It is referred to as 'level of odour annoyance'. There is no mention of other stimuli that may trigger a level of awareness. The formal procedures to be used for setting this level still need to be finalised and have not yet been published.

The EEI, Dutch and ERPG systems do not currently have values at this level.

2.4 *Difference in reference periods*

The various approaches differ with respect to the defined reference periods (Appendix I).

In the AEGL system the following exposure duration periods are used: 10 minutes, 30 minutes, 1 hour, 4 hours and 8 hours. ERPG are standardised for 60 minutes, while the TEEL

values are standardised to 15 minutes for concentration dependent chemicals and 60 minutes was assumed for dose-dependent chemicals.

The French SEL/SEI values have exposure duration periods from 1, 10, 20, 30 through to 60 minutes, and 2, 4, and 8 hours if needed.

In essence, the EEI system leaves the exposure period undefined and any value could be assigned to a given time period based on the dose-response curve. However, in the EEI examples provided in the documentation accompanying the methodology, 15, 30 and 60 minutes exposure duration are mentioned.

For DTL values, no exposure duration period is provided, but the DTL is expressed as an equation, which allows the calculation of concentrations relating to any chosen short time period.

2.5 Discussion of the differences and recommendations

The systems as currently in use are compared and discussed below with respect to their advantages and disadvantages. Recommendations and conclusions are made on which definitions should be used in the AETL system.

2.5.1 Threshold levels

2.5.1.1 Threshold level 3

At this level there is considerable agreement on the definition. Almost all approaches are targeted to set a threshold to prevent life threatening conditions or death. ERPG, AEGL, TEEL and LBW use this terminology, while EEI refers to death and permanent incapacity. The French SEL refers to the prevention of lethal effects alone.

It also seems that the EEI-3 definition is intended to express that the loss of an essential capacity (e.g. blindness) resulting in serious restrictions of normal and social or economic activity, should be rated at an equivalent level to loss of life. Such an approach might complicate a clear understanding of what this endpoint is intended to represent, as multiple endpoints are possible. For example, how would loss of hearing be rated vs. loss of eyesight? If both are possible, which threshold should be chosen? It adds subjectivity to the science of deriving an acute exposure level, and it reflects the value society places on the quality of life after a serious injury.

The approaches developed for quantitative risk assessment focus on death alone and give the means to calculate the expected number of deaths. The 50% probability estimate provides the most accurate figure. It is preferred over other percentile probability estimates. In the Netherlands, a similar approach (EPEL) has been published in the 'Green Book' (Verberk, 1975).

It is obvious that these two approaches will produce dramatically different values and that there is a need for two separate definitions to satisfy the need of our stakeholders. For this reason the following two definitions are proposed to promote harmonisation among the stakeholders:

- Threshold level 3a (certain death) focuses on the expected number of deaths, with the purpose of forecasting the number of casualties in case of an accident.
- Threshold level 3b (life threatening) focuses on the most severe exposure conditions causing no death or life threatening situations, with the purpose of preventing casualties.

Upon consideration whether there could be a distinction within threshold level 3b between life threatening effects and the onset of death, it was concluded that although ‘life threatening effects’ represents the more prudent approach, in practice a real distinction between these two endpoints is not possible for a given population.

The question remained how to deal with permanent incapacitating effects (like blindness). In line with most of the current approaches, it is proposed to include these under threshold level 2.

Recommendation

Based on the above discussion it is considered essential, in defining AETLs, to make a distinction between the highest exposure conditions causing no death (threshold level 3b) and the exposure conditions causing a number of deaths in a given scenario (threshold level 3a). It is recommended that permanent incapacitating effects should be covered under threshold level 2 not 3.

2.5.1.2 Threshold level 2

When reading the definitions for level 2 there appears to be a distinct difference between EEI on one hand and the ERPG and AEGL values on the other hand. The EEI definition focuses on the prevention of effects leading to ‘disability’, while the ERPG and AEG definitions focus on the prevention of two types of effects: irreversible and long lasting/serious health effects and impairment of the ability to escape.

In order to be complete it must be mentioned that EEI uses the following definition for disability: “The term ‘disability’ is used to indicate that persons will require assistance or that effects of exposure will be more severe and/or prolonged without it.” Thus, the scope of disability may be wider than impairment of the ability to escape. Assistance could also refer to the need to provide antidote or other first aid treatment. If lack of assistance does not lead to more severe or prolonged effects it does not fall into this class.

It can be concluded that the EEI definition focuses primarily on the identification of victims for which assistance is needed. However, as described in the explanatory text on page 10 of ECETOC (1991), it is clear that the adoption of the level is intended to ensure that permanent, long lasting effects are avoided. If this is taken into account, there is actually no difference between the three examined approaches, except that permanent impairment is considered as an effect of level 3 under the EEI scheme.

The SEI threshold clearly refers to irreversible effects alone and does not include impairment to escape. This is also the case for the SLOT, which focuses on severe health effects and the need for medical attention. Here also no reference is made to impairment to escape.

Considering these approaches at severity level 2 the following end points can be identified:

- Irreversible health effects and long-lasting/serious health effects (ERPG, AEGL, EEI, SEI, SLOT, AGW);
- Need for medical attention (EEI, SLOT);
- Impairment to escape (ERPG, AEGL, EEI, TEEL, AGW).

The approach followed by AEGL, ERPG and EEI leads to difficulties when the authorities want to know, for emergency planning purposes, at which level impairment of the ability to escape occurs or people need (medical) assistance (EEI and SLOT). The current approach does not indicate the critical effect that determined the setting of the limit value at this level. On the other hand, if authorities are interested to know at which concentrations they may see

the onset of irreversible effects, with the exception of SEI, none of the current threshold values can provide that answer either.

For clarity it may be recommended to define separate thresholds for impairment of the ability to escape (combined with the need for assistance) and the onset of irreversible (long-lasting/serious) health effects. For the sake of simplicity, it is helpful to focus on the criteria that are considered most important to stakeholders. For emergency responses, the value indicating impairment to escape is expected to be of most value. In addition, it will be of value to emergency responders to know which exposure should be prevented to avoid irreversible effects (e.g. through evacuation).

Policy and decision-makers may be most interested in the population that will be confronted with irreversible effects. It is believed that separating such levels may not be an easy task. When deciding to choose one value it should be explained what type of effect has been used to set the threshold level (irreversible effects or impairment to escape).

Recommendation

Based on the above and with the objective to keep the scheme simple, the limit value should be chosen which is of key importance for the emergency responder and which aligns with the current ERPG and AEGL value. Such a value prevents irreversible effects and the impaired ability to escape. In order to provide transparency for the end-user, it should be indicated in the listing of values whether the value was based on ‘impaired ability to escape’.

2.5.1.3 Threshold level 1

The definitions for level 1 (as provided in Appendix I) are in close agreement. Emphasis of all indices identifying a level 1 (AEGL, ERPG, EEI, TEEL) is on the prevention of discomfort. Discomfort is generally described as the occurrence of irritation and/or mild and transient health effects.

ERPG and TEEL refer to objectionable odour, while the AEGL indicates that disagreeable odour and mild irritation can occur below this value. The AEGL mentions explicitly that these types of effects are reversible and not disabling. Thus, there is no agreement on how odours should be handled.

The EEI approach defines discomfort as follows: “persons suffering discomfort, though distressed and possibly requesting assistance, will not be dependent on it.” This implies that, if nothing is done, no irreversible effects should be expected. It is not believed that this explanation makes the EEI approach different from the other two approaches.

Recommendation

As there is no clear difference between the four approaches for level 1, any of the current definitions could be used. In order to clarify the role of odour, this may need to be addressed in more detail in the definition or supporting documentation.

2.5.1.4 Sensory awareness level

Only the TEEL approach defines a level 0 but with only very little clarification in the available documentation. Recently the AEGL committee has developed an approach for setting a level of distinct sensory awareness. At this level there is no discomfort (odour is not objectionable) but it is clearly perceived by the population and may cause anxiety.

This level will be of value for emergency response. In a number of cases, health effects can occur before there is any sense of smell. Therefore, for certain substances this value might better be denoted as not applicable (n.a.).

Recommendation

A 'level of detection' would be of value for communication purposes. Therefore, it is recommended to develop a definition for this level and naming it such that the different dimension to the other 3 threshold levels is made clear. It is proposed that an approach and procedure is developed that aligns with the approach that was recently developed by the AEGL committee.

2.5.2 Reference periods

There is considerable variability in the current approaches. There seems to be a trend towards more complexity, although some of the old values (e.g. EPEL) already had multiple time frames. It appears that these multiple time frames served a purpose. Therefore, the time spans and their detail are currently the topic of discussion.

The French system currently uses the time span of 1 to 480 minutes (although there were 24-hour time frames in the past). The use of a 480-minute time span might be problematic as it may be easily confused with OEL values that typically use the same time frame.

In typical industrial accidents the release of chemicals would not last more than several minutes. But in case of fires, releases of toxic gases lasting for days or even weeks can occur. It has been questioned whether time frames of less than 10 minutes (like in use for the French system) could be developed and be of any value.

2.5.2.1 Technical feasibility of a less than 10-minute time span

For the large majority of chemicals, even commodity chemicals, there is little toxicology data for time periods less than 30 minutes. For time periods of 5 or even 1 minute there are almost no toxicology data.

The absence of data on short duration is partly explained by the major technical challenges in generating 1-, 5-, or 10-minute inhalation exposures.

Therefore, establishing guidance for these short durations will almost always involve extrapolation, usually from 1- or 4-hour data. Even assuming the use of 1-hour data, the generation of a 1-minute AETL would require a 60-fold extrapolation.

This extrapolation is even more difficult as breathing patterns may influence the very short-term exposure (e.g. the animal holding its breath). In addition, only few of these types of ultra short time studies are available due to the technical complexity of carrying out these studies. Where studies are available, critical attention should be paid to the homogeneity of the concentration offered to the animals.

2.5.2.2 Value of a less than 10-minute time span

AETL values are being developed in order to protect the general public in case of chemical releases. Generally (with the exception of transport incidents) there is some distance between the factory and the fence line and the nearby housing. Thus, clouds that are being released disperse depending on the meteorological conditions. Due to this dispersion under common meteorological conditions the exposure time for the population even from a one-minute

release will be generally rather in the order of 10 minutes than one minute. For this reason, it is believed that having shorter time frames is of minor practical value for the general population.

Even considering a situation where the victim is very close to the source, e.g. at the site of a transport incident, it is unlikely that the source will be controlled within a short time, unlike in an industrial setting.

2.5.2.3 Level of detail

For emergency response purposes it is best to have discrete numbers at discrete time intervals. For emergency response planning, land-use planning and Quantitative Risk Assessment (QRA) having a continuous curve may be more helpful and would avoid the need for interpolations.

Recommendation

- Limit the time span to a range of 10 minutes to 480 minutes.
- Determine AETL values for discrete time intervals of 10, 30, 60, 120, 240 and 480 minutes.
- Establish limit values for shorter than 10 minutes only when the toxicity data set provides the information to set these values in a valid way.

Limit values for shorter than 10-minute periods will only be established when the toxicity data provide the information to set these values in a valid way. Applying the current methodology to the 22 case studies made in the context of this project confirms that only in a few cases (mainly irritant chemicals and for AETL-3 levels) such derivation is feasible. In other cases, establishing limits for exposure less than 10 minutes is not possible for the following reasons (more details provided in TSD):

- data are not available for such short exposure durations;
- the generation of the atmosphere is not technically feasible due to physico-chemical properties of the substance;
- the basis for the derivation of the AETL is a study of relatively long duration (>60 minutes). Extrapolation/modelling to short exposure duration leads to too many uncertainties.

In addition to these observations, sensory irritation induced by chemical exposure leads to restraint of breathing. Thus, from a toxicological point of view, derivation of AETLs for exposure less than 10 minutes is not recommended. Provision of concentration-time curves in the TSD to allow for standardised interpolation should also be considered.

2.5.3 Definitions of susceptible and hypersusceptible subpopulations in the existing systems

2.5.3.1 Introduction

In this section, a description and a comparison are provided for the different definitions that are used to categorise subpopulations in the definition (e.g. susceptible or hypersusceptible).

2.5.3.2 AEGL approach

The Standing Operating Procedures contain a very comprehensive discussion on intraspecies uncertainty factors, where the range of susceptibility is reviewed and distinguishes between susceptible and hypersusceptible subpopulations.

In addition, AEGL references the NRC as follows: “The NRC guidelines for developing community emergency exposure levels (CEELs) state that the exposure levels are designed to protect almost all people in the general population...” (NRC, 1993). The NRC guidelines state that although the levels “are designed to protect susceptible individuals, some hypersusceptible individuals might not be protected...”. That distinction is based on the premise that CEELs must be set low enough to protect the general population but must also be set at levels that minimise the public health and safety risks associated with response to chemicals as a result of rare or exceptional circumstances. Consequently, the AEGL values may not be expected to necessarily protect certain individuals with unique or idiosyncratic susceptibilities. This consideration is clearly communicated in the NAC/AEGL Committee’s definition of the AEGLs.

The NAC/AEGL Committee has identified specific categories and subpopulations that may be considered susceptible while still part of the general population that the AEGL values are intended to protect. These categories include children and infants, the elderly, asthmatics, pregnant women and the foetus, and individuals with pre-existing illnesses, diseases or metabolic disorders who would not ordinarily be considered in a severe or critical medical condition. Examples of susceptible subpopulations based on pre-existing illnesses include those with compromised pulmonary function (e.g. pneumoconiosis, emphysema, respiratory infections, smoking-related diseases, immunological sensitisation due to prior exposures, and cystic fibrosis), hepatic function (e.g. alcoholism, hepatitis, and prior chemical exposures), cardiac function (e.g. dysrhythmias and coronary heart disease), and impaired renal or immunological function (e.g. acquired immune deficiency syndrome, AIDS, and systemic lupus erythematosus).

Hypersusceptible subpopulations are considered to comprise those individuals whose reactions to chemical exposure are unique and idiosyncratic; lie outside the range of distributions expected for the general population, including susceptible subpopulations; and constitute a relatively small component of the general population. For example, the AEGLs are intended to be protective of individuals with mild-to-moderate asthma but are not necessarily protective of those with severe asthma. Additionally, there are some asthmatics that, at any given time, could be suffering coincidentally acute asthmatic episodes at the time of a chemical emergency. Such subpopulations may be considered to comprise transient hypersusceptible individuals and would not necessarily be protected by the AEGLs. Examples of hypersusceptible subpopulations might include those with severely debilitating pulmonary, hepatic, or renal disorders or diseases, the elderly with serious debilities of primary physiologic systems, and those individuals with unique hypersensitivities (i.e. severe immune-type responses) to specific chemicals (e.g. 4,4N-methylene bis(2-chloroaniline)) or chemical classes (e.g. isocyanates). It is acknowledged that the AEGL values might not be protective under such circumstances.

2.5.3.3 French approach

In the French approach, the following is stated:

“It is worth recalling that this methodology’s concern is protecting the entire population with individuals of variable sensitivity. Hypersensitive persons are however excluded from the domain of application.”

2.5.3.4 EEI

In the EEI approach the following is stated:

“The general population will contain groups who may be more susceptible to chemical exposures than the average person, e.g. the elderly, the young, the pregnant and those with minor acute illness or chronic illness compatible with participation in normal daily activities. The TF considered that EEIs set should take into account such susceptible groups but not more seriously debilitated, hypersusceptible groups, e.g. those with pneumonia or myocardial infarction.

Hypersusceptible groups of people have been excluded from this exercise because:

- such individuals are considered to be in a grave and unstable health condition and the outcome of their illness will not primarily be related to the degree of exposure (when potentially exposed as a consequence of a major chemical release hypersusceptible people should, as a high priority, receive medical attention as a precautionary measure);
- data from experimental studies will not, normally, accurately predict the health effect of a chemical in a hypersusceptible individual”.

2.5.3.5 ERPG

The ERPG handbook (AIHA, 2005) notes the following: “the values derived for ERPGs should not be expected to protect everyone but should be applicable to most individuals in the general public. In all populations, there are hypersensitive individuals who will show adverse responses at exposure concentrations far below levels at which most individuals normally would respond.”

From this statement one could conclude that the ERPG limits do include the sensitive but exclude the hypersensitive population.

2.5.3.6 Dutch approach

In the Dutch approach it is indicated that people with an increased sensitivity due to age, sex or pre-existing disease are taken into account when setting the limit values. However, the effect of exposures to concentrations that are close to the limit value on very sensitive persons, like terminally ill patients and sensitised persons, is difficult to predict. For this reason, the thresholds are not primarily aimed at these types of persons.

2.5.3.7 ACUTEX approach

In the view of the ACUTEX group, a differentiation between susceptible and hypersusceptible subpopulations is not useful for the derivation of AETL values. Only few examples exist (e.g. allergic responses) which make such a differentiation plausible. In most cases of higher susceptibility, continuous distribution of susceptibility can be expected,

making it impossible to draw a line between two groups of susceptible individuals. The ACUTEX approach is described in sections 3.3.1 and 4.5.5.

3 GENERAL CONSIDERATIONS

3.1 Scientific criteria to inform prioritisation of substances for which AETLs will be developed

3.1.1 Introduction

The aim of the proposed prioritisation scheme is to provide a tool to help to make the most cost-effective choice of substances to go forward for AETL development. Essentially, this involves identifying the dangerous substances for which the risks to the general population are the greatest. This scheme focuses on substances present at installations to which the Seveso II Directive applies. The scheme does not apply to other sorts of risks associated with areas such as transport, the military or terrorism.

The scheme has been developed in consultation with Seveso II stakeholders, to ensure that it is acceptable to them and takes account of factors that are of importance to them. It aims to be transparent and cost-effective, using a level of detail that is appropriate to the task. The scheme is designed to use only information on substances that is readily available and uses objective criteria where possible, while recognising that expert judgement necessarily has an important role.

3.1.2 Proposed application of the prioritisation criteria

The selection and ranking criteria are summarised in Figure 3-1. Comprehensive information on why these criteria were chosen and how to apply them is provided in Trainor *et al* (2005).

The criteria are proposed to be applied to lists of substances nominated by Member States through their Seveso II Competent Authorities (CAs). The use of such lists ensures that each Member State's priorities will be directly reflected in the EU AETL priority list. It is envisaged that the CAs will be asked to provide a brief justification for each nomination, notably the number of top- and lower-tier sites holding the substance and approximate tonnages per site for certain substances presenting a risk because of their corrosive or irritant properties.

Initially, nominated substances will be screened to make sure that only substances with toxicological properties of concern in relation to Seveso II sites are included. Substances must be classified as toxic (for single exposures) or very toxic, or carcinogenic, or be of concern because of corrosive or irritant properties (Criterion S1). Next, substances that contribute significantly to off-site risk at top tier sites, based on the expert judgement of each CA, will be assigned to a Preliminary Higher Prioritisation List, and other substances will be assigned to a Preliminary Lower Prioritisation List (Criterion R1).

The remaining criteria will be applied, in turn, to the Preliminary Higher and Lower Prioritisation Lists. Priority will be given to substances nominated by more than one Member State (Criterion R2). Then, substances will be ranked according to their relative potential to cause adverse health effects (Criterion R3). This procedure uses readily available physico-chemical and toxicological information, taking hazard as a simple surrogate for risk. Most nominated substances will be gases or liquids. For each of these fluids the area (in km²) that would be covered by a plume from a hypothetical catastrophic release, within which the LC₅₀

(4h) could be reached, will be calculated. This area, termed the 'LC₅₀ footprint', is estimated using a source term and dispersion model. Two release quantities will be assumed for toxic and very toxic substances, a 20 t reference quantity and the Seveso II qualifying quantity for that substance. For irritant and corrosive fluids, information provided by the CA on quantities present at each site will be used as model inputs in place of the qualifying quantity. For solids it is not possible to devise hazard models that can be applied to a broad range of substances and storage conditions, so solids will be ranked according to the hazard measures '1 / LC₅₀ (4 hours)' and 'Seveso II qualifying quantity / LC₅₀ (4h)'. The ranked lists for fluids and solids will be combined using expert judgment. Optionally, for substances with similar hazard, ranking priority will be given to substances present at the greatest number of Seveso II sites (Criterion R4).

Finally, the prioritisation scheme allows factors of importance to individual Member States to be considered on a case-by-case basis (Criterion R5). For example, the prioritisation of substances may be influenced by national policy considerations.

It is recommended that the resulting Preliminary EU Higher (Stage 1) and Lower (Stage 2) Priority Lists take account of EU policy issues and are subjected to stakeholder consultation and value-for-money considerations, to produce an EU First List of Substances for AETLs Development.

There might be other regulatory needs, outside the context of the Seveso II Directive, for which no prioritisation scheme can be given. However this TGD still applies for the definitions of AETLs and their TSD.

3.2 Procedure to develop AETLs and need for peer review (case studies)

The AETL methodology detailed in this TGD is intended for use by any organisation or group of organisations that have a need to establish acute exposure threshold levels for particular substances. It is understood that, depending on the organisations involved, the process for establishing final AETL values may vary depending on the needs of the organisation or organisations supporting the work. However, at a minimum, it is recommended that the following protocols be followed:

- the technical team developing the AETLs has a strong competence in toxicology;
- the values generated by the technical team are subjected to a thorough peer review.

Beyond these recommendations, the structure and organisation of the process should be established as appropriate to meet the requirements of the methodology and to ensure a high quality output.

| Criteria for All Nominated Substances | |
|---------------------------------------|--|
| Criterion | Description |
| S1 | Substance is Toxic (for single exposure), Very Toxic, Seveso II 'Named Carcinogen', or Corrosive/Irritant |
| R1 | Substance contributes <i>significantly</i> to off-site hazard/risk at top-tier sites: if yes, assign to preliminary higher priority list |
| | Substance contributes to off-site hazard/risk at top- or lower-tier sites: assign to preliminary lower priority list |



| Criteria for Preliminary Higher Priority List | |
|---|--|
| Criterion | Description |
| R2 | Give priority to substances nominated by more than one Member State |
| R3 | Rank substances according to their relative potential to cause adverse health effects, based on physicochemical and toxicological properties and Seveso II Qualifying Quantity or Corrosives/Irritants site tonnage. Fluids: use modelled plume 'LC ₅₀ footprint' Solids: use '1 / LC ₅₀ (4h)' |
| R4 | For substances with similar ranking, give priority to those dominating hazard/risk at greatest number of top-tier sites |
| R5 | Take case-by-case account of additional factors raised by Member States |

| Criteria for Preliminary Lower Priority List | |
|--|--|
| Criterion | Description |
| R2 | Give priority to substances nominated by more than one Member State |
| R3 | Rank substances according to their relative potential to cause adverse health effects, based on physicochemical and toxicological properties and Seveso II Qualifying Quantity or Corrosives/Irritants site tonnage. Fluids: use modelled plume 'LC ₅₀ footprint' Solids: use '1 / LC ₅₀ (4h)' |
| R4 | For substances with similar ranking, give priority to those dominating hazard/risk at greatest number of lower-tier sites |
| R5 | Take case-by-case account of additional factors raised by Member States |

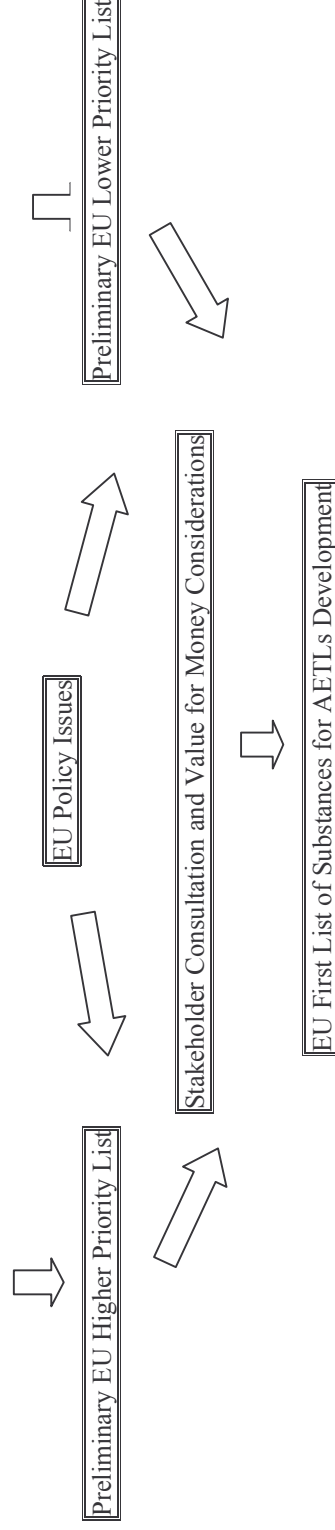


Figure 3-1: Summary of proposed criteria and other considerations to be applied to substances nominated by Member States, to produce a prioritised list of substances for AETL development

3.3 Context for the development of EU AETLs

3.3.1 Population of concern

The AETL values represent threshold levels, which are derived with the aim of protecting the general population. From the view of risk assessment, this population is a very heterogeneous group of subjects: with different ages from the unborn to elderly; male and female including pregnant women; individuals with pre-existing diseases, poor nutritional status, obesity or prior exposures, and those using medical drugs or other drugs like alcohol and tobacco.

The scenario of concern is an airborne exposure to industrial chemicals for a maximum duration of a few hours. Under these exposure conditions, particular subpopulations may be more susceptible with respect to stronger effects when compared to healthy middle-aged adults (the so-called 'normal' population). At identical levels of external exposure, this may be caused by one or more of the following three factors:

- higher inhalation uptake due to higher ventilation rates on a body weight basis leading to higher internal concentrations, independently of the particular chemical or mechanism of action;
- slower hepatic elimination of the parent compound or faster metabolism/slower renal elimination in case of toxic metabolites, leading to higher internal concentrations of the relevant compound regarding the toxic effect (differences in toxicokinetics). This leads to a higher sensitivity if the particular chemical undergoes the type of metabolism / elimination described;
- higher sensitivity of the tissue due to a shift of the concentration-effect relationship, leading to a stronger response at the same level of internal exposure (differences in toxicodynamics). This leads to a higher sensitivity, which depends on the mechanism of action of a particular chemical.

In risk assessment, the consideration of sensitive subpopulations is one of the major topics. Depending on the particular chemical of interest, sensitive subpopulations have to be identified (there are no subpopulations that are sensitive in all cases). For common types of possible airborne exposure to chemicals, sensitive subpopulations are described in section 4.5.5.4.1.

In a second step, the higher risk of sensitive subpopulations has to be quantified. This is done by the derivation of an intraspecies factor for the particular subpopulation, which has to be applied additionally to the intraspecies factor considering the 'normal' variability in healthy middle-aged (male) adults (e.g. 'normal' intraspecies factor of 3 and additional intraspecies factor of 2 for the subgroups results in a total factor of 6 in order also to protect individuals of the subgroup. For further examples, see section 4.5.5.4.2). In this context, the following questions have to be answered:

- What is the quantitative distribution of the response (mean/median and variability) in the subgroup compared to that in the 'normal' population?
- How frequent is the subpopulation?

The response of sensitive subpopulations generally represents the outer boundaries of the distribution in the total population. The total distribution may be unimodal or multimodal. Examples of the former are asthmatics in case of local effects on the respiratory tract, or patients with coronary heart disease in case of the systemic effect of an asphyxiant. In both of these groups of patients, the higher sensitivity can range from mild to extreme, depending on the level of severity. In the bimodal case, the second (distinct) distribution representing the sensitive subgroup may vary in size between relatively large (e.g. in the case of a certain

polymorphism which is, by definition, more frequent than 1%) and very small (e.g. rare metabolic disorders). In addition, unique or idiosyncratic biological reactions may occur in individuals but not in the 'normal' population (e.g. malignant hyperthermia, allergic responses). Qualitatively different responses appear also in the case of developmental effects in unborn children.

A further aspect of considering possible subpopulations is their identification during an accident (e.g. for evacuation). This is expected to be easy for the majority of subgroups, but not for some others (e.g. undiscovered coronary heart disease or early pregnancy, genetic variations).

With regard to subpopulations, the scientific problem is the derivation of the additional intraspecies factors necessary for their protection. This is a difficult problem as data are often missing, especially regarding quantitative responses in humans, and frequencies of subgroups. Therefore, in many cases only rough estimates will be possible.

Furthermore, a policy decision has to be made about what proportion of the total population (or the subgroup) should be protected. This is a question with high complexity involving amongst other things cost-benefit analyses and issues of acceptance of risks in the society. It has to balance the severity of response in a particular subpopulation and its frequency. In the case of a rare mutation occurring in e.g. 1 to 40,000 people, the decision not to protect these individuals may be easy. In the case of newborns whose frequency is less than 1 to 1000 in industrialised countries, emotional aspects play a higher role, especially in case of possible irreversible effects leading to a long period of suffering or decreased quality of life. The same applies for pre-term infants.

As no general decision has been made on this topic, and different European countries have different philosophies in risk management, the consideration of sensitive subpopulations is not uniform. To inform on the protection of a particular subpopulation during AETL development, the additional intraspecies factor for this subpopulation has to be derived. For some exposure scenarios, additional intraspecies factors are presented in section 4.5.5.4.2.

In any case, the aim of this TGD is to be transparent. In each TSD, the particular sensitive subpopulations have to be identified, and it has to be stated to which extent they will be protected by the derived AETL value (for details, see section 4.5.5). The information is also necessary for emergency and land-use planning (e.g. hospital or kindergarten nearby, with probable higher frequencies of sensitive individuals). A differentiation between sensitive and hypersensitive subpopulations, as used in some other methodologies for setting emergency threshold levels (see section 2.5.3), is not used for the derivation of AETLs.

Physical activity, hyperventilation and infant crying

For the derivation of AETL values, inhalation is the main route of exposure. Therefore, local effects on mucous membranes of the airways as well as the internal exposure relevant for systemic effects both depend much on the ventilation rate. Under normal conditions, the ventilation rate reflects the metabolic needs of the body. In the case of physical activity (e.g. heavy work, sport activities), the ventilation rate may be increased many fold. Compared to resting conditions, this may lead to much higher internal exposure of systemically acting chemicals, especially in case of short-term exposure (steady state conditions not reached), and in case of chemicals with high partition coefficients (high capacity of the body to store the chemical). This is well known from model calculations and experimental measurements during inhalation of VOCs (Åstrand, 1983). If this factor was considered in the derivation of AETL values, it would be more important than nearly any other. Heavy physical activity

would be much more severe regarding higher susceptibility than most other conditions. Therefore, subpopulations, which might be sensitive, should be compared under resting conditions.

Regarding escape from the accidental site, running away may be dangerous if the toxic cloud is large (better: calm walking), but may be advantageous if clean air can be reached within a short distance.

The same considerations as for physical activity are valid for hyperventilation and infant crying (which may also result in hyperventilation). The influence of these conditions is stronger than that of most other factors.

3.3.2 Duration, pattern and routes of exposure

3.3.2.1 Duration

For emergency response purposes it is best to have discrete numbers at discrete time intervals. Therefore the time span has been limited from 10 to 480 minutes, and AETL values will be developed for discrete time intervals of 10, 30, 60, 120, 240 and 480 minutes. In addition, concentration-time curves should be provided to allow standardised interpolation. For emergency response planning and land-use planning it may be more helpful to have a continuous curve as this would avoid the need for interpolations.

Limit values for shorter than 10 minutes will only be established when the toxicity data provide the information to set these values in a valid manner. Applying the current methodology to the 22 case studies performed in the context of the project confirms that only in few cases (mainly irritant chemicals and for AETL-3 levels) such derivation is feasible. In the others cases, establishing limits for an exposure of less than 10 minutes is not possible for the following reasons:

- data are not available for such short exposure durations;
- the generation of sufficiently high atmospheric concentrations is not technically feasible due to physico-chemical properties of the substance;
- relating to available data, the point of departure for the derivation of the AETL level corresponds to a long exposure duration (>60 minutes) and extrapolating / modelling to short exposure duration leads to too many uncertainties.

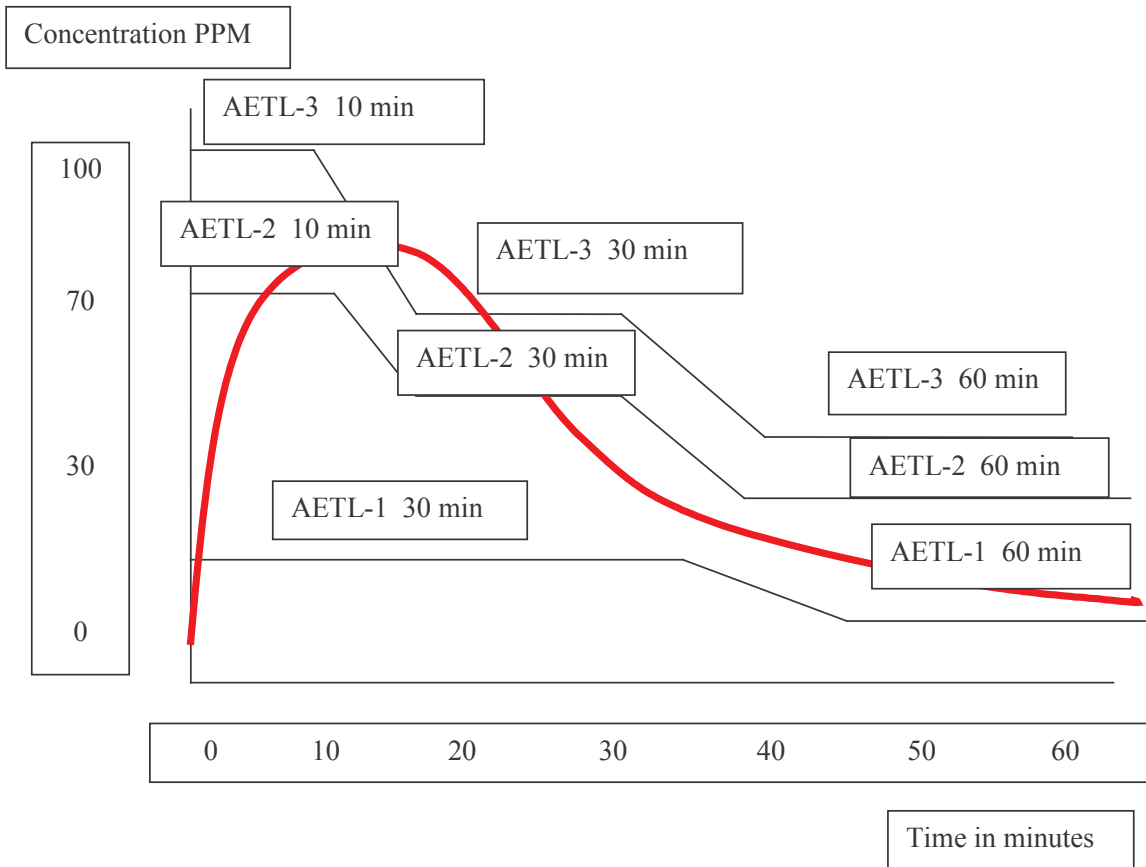
In addition to these observations, sensory irritation induced by chemical exposure leads to restraint of breathing. Thus, from a toxicological point of view, the derivation of AETLs for an exposure of less than 10 minutes is not recommended.

3.3.2.2 Pattern

AETL values have been defined for situations where constant exposure is assumed over a given period of time. In practice, the level of exposure will not be constant but is likely to fluctuate with peak concentration levels.

Emergency exposure scenarios may show the following pattern: initially there will be a rapid rise in concentration followed by an asymptotic decrease to zero.

Figure 3-2: Exposure pattern and AETL analysis



Using the exposure scenario in Figure 3-2 as an example, helps to explain how the various AETL values can be used to evaluate a given release scenario.

From the graph it can be seen that from an AETL-3 perspective, the 10-minute AETL is not exceeded. However, as the exposure prolongs the 30-minute AETL is exceeded for approximately 15 minutes. As this 15-minute exceedance is compensated for by a similar period where the level is below the AETL-3, the situation may still be barely acceptable on the basis of the average exposure over the whole period. However, it might be prudent to consider the situation sufficiently critical to take measures appropriate to the exceedance of the AETL-3.

From an AETL-2 perspective it can be seen that the 10-minute value is exceeded for more than approximately 20 minutes. Although not of importance, the AETL-2 (30 minutes) is also grossly exceeded during more than 20 minutes.

In this scenario the AETL-1 is exceeded all the time.

In order to make a proper interpretation of the scenarios using available AETLs, both concentration level and concentration duration need to be taken into account, to assess the average exposure level that may be compared to the appropriate AETL⁶.

3.3.2.3 Routes of exposure

The AETLs focus on airborne exposure. For the general population, it is assumed that the main route of exposure is by inhalation.

However, the skin is also in direct contact with the gas or vapour and absorption into the body may occur via the skin. This is especially relevant for gases or volatile liquids with a systemic mode of action and might be important for rescuers wearing respiratory protection. In some cases, skin protection is needed for them.

⁶ For a more detailed explanation see pages 14 and 15 in ECETOC (1991).

4 METHODOLOGY TO DERIVE AETLS

4.1 Definitions of the threshold levels of toxicity

Based on the discussion in chapter 2, the following definitions for the various toxicity levels have been developed.

Threshold level 3 should be split into two levels, one dealing with death for land-use planning purposes and the other one with life threatening conditions for emergency response purposes:

- AETL-3a: the airborne concentration at which it is predicted that, after a specified exposure time, a certain (i.e. 1, 5 and 50%) percentage of the general population* will die (*see definitions in Appendix II).
- AETL-3b: the maximum airborne concentration at which it is predicted the general population* could be exposed up to a specified exposure time without experiencing life threatening health effects or death (*see definitions in Appendix II).

Threshold level 2 should be represented by one exposure level that deals with the prevention of irreversible health effects and the impairment to escape. For each value it should be indicated whether it has been based on irreversible health effects or impaired ability to escape.

- AETL-2: the maximum airborne concentration at which it is predicted the general population* could be exposed up to a specified exposure time without experiencing or developing irreversible or other serious adverse health effects including symptoms that could lead to impairment to escape (*see definitions in Appendix II).

Threshold level 1 should be represented by one exposure level that deals with the prevention of reversible health effects (e.g. notable discomfort, irritation, or certain asymptomatic non-sensory effects).

- AETL-1: the maximum airborne concentration at which it is predicted the general population* could be exposed up to a specified exposure time without experiencing more than mild and reversible adverse health effects (*see definitions in Appendix II).

Sensory awareness will be represented by one exposure level that deals with the prevention of odour or other sensory stimuli (e.g. taste) that may be detected and lead to public complaints, concerns or even panic.

- Level of Distinct Sensory Awareness (LDSA): the airborne concentration at which it is predicted that a proportion of the general population* could experience sensory stimuli (e.g. odour) that may lead to public complaints, concerns or even panic (*see definitions in Appendix II).

4.2 Human health endpoints for each level of toxicity

To select the most appropriate human health endpoint to be used to derive an AETL, a detailed analysis of the effects observed in animal studies and human exposures is needed. In this section each possible target organ with their relevant toxicological endpoints is described. Also, study protocols are described which allow the investigation of specific endpoints in more detail. A grading system is proposed to describe the severity of the effect observed which allows allocation to one of the AETLs. But a general guidance for humans and extrapolation over time cannot always be given for each endpoint, as they are substance-specific.

The experience in humans is mostly based on unfortunate accidental exposures. The case studies sometimes give a good description of the effects observed after the exposures to chemicals (i.e. description of life threatening, irreversible, reversible, and nuisance effects on the workers or the exposed population). It is also possible to find information on the applied treatment after exposure. However, they rarely give reliable information on the concentrations and the duration of the exposures. When exposure information is given, this is usually based on retrospective calculations or estimates. Consequently, the information given by these case studies is more of qualitative than quantitative nature.

Some studies are conducted on human volunteers, who are exposed in defined conditions. These studies often provide both quantitative and qualitative information on the effects of moderate concentrations of duration ranging from several minutes to several hours, starting from nuisance (odour perception, neurogenical irritation) up to mild reversible effects (upper and lower respiratory irritation).

4.2.1 Effects on the nervous system

One key target system for the development of AETLs is the nervous system. This consists of the brain and the spinal chord i.e. central nervous system (CNS), and network of peripheral nerves (PNS). Since some of the peripheral nerves innervate various muscles, effects on the PNS can result in neuromuscular effects. Since AETLs can be used to evaluate accidental chemical releases and for emergency preparedness and planning, most of the following discussion focuses on nervous system effects relevant to single acute exposures. Chronic health effects resulting from prolonged and repeated exposures, such as chronic toxic encephalopathy resulting from long-term higher level exposure to organic solvents, or axonopathies resulting from long-term exposure to, for example, n-hexane, methyl n-butyl ketone, and carbon disulphide, are not addressed.

Single exposure to very high concentrations of certain chemicals can cause a spectrum of effects on the CNS. An often-reported clinical manifestation of neurotoxicity is CNS depression or narcosis. This syndrome gathers both symptoms related to non-specific, vegetative, general effects, such as nausea, vomiting, headache, dizziness, vertigo, drowsiness; and to more specific neurotoxic ones, such as cognitive deficits (i.e. reduced vigilance/alertness, poor concentration, disturbed/impaired judgement, prolonged reaction time, irritability, impaired memory function). It also covers symptoms related to behavioural effects, such as fatigue, disorientation, euphoria, excitement, lethargy (sleepiness, fatigue, lassitude), confusion, narcosis (unconsciousness, coma), loss of reflexes, lack of co-ordination, or gait disturbance.

The clinical signs and symptoms depend on the degree of exposure. They range from disorientation, euphoria, giddiness, and confusion progressing to dizziness, inco-ordination,

anaesthesia, coma, and death. Generally, these symptoms develop very rapidly indicating that they are mostly due to the chemical itself rather than a metabolite.

If death does not occur, in the majority of cases, the effects are reversible. Recovery is rapid and complete. An exception occurs when, for example, CNS effects are accompanied by prolonged hypoxia and neuronal death. In this case, the effects may be more long lasting and irreversible.

Also, severe CNS depression and narcosis may result in incapacitation and inability to escape, which is an important consideration in emergency response and therefore for the development of AETLs. Some of the methods to assess incapacitation in animals are discussed in section 4.2.1.2.

A considerable number of toxicants produce neurotoxic effects through injury to neurones. Some well-known examples are methyl mercury and trimethyl tin. The degree of damage varies with dose. However, it is important to note that in the case of severe injury, neuronal death is irreversible and is accompanied by degeneration of all neurones' cytoplasmic extensions, dendrites, axons, and myelin leaving the axon unsheathed.

Another group of toxicants produces neurotoxic effects through inhibition of enzymes present in nervous tissue. Some examples include organophosphorus and carbamate esters. These inhibit acetylcholinesterase, the enzyme responsible for the destruction and termination of the biological activity of the neurotransmitter acetylcholine (ACh). Accumulation of free unbound ACh at nerve endings results in continuous stimulation of electrical activity. Stimulation of muscarinic receptors of the parasympathetic autonomic nervous system leads to signs of poisoning. This includes increased secretions (salivation, tearing, bronchial hypersecretion, sweating), bradycardia, hypotension, bronchoconstriction, miosis, gastrointestinal cramps, diarrhoea, and urination. Stimulation of nicotinic receptors, including the ganglia of the sympathetic nervous system, as well as the junctions between nerves and muscles, leads to symptoms that include tachycardia, hypertension, muscle fasciculation, tremors, and muscle weakness. These agents also produce effects on the CNS including restlessness, ataxia, mental confusion, and convulsions.

Single high doses of certain organophosphorus esters also produce delayed neurotoxicity through inhibition of a neuronal, non-specific carboxylesterase termed neurotoxic esterase (NTE). In cases where inhibition exceeds 70%, the classic ataxia develops 7-14 days post-exposure, progressing to severe muscular weakness and paralysis. From a histopathological perspective, a dying-back degeneration of the large diameter axons and their myelinic sheaths in the distal parts of the peripheral nerves and of the long spinal core tracts is observed. Tri-o-cresyl phosphate, diisofluorophosphate, and leptophos are examples of agents that produce this type of neurotoxicity. Mechanistic and biochemical studies indicate that there are subtle structural requirements related to the binding of these esters on the NTE protein which account for their ability to produce this type of neurotoxicity.

Odour is another endpoint mediated by the nervous system worthy of consideration for AETL development. The lateral wall of the nasal cavity in mammals is innervated by the first cranial (olfactory) nerve. Chemical stimulation of this nerve produces a signal, which is transmitted to the olfactory bulb located in the very front of the brain. From the olfactory bulb, signals are relayed to both the brain's higher cortex, which handles thought processes, and the limbic systems, which handle emotions. This stimulation is separate from that occurring when nerve endings of the fifth cranial (trigeminal) nerve, located in the nasal, oral, and ocular mucosae are stimulated, resulting in irritation. This is why, at lower concentrations, a chemical may be perceived as odorous if only the olfactory nerve is stimulated. At higher concentrations, irritation may occur when the trigeminal nerve is stimulated. However, since in the brain,

inputs from olfactory cells are linked to signals from other sensory inputs, olfaction may combine with irritation or other sensory inputs to form an overall perception.

There is a high degree of individual variability to olfactory responses. Generally, olfactory sensitivity decreases with age and females have higher sensitivity than males. Odour sensitivity also varies with health status (e.g. cold, allergy) and smoking behaviour. Many of these differences are partially due to the anatomic locations of the receptors in the nasal mucosa and condition of the cilia projecting from the olfactory epithelium. However, since odour perception also involves higher-level brain function, behaviour, personality, and training may contribute in some degree to the ability to assess an odour. Odour adaptation associated with repeated exposure may also alter odour sensitivity and perception.

Four major attributes characterise the sensory perception of odorants: detectability, intensity, hedonic tone, and odour quality. Detectability refers to the minimum concentration of odorant necessary for detection by some specified percentage of the test population. Intensity refers to the perceived strength or magnitude of the odour sensation. Hedonic tone is a subjective categorisation of the relative like (pleasantness) or dislike (unpleasantness) of the odour. Odour quality refers to expressions that describe what chemicals smell like (e.g. fruity, fishy, nutty, hay, etc.).

The methods to measure odour thresholds reliably are beyond the scope of this report. Olfactometry, considered the most reliable method, employs a panel of human subjects exposed to diluted odorous mixtures and an odour-free gas as a reference. Modern olfactometry testing procedures include those described by ASTM (1968) and CEN (2003).

4.2.1.1 Human experience

There is a wide variety of methods available to evaluate the neurotoxic potential of chemicals. The purpose here is not to review the relevance of all of these tests and methods, but to focus on the more common methods used to evaluate narcotic effects resulting from acute high-level exposures.

Some methods of investigation, for example the clinical neurological examination, computerised tomography of the brain, neuropsychological examination, and electroencephalography recordings, and measurement of nerve conduction velocities, are used commonly in clinical practice. Other tests and methods are used in research or to assess the neurotoxic potential of chemicals.

Some procedures performed with animals cannot be used with humans and *vice versa*. Some tests performed with animals have little relevance for humans. In some cases, particularly where the main interest is on evaluation of acute effects of higher-level exposures such as AETL-2 or AETL-3, ethical considerations limit the use of human subjects. A brief discussion of the human health endpoints and endpoints from animal testing, respectively, which are potentially applicable to the development of AETLs, is given in this section and the next.

Many test systems have been developed to evaluate potential effects of chemicals on the human nervous system. It is designed to assess attention, memory, learning, motor performance, and perception coding. Of the many endpoints that can be assessed, only a selected number is considered most relevant to the development of AETLs. Generally, these include those tests related to attention (e.g. simple reaction time, switching attention, colour word vigilance tests) and motor function (e.g. finger tapping and hand-eye co-ordination tests).

Case reports of CNS effects in humans exposed to higher levels of chemicals subsequent to accidental chemical releases may provide information relevant to the development of AETLs. Often, the lack of quantitative exposure data, and consistent and accurate reporting of effects limit the usefulness of such data. Such reports tend to note general signs and symptoms of exposure. Some of the signs and symptoms considered most common and useful for the development of AETL values appear in Table 4-3.

4.2.1.2 Animal data

A wide variety of test systems is available to evaluate the effects of chemicals on the nervous systems of animals.

Neurobehavioural functioning is evaluated using selected measures from a standardised functional observation battery (FOB) and motor activity assessment protocol. The FOB consists of standardised observational and simple tests to evaluate gross changes in neurological and behavioural functioning in the rat using measures taken from different functional domains including: autonomic function, neuromuscular function, convulsive behaviour, excitability and sensorimotor reactivity (Table 4-1). In this sense, the FOB evaluates a much broader range of neurological effects than those occurring in the CNS alone. The endpoints considered most relevant to AETLs based on CNS effects include those related to activity (rearing, posture, and particularly motor activity), some of the neuromuscular endpoints (e.g. righting reflex) and response to auditory stimuli.

Table 4-1: Tests included in the WHO/IPCS collaborative study on neurotoxicity assessment*

| Domain | Behavioural End Point |
|---------------|--|
| Autonomic | Lacrimation, salivation, pupil response to light, palpebral closure, piloerection, defecation, urination |
| Neuromuscular | Gait*, fore limb and hind limb*, grip strength*, landing foot splay* righting reflex |
| Sensorimotor | Response to tail pinch*, click*, touch*, and approach of a visual object* |
| Convulsive | Clonic* and tonic* movements |
| Excitability | Ease of removal, handling reactivity, arousal* |
| Activity | Rearing, posture, motor activity* |

*Endpoint included in the HSPA/TNO neurobehavioural programme.

Further, the OECD has provided an overview of the recommended animal tests to evaluate the neurotoxic potential of chemicals (OECD, 2000). Similarly, the US EPA has developed guidelines for evaluating the neurotoxic potential of chemicals (US EPA, 1998). It is also important to note that standard protocols are available for many of these tests, the use of which is critical to the successful evaluation of neurotoxic effects. While some of the tests described in this guideline relate to effects from prolonged and repeated exposures, a number of tests can be used for evaluating potential effects resulting from acute higher-level exposure which may be most applicable to the development of AETLs. These tests include grip

strength, rotating rod, hind limb foot splay and foot spread and motor activity. They are, along with common measurement techniques and model agents, presented in Table 4-2.

Table 4-2: Tests commonly used to measure motor function (OECD, 2000)

| Test | Measurement | Agents |
|---|---|---|
| Grip Strength | Muscular strength in fore and hind limbs using strain gauges. | Decreased by agents that produce peripheral neuropathy; increased by agents that produce hypertonia, e.g. 2,4-D. |
| Rotating Rod (rotorod, accelerod) | Animal is placed on the circumference of a rotating rod, and the time to fall off, or the speed of the rotation when the animal falls off, is measured. | Time is decreased by agents that produce peripheral neuropathy or ataxia, e.g. ethanol or vestibular dysfunction, e.g. IDPN. |
| Hind limb Foot Splay Landing Foot Spread | Measures distance between feet; neurological response following loss of vertical support. | Increased distance by agents that produce peripheral neuropathy. |
| Motor Activity | Measures horizontal and/or vertical movement in a test chamber. | Frequency is increased by CNS stimulants cholinergic muscarinic receptor antagonists; decreased by CNS depressants, cholinesterase inhibitors, e.g. carbaryl. |

A number of animal incapacitation models, none of which are simple or internationally validated, have been developed. Examples of behavioural models are: the rotating activity wheel, the lever actuation conditioned avoidance response developed by Annau (1979), the rotarod, the greased pole, Alarie's respiratory rate model (ASTM, 1996), and hind-leg flexion conditioned avoidance response.

Since these above models have not been examined under the same conditions with the same materials, it is not possible to decide on the basis of experimental evidence what the best model is to assess the incapacitating effect of materials. As each method has both advantages and disadvantages, the choice of one over the other appears to be a matter of personal preference. It is clear, however, that no animal incapacitation or behavioural model will be equally sensitive or responsive to the broad spectrum of compounds.

With the exception of Alarie's respiratory rate model that measures sensory and pulmonary irritants (for details, see section 4.2.2), all of the above models monitor the loss of neuromuscular functions. Some standard methods are also available to evaluate time-to-incapacitation. In the experiments performed time-to-incapacitation was measured for each animal and a mean time with a standard deviation was calculated for the six animals exposed in each experiment.

Time-to-incapacitation can also be examined when the exposure duration has been set (for example, at 30 minutes) and the mean and standard deviation of the time-to-incapacitation for the exposed animals can be calculated for different concentrations of material. The lower the concentration the more time would be needed to incapacitate the animals. These points will be represented by a function that asymptotically approaches a threshold time-to-incapacitation on one axis and a concentration needed to produce incapacitation in the specified time limit on the other axis.

4.2.1.3 Grading

There are numerous endpoints related to CNS toxicity that can be measured and assessed in human and animals studies. Some of the endpoints considered relevant to AETL values are presented in table 4-3.

Table 4-3: Acute human nervous system endpoints: grading of effects

| Endpoint | LT | I | ESC | R | Comment |
|--|----|---------------------------|--|--|--|
| General effects Nausea, vomiting Dizziness, vertigo, drowsiness (light-headedness, fainting, spinning, inebriation) Headache Weakness Faintness | | | x x x | x x x x x | For some effects, the severity is critical for categorisation |
| Motor effects Muscle weakness Twitching Tremor Reflex Cramps Dystonia Inco-ordination Myoclonus, fasciculations Spasticity, rigidity Paresis Seizures (convulsions) Ataxia Paralysis | | | x x x x x x x x x x | x x x x x x x x x x | For some effects, the severity is critical for categorisation |
| Sensory effects Smell abnormalities Vision (colour vision, night blindness, miosis) Tactile disorders Hearing loss, tinnitus Equilibrium changes Numbness, tingling | | x x x x x | x x x | x x x x | For some effects (e.g. visual, hearing disturbances), function may return to normal but death of individual neurones is irreversible |
| Cognitive effects Psychomotor or attention deficits: reduced vigilance/alertness, poor concentration, impaired memory function, mental slowing, disturbed/impaired judgement, learning and speech impairment Reduced initiative | | | x x | x x | |
| Mood and personality effects Excitability, depression, anxiety, irritability, restlessness, nervousness Delirium Hallucinations | | | x x | x x | (x) |

Life Threatening (LT), Irreversible (I), Impairment to take action or escape (ESC), or Reversible (R).

For some effects, the severity is critical for categorisation. Endpoints scored in several categories are consequences of the degree of severity.

4.2.1.4 Conclusions

To evaluate acute CNS effects for purposes of defining AETLs, studies in both humans and animals can provide useful information. Due to ethical concerns, controlled exposure studies in humans will generally provide data relevant to AETL-1 and to a lesser extent AETL-2. Since studies in animals can be performed at higher exposure concentrations, such data can be used to help define AETL-2 and -3. For these latter studies in animals, those endpoints related to activity (rearing, posture, and particularly motor activity), some of the neuromuscular endpoints (e.g. righting reflex) and response to auditory stimuli appear to provide the best information for assessing higher-level narcotic effects. In addition, since both the onset of and recovery from narcosis occur relatively quickly, those tests, methods, and measures that can be performed during the exposure, or soon after, will provide the most accurate assessment of narcotic effects. In contrast, those tests performed at a later time are more likely to reflect more long lasting toxic effects such as those occurring in the peripheral nervous system.

It is important to note that for some chemicals, the effects observed for these endpoints may not be the result of impacts on the nervous system alone. Rather, at high exposure concentrations, effects on other target organs can occur concurrent with those on the nervous system.

4.2.2 Effects on the respiratory tract

The respiratory tract is the first port of entry into the body of a pollutant released into the air and inhaled. The effects on the respiratory tract are complex due to different local anatomical features that interact with the physical/physico-chemical nature of a substance and determine particle or gas deposition and clearance.

Each region of the respiratory tract has its specific vulnerabilities either due to the presence/absence of metabolic systems, the thickness of a protective mucus layer or the location and specific absence/presence of highly specialised cell-types. As a consequence, the biological responses may be site specific and complex and may vary among species.

Reactive agents may impact the mucus layers and the epithelial cells of the nasal passage and the pharynx in the upper respiratory tract. This may lead to irritation and corrosion of the respiratory mucosa. Less reactive agents are more likely to penetrate further into the respiratory tract. From here, they may be cleared by various mechanisms; mechanically by mucociliary clearance, dissolution and absorption into the systemic circulation, retained and accumulated locally in pulmonary macrophages or translocated via axons of the olfactory nerve, the lung interstitium and lung associated lymph nodes.

Given the difference in their anatomy, various species used in inhalation toxicology studies do not necessarily receive identical doses in comparable respiratory tract regions when exposed to the same external particle or gas concentration.

Retention is the actual amount of inhaled agent found in the lungs at any time and is determined by the relative rates of deposition and clearance. Retention and the toxicological properties of the inhaled agent determine the magnitude of the pharmacological, physiological, or pathological responses.

For particles, deposition mechanisms include inertial impaction, sedimentation (gravitational), diffusion, interception, and electrostatic precipitation. Generalisations regarding the site of deposition of particles of a given size are problematic due their dynamic change within the respiratory tract (Snipes 1989a, b; Snipes *et al*, 1983; Phalen, 1984; Lippman and Schlesinger,

1984; Warheit, 1989). However, in the average adult human, most particles larger than 10 μm in aerodynamic diameter are deposited in the nose or oral pharynx and are unlikely to penetrate to tissues distal to the larynx. Very fine particles (0.01 μm and smaller) are also trapped relatively efficiently in the upper airways by diffusion.

The sites of deposition of gases in the respiratory tract define the pattern of toxicity of those gases. Water solubility is the critical factor in determining how deeply a given gas penetrates into the respiratory tract. Part of the gases, which are not scrubbed by the nose reach the posterior part of the respiratory tract, the trachea and the lung, leading to inflammatory responses in the range from slight irritation to severe injury, and oedema.

The intensity of the effects is related to the concentration, deposition pattern and depth of penetration of the pollutant into the respiratory tract, and the duration of contact with the different cell types of the respiratory epithelium. The differentiation between local toxicity and systemic toxicity depends on the physico-chemical properties of the pollutant, and its ability to penetrate into the blood.

Chemicals can also be activated or deactivated in specific cells of the lung. The local density of such cells and their level of activity will determine the local toxic response. Furthermore, unsuccessful degradation of phagocytosed particles by macrophages may make them burst and release endogenous factors causing lung inflammation.

Where surface deposition is non-uniform the initial dose delivered to specific sites may be greater than when an even surface deposit occurs. This is especially important for inhaled particles that affect the tissue on direct contact, such as irritants.

Moreover, due to species-specific structures and responses to inhaled agents the most susceptible species may not necessarily be the most relevant species for human risk characterisation. Therefore, in many cases, the biological endpoint or health effect may be more directly related to the quantitative pattern of mass deposited within the respiratory tract than to the external exposure concentration.

Also, the dependence on available technologies in the dosing of experimental animals is almost unique to inhalation toxicology. This makes the comparison and selection of key-inhalation studies from different sources difficult. In inhalation studies, expected toxic potency values may not necessarily reflect the true toxic potency but may reflect technical difficulties involved with the characterisation of the test atmosphere. Thus, flaws in experimental design and inconsistencies in the results of atmosphere sampling and characterisation may call the conclusions of selected studies into question.

However, in the case of accidental exposure to chemicals, the above-described complexity is limited by the nature of the substances involved (mainly volatile liquids and gases) and short duration of exposure (no activation of metabolic systems or accumulation).

In the scope of this report the emphasis is on the effects of corrosive and irritant gases and aerosols with a local irritant effect on the respiratory tract, since these are of highest concern with regard to emergency exposures. However, the generation of particles (e.g. condensation aerosols) cannot be ruled out under such conditions and liquid aerosols (that include mists and fogs) may be readily available for inhalation exposure. Therefore it is important to remember that the mechanisms of deposition and retention, including the deposition efficiency of particles, are appreciably different from those governing the deposition and retention of gases and vapours.

4.2.2.1 Target organ sites

Upper respiratory tract

The nasal airways are the initial portion of the respiratory tract and extend from the nares (nostrils), the nasal vestibule to the nasopharynx. Posterior to the nasal vestibule the nasal mucosa contains pseudostratified ciliated columnar epithelium, which extends throughout the remainder of the upper respiratory tract and into the tracheobronchial and pulmonary regions of the respiratory tract.

Although humans are combined nose/mouth breathers, the nose represents the first line of defence of the respiratory tract for inhaled particles. Inertial properties, as reflected in measurements of aerodynamic diameter, are primarily responsible for deposition of particles in the nose. Inhalable particles with aerodynamic diameters greater than 10 μm have a high probability of depositing in the nasal airways of people during nose breathing. Particles of all sizes larger than 0.5 μm aerodynamic diameters will deposit to some extent on the nasal airways. For large particles, the predominant deposition mechanism in the extrathoracic region (head airways region) is inertial impaction.

Particles that deposit in the nasal vestibule can be removed by mechanical means, such as nose blowing or wiping. Particles that impact and become trapped in the mucus layer of the nasal airways are normally removed along with the mucus as it is cleared by cilia to the pharynx, where it is swallowed.

The nasal mucociliary system can clear deposited particles from the nose in about 12-30 minutes (Andersen and Proctor, 1983; Witek, 1993). This rate can be reduced by disease or damage to nasal cilia by inhaled agents. Clearance rate can also be altered by changes in the cilia or the rheological properties of mucus.

In the nose, the primary location for lesions of the respiratory epithelium exposed to water-soluble toxicants is in the anterior portion of the nasal passages, the mid-ventral septum and the middle turbinate.

Chemosensory effects in the nose can either be irritative or odorous. Stimulation of the olfactory nerve results in the sensation of smell, whereas stimulation of the trigeminal nerve gives rise to chemical irritation and evokes a burning sensation of the nasal passages. This inhibits normal respiration. It will also induce coughing from laryngeal stimulation.

Historically, there have been attempts to segregate chemicals into categories of pure olfactory (sensory) and pure trigeminal stimulants (irritants). However, it is now conceded there are very few chemicals that fall exclusively into either category. Because most agents, at sufficiently high concentrations, elicit both olfactory and trigeminal activation, it is important to understand the normative function and interactions of these two systems, as well as the clinical and experimental methods used to assess and quantify odour and sensory irritation that can result from exposure to airborne chemicals.

Water-soluble agents often show a typical anterior-posterior gradient of lesions of the olfactory epithelium. Morphological degeneration of the olfactory mucosa is characterised by loss of sensory and supporting epithelial cells resulting in the thinning or attenuation of the affected mucosa. In more extensive lesions, the degeneration can extend downward with loss of the entire epithelial cell layer with only a thin layer of basal cells remaining. Other changes may include loss of Bowman's glands and nerves bundles, necrosis of individual cells, and exfoliation of cellular debris. This effect results in dysosmia or anosmia, a partial or a complete loss of odour detection in the affected individuals.

Tracheobronchial tract

The tracheobronchial region begins at the larynx and includes the tracheobronchial tree which is subdivided into:

- trachea:
 - primary (principal) bronchi;
 - secondary (lobar) bronchi;
 - tertiary (segmental) bronchi;
 - subsegmental bronchi (the number of bronchial generations is species-specific);
- bronchioles;
- terminal bronchioles;
- respiratory bronchioles;
- alveolar ducts;
- alveoli.

Tracheobronchial deposition is caused by mechanisms of inertial impaction at bifurcations, sedimentation and, for small particles, Brownian diffusion. Interception can be an important deposition mechanism for fibrous dusts. During mouth breathing of aerosols the benefits of the collection of larger particles in the nose are lost and these larger particles tend to deposit in the tracheobronchial region with high efficiency. A relatively small fraction of all sizes of particles, which pass through the nasal pharyngeal region will deposit in the tracheobronchial region.

An important characteristic of this region is that it is both ciliated and equipped with mucus secreting elements. Thus, clearance of deposited, poorly soluble particles occurs rapidly by mucociliary action to the throat for swallowing. The rate of mucus movement is slowest in the finer airways and increases toward the trachea. Since particles depositing in the tracheobronchial tree are probably distributed differently with respect to size, with smaller particles tending to deposit deeper in the lung, it is expected that larger particles will clear more quickly.

Liquid aerosols and water-soluble particles are likely to be dissolved in the lining fluids of the respiratory tract and, accordingly, may lose their particle-specific characteristics and, as a consequence, their rate of uptake follows diffusion gradients.

The deposition site and rate of uptake of a volatile chemical are determined by its reactivity and solubility characteristics, including the water-air partitioning. Therefore, the pharmacokinetics of gases and vapours are governed by the rate of transfer from the environment to the tissue, the capacity of the body to retain the material, and the elimination of the parent compound and metabolites by chemical reaction, metabolism, exhalation, or excretion. The mechanisms affecting the transport and deposition of gases involve convection, diffusion, absorption, dissolution, and chemical reactions.

Overall in the tracheobronchial region, deposited and relatively soluble material is rapidly cleared into the blood, while for poorly soluble agents physical clearance by mucociliary transport to the throat for subsequent swallowing is predominant.

Bronchoconstrictors are chemicals, which when inhaled will induce an increase in resistance to airflow within the conducting airways of the lung. The action can be via a direct effect on smooth muscles of the conducting airways by axonal reflex, vago-vagal, or trigeminal-vagal reflexes following stimulation of nerve endings belonging to these systems or by liberation of

histamine. Most of these chemicals are also sensory irritants. Their action on the bronchial mucosa produces a painful sensation.

Lower respiratory tract irritants (deep lung irritants) are chemicals which, when inhaled, will stimulate sensory receptors within the lung. They increase respiratory rate and decrease tidal volume resulting in rapid shallow breathing. Their action, as opposed to that of sensory irritants or bronchoconstrictors, is to evoke a sensation of dyspnoea and breathlessness rather than a conscious painful sensation.

The type of lesions in the medium respiratory tract epithelium range from areas of deciliation, slight epithelial hypertrophy or hyperplasia, small areas of epithelial cell damage with exfoliation, to epithelial erosion, ulceration and necrosis with variable inflammation of the sub-epithelial tissues.

Gas-exchange region

The third compartment, the pulmonary region, includes the functional gas exchange sites of the lung. The most important structure in this region is the alveolus. Each alveolus in the lung parenchyma opens directly into an alveolar duct or sac. Alveoli and alveolar ducts arising from a single conducting airway constitute a pulmonary acinus. A thin tissue barrier consisting of type I and type II alveolar cells provides an extremely efficient means of gas transfer over a large surface area.

Type I cells cover a large surface area (approximately 96% of the alveolar surface). These thin cells serve to minimise the distance between the alveolar air space and pulmonary capillaries, thus, maximising gas exchange. Preferential damage to type I cells by various agents may be explained by their large surface area in relation to cell mass. In the case of damage to the type I epithelium, the mucus may undergo mitotic division and replace damaged cells.

In contrast, type II cells are spherical (cuboidal) pneumocytes. Although these pneumocytes constitute only 4% of the epithelial surface area, they represent 60% of the epithelial cells by number. Type II cell plays an important role in the repair of damaged epithelium, in surfactant synthesis and secretion and metabolic activation of toxic intermediates.

A typical rat alveolus (14,000 μm^2 surface area) contains an average of two types I cells and three type II cells, whereas a human alveolus with a surface area of 20,000 to 30,000 μm^2 contains 32 type I cells and 51 type II cells.

The integrity of the delicate alveolar septa is maintained, in large measure, by a network of mesenchymal interstitial cell populations that produce collagen and elastin fibres. Clara cells are located in the terminal bronchioles and have a high content of xenobiotic metabolising enzymes.

The usually slow turnover of alveolar epithelium is speeded considerably after injury and the number of type II cells in the lung doubles within a short period of time. The mechanisms that control the orderly differentiation of type II cells to attenuated gas-exchanging type I cells remain a mystery. The damage of type I cells is accompanied by type II cell proliferation which also has stem cell function.

Extracellular fluid compartments

The lung incorporates three extracellular fluid compartments: the vascular, the interstitial and the epithelial lining fluid.

The alveolo-capillary barrier is normally extraordinarily effective at partitioning lung and plasma proteins. The low-molecular weight proteins are in equilibrium with plasma. Larger proteins are either produced locally or gain access to the alveoli through highly controlled paracellular (tight junctions) and transcellular (vesicular transport) mechanisms.

Increased fluid flux from the vascular system into the septal interstitium is associated with an early oedematous effect. In adults, the epithelial lining fluid volume is approximately 20 ml with a total protein concentration of approximately 20 g/l. If the barrier is sufficiently impaired with compromised permeability, plasma proteins also enter cross into the alveolus by diffusion in a size-dependent manner and the epithelial lining fluid takes on a composition similar to that of plasma.

Alveolar plasma clearance is slow (approximately 1-2%/hour) during lung oedema resolution and is dependent on water clearance and the creation of favourable concentration gradients. Clearance rates are inversely proportional to the size of the protein molecules and reflect restrictive diffusion of the intact molecule.

Pulmonary surfactant

Mammalian pulmonary surfactant is a complex mixture of phospholipids, neutral lipids and specific proteins that forms a monolayer at the gas/liquid interface, reducing surface tension and the work of breathing. In addition, surfactant stabilises the lung by varying the surface tension directly with the radius of curvature. This property is also important in the maintenance of fluid balance.

Dipalmitoylphosphatidylcholine, the surface-active component credited with reducing surface tension to extremely low values during compression at low lung volumes, comprises approximately 65% of surfactant by weight. Surface tension at the air/water interface produces forces that tend to reduce the area of the interface leading eventually to a collapse of alveoli.

Pulmonary surfactant also reduces the pressure gradient between the vascular system (high hydrostatic pressure) and alveolus (sub-atmospheric pressure), thus preventing extravasations of plasma into the alveolus. It is highly insoluble and floats on the surface of the alveolar lining fluid. The surfactant is primarily synthesised in alveolar type II cells, which consume approximately 50% of the lung's ATP.

Once the amphiphatic mixture is released into the alveolar hypophase (the aqueous lining of the lung), the content of the lamellar bodies hydrate to form a three-dimensional lattice structure known as tubular myelin. In turn, tubular myelin supplies the monolayer at the gas/liquid interface.

For reasons not well understood, the monolayer constantly becomes inactive, possible through oxidation. Re-uptake of inactive material and release of fresh surfactant are essential for maintaining a viable lung. In rats, dipalmitoylphosphatidylcholine has a half-life of approximately 85 minutes, with as much as 85% being re-utilised.

The chemical reactions of a gas with both the lining fluids of the respiratory tract and tissue layers are important. If the gas was the only toxic molecule, then this first-pass reaction would protect the tissue. Conversely, if the reaction products were toxic, then reactions which the tissue layer would increase the delivery of toxic molecules to the tissue. Chemical reactivity with the biological constituents of the tissue is similarly important to the gases' toxic potential to the respiratory tract tissue and to the amount of gas and reaction products that enter the blood for potential extrarespiratory toxicity.

Disturbance of the surfactant system by noxious agents can take place at different stages: the intracellular synthesis of surfactant, the packaging and transport via multivesicular and lamellar bodies, the exocytosis at the cell membrane, the unfolding of the surfactant finally to a thin alveolar lining layer, and the recycling/removal of surfactant.

The lung appears to be one of the most sensitive organs for changes in phospholipid homeostasis. The turnover of phospholipids is highest in this organ due to the anabolism, catabolism, storage, and recycling of pulmonary surfactant. A compromised surfactant layer may lead to an increased permeability of the air-blood barrier and subsequent extravasation of plasma proteins.

In this context it has been hypothesised that surfactant dysfunction occurs as a result of chemical modification by direct chemical reactions or by extravasated plasma proteins causing proteolysis of surfactant-associated proteins. Either mechanism may lead to surfactant dysfunction and increased alveolar surface tension, which increases the transmural capillary pressure gradient sufficiently to result in pulmonary oedema.

4.2.2.2 Primary effect assessment

Traditionally, pulmonary disease has been evaluated by techniques such as histopathology and pulmonary function testing. Acute injury of the airways of the lower respiratory tract of rodents by reactive, moderately water-soluble gases (e.g., HCl, SO₂, some aliphatic isocyanates) is often associated with a biphasic onset of mortality.

Exposure to high concentrations of irritant gases may cause epithelial desquamation, fibroproliferative responses, associated with excessive mucus production. This eventually leads to plugging of airways and delayed-onset in mortality due to an obliterating bronchiolitis. The obstruction of airways, in turn, causes a severe mismatch of the ventilation-perfusion relationship. The alveoli linked to the damaged airway regions are still perfused, but not ventilated due to the plugging and obstruction of the conducting airways. This leads to venous admixture ('shunt blood') and death as a result of hypoxia.

Intermediate concentrations cause a typical delayed-onset type of mortality whereas higher concentrations of reactive gases may penetrate the alveoli and cause immediate mortality through direct damage of the blood: air barrier and ensuing intra-alveolar oedema.

4.2.2.2.1 Human studies

Upper respiratory tract

A wide range of approaches is used in clinical practice and research to evaluate the health status of the nasal portion of the respiratory tract (O'Donoghue *et al*, 2000; Seiden *et al*, 2002). This includes elicitation of subjective perceptions of functional status and pain, direct visual observations, and histological and biochemical examination of nasal tissue and secretions. In addition, various functional measurements may be made including airway dimensions, airflow, and mucociliary clearance.

Irritation of the nasal cavity is classically evaluated by visual observations (both gross and microscopic) and alterations in biochemical parameters in the cells or secreted fluids. As the upper respiratory tract shows an extreme heterogeneity of airflows and cell types, which leads to highly localised and focal damage, nasal lavage is not considered to be a reliable technique.

For other types of effects, subjective perceptions of nasal congestion, secretion, dryness, irritation, unpleasant smell, and pain are appropriate starting points for evaluating the health status of the nose. This includes evaluating the patients' perceptions of nasal airflow or

obstruction, and the extent and nature of secretions, pain or altered olfaction. However, such subjective reports are not always reliable and are marked by great variability, as patients find it difficult to describe the sensation they feel.

The difficulty in eliciting and interpreting subjective findings has led to efforts to identify objective criteria for evaluating nasal function. Two areas have received substantial attention: (1) evaluation of nasal mucociliary clearance, and (2) evaluation of nasal cavity dimensions and airflow.

Two of the critical functions of the nasal portion of the respiratory tract, filtering of material in the inspired air and its clearance, require a functional mucociliary apparatus. Of special concern is a slowing or absence of clearance due to dysfunctional cilia or mucus. This can result in accumulation of noxious materials including inhaled infectious agents.

The Saccharin Transport Time (STT) test procedure was developed by Andersen *et al* (1974) for studying mucociliary function. In the STT test, a small amount of saccharin is placed on the anterior portion of the nasal mucosa. The subjects are told to avoid coughing, sneezing or blowing their nose and at one-minute intervals are asked to swallow and relate if they can detect in their mouth the unique sweet taste of saccharin. The STT is the time interval from administration of the saccharin until it is detected in the mouth.

The normal STT is quite variable from 3-20 minutes with values greater than 30 minutes considered pathologic and indicative of altered mucus flow (Andersen and Proctor, 1983; Tami, 2002). In any event, there is considerable variation in STT values, both intra- and interindividual, with the STT varying between about 4 and 23 minutes.

Relative humidity is a factor to consider in evaluating nasal clearance rates. The air above the nasal epithelium is normally saturated with water vapour. Dry air may cause excessive water loss from the nasal mucosa and increased viscosity of the nasal mucus. The nasal cilia may not move the more viscous mucus along the nasal airways at the same rate that mucus subjected to humid air is moved.

The correlation between nasal and tracheobronchial clearance is controversial and findings on nasal clearance should not be extrapolated to tracheobronchial clearance. In general, the nasal region is very different from the tracheobronchial and pulmonary regions with regard to function, anatomy and responses to inhaled agents. Hence, caution should be exercised in assuming that findings in the nose can be used to predict responses in the tracheobronchial and pulmonary regions or *vice versa*.

Rhinomanometry is a method of evaluating nasal airway resistance, in other words the obstruction of airflow through the nasal passages (Tami, 2002). Nasal resistance is calculated by measuring the airflow through the nostrils of the nose and also the pressure of force required to cause the airflow. The airflow is typically reported for a given pressure differential.

There is inadequate evidence of the clinical utility of rhinomanometry and caution is required in interpreting subtle changes in measurement values in a given individual or group of individuals in research studies. Different results may reflect the range of normal physiological response or may indicate adverse health effects.

Bronchoalveolar lavage

If an inhaled injurious agent reaches the bronchoalveolar region, the lung has only limited ways of responding. If the material is an insoluble particle, or if the material causes an injury resulting in intraluminal debris, the resident macrophages will be activated. This results in the

release of reactive forms of oxygen or nitrogen, pro-inflammatory mediators such as cytokines and chemokines, growth factors and mitogens, metabolites of cellular debris (e.g. arachidonate metabolites) and a great number of potentially tissue-destructive enzymes. The interplay of factors may attract and activate further cells resulting in an amplification and perpetuation of the inflammatory response. This can cause an increase in the permeability of the alveolar/capillary barrier that allows greater extravasation of serum components into the interstitium or alveolar lumen.

The progress of an inflammatory response can be monitored by the analysis of the Broncho Alveolar Lavage (BAL). Bronchoalveolar lavage is a method of sampling the epithelial lining fluid of the bronchoalveolar region of the lung, including the airways. Responses of the lung related to the degree of cytotoxicity or inflammation induced by the inhaled material should be apparent from analysis of the BAL. The precise composition of the exudates in the BAL depends on the cause and extent of injury and the site at which it occurs. However, the use of BAL as a technique for evaluating responses to inhaled toxicants is useful to characterise intraluminal events whilst interstitial sites are inaccessible by the lavage fluid, and, therefore, BAL complements the more conventional histopathological techniques. Experimental problems related to dilution or fixation artefacts, which make the histopathological quantification of subtle intra-alveolar lung oedema difficult, can be overcome by BAL.

In toxicology, BAL has been used in three major ways:

- to detect an inflammatory response in the bronchoalveolar region of the lung;
- to follow sequentially the progress of a disease;
- to elucidate pathogenic mechanisms;

which can be achieved by determination of a variety of markers of pulmonary damage within the BAL fluid (BALF) and BAL cells (BALC).

The markers commonly selected to address changes in the bronchoalveolar region are the following:

- Increase of BALC:
 - Immediately after the exposure to irritant gases an increase of Poly-Morphonuclear Neutrophils (PMN) is observed, which persists several hours post exposure.
- Determination of proteins which indicate early acute injury of the air-blood barrier:
 - Alkaline phosphatase (AP) serves as an endpoint to assess the degree of pneumocyte type II activity, which may be secreted along with discharge of surfactant contained in lamellar bodies that are synthesised in this cell type.
 - The lysosomal enzyme β -N-acetylglucosaminidase is most probably released from resident alveolar macrophages, which are known to secrete the enzyme when stimulated.
 - The activity of the cytosolic enzyme, lactate dehydrogenase (LDH), to detect increased lysis of cells or general cytotoxicity, although some of the LDH and AP present in BALF can be accounted for by the amount of transudated serum present in the alveolar lumen.
 - Tryptase released by mast cells is also increased in the BAL fluids immediately post exposure, however it does not persist.
 - Increased alveolocapillary permeability is associated with increased transudation of high-molecular weight proteins, such as alpha2-macroglobulin (molecular weight 720 kDa). This has been utilised in humans to differentiate homeostatic responses

(increased transudation of albumin, molecular weight 67 kDa) from early adverse effects as a result of plasma leakage in the airways with loss of filtration control.

- Determination of phospholipids (PLIP) in the lavage fluid or within BALC as surfactant constituents may be useful to assess surfactant dysfunction because these endpoints appear to be good indicators of these changes (Henderson 1989; Henderson and Belinsky, 1993).

The usefulness of the BAL technique to detect early lung injury and inflammation has been subject to many reviews.

In the context of the derivation of AETL-2 values, the analysis of the time-course and concentration-dependence of endpoints indicative of early lung oedema by the BAL technique is particularly useful (Pauluhn, 2000a, b and 2004, Pauluhn and Mohr, 2000). Moreover, as most lower respiratory tract irritant agents cause a breach of the blood-air barrier, the analysis of the concentration-dependence of BAL-protein has received increased attention for the estimation of irritant threshold concentrations following acute exposures.

Benchmark procedures can readily be applied to estimate acute irritant threshold concentrations. However, due to the dynamics of the exchange of protein across the blood-air barrier through reflexively induced changes (stimulation of vascular receptors; Paintal reflex) (Jacquez, 1979), BAL-protein concentrations need to exceed 25-50% levels observed in the control group by 25-50% to be of pathodiagnostic significance.

Another important aspect is that plasma exudation in human airways is more specific to inflammation than in rodent airways. This is because simple neural reflex mechanisms may not produce this response.

The selection of the most appropriate time-point for taking the sample is critical for the outcome of the test. This is particularly important when exposure is acute. Serial sacrifices revealed that most endpoints indicative of increased transepithelial fluid flux were significantly increased shortly after cessation of exposure to pulmonary irritants. The maximum values commonly occurred one day after exposure.

Respiratory function test

Pulmonary function tests provide a non-destructive means of assessing the functional impact of alterations of lung structure, including those originating from intraluminal effects within the airways and alveoli or restrictive processes originating at the more interstitial site. The tests provide information and the presence (whether or not function is impaired), nature (type of impairment), and extent (magnitude of impairment) of function loss. The tests are used in inhalation toxicology as an indicator of toxic response, to characterise the pathogenesis of lung disease, and to extrapolate the impact of toxicant-induced lung disease from laboratory animals to man.

Much is known about interrelationships among measured lung function, subjective feelings of respiratory illness, clinical lung disease, and occupational disability in humans. Inhalation toxicological studies and studies using specific experimentally induced lung diseases in animals have shown that functional responses of man and animals to different types of lung injury are similar. Based on these findings, pulmonary function tests can be used to estimate the impact in man of responses to inhaled materials studied in laboratory animals.

Although a functional change implies the presence of an underlying structural change, function tests themselves do not describe these underlying changes. As an example, lung compliance can be reduced by surfactant dysfunction and many structural changes, among

which are fibrosis, oedema, haemorrhage, smooth muscle constriction, cell proliferation, and reduced lung volume. A finding of reduced compliance would not reveal the nature of the morphological cause.

Furthermore, although changes in function result from changes in structure, it is possible to find changes in one without demonstrable changes in the other. Pulmonary function tests cannot be considered as a substitute for histopathological evaluations. For example, lung tumours characteristically have little effect on lung function unless they occupy a large portion of the lung tissue.

Finally, most pulmonary function tests, as does bronchoalveolar lavage, evaluate the integrated function of the entire organ. Focal lesions can exist without measurably affecting total organ function and, accordingly, remain undetected by these more integrated measurements.

The question of the relative sensitivity of pulmonary function tests and histopathology is irrelevant in a general sense. Either assay could be the more sensitive under a given circumstance. The two approaches are complementary and are used to best advantage in concert. The examination of statistical correlations between pulmonary function data and data for morphological changes derived from the scoring of lesions is a particularly powerful approach to understanding structure-function relationships. As a general principle, qualitative changes in lung structure detected by light microscopy and quantitative changes in lung function determined by appropriate tests are detectable at about the same time in chronic, progressive lung diseases (Mauderly, 1986).

Under certain circumstances, pulmonary function tests might be the sole indicator of a response to an inhaled material. Transient airflow limitation from acute bronchoconstriction, for example, can have a severe impact on lung performance but is very difficult to demonstrate by histopathology.

Although changes in pulmonary function are not diagnostic for specific lesions, much about lung structure can be implied from functional changes. A battery of tests of different facets of lung function is usually applied. Results are expressed as classes of function disorders (e.g. obstructive or restrictive) that are consistent with classes of morphological changes (e.g. emphysema, bronchitis, and fibrosis).

The functional measurements can be categorised into tests to analyse: (1) the breathing patterns during tidal breathing, and (2) measurements that focus on the identification of changes in lung mechanics, diffusing capacity and differentiation of chronic obstructive and restrictive lung disease. Not all changes observed during spontaneous tidal breathing may necessarily reflect true changes in lung mechanics. For a more in-depth evaluation of airway resistance, forced manoeuvres have a markedly higher sensitivity to probe for changes in the dynamic and static changes of lung function parameters.

Measurements and endpoints commonly included to evaluate the breathing patterns during tidal breathing are the following:

- respiratory rate;
- (pseudo) tidal volume;
- inspiratory and expiratory times (T_i and T_e);
- relaxation time (RT);
- apneic pause ($\text{Pause} = R_e/RT - 1$);
- peak inspiratory and expiratory flows (PIF and PEF);

- enhanced pause ($P_{enh} = \text{Pause} \times \text{PEF}/\text{PIF}$).

It has been shown that the changes in P_{enh} parallel those of pulmonary parenchymal damage, rather than ‘airway resistance’ as commonly believed (Hamelmann *et al*, 1997; Chong *et al*, 1998). P_{enh} appears to integrate several physiological endpoints in a wholly non-invasive and non-disturbing manner so that non-specific functional changes can readily be identified. However, caution is advised to link these changes to specific pathophysiological effects. Apparently, P_{enh} reflects expiratory pattern-related changes and therefore depends on the control of breathing rather than airway constriction (Mitzner and Tankersley, 1998; Mitzner *et al*, 2003; Hantos and Brusasco, 2002; Peták *et al*, 2001).

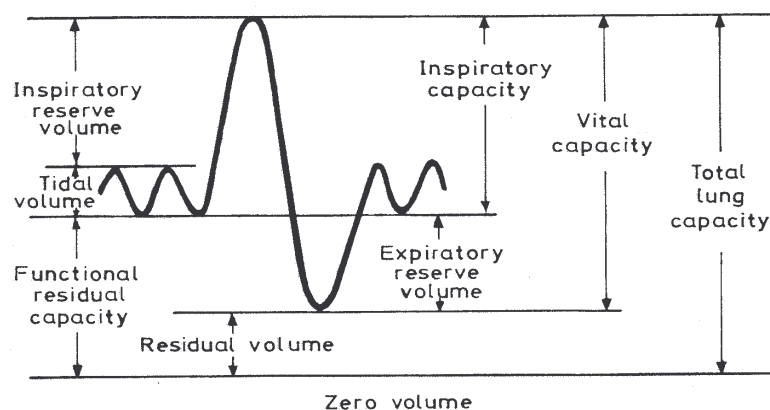
Irritant related increased protein extravasations into alveoli, as probed for example by measurements of protein in BALF, may cause changes in the elastic recoil of lung tissue and associated changes in inspiratory and expiratory breathing patterns, correlate with increased P_{enh} . However P_{enh} displays a substantially greater variability.

When peak expiratory flow (PEF) is measured repeatedly over a period and plotted against time (e.g. by asthmatic patients), the pattern of the graph can be very important in identifying particular aspects of the patient’s diseases. Typical patterns are the fall in PEF during the week with improvement on weekends and holidays, which occurs in occupational asthma; and the ‘morning dipper’ pattern of some asthmatic patients due to a fall in PEF in the early morning hours. Isolated falls in PEF in relation to specific allergens or trigger factors can help to identify and quantify these for the doctor and patient. A downward trend in PEF and an increase in its variability can identify worsening asthma and can be used by the doctor or patient to modify therapy. PEF monitoring is particularly useful in the substantial number of asthmatic people with poor perception of their own airway calibre. An increase in PEF and a decrease in its variability usually accompany response to asthma treatment.

The measurements which are usually made for a more in-depth evaluation of airway resistance and which include some forced manoeuvres are the following:

- VC (vital capacity, see figure 4-1) is the maximum volume of air, which can be exhaled or inspired during either a forced (FVC) or a slow (VC) manoeuvre.

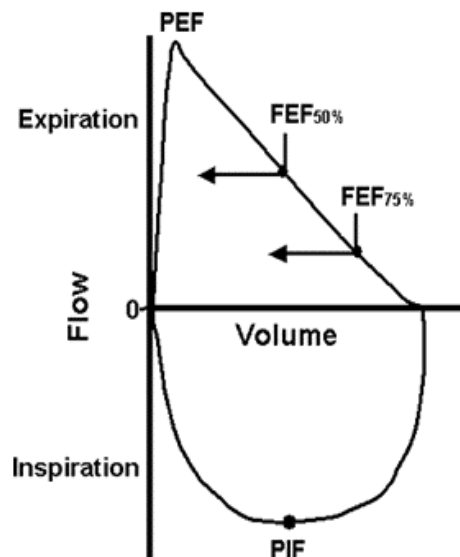
Figure 4-1: Static lung volumes



- FEV₁ (forced expired volume in one second) is the volume expired in the first second of maximal expiration after a maximal inspiration and is a useful measure of how quickly full lungs can be emptied.

- $FEV_1/VC\%$ is the FEV_1 expressed as a percentage of the VC or FVC (whichever volume is larger) and gives a clinically useful index of airflow limitation.
- $FEF_{25-75\%}$ is the average expired flow over the middle half of the FVC manoeuvre and is regarded as a more sensitive measure of small airways narrowing than FEV_1 .
Unfortunately $FEF_{25-75\%}$ has a wide range of normality, is less reproducible than FEV_1 , and is difficult to interpret if the VC (or FVC) is reduced or increased.
- PEF (peak expiratory flow) is the maximal expiratory flow rate achieved and this occurs very early in the forced expiratory manoeuvre.
- $FEF_{50\%}$ and $FEF_{75\%}$ (forced expiratory flow at 50% or 75% FVC) is the maximal expiratory flow measured at the point where 50% of the FVC has been expired ($FEF_{50\%}$) and after 75% has been expired ($FEF_{75\%}$). Both indices have a wide range of normality but are usually reproducible in a given subject provided the FVC is reproducible.

Figure 4-2: Normal spirogram showing peak expiratory and inspiratory flow (PEF, PIF) curves during the measurements of forced vital capacity (FVC), forced expired volume in one second (FEV_1) and forced expiratory flow over the middle half of the FVC ($FEF_{25-75\%}$).



For the correct interpretation of the data all indices of ventilatory function should be reported at body temperature and pressure saturated with water vapour (BTPS). If this is not done the results will be underestimated, because when the patient blows into a ‘cold’ spirometer, the volume recorded by the spirometer is less than that displaced by the lungs.

To interpret ventilatory function tests in any individual the results should be compared with reference values obtained from a well-defined population of normal subjects matched for gender, age, height and ethnic origin and using similar test protocols. Carefully calibrated and validated instruments should be used.

Normal predicted values for ventilatory function generally vary as follows:

- Gender: for a given height and age, males have a larger FEV_1 , FVC, $FEF_{25-75\%}$ and PEF, but a slightly lower $FEV_1/FVC\%$.

- Age: FEV₁, FVC, FEF_{25-75%} and PEF increase, while FEV₁/FVC% decreases, with age until about 20 years old in females and 25 years in males. After this, all indices gradually fall, although the precise rate of decline is probably masked due to the complex interrelationship between age and height. The fall in FEV₁/FVC% with age in adults is due to the greater decline in FEV₁ than FVC.
- Height: all indices other than FEV₁/FVC% increase with standing height.
- Ethnic Origin: Caucasians have the largest FEV₁ and FVC and, of the various ethnic groups, Polynesians are among the lowest. The values for black Africans are 10-15% lower than for Caucasians of similar age, sex and height because, for a given standing height, their thorax is shorter. Chinese have been found to have an FVC about 20% lower and Indians about 10% lower than matched Caucasians. There is little difference in PEF between ethnic groups.

Measurements of ventilatory function may be very useful in a diagnostic sense but they are also useful in following the natural history of disease over a period of time, assessing preoperative risk and in quantifying the effects of treatment. The presence of ventilatory abnormality can be inferred if any of FEV₁, VC, PEF or FEV₁/VC% is outside the normal range.

The inter-relationships of the various measurements are important diagnostically:

- A reduction of FEV₁ in relation to the forced vital capacity will result in a low FEV₁/FVC% and is typical of obstructive ventilatory defects (e.g. asthma and emphysema). The lower limit of normal for FEV₁/FVC is around 70-75% but the exact limit is dependent on age. In obstructive lung disease, the FVC may be less than the slow VC because of earlier airway closure during the forced manoeuvre. This may lead to an overestimation of the FEV₁/FVC%. Thus, the FEV₁/VC% may be a more sensitive index of airflow obstruction.
- The FEV₁/FVC% ratio remains normal or high (typically >80%) with a reduction in both FEV₁ and FVC in restrictive ventilatory defects (e.g. pulmonary fibrosis, respiratory muscle weakness, and thoracic cage deformities such as kyphoscoliosis).
- A reduced FVC together with a low FEV₁/FVC% ratio is a feature of a mixed ventilatory defect in which a combination of both obstruction and restriction appears to be present. Alternatively, this may occur in airflow obstruction as a consequence of airway closure resulting in gas trapping, rather than as a result of small lungs (e.g. carcinoma of the trachea). It is necessary to measure the patient's total lung capacity to distinguish between these two possibilities.

The shape of the expiratory flow-volume curve varies between obstructive ventilatory defects where maximal flow rates are diminished and the expiratory curve is scooped out or concave to the x-axis, and restrictive diseases where flows may be increased in relation to lung volume (convex). A 'tail' on the expiratory curve as residual volume is approached is suggestive of obstruction in the small peripheral airways. Examination of the shape of the flow-volume curve can help to distinguish different disease states, but note that the inspiratory curve is effort-dependent. For example, a plateau of inspiratory flow may result from a floppy extrathoracic airway, whereas both inspiratory and expiratory flows are truncated for fixed lesions. Expiratory flows alone are reduced for intrathoracic obstruction.

Fig 4-3: Schematic diagram illustrating idealised shapes of flow-volume curves and spirometers for obstructing, restrictive and mixed ventilatory defects

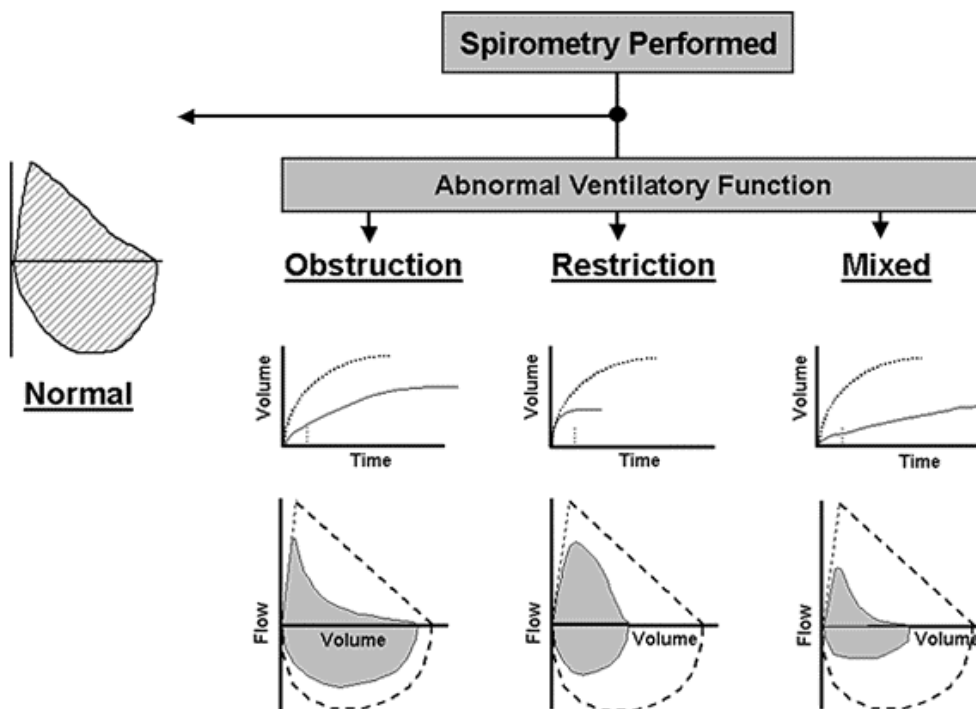


Fig 4-4: Classification of ventilatory abnormalities by spirometry

| | OBSTRUCTIVE | RESTRICTIVE | MIXED |
|-----------------------|----------------|----------------|-------|
| FEV ₁ | ↓ | ↓ or Normal | ↓ |
| FVC | ↓ or Normal | ↓ | ↓ |
| FEV ₁ /FVC | ↓ | Normal or ↑ | ↓ |

To measure the degree of reversibility (typically increased in asthma) of airflow obstruction, perform spirometry before and 10 to 15 minutes after administering a bronchodilator by metered dose inhaler or jet nebuliser. Beta₂ agonists (e.g. salbutamol, terbutaline, etc.) are generally considered the benchmark bronchodilators. To express the degree of improvement:

- calculate the absolute change in FEV₁ (i.e. post-bronchodilator FEV₁ minus baseline FEV₁) and
- calculate the percentage improvement from the baseline FEV₁.

There is presently no universal agreement on the definition of significant bronchodilator reversibility. According to the ATS the criterion for a significant response in adults is: $\geq 12\%$ improvement in FEV₁ (or FVC) and an absolute improvement of ≥ 0.2 L.

Normal subjects generally exhibit a smaller degree of reversibility (up to 8% in most studies). The absence of reversibility does not exclude asthma because an asthmatic person's response can vary from time to time and at times airway calibre in asthmatic subjects is clearly normal and incapable of dramatic improvement.

4.2.2.2.2 Animal models

Animal models have been established to determine the relative potency of upper respiratory tract irritants.

Tidal breathing measurement

Measurements during tidal breathing do not require any intervention and therefore can also be applied to small laboratory rodents. In this context it is important to recall that in small laboratory rodents the nasal airway resistance is the largest component of the total airway resistance in obligate nasal breathing rodents.

Measurements in anaesthetised animals using a non-invasive orotracheal cannula are paramount to understand pathophysiological events. However, these measurements require extensive manipulation of the animals. Lung function measurements during tidal breathing in non-cannulated, spontaneous breathing animals are commonly performed to analyse for reflexively induced changes in breathing patterns or a more precise measurement of the respiratory minute volume and the inhaled dose.

Published data on respiratory minute volumes vary extensively because of their dependence on manipulations (imposed stress), excitement, sedative measures, age and mode of exposure (restraint) as well as the method used for measurement. The respiratory minute volumes of young-adult mice, rats and dogs are approximately 2.5, 1, and 0.35 L/min/kg body-weight. For agents exclusively deposited and retained in the alveolar region the alveolar ventilation is 2/3 of the total ventilation due to the respiratory dead space of the lung.

Due to its ease of handling, measurements in unrestrained, spontaneously breathing animals using barometric whole-body plethysmography has gained popularity.

RD₅₀ test

This sensory irritation test has been developed based on trigeminal nerve stimulation in the nasal mucosa of rodents, which results in a decreased respiratory frequency.

The RD₅₀, the concentration inducing a 50% decrease in the respiratory rate, was proposed for the assessment of relative sensory irritation potency of different test agents (Alarie 1966, 1973, 1981; Jaeger and Gearhart, 1982; Schaper, 1993; Bos *et al*, 1991; ASTM, 1984). This mouse bioassay is particularly useful to categorise gases or volatile agents into upper and lower respiratory tract irritants. The onset of the response, that is, the decrease in breathing frequency in small laboratory rodents (*vide infra*), is usually observed within a few minutes

and is characterised by a stereotypic bradypneic pause during the expiratory phase of respiration.

For volatile agents, this endpoint is useful both to rank the relative sensory irritation potency based on RD₅₀-values as well as to estimate irritant threshold concentrations (RD₀) (Nielsen *et al*, 1985; Pauluhn, 1998). However, for state-of-the-art interpretation of this endpoint it is important to understand the site of predominant deposition and ensuing local irritation of the agent under consideration.

Table 4-4: Grading of the severity of the sensory irritation effects in mice (ASTM E 981, 2004)

| <u>Respiratory rate decrease</u> | <u>Endpoint</u> |
|----------------------------------|---------------------------------|
| less than 12% | should be considered background |
| 12-20% | slight response irritation |
| 20-50% | moderate response |
| 50-85% | extreme response |

In rodents, reflex reactions are considered to be of a protective nature, i.e. to limit contact to a variety of potentially hazardous chemicals. Although other nerves are involved with the effects of sensory irritants on heart rate and blood pressure, e.g. vagal nerves (heart rate), the trigeminal nerve appeared to be the only nerve directly involved with the decrease in respiratory rate. This decrease is concentration-dependent and generally appears within a few minutes, depending on the chemical. When mice and rats are exposed to the same atmosphere, the response observed in mice appears generally more pronounced and more stable than in rats. Despite the potential of different susceptibilities of rats and mice, the change in respiratory pattern is stereotypic in both species.

While upper respiratory tract sensory irritants evoke a reflexively induced pause between the end of inspiration and expiration, pulmonary tract irritants appear to evoke an apneic pause between end of expiration and inspiration of the following breath. This allows ready identification of lower and upper respiratory irritants. The strength of this methodology is to quantify the extent of upper respiratory tract sensory irritation of volatile agents with sufficiently high water solubility for a substance to penetrate the respiratory mucosa.

There is no direct relation between the RD₅₀ and other toxic effects like respiratory tract irritation, systemic effects or mortality. These effects may occur at or below RD₅₀ concentrations. The benefit of this endpoint has often been misjudged (Bos *et al*, 1991), because of the negligence of technical ramifications or the physico-chemical properties of the test substance. Therefore this endpoint is of limited relevance in the absence of complementary histopathological data and lung function parameters addressing the lower airways, including alveoli.

The assessment of alveolar-capillary gas exchange efficiency

DL_{co} (single-breath CO-diffusing capacity) in rats has been described by Takezawa *et al* (1980). Methodological details are published elsewhere: Likens and Mauderly, 1982; Mauderly 1984, 1988, 1989; Costa *et al*, 1983, 1992; Costa and Tepper, 1988; Diamond and O'Donnell, 1977; Pauluhn *et al*, 1995; Pauluhn and Mohr, 2000.

4.2.2.3 Long-term effects

Short exposure to a high concentration of a highly irritant or corrosive chemical in the inhaled air may cause several types of longer term and irreversible effects to the respiratory tract. For example:

- Chronic inflammation of the nasal or lung mucosa.
- Loss of the function or dysfunction of this altered mucosa following recovery of the mucosa after corrosion damage. For example the destruction of the olfactory epithelium, replaced during the cicatrisation process by nasal mucosa if followed by anosmia or dysosmia.
- Emphysema may permanently affect the functioning of the respiratory system.
- Pulmonary fibrosis due to the abnormal formation of fibre-like scar tissue in the lungs. The scar formation is preceded by, and associated with, inflammation. If the disease progresses, the lung tissues eventually thicken and become stiff. Breathing then is impaired because of reduced compliance. The disease can run a gradual course, remain unchanged or run a rapid course. It can also be fatal. When the alveoli are affected fibrosis twists them out of shape. Lung capillaries can also be distorted by pulmonary fibrosis. In addition, the tissues between and surrounding the alveoli are changed by fibrosis, thus completely changing the basic architecture of the interstitium. Lung fibrosis may lead to pulmonary hypertension with poor prognosis because of untreatable right heart failure.

Rats subjected to acutely toxic agents can experience obstructive or restrictive lung disease as long-term consequence of a single exposure (bronchiolitis obliterans with organising pneumonia). This is indicated by increased lung volumes, decreased maximal forced expiratory manoeuvres and a left shift of the pressure-volume curve. On the other hand, this set of changes induced by ionising irradiation, can clearly be differentiated from the above effects. The fibrogenic response of the lung is characterised by decreased lung volumes and right-shift of the pressure-volume curve, and decreased maximal forced expiratory manoeuvres. These were indistinguishable from the rats experiencing a mixed response (O'Neil and Raub, 1984; Pauluhn, 2000b; Pauluhn *et al*, 2001).

Reactive airways dysfunction syndrome (RADS)

RADS is an asthmatic like illness that develops within minutes to hours after an acute exposure to dust, smoke, or solvent. It is characterised by a persistent bronchial hyperactivity with positive methacholine challenge. The asthma becomes chronic after the initial exposure and can be difficult to treat.

Reactive upper-airways dysfunction syndrome (RUDS)

RUDS is a hypersensitivity to chemicals in a subpopulation resulting in persistent rhinitis, following exposure to a chemical. Morphologically, the nasal mucosa shows lymphocytic infiltrates, thickening of the basement membrane and desquamation of the respiratory epithelium. The desquamation of the respiratory epithelium may remove a barrier to chemical irritants, which may reach and trigger the irritant receptors at lower concentration.

RUDS probably affects only a very small proportion of people. It deals with a sensitive subpopulation and therefore cannot be used for setting AETLs.

4.2.2.4 Deposition and clearance of inhaled materials

Theoretically, knowledge of all the chemical species involved and the reaction rates of the reactants and products is necessary to characterise a system for dosimetry. Gases that are not soluble or reactive are relatively inert to the airways and penetrate to the alveoli. The major factor driving the uptake of these gases is the diffusion of the gas from alveolar air to the capillary blood. The concentration in alveolar air and capillary blood is generally considered to reach equilibrium. Therefore, uptake of alveolar gases depends on blood-air partitioning, ventilation/perfusion ratio, and air and blood concentrations. For gases that are soluble, uptake is linearly related to solubility.

Because uptake and deposition of inhaled vapours and gases are driven by the equilibration of their partial pressures in tissues with their partial pressures in ambient air, solubility may be appropriately described by the Ostwald solubility coefficient at body temperature.

The blood-air (or blood-gas) partition coefficient is a critical determinant in the uptake and achieved blood concentration of volatile organic chemicals.

Interspecies comparisons necessitate consideration of the effects of the differences in anatomy and physiology described previously. However, it can generally be stated that the less water soluble and less reactive the gas, the more similar the deposition will be between humans and laboratory animals. The tissue-gas partition coefficient of a chemical has been shown to correlate with its fat-gas and blood-gas partition coefficients so that linear correlation equations may provide a useful means of estimating tissue-gas and blood-gas partition coefficients.

In rats the layout of the upper respiratory structures is such that the air could travel in nearly linear fashion from the nose to the bifurcation of the trachea. The linear arrangement of the upper airways allows the larynx of rats to lie close to the posterior edge of the oral and nasal cavities. The apposition of the epiglottis to the soft palate in the resting condition isolates the oral cavity from the respiratory airways and makes small laboratory rodents obligatory nose breathers (they have virtually no oropharynx). Thus, the direction of flow in rats is almost linear whereas in humans it is rectangular. Furthermore, in rats laryngeal deposition is enhanced by the 'laryngeal jet'.

Animal models that use interventions to decrease the extent of deposition in the extrathoracic airways by using cannulas have been of limited success due to changes in respiration, the lack of humidification of the inhaled air and/or a high water-air partition coefficient of the inhaled gas.

Numerous model structures have been used to describe toxicant uptake in the respiratory tract. The type of model often reflects the physico-chemical characteristics of the gases to which they are applied. The two categories of gases with the greatest potential for respiratory effects are:

- gases that are highly water soluble and/or rapidly irreversibly reactive;
- water-soluble gases, which may also be rapidly reversibly reactive or moderately to slowly irreversibly metabolised in respiratory tract tissue.

The objective of the default modelling approach is to describe the effective dose to the three major regions of the respiratory tract by addressing the absorption or 'scrubbing' of a relatively water soluble and/or reactive gas from the inspired airstream as it travels from the extrathoracic to the pulmonary region. At low concentrations, observed effects are largely confined to the extrathoracic region. At higher concentrations, more severe effects occur in

the extrathoracic region and toxicity is also observed to progress to the peripheral regions. The severity of toxicity also progresses distally with increased exposure concentrations.

The defining characteristic of Category 1 gases is that they do not significantly accumulate in the blood, which would reduce the concentration driving force, and, hence, reduce the absorption rate. The default model structure is based on these characteristics. Examples of gases classified as Category 1 are hydrogen fluoride, chlorine, formaldehyde, and the volatile organic acids and esters.

Ozone, sulphur dioxide, xylene, propanol, and isoamyl alcohol are examples of Category 2 gases.

The boundaries between categories are not clear-cut. Some compounds may be either Category 1 or Category 2 because water solubility and reactivity are continua. Thus, although sulphur dioxide is reversibly reactive, which would make it a Category 2 gas, it is also highly soluble such as to be a Category 1 gas. Similarly, ozone is highly reactive yet only moderately water-soluble. More explicit delineation of categories will be made upon review of the empirical data and the predictability of the model gases that may appear to fit more than one category.

Gases in Category 3 are relatively water insoluble and unreactive in the extrathoracic and tracheobroncho surface liquid and tissue, and, thus, result in relatively small dose to these regions. The uptake of Category 3 gases is predominantly in the pulmonary region and is perfusion limited. Styrene is an example of a Category 3 gas. The site of toxicity of these gases is generally remote to the respiratory tract. A compartmental approach can be used to describe distribution to various systemic tissues (US EPA, 1994a).

4.2.2.5 Grading

The elements of an extensive protocol for measuring the sensory response of human subjects are presented in the dissertation of Anglen (1981). He exposed a number of volunteers to low concentrations of chlorine in air for several exposure periods and recorded the intensity of several sensations like smell, taste, burning of the eyes, nose and throat, urges to cough, runny nose, nausea, headache, general discomfort, dizziness, drowsiness and shortness of breath.

Meldrum (2001) has recently presented the approach to the European Scientific Committee of Occupational Exposure Limits (SCOEL). It is noted, that irritant effects on the eyes and the respiratory tract can produce symptoms ranging from the trivial to the serious, and that responses to irritants may be viewed as belonging to a continuum:

1. No effects observed; no awareness of exposure.
2. Very slight effects; awareness of exposure.
3. Slight irritant effects or nuisance (e.g. smell), easily tolerable.
4. Significant irritation/nuisance, overt health effects, barely tolerable.
5. Serious health effects (e.g. pulmonary oedema), intolerable.

Consideration was given to symptoms such as eye and/or nasopharyngeal discomfort, headache and decreased performance, which should be regarded as 'adverse' effects on health and well-being. Effects may be considered to satisfy the criterion for nuisance at somewhere between 2 and 3 on the above continuum.

Table 4-5: Respiratory system endpoints: grading of effects*

| Human Endpoint | LT | I | ESC | R | A |
|---------------------------------------|----|---|-----|---|---|
| Nose irritation | | | | x | |
| Olfactory epithelium degeneration | | | | x | |
| Odour detection | | | | | x |
| Sneezing | | | | x | x |
| Epistaxis | | | | x | |
| Stuffiness | | | | x | |
| Larynx / pharynx irritation | | | x | x | |
| Dysosmia or anosmia | | x | | | |
| Trachea irritation | | | | x | |
| Lung Irritation | | | x | x | |
| Lung Oedema | x | x | | x | |
| Reduced respiratory function / asthma | x | x | | | |
| Emphysema | | x | | | |
| Chronic inflammation | | x | | | |
| Fibrosis | | x | | | |
| Sensory irritation | | | | x | x |
| RADS | | x | | | |
| RUDS | | x | | | |

* Life Threatening (LT), Irreversible (I), Impairment to take action or escape (ESC), Reversible (R), Awareness (A)

4.2.2.6 Conclusion

In many inhalation studies with irritant agents mice appear more sensitive when compared to species having a more human-like breathing physiology and lung anatomy as detailed elsewhere (Irvin and Bates, 2003). The assessment of inhalation data should, preferentially, be based on the commonly used rat. In an attempt to demonstrate the effort that goes into selecting or evaluating animal models, certain aspects of specific species susceptibilities, technical exposure artefacts and procedures available to monitor specific endpoints have been taken into account.

4.2.3 Eye effects

The eye is a vital organ for contact with and perception of the outside world. The eye houses the visual system, which also supports precise movement and co-ordination. Any effect on the eye that interferes with the visual system will lead to problems of co-ordination and orientation, which are both vital in reaction to an accidental exposure and may impair the capacity to escape from that exposure.

The visual system can be affected in various ways: image formation, accommodation, dark and light adaptation, colour vision and the pupillary light reflex. These effects can be direct (tissue damage) and immediate (tearing, stinging, pain) or delayed. Alternatively, they can occur as a result of neuromuscular effects or of changes in the blood supply of the visual system.

4.2.3.1 Tissue damage

Accidental splashing of substances into the eyes is the commonest cause of toxic eye injuries. Some substances produce serious injury or pain almost immediately. Others produce superficial reversible damage or induce injury that may appear unimpressive at first, but becomes progressively worse after a latent period. There is a paradox in that the most serious

chemical burns may produce little pain, because the injury also destroys the sensory nerves of the cornea. On the other hand relatively slight superficial and reversible injuries may cause great discomfort apparently by exposing corneal nerve endings to irritation rather than destroying them. It is important to recognise these different types of toxic action, because of their bearing on prognosis and treatment.

Immediate effects

Immediate effects on the eye are felt as tearing, stinging or pain.

Rapid, deep penetrating injuries of cornea, conjunctiva, sclera and even lens and iris are most notoriously produced by alkalis and acids as a result of extreme change of pH in the tissues. The immediate effects are:

- Dissolution of the epithelium and mottled clouding of corneal stroma (alkalines) or
- Coagulation of the epithelium (acids).

Other effects appear later including oedema, loss of mucous from the corneal stroma, further opacification, vascularisation and degeneration of the cornea.

A splash of a chemically inert solvent usually causes immediate stinging and smarting pain, and it may cause loss of some or all the corneal epithelium mainly due to fat solubilisation. This injury generally regenerates within a few days without residual permanent damage.

Contamination of the eyes with a surfactant, like ordinary soap, causes immediate stinging or burning with little or no injury.

Lachrymators or tear gases are substances that at low concentration in air cause immediate stinging and smarting sensation in the eyes with tearing, but in general without evident injury to the cornea.

The slightest effect on the eye is blinking which is observed by contamination with inert dust or as a response to slight irritation.

Delayed corneal and conjunctival effects

People exposed to amines in vapour form may, after several hours, start to see coloured haloes around lights as a result of diffraction effects from the myriads of swollen corneal epithelial cells. This condition does not cause discomfort and is spontaneously reversible within a couple of days. Only in the case of excessive exposure, eye discomfort or pain may result.

Exposure to this type of chemicals may cause a delayed onset of discomfort. This effect appears to be similar to the reaction of the skin to excessive exposure to ultra violet light. The eyes develop a sensation of burning and irritation, with conjunctival hyperaemia, tearing, photophobia or discomfort from bright light, blurred vision and a defensive blepharospasm or closure of the eyelids. Upon inspection, the cornea may appear slightly hazy and the surface finely irregular. Characteristically, the surface lacks its normal shiny smoothness and distinctive reflection properties.

In the most extreme cases, the damaging action can involve the corneal stroma and endothelium as well as the epithelium. The stroma may undergo swelling with wrinkling of the posterior surface, infiltration with inflammatory cells, invasion by interstitial vessels and fibrous tissue. Finally permanent scarring, vascularisation and opacification may occur.

4.2.3.2 Coloration

Some substances or preparations are reported to cause irreversible coloration of the eye. When such coloration concerns the cornea or iris, this is considered as severe ocular damage likely to impair vision. When coloration is confined to the conjunctiva and persists, whilst this is an irreversible effect, it is only a cosmetic one.

Brown discoloration of the conjunctiva and cornea in the palpebral fissure has been produced from exposure to dust or to aniline and hydroquinone vapours.

4.2.3.3 Neurophysiological effects

Chemicals with neurotoxic properties and which are able to penetrate the eye can have a direct effect on vision and colour perception.

When exposed to a low dose of nerve agents, the pupil of the eye becomes contracted (miosis) which impairs night-vision. The accommodation capacity of the eye is also reduced so that short-range vision deteriorates and the victim feels pain when trying to focus on an object nearby.

The primary tests used to evaluate colour vision involve colour arrangement (which requires the subject to distinguish caps of different colours and then place them in order) or pseudo-isochromatic plates (which requires the subject to identify a coloured symbol embedded in a background of a different colour).

Iregren *et al* (2002) provided a review of the effects of chemicals on colour vision and the tests used to evaluate colour vision. Alteration in colour perception has been reported following chronic exposure to certain industrial chemicals including ethyl alcohol, styrene, toluene, perchloroethylene, carbon disulfide, n-hexane and organic and inorganic mercury. These changes are frequently observed in the blue-yellow axis. The data supporting similar effects following acute exposure are more limited. Inhalation of 100 ppm toluene for 6.5 hours was suggested to affect both visual acuity and colour discrimination in one study. However, this was not confirmed by others.

4.2.3.4 Human experience

Most case reports relate to liquid splashes in the eyes. There are only a few cases described in the literature where atmospheric exposure resulting from accidental releases of chemicals led to serious effects on the eyes.

With eye exposure to gaseous methyl isocyanate, the victim will have intense burning of the eyes, photophobia, blepharospasm, profuse lacrimation, lid oedema, and superficial corneal ulceration with a resulting reversible blindness. In the Bhopal accident in 1984, burning eyes was the most frequently reported first symptom. This led to temporary blindness in many victims.

The eyes are very sensitive to mustard gas, although no clinical indication of injury may become evident until several hours later. The corneal epithelium may become oedematous. The lids and conjunctiva become red and swollen, accompanied by burning, discomfort, photophobia, lacrimation, and blepharospasm. Exposure to vapour induces extreme discomfort and temporary disablement, but in most cases recovery is complete. In more severe cases, injuries have involved not only the epithelium but also deeper layers. Corneas may become cloudy and infiltrated and, in extreme cases, eyes may become totally opaque. Long-term effects include corneal opacities and chronic conjunctivitis (Hughes, 1942).

There is evidence that the rabbit eye is more sensitive in its response to many irritants than the human eye (Freeberg *et al*, 1986; Cormier *et al*, 1996; Beckley, 1965). Thus, if good quality data exist, which show that the substance is less irritant in humans than the animal data indicate, this should be taken into account.

4.2.3.5 Animal data

Only a few cases are described in the literature where, in acute studies, contact with vapour or gas gives clear effects on the eyes.

During inhalation studies it is often observed that the animals keep their eyes closed. This, of course, reduces the exposure to the eye. Furthermore, detailed examination of the eyes is not part of a standard acute inhalation protocol. The *in vivo* rabbit eye test (OECD, 2002b) is used to assess the relative ocular hazard of materials and to meet regulatory requirements for classification of a material for its potential eye irritancy. In this test, a certain amount of the test substance is applied directly to the eye. In this way, the test provides a grading of the irritating potency to the eye. No examples were found of acute inhalation studies in which such grading was used to assess the impact of a vapour or gas on the eye.

4.2.3.6 Grading of ocular effects

In the case of an accidental exposure, the material is dispersed in the atmosphere. Therefore, the eye will be exposed to a concentration of the substance in air rather than to a given quantity. Furthermore, as substances have to be taken up by the eye fluids to become reactive, the partition between air and water will determine the concentration in the eye fluids to which the eye will be exposed. However, a grading system describing the effect and its severity in a standardised way could also be useful to evaluate the consequences of accidental airborne exposures where effects on the eye are observed.

The EU and US regulatory authorities use a scoring system to scale for the effects on the eyes as shown in Appendix IV (Tables A-1 and A-2). This can be used to describe the impact on the eye, its severity by a numeric scoring system and the reversibility of the effect.

In the EU, there are two categories of classification for irritancy to eyes, which for the purpose of setting AETLs can be used to establish AETL-2 and AETL-1 (EU, 1993).

The scoring system described in Appendix IV is used to identify significant irritation (AETL-1) and severe damage (including its reversibility; AETL-2) to the eye. The system described is, however, not suitable for describing sensory awareness resulting from neurophysiological effects and those resulting in blinking.

Neovascularisation and pannus

Neovascularisation and pannus are often used synonymously leading to the automatic assignment of irreversibility when either effect is recorded. However, some cases of neovascularisation might be considered as reversible, using routine observation techniques. Therefore, this response should be carefully monitored for a sufficient period to determine whether the intensity increases or decreases with time. In general, poor reversibility may be expected if neovascularisation clearly increases with time. If this occurs and the study was terminated for reasons of animal welfare, the effect should be considered as irreversible. In severe cases, neovascularisation of the cornea can lead to the proliferation of vascular connective tissue. This veil of fibrovascular tissue is referred to as pannus (Peiffer *et al*, 2000). Pannus is always irreversible.

Grading of other effects on the eyes

Lacrimation and neuromuscular effects on the eyes can severely impair vision and the capacity to escape (AETL-2 and AETL-1). Slight eye irritation and lacrimation often accompanied by increased blinking has no health impact but can be uncomfortable and lead to anxiety. Scoring systems have been developed based on the increase in blinking frequency (van Eick, 1977).

4.2.3.7 Conclusion

Although grading systems exist to describe the type and severity of the effect of chemicals on the eye, these systems have, apparently, not been applied in inhalation studies. It is recommended that the use of these scoring systems is included in protocols for future acute inhalation studies. This will allow the generation of new information, which will permit better characterisation of impacts on the eye resulting from accidental airborne exposure.

4.2.4 Skin effects

In general, the skin as a target organ in case of accidental airborne exposure is of limited significance as more sensitive tissues, like the eye and the respiratory tract, will respond and/or be affected at much lower concentrations. The effect on the skin, especially in the case of pH dependent irritation, will be influenced by the presence of moisture (sweat).

The relevant effects on the skin as a consequence of an accidental airborne exposure are limited to direct injuries i.e. skin irritation and corrosion. Whereas irritation is a reversible inflammatory response, corrosion will lead to tissue destruction and scarring. Milder responses of the skin include redness (erythema, rashes) produced by vascular congestion or increased perfusion.

4.2.4.1 Human experience

The classical example of effects on the skin by airborne exposure is that of the alkylating agent mustard gas.

Whether in gas or liquid form, mustard gas binds to skin tissue and reacts irreversibly within minutes. The action of mustard gas as alkylating agent on the skin results in dermal oedema and epidermal necrosis with blister formation. Skin irritation occurs from 20 minutes to several hours after the chemical exposure. The first skin symptom is often itching, and with heavy gas exposure this is accompanied by nausea and vomiting. The skin then develops an erythema not unlike that seen with moderate sunburn. Histological changes within the epidermis are not evident until 30 to 60 minutes after exposure and do not become fully manifest until two to three days after exposure and develop progressively over 10 days. After one to four days, blisters appear on exposed areas of the extremities. Clinically, mustard gas blisters result in superficial ulcerations not unlike those that develop in chemical or thermal burns. In some patients, the blisters coalesce to form large bullae that break, leaving large superficial ulcers that can cover in excess of 85% of the body surface area. Although the ulcers look like burns, re-epithelialisation and the propensity to infection are altered. These effects include prolonged healing due to the suppression of cell division and systemic immuno-suppression via immune cell damage and death. The affected skin develops black and blue discoloration and may become infected (Graham *et al*, 2005).

Although skin absorption is not an important route for methyl bromide intoxication, the skin is affected by contact with this chemical. Methyl bromide can cause enormous blisters that are, however, rarely deep enough to destroy the entire skin layer. For example, exposure to high concentrations of methyl bromide (about 40,000 mg/m³) for 40 minutes leads to redness and blistering of the skin. The effect of methyl bromide is different on various parts of the skin, probably depending on absorption (Zwaveling *et al*, 1987).

4.2.4.2 Animal data

Different national/international schemes exist for the grading (classification) of the irritancy/corrosivity hazard to the skin. Data obtained from *in vivo* rabbit skin irritation tests (OECD, 2002a) are used to assess the potential of materials to cause skin irritancy or corrosion in man. They can also be used to help meet regulatory requirements on classification and appropriate labelling of potentially irritant or corrosive substances. In this test, a given amount of the substance is applied directly to the shaved animal skin. However, in the case of accidental exposures, the material is dispersed in the atmosphere and therefore the skin will be exposed to a concentration of the substance in air rather than to a given quantity. Furthermore, in standard inhalation studies, animals are used whose skin is almost entirely covered with fur, which is protective and hinders detailed observations. However, a grading system describing the effect and its severity in a standardised way could also be useful to evaluate the consequences of accidental airborne exposures where effects on skin are observed.

4.2.4.3 Grading

The grading scale for irritant effects on rabbit skin, originally proposed by Draize and adapted by the US and EU regulatory agencies, is shown in Table 4-6.

Dermal irritation is scored and recorded according to this table. Further observations may be needed to establish reversibility. The skin irritation potential of chemicals is often summarised as the “Primary Irritation Index” (PII) calculated from erythema and oedema grades.

Table 4-6: Grading scale for skin irritation effects according to EC and US regulatory agencies following OECD (2002a)

| Erythema and eschar formation | Grade |
|---|--------------|
| No erythema | 0 |
| Very slight erythema (barely perceptible) | 1 |
| Well-defined erythema | 2 |
| Moderate to severe erythema | 3 |
| Severe erythema (beet redness) to slight eschar formation (injuries in depth) | 4 |
| Oedema formation | Grade |
| No oedema | 0 |
| Very slight oedema (barely perceptible) | 1 |
| Slight oedema (edges of area well defined by definite raising) | 2 |
| Moderate oedema (raised approximately 1 mm) | 3 |
| Severe (raised >1 mm and extending beyond area of exposure) | 4 |

In the EU, there are two categories of classification for irritancy to the skin (e.g. irritant and corrosive), which for the purpose of setting AETLs can be used to establish AETL-2 or AETL-1 (EU, 1993).

The scoring system described is used to identify significant irritation (AETL-1) and severe damage (including its reversibility) to the skin (AETL-2).

4.2.4.4 Conclusion

Although grading systems exist to describe the type and severity of the effect of chemicals on the skin, these systems have, apparently, not been applied to acute exposure cases.

4.2.5 Systemic effects on the heart, liver, kidney and muscles

Systemic effects might be caused by short-term high exposure to gases and vapours, accidentally released during industrial activities. The relevant target organs for systemic effects in short-term releases are the heart, the liver, the kidney and the muscles. These organs are covered separately in the following three sections.

4.2.5.1 Effects on the heart

In the case of an accident, a condition of acute stress may be initiated. Adrenaline is released into the bloodstream, which increases the heart rate, the conductivity, the excitability and the contraction power. This increases the cardiac output and prepares the organism for increased efforts to escape the dangerous situation. A healthy heart can cope with the elevated level of adrenaline, which increases the heart activity within its physiological capacity.

Chemicals may influence the system of nerve fibres in the heart muscle and modify actions on heart rate (chronotropic), conductivity (dromotropic), excitability (bathmotropic) or contractility (inotropic). The enhancement of the excitability, in combination with increased adrenaline levels may increase the incidence of arrhythmia.

Cardiotoxicity from industrial chemicals such as halogenated alkanes and hydrocarbons can involve multiple mechanisms. Their inherent lipophilicity enables them to affect the nervous system, which also regulates the cardiac electrical activity. They may be absorbed into the cell membranes and affect membrane fluidity, which is crucial for cellular functions such as signal transduction and oxidative phosphorylation. Generally, they produce a depressant effect on the central nervous system and an attenuation of the myocardial contractility. Halogenated alkanes sensitise the heart to the arrhythmogenic effects of beta-adrenergic receptor agonists such as endogenous adrenaline.

These cardiotoxic effects are rather concentration- than dose-related. This has to be taken into account when extrapolating over exposure time.

Certain chemicals may damage the heart muscle by exerting direct cytotoxic effects on heart muscle cells. Chemicals like aldehydes, which deplete glutathione in cardiac tissue, may contribute to oxidative stress.

4.2.5.1.1 Arrhythmia

Human experience

Fluorohydrocarbons have an effect on the heart rate in the case of increased physiological levels of adrenaline in blood. Emmen *et al* (2000) exposed volunteers to 1,1,1,2-tetrafluoroethane (HFC 134a), 1,1,1,2,3,3,3-heptafluoropropane (HFC 227ea) and dichlorodifluoromethane (CFC 12) in order to study and verify cardiotoxic effects in man

reported elsewhere. Tong *et al* (1998) exposed volunteers in order to study the toxicokinetics of 1,1-dichloro-1-fluoroethane (HCFC 141b). In these studies, the volunteers did not exercise on an ergometer during exposure and were not exposed to an increased level of adrenaline. Exposure of healthy volunteers to exposure levels up to 8000 ppm (HFC 134a and HFC 227ea), up to 4000 ppm (CFC 12) and up to 1000 ppm (HCFC 141b) did not result in any adverse effect on pulse rate, blood pressure, electrocardiogram or lung function.

Animal data

Adrenaline in blood is increased by sudden stress and can induce arrhythmia. The ability of a substance to induce an increased sensitivity to adrenaline induced arrhythmia can be investigated in dogs dosed with adrenaline resulting in a blood level of about 10 times the normal physiological level of adrenaline. ASTM method 1674 (1999) provides an extensive description of the experimental protocol for studying acute arrhythmia in dogs caused by inhalation of toxicants.

Grading of effects on heart arrhythmia

There is not a single symptom characteristic of an arrhythmia abnormality.

The more common symptoms that suggest cardiac arrhythmia are the following:

- hazardous: anxiety, chest pain, back pain, dizziness, fainting and shortness of breath;
- reversible: palpitations;
- innocuous: increased heart beat.

Table 4-7: Acute heart arrhythmia endpoints: grading of effects*

| Type of effect | LT | I | ESC | R | A |
|------------------------------|----|---|-----|---|---|
| Increased heart beat 140-180 | x | | | x | |
| Increased heart beat <140 | | | | | x |
| Palpitations | | | x | x | |
| Fluttering | | x | x | x | |
| Shortness of breath | | | x | x | |
| Anxiety | | | | x | |
| Chest Pain | | | x | | |
| Back Pain | | | x | | |
| Dizziness | | | x | x | |
| Fainting | | | x | x | |
| Nausea | | | x | x | |

* Life Threatening (LT), Irreversible (I), Impairment to take action or escape (ESC), Reversible (R), or Awareness.

For some effects, the severity is critical for categorisation. Endpoints scored in several categories are consequences of the degree of severity.

4.2.5.1.2 Structural tissue lesions

Human experience

In relation to acute exposure, no established protocols exist for measuring structural tissue lesions in the heart *in vivo*. The extent of heart failure becomes evident from a patients' complaints that are caused by a sudden limitation of blood supply to the heart, e.g. in case of atherosclerosis. Effects might be chest pain, back pain, pain or deep aching and throbbing in

one or both arms, breathlessness, clammy sweating, dizziness, anxiety, fluid retention usually in the lower legs or ankles, fluttering, rapid heartbeats, palpitations, nausea, feeling of heaviness or pressure-like chest pain between the breasts that may radiate to the left arm or shoulder.

Animal data

Structural damage to the cardiac muscle in animals may cause symptoms of heart failure. In rodents this may lead to skin oedema, ascites, sweating and fatigue. Protocols that would provide useful information are OECD protocols for acute inhalation with the histopathology and clinical chemistry investigations. These are usually conducted on subchronic inhalation studies, focused on damage to the heart (OECD, 1981a and b).

Grading of effects

Structural damage to the cardiac muscle is unlikely to be an immediate response to acute inhalation of a chemical. Thus, the ability to escape is not impaired in the first period of acute exposure. However, after intense exposure and after repeated high exposure, structural damage comparable to atherosclerosis might develop with consequences on the function of the heart muscle. Therefore, related symptoms could include those mentioned under the paragraph 'human experience' above. Damage to the heart can be assessed by analysing plasma for increased levels of aspartic aminotransferase, lactate dehydrogenase and creatine phosphokinase.

A sudden change in condition after the acute exposure may trigger heart diagnostic examinations. It is difficult to indicate gradation of effects, because slight effects will mostly not be recognised.

Conclusion

Acute systemic effects on the heart caused by inhalation exposure may be recognised as cardiac arrhythmia, and possibly increased by heart sensitisation due to enhanced release of catechol amines under stress conditions.

Structural damage to the cardiac muscle due to one single short-term exposure to volatile chemicals as the only clinical sign is highly improbable.

4.2.5.2 Effects on liver

Acute hepatic damage to chemicals may result in liver necrosis, steatosis and cholestasis.

Liver necrosis is characterised by cell swelling, leakage, nuclear disintegration and an influx of inflammatory cells. When necrosis occurs in hepatocytes, the associated plasma membrane leakage can be detected biochemically by assaying plasma or serum for the increased level of enzymes in the cytosol of the hepatocytes. Example substances are dimethylformamide and ethanol.

Steatosis is fatty liver. It is defined biochemically as an appreciable increase in the hepatic lipid content, which is normally less than 5% in a normal human liver. Histologically, in standard paraffin-embedded and solvent-extracted sections, hepatocytes containing excess fat appear to have multiple, round, empty vacuoles, which displace the nucleus to the periphery of the cell. It is a common response to acute exposure to many liver toxins. It often appears to be reversible and does not result in the death of hepatocytes.

Cholestasis is the decrease of bile production and the concomitant accumulation of bile compounds (e.g. bile salts and bilirubin) in the blood. The cause might be a decrease of secretion of these bile compounds into the bile. In this case, the activity of alkaline

phosphatase in the serum is increased by leakage of the cytosol. The increased level of bilirubin pigments in the blood results into accumulation of pigments in skin and eyes, producing jaundice.

Clinical effects of liver damage can be checked by clinical chemistry analyses. Signs of intoxication are increased serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl-transpeptidase and/or bilirubin. In acute toxicity studies in animals, clinical chemistry is normally not done. For this reason, a direct comparison of sensitivity for acute toxicity between man and animal is rarely possible. Alcohol abuse may enhance the effects of liver toxicants.

4.2.5.2.1 Human experience

Liver toxicity can occur as a consequence of acute high exposure to volatile organic substances like carbon tetrachloride. In most situations, depression of the central nervous system is the first sign of intoxication. Liver toxicity by acute exposure is difficult to find unless it is actively sought. Liver toxicity usually occurs after repeated exposure and therefore will only rarely be of relevance to the setting of an AETL. The finding of an increased level of liver enzymes is a sign of liver damage due to cytotoxicity to the liver cells. Acute hepatic failure may occur by lethal exposure levels for at least one hour (Manno *et al*, 1996).

4.2.5.2.2 Animal data

Adams *et al* (1952) have studied the hepatotoxicity of carbon tetrachloride, which provides an example of data that may occasionally be available.

Gross and microscopic examination of rats, killed between 16 and 24 hours after acute inhalation exposure, revealed marked hepatic injury. This was also demonstrated by:

- an increase of plasma prothrombin clotting time;
- an increase of serum alkaline phosphatase;
- an increase of lipid content of the liver;
- central fatty degeneration of the liver.

4.2.5.2.3 Grading of effects

Protocols for testing liver toxicity

Protocols for studying experimental liver toxicity in man do not exist. However, diagnostic tests are available, for example the rate of elimination of exogenous substances like bromosulphthalein or indocyanine green from the blood after intravenous application is no longer used to test for active liver function. The level of clotting factors as well as albumin concentrations as measured by standard clinical tests inform on the capacity of the liver to synthesise these proteins.

Specific protocols for studying liver toxicity in animals do not exist. Study designs that could provide useful information are OECD protocols for acute inhalation combined with the histopathology and the clinical chemistry investigations usually conducted on a subchronic inhalation study, focused on liver cytotoxicity (OECD, 1981a and b).

Effects on the liver like increased serum cholesterol, cirrhosis, induced activity of metabolising enzymes, regenerative hyperplasia, hepatocyte hypertrophy, fibrosis or sclerosis are mostly effects of prolonged exposure and not of acute exposure. All other effects on the liver are considered to be caused by acute inhalation exposure (JMPR, 2004).

In the case of liver toxicity the following symptoms may occur: sensation of heat, flushing and anxiety, pulsating headache, nausea, abdominal pain, vomiting after an alcoholic drink or disulfiram treatment and jaundice.

Clinical chemistry may be affected as follows:

- Increased liver enzymes in serum like:
 - transaminases ALT (alanine-aminotransferase), AST (aspartic-aminotransferase), indicating damage to the hepatocytes.
 - alkaline phosphatase especially increased in case of cholestasis.
 - gamma-glutamyltranspeptidase often increased in the case of high intake of alcohol.
- Increased bilirubin in serum causing jaundice.

Table 4-8: Acute liver endpoints: grading of effects*

| Type of effect | LT | I | ESC | R |
|---------------------------------|----|---|-----|---|
| Abdominal pain | | | x | x |
| Jaundice | x | x | | x |
| Increase liver enzymes in serum | | x | | x |
| Liver failure | x | x | | |

* Life Threatening (LT), Irreversible (I), Impairment to take action or escape (ESC), or Reversible (R).

For some effects, the severity is critical for categorisation. Endpoints scored in several categories are consequences of the degree of severity.

4.2.5.2.4 Conclusion

Short-term inhalation exposure may cause severe liver damage. This liver damage will rarely result in impairment of escape, unless the exposure triggers acute abdominal pain.

4.2.5.3 Kidney effects

The proximal tubule is the most common site of toxicant induced renal injury, as 75% of re-absorption takes place in this part of the tubule. Not only the permeability, but also the biotransformation activity is variable in the tubule. The activities of cytochrome P450 and of cysteine conjugate beta-lyase are mainly located in this proximal part of the tubule. The high levels of glutathione in the kidney contribute significantly to the detoxification process. Sometimes, glutathione metabolites (for instance of halogenated alkanes and alkenes) are catalysed by cysteine conjugate beta-lyase. This can result in nephrotoxic metabolites.

Urinary excretion of high molecular weight proteins like albumin is indicative for glomerular damage. Excretion of low molecular weight proteins as beta2-microglobulin suggests proximal tubular injury.

Heavy metals may cause toxicity through their ability to bind to sulphhydryl groups and the concomitant disruption of structural membranes or critical enzymes in the cell. The inorganic soluble form generally causes the greatest damage to the kidney.

4.2.5.3.1 Human experience and animal data

Uranium hexafluoride is an example of a kidney toxin that, due to its high volatility, has the potential to become airborne following an accidental release. Uranium hexafluoride is hydrolysed in the body to the uranyl ion (UO_2^{2+}). About 50% of plasma uranium is bound, as

the uranyl ion, to bicarbonate, which is filtered by the glomerulus. As a result of the acidification in the proximal tubule, the bicarbonate complex dissociates, followed by reabsorption of the bicarbonate ion. The uranyl ion is adsorbed to the membrane of the proximal tubule cells with subsequent cell damage and increased concentration of glucose, amino acids and proteins in the urine.

Uranium hexafluoride has been investigated in a study with dogs (Morrow *et al*, 1982). The inhaled dose was stored in the kidney to a maximum of 40%. After six days, about 10% was still present in the kidney. The renal injury was dose dependent. Between one and three days after exposure, the kidneys showed widely scattered necrosis of segments of the deep convoluted tubules and the linear part of the tubules. Regeneration of the tubuli started between three and six days and occurred earlier for the lower deposited dose levels.

Another example of a substance that causes kidney toxicity on acute exposure is tetrafluoroethylene. Odum and Green (1984) reported that rats exposed to 6,000 ppm tetrafluoroethylene for six hours showed marked damage to the proximal tubule of the kidney with no effect on the liver. The toxicity was characterised by very high concentrations of urinary glucose and by marked increases of several urinary enzymes. The nephrotoxicity of tetrafluoroethylene is believed to derive from the hepatic glutathione conjugate of this compound. Following excretion and degradation of this conjugate in bile, the cysteine conjugate is reabsorbed and further metabolised in the kidney by the enzyme beta-lyase to a cytotoxic species. The effects of tetrafluoroethylene will generally not impair escape capability during exposure. The nephrotoxic effect developed within hours to days after the exposure.

Protocols for testing nephrotoxicity

Protocols for the experimental investigation of kidney toxicity in humans do not exist. However, kidney function can be tested by measuring the concentration of urea and creatinine in blood and urine sediment, calculating creatinine clearance, and the presence of low and high molecular weight proteins in the urine, as well as in the casts.

For investigating nephrotoxicity, well-known protocols can be used, e.g. OECD (1981a and b) for acute and subchronic inhalation toxicity. The clinical chemistry testing of blood and urine, and the histopathological examinations after acute exposure should be carried out according to protocol OECD 413 (1981b) as far as it is related to nephrotoxicity.

Increased kidney weight, regenerative hyperplasia and altered serum calcium and phosphate concentrations are mostly caused by prolonged exposure to pesticides. All other effects on the kidney are considered to be caused by acute toxic inhalation exposure (JMPPR, 2004).

4.2.5.3.2 Grading of effects

The effects of nephrotoxic agents by inhalation will generally not impair escape capability during exposure. The nephrotoxic effect develops within hours to days after the exposure. Depending on the level of the exposure the following effects may occur:

- Complete kidney failure with anuria.
- Decreased excretion of urea and creatinine.
- Excretion of high molecular proteins, indicating damage to the glomerulus.
- Excretion of low molecular proteins, indicating damage to the proximal tubuli.

The damage caused by toxic substances does not necessarily result in irreversible loss of renal function. Much depends on the dose level and the duration of exposure. If the basement membrane is still intact, full regeneration may occur.

Table 4-9: Acute kidney endpoints: grading of effects*

| Type of effect | LT | I | ESC | R |
|--------------------------|----|---|-----|---|
| Kidney failure, anuria | x | x | | |
| Decreased excretion of: | | | | |
| - urea | x | x | | x |
| - creatinine | x | x | | x |
| Excretion of: | | | | |
| - High molecular protein | x | x | | x |
| - Low molecular protein | x | x | | x |

* Life Threatening (LT), Irreversible (I), Impairment to take action or escape (ESC), or Reversible (R).

For some effects, the severity is critical for categorisation. Endpoints scored in several categories are consequences of the degree of severity.

4.2.5.3.3 Conclusion

Acute exposure to volatile organics may cause kidney failure. This kidney failure will never result in impairment to escape, but will develop within hours to days after exposure and should be reversible in most cases.

4.2.5.4 Effects on muscles

4.2.5.4.1 Inhibiting acetylcholinesterase

Nerve gases used in warfare (e.g. sarin) and the organophosphates insecticides (e.g. parathion) achieve their effects by inhibiting acetylcholinesterase. Thereby, acetylcholine is not hydrolysed and continues to stimulate involuntary contraction. Atropine is used as an antidote because it blocks the acetylcholine receptors.

A number of acute high exposures have resulted in debilitating muscle weakness, particularly in the legs. These cannot be explained on the basis of nervous tissue acetylcholinesterase inhibition alone. It is assumed that the excessive amount of undestroyed acetylcholine is involved by an action at nicotinic and muscarinic acetylcholine receptors. The acetylcholine accumulating at the nicotinic acetylcholine receptor elicits stimulation of the neuromuscular junctions, causing fasciculations (repetitive stimulation) followed by a depolarising blockade if the acetylcholine levels remain elevated. Subsequently, this leads to a desensitisation process with a more or less permanent reduction in nicotinic acetyl cholinesterase receptors. In turn, this causes the persistent muscle weakness observed through a lack of response to stimuli. These processes are supported by chronic abnormal electromyographic activity in human intoxication by anticholinesterase insecticides.

4.2.5.4.2 Direct tissue damage

After absorption through inhalation, damage to muscular tissue may occur by reactive or cytotoxic chemicals, e.g. phenol.

4.2.5.4.3 Grading of effects

Procedures for studying effects on muscles

The type of damage caused by acute exposure to chemicals affecting muscle functions may be investigated using electromyography. This involves the detection, recording and interpretation of the electrical activity of groups of muscles at rest and during activity. It assesses the integrity of upper motor neurones, lower motor neurones, neuromuscular junction and the muscle itself. This is achieved by computer analysis of the frequency spectrum, amplitude, or root mean square of the electrical action potential. The procedure is seldom diagnostic of a particular disease entity. The major use lies in differentiating between the following disease classes:

- primary myopathy;
- peripheral motor neuron disease;
- disease of the neuromuscular junction.

In animal studies, the OECD protocols (1981a and b) may be followed for acute and subchronic inhalation exposure, respectively. Animals are subjected to electromyography in the observation period after acute exposure. Finally, the experimental animals are subjected to macroscopic and microscopic examinations following the protocol of the subchronic inhalation study. This includes extensive analysis of muscle tissue.

The variability of effects on muscles and especially of the respiratory system is presented in Table 4-10.

Table 4-10: Acute muscle endpoints: grading of effects*

| Type of effect | LT | I | ESC | R |
|-------------------------|----|---|-----|---|
| Chest | | | | |
| Dyspnea | x | x | x | x |
| Cough | | x | x | x |
| Chest Oppression | | x | x | x |
| Wheezing | | x | x | x |
| Tachypnea | x | x | x | x |
| Muscles | | | | |
| Weakness | | x | x | x |
| Fasciculations | | | x | x |
| Convulsions | x | x | x | x |
| Numbness of extremities | | x | x | x |

* Life Threatening (LT), Irreversible (I), Impairment to take action or escape (ESC), or Reversible (R).

For some effects, the severity is critical for categorisation. Endpoints scored in several categories are consequences of the degree of severity.

4.2.5.4.4 Conclusion

Exposure to cholinesterase inhibitors may cause impairment to escape due to muscle weakness and may, in very severe cases, result in death by weakness of muscles needed for respiratory ventilation.

4.2.6 Effects on blood

Haematotoxicity: basic concepts and background

Haematotoxicity essentially involves two basic homeostatic functions:

- Red blood cells (RBC)-mediated oxygen transport;
- the production of red and white blood cells and platelets (Budinsky, 2000).

For convenience, Table 4-11 provides definitions for various clinical terms used to describe the abnormal number of circulating red blood cells, neutrophils, lymphocytes, and platelets. Some terms describe the same condition and may create confusion when used interchangeably. The suffix *poenia* means an abnormal reduction, and the suffix *cytosis* refers to abnormal excess in number of blood cells.

Effects considered in this section are mainly related to oxygen transport by blood from lung to tissue cells. Occurrence of such effects may be associated with a lack of oxygen, or of oxygen transporter (haemoglobin).

Table 4-11: Definitions of haematological clinical terms (normal adult)
(Adapted from Budinsky, 2000)

| Clinical Term | Definition/Description |
|-------------------------------|---|
| Anaemia | A reduction in either the number or the volume of red blood cells, i.e. less than 3,500,000 RBC/mm ³ or 14 g of haemoglobin per 100 ml of blood. |
| Anoxia | Condition in which there is an absence of oxygen supply to an organ's tissues although the blood flow to the tissues is adequate. |
| Aplastic anaemia | A cessation of the normal regenerative production of red blood cells in the bone marrow. |
| Agranulocytosis | A reduction in the number of polymorphonuclear leucocytes (PMNs) less than 500/mm ³ . |
| Granulocytopenia, neutropenia | A reduction in the normal number of granulocytic leukocytes in the blood; normal granulocytes number around 3000-4000/mm ³ . |
| Granulocytosis | An increase above normal of the number of circulating granulocytic leucocytes. |
| Hypoxia | A decreased concentration of oxygen in inspired air, oxygen content in arterial blood (less than 80mmHg (10.6kPa)), or oxygen content in tissue. |
| Leucopenia | A reduction of the number of circulating leucocytes (white blood cells) below 5000 cells/mm ³ . |
| Leucocytosis, neutrophilia | An increase in the number of leucocytes, typically PMNs, above 10,000 cells/mm ³ . |
| Lymphopenia | A reduction in the number of circulating lymphocytes less than the normal 2500/mm ³ . |
| Lymphocytosis | An increase in the number of circulating lymphocytes from their normal number of around 2500/mm ³ . |
| Eosinophilia | Increase number of eosinophils above 200/mm ³ . |
| Thrombocytopenia | Abnormal number of circulating platelets less than 250,000-500,000/mm ³ . |

Direct toxicological effects on the RBC

Two types of toxicity essentially affect red blood cells:

- competitive inhibition of oxygen binding to haemoglobin;
- chemically induced anaemia in which the number of circulating erythrocytes is reduced in response to red blood cell damage.

Inhibition of oxygen transport is the more commonly observed toxicity directly affecting the RBC (Budinsky, 2000).

4.2.6.1 Competitive inhibition of oxygen binding to haemoglobin

4.2.6.1.1 Methaemoglobin inducing agents

Methaemoglobinaemia is a particular form of hypoxia and is a condition where the oxygen-carrying capacity of the blood itself is reduced. Oxygen transport depends on the maintenance of intracellular haemoglobin in the reduced $\text{Fe}^{(2+)}$ state. When haemoglobin is oxidised to methaemoglobin the haem iron becomes $\text{Fe}^{(3+)}$ and is not capable of binding oxygen. Ferric haem groups impair the release of oxygen from ferrous haem groups on the same haemoglobin tetramer. Thus, oxygen delivery to tissues is impaired. In addition, one iron in the ferric state in a particular haemoglobin molecule causes the other iron molecules to hold onto oxygen more tightly, thus shifting the oxygen dissociation curve to the left and decreasing oxygen delivery to the cells (Budinsky, 2000; Bradberry *et al*, 2001; Kiese, 1974).

The mechanism of methaemoglobin formation has not been elucidated fully for all agents, but can be divided broadly into three processes. Firstly, direct oxidation of ferrohaemoglobin, which involves the transfer of electrons from ferrous haem to the oxidising compound. This mechanism proceeds most readily in the absence of oxygen. Secondly, indirect oxidation, a process of co-oxidation which requires haemoglobin-bound oxygen, is involved, for example, in nitrite-induced methaemoglobinaemia. Thirdly, biotransformation of a chemical to an active intermediate initiates methaemoglobin formation by a variety of mechanisms. This is the means by which most aromatic compounds, such as amino- and nitro-derivatives of benzene, produce methaemoglobin (Bradberry *et al*, 2001).

Normally, red cells contain less than 0.5% methaemoglobin, as a result of autooxidation of haemoglobin as red cells circulate (probably by dissociation of the superoxide anion from oxyhaemoglobin). Erythrocytes (RBCs) are also subjected to oxidant stress as a result of exposure to various xenobiotics or to certain toxins (Budinsky, 2000).

Methaemoglobinaemia is clinically important because haemoglobin is unable to transport oxygen in the ferric state. Methaemoglobin formation results in a noticeable change in the colour of blood from its normal red colour to a brownish hue. In humans and animals, significant methaemoglobinaemia creates a bluish discoloration of the skin and mucous membranes. The clinical features observed at increasing methaemoglobin concentrations are summarised in Table 4-12.

Table 4-12: Symptoms associated with varying levels of methaemoglobinaemia*
(Adapted from Brightly et al, no year)

| Endpoint | LT | LT | I | ESC | R | A | |
|--------------------|--------------------------------------|---|--|---|---|---|---------------------------|
| MetHb in blood (%) | >70 | 60-70 | 45-60 | 20-45 | 15-20 | 3-15 | 0-2 |
| Signs or symptoms | Rapidly fatal Respiratory failure | Seizures Cardiac arrhythmias Haemodynamic instability and Shock | Decreased level of consciousness Metabolic acidosis, Tachypnea | Symptomatic cyanosis: Weakness, Fatigue Headache Dyspnea, Exercise intolerance Gastric upset Lethargy, Dizziness Syncope | Asymptomatic cyanosis" (‘chocolate brown blood’) | Asymptomatic; may have slate-grey skin colour | Normal level, no symptoms |

* Life Threatening (LT), Irreversible (I), Impairment to take action or escape (ESC), Reversible (R), Awareness (A).

The presence of methaemoglobin leads to the formation of aggregates of haemoglobin degradation products called Heinz bodies. These consist of haemoglobin covalently linked to cytoskeletal proteins on the inner side of the red blood cell membrane. The presence of Heinz bodies is a sensitive indicator of blood toxicity as it indicates that some haemoglobin has been destroyed. High levels of methaemoglobin are removed by catabolism leading to the development of anaemia. The body compensates for the destruction of red blood cells by increasing erythrocyte production, resulting in large numbers of immature erythrocytes, called reticulocytes, in the blood. If the toxic dose is not too severe, these compensatory mechanisms suffice.

Some subpopulations are particularly susceptible to oxidant stress (Griffin, 1997; Kumar and Verive, 2003):

- Children, especially those younger than 4 months, are particularly susceptible to methaemoglobinaemia. In infants, the NADH system has not fully matured, and the NADH MetHb reductase activity and concentrations are low.
- Infections, especially GI infections, may cause a build-up of systemic oxidants by an overgrowth of gut bacteria.

The aetiology of methaemoglobinaemia may be congenital:

- Congenital deficiencies in protective cellular capability;
- NADH reductase deficiency;
- Individuals with haemoglobin M disease have abnormal haemoglobin that is not amenable to reduction;
- Individuals with pyruvate kinase deficiency may have an impaired glycolytic pathway, which results in deficient NADH production;
- Individuals with G-6-PD deficiency may have impaired production of NADPH in the hexose-monophosphate shunt.

Alternatively, it may be acquired (caused by exposure to oxidising chemicals or drugs). Typical examples include:

- Ferricyanide, hydroxylamine, chromate, chlorate are examples of the numerous compounds which can have this effect. Effects of nitrites, although they are normally not inhaled, are important to understand the formation of MetHb.

- Hydroxylamine is a direct-acting haematotoxic agent leading to haemolytic anaemia in animals and man. During the reaction of hydroxylamine with Hb, a large proportion is transformed to ammonia (NH₃) and nitrogen (N₂). The ratio of reaction products is affected by oxygen. Hydroxylamine does not only react to the iron of Hb but it also transforms it into a green derivative, a verdoglobin, which shows properties related to sulphhaemoglobin. In dogs at 20 mg/kg hydroxylamine, HCl oxidises about 20% of Hb. A variety of primary and secondary aliphatic amines are oxidised to this compound by liver microsome enzymes (Kiese, 1974).
- Inorganic nitrites such as sodium nitrite (NaNO₂) and chlorates (ClO₃⁻) oxidise ferrous haemoglobin (Fe²⁺) to ferric haemoglobin (Fe³⁺ or methaemoglobin). Nitrite and chlorate directly oxidise haemoglobin. Nitrate, however, must first be reduced to nitrite by nitrifying bacteria in the gut. Exposures to nitrates, nitrites, and chlorates occur mostly in industrial settings or from contaminated drinking water (Budinsky, 2000).
- Binding of nitrite to oxyhaemoglobin displaces the bound oxygen and yields methaemoglobin, hydrogen peroxide, and nitrogen dioxide in a free radical chain initiation step. The nitrogen dioxide oxidises ferrous haemoglobin to methaemoglobin, whereas hydrogen peroxide oxidises methemoglobin to a ferryl haemoglobin radical. Reaction of ferryl haemoglobin with nitrite also produces methaemoglobin and nitrogen dioxide. These last two reactions are the free radical chain propagation steps. Splitting of two nitrogen dioxide radicals produces a nitrate anion, regenerates a nitrite anion, and this constitutes the free radical chain termination step (Kohn *et al*, 2002).
- The primary mechanism of chlorate toxicity is the rupture of the red blood cell membranes with intravascular haemolysis. The formation of methaemoglobin is secondary to lysis of red blood cells, and is caused by autooxidation of the free haemoglobin. The formation of methaemoglobin from free haemoglobin is irreversible, and may cause life-threatening effects. (Within the red blood cells, methaemoglobin is rapidly reduced by methaemoglobin reductase, but this activity is lost with cell lysis). Potassium chlorate is also a relatively powerful irreversible inhibitor of catalase (OEHHA, 2002).
- Arsine, the hydride of arsenic (AsH₃), is a potent haemolytic agent and a recognised industrial hazard. Typical cases of acute poisonings, predominantly in workers accidentally exposed, resulted in haemoglobinuria, jaundice, and haemolytic anaemia. The rapid and unique haemolysis caused by arsine can progress to oliguric renal failure, which can be fatal without proper therapy. It has been reported that a half-hour exposure to 25-50 ppm can be lethal. Haemolytic anaemia, however, is the most consistent clinical finding in humans. Observed haemolytic effects in humans are consistent with effects observed in laboratory animals and include increased Hb concentrations; reticulocytosis; leucocytosis; and altered RBC morphology characterised by basophilic stippling, anisocytosis, poikilocytosis, red-cell fragments, and ghost cells (US EPA, 1994b).
- It has been proposed that arsine interacts with haemoglobin to form toxic haemoglobinoxidation products, and this was also investigated as a potential cause of haemolysis. Arsine does not affect Hb in absence of O₂, and is readily oxidised by oxyHb with formation of ferriHb. Intermediates in the oxidation of arsine to arsenic are hydrarsine (As₂H₄) or hydroxylarsine (AsH₂OH). Most likely, the formation of the latter follows the general scheme of ferriHb by reducing agents.
- Aromatic amines and nitro compounds such as aniline and nitrobenzene cause methaemoglobinaemia by initiating a redox cycle in the RBC. However, unlike those for nitrites and chlorates, the potential hazards of aromatic amines are not limited to methaemoglobinaemia. RBC changes occurring during or after methaemoglobin formation may result in damage to the RBC membrane. The damaged RBCs are recognised by

splenic macrophages, which remove and destroy them. Haemolytic anaemia can result if the number of red blood cells destroyed exceeds the bone marrow's capacity to replenish them; for example, by amplification of RBC production in response to increased release of erythropoietin (Budinsky, 2000).

- Reactive metabolite(s) of the parent aromatic amine compound, formed via cytochrome P450 metabolism, are also capable of causing methaemoglobinaemia and haemolytic anaemia. Aromatic nitro compounds, like inorganic nitrate, must first be reduced to their respective aromatic amine by gut bacteria before being metabolised to an arylhydroxylamine. It is the *N*-hydroxyl metabolite that is directly responsible for initiating haemoglobin oxidation via a redox cycle. The redox cycle results in the formation of reactive oxygen species in the RBC (i.e. hydrogen peroxide). The reactive oxygen species oxidise proteins in the RBC cytoskeleton and damage the RBC membrane by crosslinking adjacent proteins. The crosslinked proteins can be visualised in the form of Heinz bodies. RBC membrane damage may also alter the normal RBC discoid morphology (Budinsky, 2000).

4.2.6.1.2 Carboxyhaemoglobin inducing agents

a) Carbon monoxide (CO)

Once absorbed, CO diffuses through the plasma, passes across the red blood cell membrane, and finally enters the red blood cell stroma where CO binds to haemoglobin forming carboxyhaemoglobin (COHb). Such binding reduces the oxygen carrying capacity of blood and interferes with oxygen release at the tissues. The resulting impaired delivery of oxygen can interfere with cellular respiration and cause tissue hypoxia. The affinity of Hb for CO is 210-300 times greater than its affinity for oxygen, and Hb is incapable of combining with oxygen. The presence of CO also alters the dissociation of oxygen from other haemoglobin sites, and compromises the delivery of oxygen to the tissues. At the cellular level, carbon monoxide binds with haemoproteins such as myoglobin, cytochrome oxidase, mixed-function oxidases (cytochrome P-450), tryptophan oxygenase, and dopamine hydroxylase. The protein most likely to be inhibited at relevant levels of COHb is myoglobin, which abounds in skeletal muscle and the myocardium. Lower myoglobin levels cause dysfunction by impairing the blood's oxygen carrying capacity and the transport of oxygen from the blood to the mitochondria. CO also binds with cytochrome oxidase, the terminal enzyme in the mitochondrial electron transport chain catalysing the reduction of molecular oxygen to water, thus inhibiting cellular respiration and resulting in anaerobic metabolism and lactic acidosis (Fierro *et al*, 2001).

The adverse health effects associated with CO vary with concentration and duration of exposure. Clinical symptoms range from subtle cardiovascular, respiratory, and neurobehavioural effects at low concentrations (10-70 ppm) to unconsciousness and death after prolonged exposures or after acute exposure to high concentration of CO (>500 ppm) (Table 4-13).

A number of models with varying degrees of sophistication have been derived from human experimental data, for predicting time to attain a particular COHb concentration. The effects of CO and its interactions considered here represent emergency situations such as fires, where the inhaled CO concentration is well in excess of equilibrium with the COHb concentration. In such cases the departure from a linear uptake rate is not great for a constant CO concentration, so that a reasonable prediction of COHb concentration can be obtained from

$$\% \text{ COHb} = (33.7 \times 10^{-5})(\text{ppm CO})^{1.036}(\text{RMV}) (t)$$

where
ppm CO = CO concentration (ppm)
RMV = minute volume (l/min)
t = exposure time (min).

Table 4-13: Symptoms associated with varying levels of carbon monoxide poisoning*
(Adapted from Winter and Miller, 1976; IPCS, 1999; Fierro et al, 2001)

| Endpoint | LT | I | ESC | R | A | A | |
|---------------------------|----------------|--|--|---|--|---|---------------|
| CO in atmosphere (ppm) | 1950 | 800-1220 | 350-520 | 220 | 120 | 70 | 10 |
| Maximum COHb in blood (%) | >80 | 60-70 | 40-50 | 30 | 20 | 10 | 2 |
| | Rapidly fatal. | Unconsciousness; Intermittent convulsion; Respiratory failure, death if exposure is continued for a long time. | Headache, Confusion; Collapse; Fainting on exertion. | Headache; Irritable; Easily fatigued; Judgement disturbed; Possible dizziness; Dimness of vision. | Shortness of breath on moderate exertion; Occasional headache with throbbing in temples. | No appreciable effect, except shortness of breath on vigorous exertion; Possible tightness across the forehead; Dilation of cutaneous blood vessel. | Asymptomatic. |

*. Life Threatening (LT), Irreversible (I), Impairment to take action or escape (ESC), Reversible (R), Awareness (A)

To predict the time to incapacitation, the % COHb achieved after a time *t* is expressed as a fraction of the % COHb causing incapacitation. This constitutes the FED. As a default condition, it is assumed that subjects are engaged in light activity (minute volume = 25 l/min) and that incapacitation (loss of consciousness) occurs when a COHb concentration of 30% is achieved. The FED for incapacitation (FED_[co]) at time *t* is then given by

$$FED_{[co]} = (8.2925 \times 10^{-4} \times \text{ppm CO}^{1.036}) \times t/30$$

The FED can then be correlated with the FEDs for other gases depending on the nature of the toxic interactions between CO and the other gases present (Purser, 1995).

b) Dichloromethane

Dichloromethane (DCM) is metabolised by oxidative metabolism mediated by the ethanol inducible CYP2E1 leading to formyl chloride which decomposes to carbon monoxide that binds strongly to haemoglobin to form COHb. An alternative pathway involves the conjugation with reduced glutathione catalysed by GSTT1. The conjugate, S-chloromethylglutathione is highly reactive (ATSDR, 2000).

However, CYP2E1 has a much higher affinity for DCM compared to GST, and is the most important pathway at relevant human exposure levels, whereas the GSH dependent pathway becomes qualitatively relevant at high exposure concentrations. Difference in the metabolism of DCM is assumed to play an important role in the interspecies differences seen in the toxic response (ATSDR, 2000).

Studies in man have demonstrated that 8-hour exposures to concentrations of 100 to 200 ppm of DCM vapour produce carboxyhaemoglobin (COHb) concentrations of about 3 to 7%. This is very similar to levels produced by CO at 50 ppm but well below COHb levels generally

required to produce symptoms. As the metabolic pathway is saturated at high concentrations, a maximum of <10% COHb in blood is normally reached, although still higher levels have been measured occasionally (SCHER, 2005; ATSDR, 2000).

Inhalation of high concentrations of DCM can result in death. Predominant acute effects in human beings are CNS depression and elevated blood carboxyhaemoglobin (COHb) levels. These effects are reversible. Other targets can be the liver and, occasionally, the kidneys (IPCS, 1996). Mild CNS effects in humans have been reported following exposure to concentrations as low as 200 ppm for 1.5 to 3 hours (Putz *et al*, 1976). More significant effects occur at concentrations in excess of 600 ppm. Narcosis has been reported to occur following exposure to 20,000 ppm. Fatal accidents have been reported under unknown but most probably extremely high concentrations, e.g. when using DCM paint remover in unventilated cellars (UNR, 1999).

Table 4-14: Symptoms associated with varying levels of dichloromethane poisoning*
Adapted from Tukker and Simons, 1999

| End-point | LT | I | ESC | A |
|----------------|------------|------------------------|--|-----------------------------|
| Level | 20,000 ppm | >600 ppm | 200 ppm | 30-160 ppm |
| Health effects | Narcosis | Significant CNS effect | Mild CNS effect; generally used as a LOAEL for non-smoking healthy individuals | COHb levels in blood 3.5-5% |

* Life Threatening (LT), Irreversible (I), Impairment to take action or escape (ESC), Reversible (R), Awareness (A).

4.2.6.1.3 Tissue hypoxia inducing agents

Hypoxia is defined as a decreased concentration of oxygen in inspired air, oxygen content in arterial blood (less than 80mmHg (10.6kPa)), or oxygen content in tissue. Anoxia, on the other hand, is the complete absence of oxygen (Budinsky, 2000).

Hypoxia can result from a variety of conditions including anaemia; a reduction in the iron carried by the RBC; ischemia (physical barrier to blood flow) caused by occlusion or vasoconstriction of an artery; or by an increased oxygen affinity (shift to the left of the oxygen-haemoglobin binding curve) that reduces the release of oxygen. In situations involving oxygen-deficient atmospheres, the blood oxygen concentration can drop to a level at which the central nervous system and cardiovascular system risk impairment (Budinsky, 2000).

Different types of hypoxia can be distinguished:

- Histotoxic hypoxia: This form results from tissue poisoning e.g. by alcohol, narcotics (like CO₂), and certain poisons. The use of oxygen by the tissues is interfered with and the tissues are unable to metabolise the delivered oxygen.
- Hypemic hypoxia: The inability of oxygen to bind to the haemoglobin, as a result of a large blood loss, of chronic anaemia (with decreased haemoglobin content), or the formation of compounds with haemoglobin (methaemoglobin, carboxyhaemoglobin) in case of exposure to e.g. carbon monoxide, nitrites, sulfa drugs, that reduces the amount of haemoglobin available to form oxyhaemoglobin.

- Hypoxic hypoxia: This is a lack of oxygen as a result of a high altitude (decreased oxygen pressure) or by conditions that prevent or interfere with the diffusion of oxygen across the alveolar membrane (asthma, pneumonia, tumours, and arterial venous shunts).
- Stagnant hypoxia: This is attributable to a malfunction of the circulatory system resulting in a decrease in blood flow. Causes include shock and exposure to extreme hot or cold temperatures.

Hypoxia may lead to death in severe cases whatever the cause. The patient is often stuporous or comatose (in a state of unconsciousness) for periods ranging from hours to days, weeks, or months. Seizures, myoclonic jerks (muscle spasms or twitches), and neck stiffness may occur. If the patient's respiratory and cardiovascular systems can be supported adequately, recovery may occur, but it depends upon the severity of injury. As recovery proceeds, a variety of psychological and neurological abnormalities may appear, persist for a time, and then disappear. Mental changes such as dementia or psychosis may occur. Mental confusion, personality regression, parietal lobe syndromes, amnesia, hallucinations, and memory loss may also occur.

Although all tissues in the body are altered under conditions of hypoxia, the brain is by far the most sensitive tissue to a mild oxygen deficit. The effects of hypoxia range from subtle to deadly, particularly in situations where sound judgement, reasoning and physical coordination are required.

Hydrogen cyanide

Cyanide inhibits cytochrome oxidase, thus halting electron transport, oxidative phosphorylation, and aerobic glucose metabolism. Inhibition of glucose metabolism results in the build-up of lactate (lactic acidemia) and the increase in the concentration of oxygenated haemoglobin in venous blood returning to the heart. Increased oxyhaemoglobin in the venous circulation reflects the fact that oxygen is not being utilised in the peripheral tissues. The most serious consequences of oxidative phosphorylation inhibition are related to neurological and cardiovascular problems, including adverse neurological sequelae, respiratory arrest, arrhythmia, and cardiac failure. Cyanide exposure can occur via inhalation of hydrogen cyanide gas or through ingestion of sodium or potassium cyanide. Approximately 100 mg of sodium or potassium cyanide is lethal. Sublethal doses of cyanide are quickly metabolised to thiocyanate via the enzyme rhodenase (a sulphurtransferase):



The detoxification of cyanide to thiocyanate is facilitated by adding the substrate sodium thiosulphate, which reacts with cyanide through the action of rhodenase. Thiocyanate (SCN^-) is a relatively nontoxic substance eliminated in the urine (Budinsky, 2000).

The dose-effect curve of the acute effects in humans is steep. Whereas slight effects occur at exposure to hydrogen cyanide levels of 20-40 ppm, 50-60 ppm can be tolerated without immediate or delayed effects for 20 minutes to one hour, 120-150 ppm is dangerous to life and may lead to death after 30-60 minutes, 150 ppm is likely to be fatal within 30 minutes, 200 ppm is likely to be fatal after 10 minutes, and 300 ppm is immediately fatal. It should be emphasised that this represents crude average exposure estimates, based on various studies (DECOS, 2002).

Hydrogen cyanide can cause rapid death due to metabolic asphyxiation. Exposure to high concentrations of hydrogen cyanide produces hyperventilation within seconds, followed by loss of consciousness, convulsions, and fixed and dilated pupils. Death from respiratory and/or cardiac arrest may occur within minutes. Despite the presence of tissue hypoxia,

cyanosis may not occur and the skin may remain pink. A recent study reports an estimated LC₅₀ in humans of 3,404 ppm for a 1-minute exposure. Other sources report that 270 ppm is fatal after six to eight minutes, 181 ppm after 10 minutes and 135 ppm after 30 minutes (Hathaway *et al*, 1991). Exposure to low concentrations may be associated with dyspnoea, headache, dizziness, anxiety, sinus tachycardia, nausea and drowsiness. A metallic taste has been reported.

Cyanide directly stimulates the chemoreceptors of the carotid and aortic bodies, causing hyperpnoea. Cardiac irregularities are often noted (Amdur *et al*, 1991). Liquid hydrogen cyanide, hydrogen cyanide in aqueous solution (hydrocyanic acid), and the concentrated vapour are all absorbed rapidly through the intact skin and may cause systemic poisoning with little or no irritant effect on the skin itself. The liquid in contact with the eye may cause only local irritation; however, the attendant absorption may be hazardous (Hathaway *et al*, 1991). Industrial exposure to hydrogen cyanide solutions has caused dermatitis, itching, scarlet rash, papules, and nose irritation and bleeding. Perforation of the nasal septum has also occurred (NLM, 1995).

At low concentrations of HCN (up to around 100 ppm) the time to incapacitation is long (>20 minutes). At higher concentrations (approaching 200 ppm) the time to incapacitation is short, around two minutes. This means that while exposure to low cyanide concentrations is relatively safe, at around 200 ppm and above, incapacitation occurs very rapidly.

From the primate exposure data, an expression has been developed for the prediction of time to incapacitation (loss of consciousness) at different inhaled HCN concentrations. It is considered that this might be somewhat conservative for direct comparison with a resting adult human but could be reasonably predictive of time to incapacitation in children or exercising adults.

For a constant HCN concentration time to incapacitation ($t_{[en]}$) is given by

$$t_{[en]} = 220 / \exp ([CN] / 43)$$

so that

$$FED [en] = (\exp ([CN])/43) t/220.$$

Despite the differences between exposures producing incapacitation at different HCN concentrations, for simple rat lethality estimations, a 30-minute exposure time is assumed and the LC₅₀ concentration is 165 ppm. In rats, less-marked differences in exposure times leading to incapacitation and lethality were observed at different exposure concentrations.

The modelling of toxicological effects of gas combinations was derived from the CO mathematical model (Hartzell, 1985) and applied to a mixture of CO and irritants. It has been also shown that Haber's rule does not address sufficiently possible high rate of detoxification, which can negate effects from extended exposure to low concentrations of substances such as hydrogen cyanide (Miller *et al*, 2000a).

4.2.6.1.4 Simple asphyxiants

If the atmosphere composition is modified by an accidental gas release, which results in low oxygen content, hypoxia can develop in the exposed persons. Hypoxia may be caused by a number of other events, such as smoke or carbon monoxide inhalation, high altitude exposure, strangulation, anaesthetic accidents, or poisoning.

Some gases and vapours (e.g. argon, carbon dioxide, ethane, helium, hydrogen, methane, nitrogen) when present at high concentrations in air, act as simple asphyxiants by reducing the oxygen to such an extent that life cannot be supported. Many asphyxiants are odourless and

colourless and not readily detectable. The toxicity of this group of substances depends on concentration, duration of exposure, and ventilation. Indeed, no toxicity will develop if the fraction of inspired oxygen is adequate; they are not irritating to the respiratory tree or systemically toxic.

Hypoxia typically occurs when the atmospheric oxygen (normally at 21%) is too low to sustain the oxygen saturation of haemoglobin above 80%. Under circumstances of reduced oxygen delivery to the lungs, serious cardiovascular and central nervous system impairment can develop. The symptoms range in severity from euphoria to loss of consciousness, seizures, and cardiac arrhythmias. Haemoglobin oxygen saturation less than 80% results in a sense of euphoria, impaired judgment, and memory loss. As the oxygen content of haemoglobin decreases, the extent of the effects on the central nervous system increases. If oxygen pressure drops to 30mm Hg, a level corresponding to approximately 55-60% oxygen saturation, consciousness may be lost. Individuals with ischemic heart disease, such as atherosclerotic coronary vascular disease, may be more sensitive to hypoxic conditions than in healthy individuals. Individuals with atherosclerosis may be more prone to hypoxia-induced ischemia, which may lead to arrhythmia or ischemia-like pains. Subjects with serious atherosclerosis of the cerebral vasculature are more likely to develop CNS impairment related to hypoxia than are healthy subjects. Hence, hypoxia resulting from either low oxygen concentrations or interference with oxygen transport must be assessed according the subject's cardiovascular status (Budinsky, 2000).

Table 4-15: Relationship between environmental oxygen levels and health effects*
Adapted from: EBME, no date

| | LT | I | ESC | R | A |
|--|--|---|--|--|-----------------------------------|
| Oxygen level in air (%) (Normal value is 20.93 %) | 0-6% | 6-8% | 8-11% | 11-14% | 14-21% |
| Estimated O ₂ saturation level # | | | <56% | 56-97% | 97-99% |
| Health Effects | Fainting almost immediate; Death or severe brain damage. | Fainting within a few minutes; Resuscitation possible if carried out immediately. | Possibility of headaches, dizziness and fainting after a short period of time. | Physical and intellectual performance becomes difficult. | Increasing pulse rate; Tiredness. |

* Life Threatening (LT), Irreversible (I), Impairment to take action or escape (ESC), Reversible (R), Awareness (A).

In arterial blood during moderate activity as estimated by SatCur model.

Determination of the cause of death in hypoxic gas cases is very difficult because of the variation in circumstances surrounding such deaths. To clarify the cause of death and identify the factors involved in hypoxia, i.e. the concentration of gases at the time of respiratory arrest, the time until death and the concentration of gaseous substances in the tissues, were studied by Watanabe and Morita (1998) in rats exposed to six different gases. Three hypoxia conditions were used:

- rapid hypoxia (2-3 minutes) in an exposure chamber in which the oxygen was depleted completely;
- prolonged hypoxia (20-25 minutes) by gradually depleted oxygen;
- hypoxia by inhalation of gases saturated with a critical gas concentration, maintaining the O₂ at 20% (60 minutes).

In the first group, respiratory arrest occurred within 30 to 40 seconds, followed by cardiac arrest 2 or 3 minutes thereafter. Severe convulsions were observed only with the use of nitrogen.

In the prolonged hypoxia groups, respiratory arrest occurred at the concentration of 4-5% O₂, with non-toxic gases (N₂, N₂O₂ and propane).

The toxic gases CO₂ and Freon-22 produced respiratory arrest at the concentration of respectively 6.6-8.0% O₂ (60-67% CO₂) and 13-14% O₂ (30-35% Freon-22).

Variations in the gas concentrations among the tissues were observed according to the type of hypoxia, type of gas and the duration of exposure.

The concentration of the fat-soluble gases in the adipose tissue showed marked variation according to the duration of the exposure.

The distribution pattern of methane was different from those of the other gases, in which the variation of concentrations among the tissues except lung were small in both rapid and prolonged hypoxia. This phenomenon was considered to be attributable to the relative solubility of gaseous substances in blood and tissues.

Atrophy in the alveoli was observed after the rapid hypoxia with CO₂ and N₂O. Local haemorrhage in the lungs was also observed, especially in CO₂ hypoxia.

The risks of oxygen-depletion hypoxia are the rapid loss of consciousness and respiratory and cardiac arrest.

4.2.6.2 Conclusion

Interaction of toxic substances with blood, oxygen uptake and delivery to tissue cells can lead to direct or indirect hypoxia through different modes of action like methaemoglobinaemia. Caution must be taken for extrapolation from animal data to deleterious graded levels in humans, even though some mathematical models provide support to derive some critical levels.

Grading of effects should take both blood parameters and symptoms into account. This might be relatively easy in cases of short-term exposure to high doses, but will be more difficult for low doses and longer exposures, where models do not take detoxification into account.

4.2.7 Reprotoxicity / Developmental effects and fertility

4.2.7.1 Developmental / Prenatal effects

Pregnant women as well as their developing children are considered as potential subgroups in terms of sensitivity and vulnerability. This default position (in the absence of specific data) takes into account two different aspects:

- Pregnant persons may differ from the non-pregnant population in terms of metabolic rate, ventilation and circulation rates. These are mainly kinetic deviations from the average population and may be treated sufficiently by assessment factors for intraspecies variation.
- Few chemicals have the potential specifically to affect the foetus or embryo and interfere with its normal development and differentiation even if the maternal organism is not adversely affected. Such selective (developmental) toxicity is a category by itself and

should be considered as a potentially relevant endpoint for setting acute exposure levels, especially if there is evidence that the effect may occur after one single exposure.

The following is exclusively dedicated to the aspect of selective developmental toxicity.

The endpoint 'developmental toxicity' can be differentiated into a number of sub-endpoints according to the effects obtained in experimental animals (e.g. intrauterine death, malformations, variations, retardations, or reduced foetal weights). The relevance of these observations for setting acute exposure limits has been proposed (Dewhurst, 2000; Billington and Carmichael, 2000) and needs to be discussed in the light of the question whether or not they may occur after a single exposure. This issue has been recently addressed in a more comprehensive paper by van Raaij *et al* (2003).

Developmental toxicity affects tissues in various stages of differentiation and growth and is frequently linked to certain specific time windows of vulnerability during intrauterine life. Different stages of embryogenesis are susceptible in their unique way. In order to cover all potential stages, developmental toxicity is normally investigated under repeated administration. From a thoughtful analysis of literature examples, van Raaij *et al* (2003) have compared the data for chemicals that had been investigated for single and repeated administration. They have shown that, in the absence of data obtained from specific administration during the proper time window(s), NOAELs taken from conventional inhalation studies using repeated administration are meaningful and sufficiently conservative starting points for setting acute exposure limits.

Most developmental studies use gavage administration. This leads to a bolus resorption resulting in higher tissue levels than achieved after dietary or inhalation exposure. For the inhalation route (mostly 4-8 hours of exposure) tissue levels are normally intermediate between those resulting from gavage and dietary exposures. Nevertheless, the route-to-route extrapolation (from oral to inhalation) is often difficult and requires an individual assessment for every compound.

The effects relevant for acute exposure risk estimation need to be identified from each study. It is pointed out that this cannot be obtained from the EU classification system, which is mainly hazard-driven and often based on oral studies over 10-19 days.

4.2.7.1.1 Animal data

Single exposure with adverse effects on the developing foetus or embryo has clearly been shown in animal experiments (Hauck *et al*, 1989; Hishida and Nau, 1998; Piersma *et al*, 1996; van Raaij *et al*, 2003). The usual guideline testing protocols require repeated administration in order to cover as many potentially susceptible time windows as possible with an effective bioavailability (e.g. OECD 414 guideline today requires exposure from gestation day 6 through 19 for rats and 7-29 for the rabbit (OECD, 2001a)). Most experimental investigations are carried out after oral administration via gavage (bolus administration). Administration via the diet or the inhalation route leads to a slower resorption and lower plasma peak levels in most cases but these routes are less frequently used. Bolus administrations expose the embryonic tissues to higher peak concentrations, at least of the parent compound and in some cases also its relevant metabolites. For some compounds, peak concentrations (C max after bolus) appear to have a higher impact than the same dose (time-integrated in mg/kg bw) received over a longer period (e.g. inhalation). However, for other chemicals AUC-dependence has been shown. The picture is further complicated by the fact that some compounds (or their metabolites) show cumulative properties, which lead to sustained plasma levels after repeated administration.

EU classification is not a sufficient criterion to decide on AETL relevance. It has been shown for several chemicals of EU Category 2 and 3 that they do not necessarily pose a risk to the foetus when inhaled or they may simply not be inhaled in amounts that lead to concentrations causing an effect in the blood (e.g. di-2-ethylhexylphthalate in Klimisch *et al*, 1992). In such cases it is not necessary to base AETL levels on developmental effects. Where only results from oral studies are available, the route-to-route extrapolation from oral to inhalation pathway remains a principal difficulty.

Many teratogenic solvents, such as dimethylformamide and monomethylformamide or methoxyethanol and 2-methoxypropanol-1, produce long-lasting metabolites (Mraz and Nohová, 1992; Klug *et al*, 1998; Groeseneken *et al*, 1989; Carney *et al*, 2003). They appear to be similarly active after inhalation as they are after gavage administration. Furthermore, chemicals responsible for developmental effects, which are only slowly excreted, are possibly more effective in humans (as the larger species with a slower metabolic rate) than in experimental animals. On the other hand, the development of the human embryo and foetus is slower and a short time of exposure may be less critical than for the more rapid intrauterine development of rodents.

In most cases, the sensitive time window is not known from studies using single exposure. The NOAEL or LOAEL from an OECD 414 (2001a) study (inhalation) may then serve as a conservative assumption also for the NOAEL for single administration at the critical time point.

Decisions need to be taken concerning which sub-endpoints in developmental toxicity studies are relevant for single exposure scenarios. van Raaij *et al* (2003) propose the following: resorptions (especially early resorptions) and malformations (though there are large differences) are more relevant, whereas late resorptions and decreased foetal weights, in the absence of malformations, are less likely to result from a single exposure (unless it is to a compound with an unusually long persistency).

4.2.7.1.2 Grading

Grading should be made on individual assessments of the toxicological data and not on EU categories as those are mainly hazard-driven and not risk-based.

In contrast to gavage administration, effective plasma levels in the maternal organism may not be achieved via the inhalation route. However, inhalation studies, dosimetric aspects, PBPK modelling and other data may show whether in such cases (single) inhalation up to AETL-2 levels would present a danger to intrauterine development.

For chemicals requiring maternal toxicity in order to exert adverse effects on embryonic and/or foetal tissues (mostly EU Category 3), AETL-2 levels based on subchronic NOAELs would often also protect the unborn child.

For chemicals showing selective developmental effects at dose levels below maternal toxicity the foetal NOAEL should be linked to AETL-2 or AETL-3 level depending on the type and severity of effects.

4.2.7.1.3 Conclusion

When assessing developmental effects in experimental studies for AETL relevance, a high relevance is seen for malformations, prenatal death and/or intrauterine resorptions. This is especially the case, when this occurs in inhalation studies and independent of whether the exposure was repeated (which is a default position).

If malformation, foetal or embryonic deaths were observed in an oral study, and an inhalation study is not available, an assessment has to be made whether an equivalent (and thus relevant) bioavailability can also be expected via the inhalation route.

For effects which typically occur as a result of repeated administration or which may only occur after oral administration an AETL relevance is very unlikely.

4.2.7.2 Fertility / Reproductive effects

In this section, reproductive effects are considered as specific adverse effects on male and/or female fertility or fecundity. The classical experiment for their detection is the 1- or 2-generation study, which investigates the fertility indices. However, these expensive studies have not been completed for most chemicals. Therefore, adverse effects on the sexual/reproductive organs in subacute or sub-chronic studies must be used as surrogates. These effects need to be specific in order to be relevant for AETL and should not be the results of general toxicity. For instance, narcotic effects preventing animals from pairing would be unspecific in this context, as would a moderate testicular atrophy resulting from severe malnutrition.

If the data allow differentiation, it is important for the AETL relevance to know whether an effect on fertility is linked to repeated administration only (not expected after single exposure) and oral exposure only (not inhalation). A further relevant distinction is whether these effects are reversible (presumably, in most cases) or irreversible (rare).

Testes are a frequent target organ in sub-chronic experimental studies and may be affected at dose levels below toxicity to other target organs.

The pattern that is observed in most cases is a testicular atrophy with lower testes weights and focal or generalised losses in germinative epithelium. (However, in older sub-chronic studies the reproductive organs may not have been adequately investigated).

Testicular atrophy is also linked to inhibition or impairment of fertility depending on the extent. In rodents a slight to moderate testicular atrophy with little reduction in sperm populations has often not much effect on reproductive performance and fertility parameters. However, slight reductions in sperm population may adversely affect fertility in humans.

Occasionally, the ovaries may also be the specific target organs of a chemical.

Certain chemicals (including poly-halogenated aromatic compounds) may adversely affect fertility for other reasons than testicular or ovarian toxicity. The full investigation of all reproductive and fertility parameters is therefore only provided with the 2-generation study (OECD, 2001b). This exposes two consecutive generations from the time point of origin of the first. However, for the majority of chemicals, effects on fertility without morphological effects on the reproductive organs are rare. Hence, the NOAELs from sub-chronic studies should also indicate the thresholds for potential fertility effects for most chemicals.

Morphological effects on reproductive organs may be encountered after just a single administration. Therefore, the fertility endpoint must also be addressed for AETL considerations. By definition, effects on reproductive organs and fertility are related to the AETL-2 level. There is a difference whether such effects are reversible or irreversible, even though this is not reflected in the grading of the three existing AETL levels. It is proposed that such differences in severity are treated by using different assessment factors. For many chemicals, the question of reversibility is also a matter of dose. Slight effects with oligospermia, which are also much more common, are more prone to full reversibility than complete azoospermia.

4.2.7.2.1 Human exposure

Adverse effects on male reproductive capabilities (e.g. as reflected in spermatograms) have been shown in epidemiological studies for a number of chemicals, though presumably not after a single exposure.

Occupational exposure to certain glycol ethers was alleged to cause lower sperm counts.

The pesticide (nematocide) 1,2-dibromo-3-chloropropane influenced the spermatogenesis (no effects on sexual function) of men involved in production of this substance or in application by spraying the material (Kharrazi *et al*, 1980; Lipshultz *et al*, 1980; Whorton *et al*, 1979). Men repeatedly exposed to this pesticide showed an increased incidence of oligospermia and azoospermia. Oligospermia (<20 million sperm/ml) was reversible, but azoospermia (0-1 million sperm/ml) was not. Azoospermia could only be detected after at least three years of repeated occupational exposure.

Medical treatments e.g. with cytostatic drugs often affect and deplete the testicular germinative epithelium. Increasing experience, however, shows that such effects were mostly reversible and affected persons had normal and healthy children later in life.

High amounts of PCBs and furans in rice oil consumed some 25 years ago were considered as the principal cause for low sperm quality among 37-50 years old men exposed at that time. It was noted that their sperm cells bind less effectively to oocytes than those of unexposed control persons.

Gossypol, a polyphenol isolated from cotton plants shows a two-week half-life in man. Male infertility and reduced spermatogenesis or even azoospermia was observed at blood levels of this substance as low as 100-150 ng/ml (Coutinho, 2002).

Adverse effects on female fertility are more difficult to detect. A differentiation into functional interference (e.g. in the case of contraceptive pharmaceuticals) and ovarian (cyto)toxicity should be considered (the latter being presumably less reversible than the former). Stillbirths (equivalent to resorptions in animal studies) are mostly due to foetal toxicity rather than to impact on fertility.

4.2.7.2.2 Animal data

Adverse effects on reproductive tissues are common findings in the course of subacute and subchronic toxicity studies. Testicular toxicity is much more frequent than ovarian toxicity, the latter being exhibited, for example, by vinylcyclohexene (and other mutagens). Concerning the mechanism(s) of testicular toxicity, such effects are sometimes detected only at dose levels causing general toxicity including malnutrition and/or liver toxicity. The occurrence of a dose range, where detrimental effects are also obtained in the absence of other toxicity, is a more specific case. This has consequences also for the EU categorisation (Category 2 vs. 3).

A number of chemicals were shown to exert adverse effects towards testicular/spermatogenic tissues after just a single administration. This observation is much more common after oral (gavage) administration than after inhalation. Not all chemicals classified into Category 2 or 3 (EU) for testicular toxicity and/or fertility impairment after oral administration present a fertility risk for the inhalation route especially not after just a single exposure. An assessment on the basis of individual toxicity data and not classification is proposed.

4.2.7.2.3 Grading

NOAELs and LOELs from subchronic studies should be used as the basis for establishing AETL-2 (unless NOAELs after single administration are available with post-observation times over seven weeks including at least one spermatogenic cycle). Data indicating that inhalation exposure would not lead to critical internal doses (in contrast to oral studies) should be considered.

Small decreases in sperm count are to be treated differently from full sterilisation. Reduced fertility by oligospermy is reversible in most cases, whereas severe grades of sterility may be irreversible. Both responses, however, may be observed with the same chemical at different dose levels. A more refined discussion of grading and assessment factor is recommended. Both the reversible and irreversible effects are related to the AETL-2 level, but should be treated differently in terms of extrapolation / assessment factor.

Table 4-16: Reprotoxicity / developmental endpoints: grading of effects

| Type of effect | Irreversible damage | Reversible effects |
|---|---------------------|--------------------|
| Slight and moderate testicular atrophy | - | + |
| Severe testicular atrophy | (+) | + |
| Ovarian toxicity | (+) | + |
| Decrease in fertility | - | + |
| Complete infertility | (+) | + |
| Hormonal effects in adults | - | + |
| Perinatal hormonal imprinting with sexual organs (related to developmental toxicity or brain regions persistently affected) | + | (+) |

4.2.7.2.4 Conclusion

Testicular atrophy is a frequent finding in experimental animals and sometimes observable also after just a single administration. Ovarian toxicity is comparatively rare. Adverse effects on fecundity may occasionally occur without morphological effects in the reproductive organs. However, in most cases, there is at least a coincidence with such morphological effects and therefore these effects can be visible in sub-chronic toxicity studies.

Irreversible sterility after a single inhalation is regarded as a very rare event. Slight or moderate effects are, presumably, reversible. As a conservative approach, NOAELs and LOELs from sub-chronic studies may be used as starting points for establishing the AETL-2 levels.

4.2.8 Carcinogenic / Mutagenic effects

4.2.8.1 Carcinogenicity

Chemicals are classified and labelled for carcinogenicity if they show evidence for such effects either in humans (Category 1) or in animals (Category 2). The effective doses and the route of exposure are not relevant for classification since this is based on hazard and not risk. In specific cases, a classification for carcinogenicity into Category 2 may also be adopted in the absence of valid carcinogenicity data if the evidence is otherwise convincing by chemical analogy or screening experiments.

Category 3 is the option either for suspected carcinogens or for established carcinogens of non-genotoxic, threshold-related mechanism, mostly confined to high dose levels.

Certain compounds are carcinogenic only upon inhalation (e.g. nickel or cadmium compounds) and not via oral administration; they are labelled with R49.

Some compounds have been shown to exert carcinogenic effects only upon oral (sometimes only bolus) administration and not by inhalation. These are mostly carcinogens of Category 3 requiring high dose levels (e.g. 1,4-dioxane).

Most carcinogens of Category 1 and 2 are genotoxic and cause somatic mutations. But others are only weakly or not mutagenic at all. Long half-life times and cumulative properties are important factors (e.g. for asbestos). On the other hand, some genotoxic compounds exist with no or only weak carcinogenic potential.

Animal studies for carcinogenicity are usually performed, according to current testing guidelines, by exposure in rodents over the major part of their lifetime from the age of six weeks to two years. The most common exposure routes are dietary, drinking water or by inhalation. The potency of a carcinogen is usually expressed by the number/incidence of treatment-related tumours against the natural background frequencies. The time of their manifestation (latency period) after the beginning of treatment is also a significant determinant.

In terms of their mechanism of action, two major classes of carcinogens have been identified:

- Non-genotoxic carcinogens requiring (cyto)toxic doses or acting through other mechanisms of carcinogenicity such as hormonal, receptor-mediated or a combination of several factors.
- Genotoxic carcinogens which in most cases follow a more or less linear dose-response relation and for which, in most cases, it is not possible to identify a non-effective dose.

Carcinogenic effects after chemical exposure are often perceived as late sequelae and (in most cases) require chronic or at least repeated administration. The conventional risk assessment process is, therefore, mostly based on a modelling/calculation of the lifetime low-dose exposure, which is addressed e.g. for work place or food exposure or other forms of environmental contamination and exposure. There are, however, some identified chemicals with a propensity to cause tumours even after a single ('peak') administration with a certain latency period. This phenomenon needs to be addressed for the assessment of unforeseeable risks e.g. after accidents. Tumours after peak exposure have been shown experimentally only for a limited group of chemicals, predominantly after administration to immature animals. But there is a possibility that this may also be valid for larger numbers of chemicals (Calabrese and Blain, 1999; see section below on 'Animal data'). There are also some spurious human case reports indicating that single high exposure to certain chemicals may result in tumours (see section below on 'Human experience').

Druckrey (1964), Druckrey *et al* (1964), Druckrey 1967), who worked with genotoxic compounds (PAHs), have shown that for each genotoxic carcinogen there is a constant relation between daily dose and the tumour induction time t (or latency period). More recently, this principle has been confirmed with nitrosamines (Gray *et al*, 1991; Peto *et al*, 1991a and b; Swenberg, 1991). This means that a genotoxic dose may be too low to induce tumours because the latency time is longer than life expectancy (Guess and Hoel, 1977). However, considering the stochastic nature of mutagenicity and tumour development in somatic cells a mathematical calculation of the lifetime risk today is often carried out if valid studies with defined exposure levels and tumour data are available (e.g. linear/multistage extrapolation models; TD₂₅ approach). Thus an estimation of a one-day exposure risk may be

based on a two-year study by simply dividing the daily dose by 720. Mechanistic and kinetic data may refine this assessment (e.g. dosimetry of responsible metabolite(s), requirement of a necessary enzyme induction.).

The Health Council of the Netherlands (Verhagen *et al*, 1994) has proposed a Dose-Rate Correction Factor (DRCF) with the definition of “a factor by which the tumour incidence caused by a specific dose of a chemical carcinogen at low rates is multiplied to derive the tumour incidence at high dose rates”. The key question to be treated has been formulated in this way: “What is the estimated cancer risk of peak exposure to a genotoxic carcinogen relative to the cancer risk of the same total dose distributed over an entire lifetime?”

The Standing Operating Procedures (SOPs) for developing AEGL values in the US for hazardous substances (NRC, 2001) aim to assess carcinogenic effects after short-term exposure and to calculate the one-day dose for cancer risk levels of 10^{-4} - 10^{-6} (Howe *et al*, 1986; Schumacher-Wolz, 2003). These values may serve as a starting point to compute concentration risks of even shorter exposure times (e.g. 30 minutes to 8 hours). An adjustment factor for the stage of carcinogenesis (cell kinetic multistage model) has also been suggested (Kodell *et al*, 1987). However, the NAC/AEGL Committee has, as yet, not based its AEGL values on potential carcinogenicity. Uncertainty of the assumptions in extrapolating from lifetime to acute exposure and shortcomings of data on single exposure carcinogenicity has been pointed out. Furthermore, the estimated low excess risks at levels of 10^{-4} - 10^{-6} versus relatively small populations potentially exposed in chemical accidents (e.g. 1,000-5,000 persons) were outweighed against risks associated with evacuation. Carcinogenicity data, however, are included in AEGL documents and evaluated in order to provide this information to the public.

4.2.8.1.1 Human experience

There is a long history of cancers from occupational exposure. In most cases, such cancers were associated with long and repeated exposure. However, a limited number of reports indicate that certain materials have the propensity to induce cancer after a single exposure. These were mostly strongly genotoxic compounds with an additional cytotoxic component or materials with long biological half-life times and persistency in the tissue. The latter scenario, due to the long and persistent internal dose, is practically equivalent to a repeated dose (examples are asbestos and TCDD).

A spill of 1,3-dichloropropene was associated with the occurrence non-Hodgkin lymphoma in two out of nine firemen 6-7 years after exposure and another lymphoma occurred a few months after a 30-day exposure (Markovitz and Crosby, 1984).

A considerable amount of literature shows genotoxic lesions in peripheral lymphocytes after a single or few exposure(s) to genotoxic carcinogens. Such effects may also indicate that other target tissues may be affected by genotoxic lesions. Therefore, at least theoretically, a certain possibility, even if low, remains that such cells are transformed or advanced towards a malignant or pre-malignant state. The predictive value of such genotoxic events is, as yet, not sufficiently elaborated (methodological difficulties: reversibility or gradual ‘dilution’ of effects by cell-replication or cell replacements?).

Nevertheless, as a result of exposure to genotoxic compounds typical alterations in (cyto)genetic biomarkers e.g. in peripheral blood lymphocytes may occur. The most common genetic endpoints investigated are chromosomal aberrations. Other endpoints, which may be used, include: sister chromatic exchanges, micronuclei, mutants of hypoxanthine guanine phosphoribosyltransferase or DNA adduct levels (Albertini *et al*, 1982; Becker *et al*, 2001;

Bonassi *et al*, 2000; van Delft *et al*, 1998; Hagmar *et al*, 1998a, 1998b, 2004; Hou *et al*, 1999).

From these studies it has been found that in cohorts with a high frequency of chromosomal aberrations an (approximately twofold) elevated standard incidence ratio (SIR) for all cancer could be observed. No such cancer association was shown for endpoints other than chromosomal aberrations (sister chromatic exchanges, micronuclei) nor for chromosomal aberrations with medium or low frequencies (Hagmar *et al*, 1998a, 1998b, 2004; Bonassi *et al*, 2000). This, however, is only indicative evidence and for a more sophisticated risk estimation, the sensitivity of the different endpoints for each chemical needs to be taken into account (van Delft *et al*, 1998). Smoking and alcohol misuse may be regarded as potential confounding factors, which may contribute to alterations in these biomarkers but also to cancer incidence in their own right.

Since peripheral lymphocytes are rather long lasting and non-cycling cells, it is difficult to discern between a recent single event and repeated exposure. On the other hand, a number of strong human carcinogens are known, like bis-(chloromethyl)ether (BCME) or certain nickel dusts, which cause tumours in the respiratory organs and have low systemic bioavailability and no significant effect on bone marrow or peripheral lymphocytes. For that reason, positive or negative cytogenetic effects after a single expose are difficult to correlate with cancer risk. Following a single exposure with minor cytogenetic effects, the chance of a cancer developing at a later time, in fact, appears low.

DNA adducts analysis and also Ames tests with urine samples are more sensitive measures at least for certain classes of compounds with systemic availability. There are reports on increased DNA adducts levels and Ames-positive urine samples after passive smoking in restaurants.

In general, it does not appear possible to draw firm conclusions on eventual cancer risks from a single sample investigated for genetic endpoints.

Carcinogens in Annex I of 67/548/EC, which may be released into the atmosphere

Several Category 1 and 2 carcinogens are more or less continuously released into the atmosphere and may potentially contribute to an environmental background risk for tumorigenic effects.

Among those are:

- Benzene (cat. 1);
- Tobacco smoke (cat. 1);
- Asbestos (cat. 1)
- Certain gases and vapours from petroleum and tar distillation (cat. 1);
- Benzo- α -pyrene (cat. 2);
- Benz-[a]-anthracene (cat. 2);
- Cadmium compounds (cat. 2);
- Hydrazine (cat. 2).

The natural radioactive gas radon is recognised as the most prominent cause for lung cancer among non-smokers. Furthermore, ethylene oxide (cat. 2) is continuously produced in the metabolic pathways of certain organisms, and related to a consistent level of certain DNA adducts with potential toxicological significance. Other materials like nitrosamines are present in food. However, considerable and successful actions have been taken to minimise these.

The theoretical carcinogenic risks of these exposures have been subject to mathematical calculations (linear low dose risk extrapolation models). As these risks are higher than zero, they are not to be ignored. Nevertheless, they are not perceived as relevant to AETLs.

4.2.8.1.2 Animal data

Information from valid lifetime carcinogenicity studies derives mainly from rodent long-term bioassays. In the main, these use oral administration (via feeding, drinking water or gavage) and not the inhalation route.

Only a few chemicals have been investigated via both routes (oral and inhalation). Sometimes inhalation proved to be less effective or even without tumorigenic effects observable in the frame of a two-year bioassay (e.g. 1,2-dichloroethane). Route-to-route extrapolation is critical when a chemical has been tested by the oral route only.

Carcinogenic effects are often only observed at dose levels at, or close to, those concentrations, which cause systemic toxicity. In such cases, where the chemical is not a mutagen (cytotoxic carcinogen), the NOAEL for cytotoxicity should also protect against carcinogenic effects even many years after the event (e.g. 1,4-dioxane).

Another major difficulty arises from the need to extrapolate results of two-year bioassays, which normally start with weanling rodents. This is necessary to cover the earlier life stages, which include organogenesis and may be more vulnerable to the impact of chemicals. The more rapidly dividing tissue cells may facilitate the mutagenic fixation of DNA alterations. Furthermore, young organisms have a longer lifespan ahead, which may allow the manifestation of a tumour after the dose related latency time of an exposure has been exceeded. Limited data are available from studies on vinylchloride and other chemicals investigated after transplacental or perinatal exposure (Maltoni *et al*, 1981, 1984; Anderson *et al*, 2000; Peto, 1984; Vesselinovitch *et al*, 1979; McConnell and Bhoola, 1973; Miller *et al*, 2000b; Ginsberg, 2003) indicate a higher tumour incidence and shorter latency time after early life exposure. Weanling these animals show a greater susceptibility to formation of DNA adducts (Morinello *et al*, 2002a and b; Laib *et al*, 1989). Foetal tissues appear to show a higher sensitivity in transplacental micronucleus assays (Hayashi *et al*, 2000). A neonatal mouse model employing two dosings prior to weaning with a post-observation period of one year showed carcinogenic responses only for genotoxic carcinogens and not for non-genotoxic mechanisms (Flammang *et al*, 1997; McClain *et al*, 2001). An extensive review of this topic and the available database has recently been presented by US EPA (2003a). For exposure up to the age of two years, the US EPA proposes a 10-fold adjustment factor. However, one should not generally exclude the possibility that aged organisms may be more vulnerable since DNA damage has already accumulated during life and/or may be less efficiently repaired; this may possibly shorten the latency period.

Animals provide some evidence of carcinogenic effects induced even after a single administration. Calabrese and Blain (1999) have reviewed studies on carcinogenic effects after single administration and via different routes, on different species and age groups. The agents had been administered by the oral route in 12% of all experiments, typically via gavage (bolus) and in 4% via dermal exposure. Most experiments were made via parenteral injection; inhalation route was used only in 0.5% of all experiments. Tumorigenic doses used were generally not acutely life threatening. Among 818 carcinogens from different chemical classes (including PAHs, inorganics, nitrosamines and mineral fibres) carcinogenic effects were reported on 426. Many of the test materials were solid and had little water solubility (e.g. metals or metal compounds). These formed depots close to the injection site, which provided

continuous and long lasting bioavailabilities. This, of course, is different from a chemical with short and transient bioavailability and resembles more a (sub)-chronic exposure.

The conclusion of the authors is that single-exposure related carcinogenicity might, in fact, be quite common and not limited to specific animal models with increased susceptibility. Humans would probably respond to a comparable extent, though information on single-exposure related carcinogenesis in humans is very limited.

Limited data from inhalation exposure was obtained for BCME, which showed in rats after 10 exposures to 0.1 ppm and lifetime post observation 1/41 nasal tumours and after 20 exposures 3/46 (Kuschner *et al*, 1975). The inhalation LC₅₀ of this material in rats and hamsters was 7 ppm according to a further study. Three years after a single exposure to 1 ppm, a malignant nasal tumour in hamsters was recorded (number of surviving animals unclear). After a single exposure to 2.1 ppm, a high incidence of hyperplasia and hyperplastic atypia in hamsters and squamous metaplasia in rats and a strong reduction in mean life span of both species was observed. The example of BCME shows for this highly carcinogenic compound that a single exposure in the AETL-3 range may be associated with a non-negligible risk of carcinogenicity. Whether this also holds true for a hypothetical AETL-2 range is unclear from the data available at present.

Asbestos has induced lung carcinoma and mesotheliomas in Wistar rats exposed seven hours per day to 12 mg/m³ respirable dust for 3-24 months. Such neoplasms have also been reported from rats exposed for only one day to this concentration (HSDB, 2005).

Nickel subsulphide (Ni₃S₂) is an example of a metal compound with pronounced carcinogenicity in humans after occupational exposure (nasal and lung carcinomas after dust inhalation) and local carcinogenic effects at the injection site in female Wistar rats. A single intraperitoneal (ip) injection of 6 mg nickel subsulphide resulted in local mesothelioma (incidence = 4 out of 36 rats) or sarcoma (16/36) vs. none in treated controls (Pott *et al*, 1991). No single-exposure data are available for the inhalation of nickel subsulphide dusts; a dose dependent increase in lung tumours, however, occurred in female Wistar rats after 15 tracheal instillations of 0.063-0.25 mg/rat (n = 40-47; incidence 15, 29, 30%; Pott *et al*, 1987).

4.2.8.1.3 Grading

For non-genotoxic carcinogens (today, probably the majority of chemicals tested positive in long-term bioassays) a threshold for carcinogenic activity may be established, either from the bioassay data or mechanistic studies. Due to the non-stochastic nature of the process, mathematical extrapolations into the low dose range would not be appropriate. Potential carcinogenic effects after single exposure need not be considered unless a material is persistent in the organism.

For genotoxic carcinogens, due to the stochastic nature of genotoxic impact, tumour data from long-term animal studies or epidemiologic studies in humans may be subjected to mathematical extrapolation models. These are conceived as a default strategy for a conservative risk assessment even though the scientific basis of this is not very firm. Within the validity of such a model, a single day exposure may be regarded as a proportion to the total lifetime dose administered in a long study. When a virtually safe dose over a life time (VSD; µg/kg bw/day) can be calculated from the study results, it may be extrapolated to a 1-day exposure time by multiplying the VSD by the number of days, either 720 for the rat or 25600 for humans (Verhagen *et al*, 1994; Bos *et al*, 2004). Both factors may be debatable, but the rat number is more conservative. On the other hand, in relation to basic metabolism, i.e. cell turnover and total lifespan, a rat-day is a longer exposure period than a man-day, and

25600 may therefore be more appropriate. These calculations may be refined for aspects of metabolism and kinetics, overload and MTD phenomena and whether an effect is more related to the systemic dose (AUC) or more to a peak concentration (e.g. important for strongly irritant carcinogens). A dose-rate correction factor, (DRCF) as suggested by the Dutch Health Council (Verhagen *et al*, 1994), which is compound-specific, is another valuable tool and may either vary from one to eight, or can even be lower than one (Hattis, 1990).

A first step may be to differentiate chemical carcinogens into the following four categories of increasing hazard after single exposure to an AETL-2 or -3 dose.

1. Non-genotoxic carcinogens with short half-life times (e.g. chloroform).
2. Weakly genotoxic carcinogens and substances for which genotoxicity is not much related to carcinogenicity (e.g. Category 3 of the EU classification) with short half-life times (e.g. aniline, methylchloride, formaldehyde).
3. Highly genotoxic or notoriously potent carcinogens with short half-life times (vinylchloride, aziridine).
4. Established or suspected carcinogens with long half-life times (depot effect; 'pseudo-single-exposure') (e.g. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), nickel tetracarbonyl).

Group (1) and (2) chemicals may be dismissed from concerns about a tumorigenic risk after a single transient exposure. For these chemicals, AETL-2 values (and even AETL-3) may be based on conventional acute and subacute toxicity data and are considered as protective against carcinogenic effects.

Group (3) chemicals need case-by-case deliberations that may be supported by mathematical risk calculations starting from long-term bioassay data and projecting the risk that has been linked to a daily lifetime dose from a single inhalation exposure. Dosimetric data and, if available, data on carcinogenicity after a single exposure, should be taken into account (Verhagen *et al*, 1994). A considerable number of these materials may turn out to be very low in risk after a one-day inhalation (e.g. INERIS, 2002), others not.

For chemicals of group (4) the risk after single exposure may be considerable and possibly underestimated from long-term bioassay data alone. Nevertheless, the dose received from a single exposure may be compared with the total dose received in a lifetime study (if available) and with data on the bioavailability of the chemical in the body after a single exposure.

For genotoxic carcinogens mathematical extrapolations of risks from chronic exposure to the single exposure situation could be performed. To perform such an assessment, a decision must be made as to the level of incremental risk (e.g. 10^{-6} , 10^{-5} , 10^{-4}). In case of accidents, one could consider using a higher incremental risk trigger (e.g. 10^{-4} or 10^{-5}) than normally used in chronic exposure situations (10^{-6}). The enhanced susceptibility of early life phases may be allowed for by using an application factor of 10.

4.2.8.1.4 Conclusion

Scientific knowledge on carcinogenic effects and risks after short transient exposure is still in an early stage. However, the endpoint carcinogenicity should be addressed in all AETL determinations.

Cancer, as a potential life threatening disease, may be related to AETL-3 by definition. If, however, AETL-3 is determined by acute lethal effects, it needs to be made clear whether AETL-2 is linked to any potential cancer-risk later in life, and whether or not an AETL level

protects also against a carcinogenic event with a adequate reliability. For carcinogens in animal experiments the risk after a short transient exposure is low or practically absent (see section 4.2.8.1.3). If this is not the case, the foreseeable likelihood of a dose causing cancer later in life should be the starting point for AETL-3. This, however, only applies to a small proportion of the group of chemicals that have been tested positive for carcinogenicity.

4.2.8.2 Mutagenic effects (comments in addition to 4.2.8.1 on carcinogenicity)

Experimental evidence has shown that some chemicals may cause germ cell damage after only one or a few administrations (as usually done in the dominant lethal assay). In humans, manifestation of germ cell mutation via chemical exposure (including medical treatment) has, so far, not been found. This is the case, even though a considerable number of people have undergone tumour chemotherapy with mutagenic chemicals at a young age. The available observation is that their children appear to be healthy and have no higher rates of mutation or birth defects. The reason for this lack of genotoxic manifestation may be that gross genotoxic damage is frequently not survived by haploid germ cells and therefore is not transmitted into the following generation. Whether this is also true for recessive mutations is doubtful, since they mostly go undetected as long as their incidence in the population is low.

Human genetic risks from exposure to chemicals have been evaluated for a number of compounds such as ethylene oxide, cyclophosphamide, acrylamide and 1,3-butadiene (Waters and Nolan, 1995). However, the database appears to be very limited concerning the specific question of genetic risks associated with only single administration. Presently, it is not technically possible to set AETLs on the basis of mutagenicity. The available standard mutagenicity test rarely gives any information on dose response relationships. Furthermore, the standard regulatory approach is to treat mutagenicity as a non-threshold endpoint.

The following compounds have been classified for mutagenicity (Category 2), which means that there is convincing evidence for germ cell mutagenicity (transmittable) or genotoxicity.

- Aziridine;
- Acrylamide;
- Benzene;
- Benzo- α -pyrene;
- 2,2'-Bioxirane (Di-epoxybutane);
- 1-n-Butoxy-2,3-epoxypropane;
- Dimethylsulfate;
- Diethylsulfate;
- Chromates;
- Soluble cadmium compounds;
- Ethylene oxide;
- Propylene oxide;
- Nitrotoluene;
- Olanquinox;
- 4,4'-Oxidianiline;
- Hexamethylphosphoramide;
- Trimethylphosphate;
- 1,3,5-Tris(oxiranylmethyl)-1,3,5-triazine-2,4,6(1H,3H,5H)trione;

- Carbendazime;
- Benomyle.

So far, no chemical has been classified as Category 1. Many compounds are classified as Category 3 on the basis of a positive effect in somatic cells. Some of the materials listed as Category 2 mutagens are rather weak mutagens for germ cells in the sense that there is only a small or even remote individual risk of a short, transient exposure resulting in a transmittable mutation. Nevertheless, these chemicals need to be firmly controlled in order to protect the gene pool of humans and wildlife.

4.3 Data collection, evaluation, selection and documentation

4.3.1 Data sources

4.3.1.1 Search strategy

The literature search and the review of published and unpublished toxicological or general data and the official/existing acute exposure thresholds should include:

- Primary sources obtained through searches in e.g. MEDLINE, TOXLINE, IUCLID;
- Monographs from WHO and IPCS; ECETOC JACC reports; AEGL TSDs;
- Published books or documents from public and private sectors;
- Unpublished data from private industry or organisations. With respect to confidential data, permission must be granted from the appropriate individual or body before such data can be included. If only non-confidential parts of a study are available, the Klimisch score would be 3, even if the study appears to be well conducted (Klimisch *et al*, 1997). In that case, the confidential data should not be used. However, when robust summaries are available and reviewed by competent authorities, the data can be used if it received an appropriate Klimisch score.

The CAS registry number of the chemical is used in a first step to initiate the search. For specific searches of primary sources (e.g. TOXLINE, MEDLINE), search terms could be used such as the name of the chemical, acute toxicity, inhalation or respiratory tract related terms, short-term, threshold limit.

Information on target organs is normally not available from acute studies. Therefore, search parameters should be extended in order to obtain more general toxicological information, such as repeated exposure studies, which allow the identification of target organs and their toxicological endpoints.

4.3.1.2 General electronic databases

The following international databases should be searched:

- PUBMED including MEDLINE and PREMEDLINE (produced by National Library of Medicine – NLM);
- TOXLINE database from the US NLM;
- Hazardous Substances Data Bank (HSDB) from TOXNET;
- Registry of Toxic Effects of Chemical Substances (RTECS) compiled by NIOSH (US National Institute for Occupational Safety and Health);
- Integrated Risk Information System (IRIS) from US EPA;

- National Technical Information Service (NTIS) providing access to the results of US government-sponsored research.

Other national general databases could be used as sources (for example, INRS (Institut National de Recherche et de Sécurité) documents in France).

4.3.1.3 Published books or documents

General references for regulatory or toxicology information may be obtained from:

- ACGIH (American Conference of Government and Industrial Hygienists) producing TLV (Thresholds Limit Values) and biological exposure indices;
- AIHA (American Industrial Hygiene Association) producing Emergency Response Planning Guidelines (ERPGs);
- AEGL (Acute Exposure Guideline Levels) values from US National Research Council;
- ATSDR (US Agency for Toxic Substances and Disease registry) given toxicological profiles for chemicals;
- IARC (International Agency for Research on cancer) for the evaluation of the carcinogenic risk of chemicals;
- NIOSH and the documentation of Immediately Dangerous to Life and Health (IDLH);
- NTP (US National Toxicological Program) providing scientific reports on toxicological research and testing;
- Patty's Industrial Hygiene and Toxicology;
- SEI/SEL (Seuils des Effets Létaux/Seuils des Effets Irréversibles) values of the French Government;
- IPSC Environmental Health Criteria (EHC) monographs;
- IPSC Concise International Chemical Assessment Documents (CICADs);
- ECETOC JACC reports;
- There are a several series of European documents supporting occupational exposure limits, from the MAK Commission (Germany), DECOS (The Netherlands), Nordic Council, and HSE (UK), which all can provide useful information (and sometimes superior to ACGIH) on the available toxicity data;
- IUCLID (Inventory of toxicological information of high production volume chemicals in the EU).

In the Technical Support Document (TSD), all the data sources should be mentioned, e.g. the Internet sources consulted and all the literature sources available and used for the TSD.

4.3.2 Evaluation and selection of 'key study', critical effect and supporting data

4.3.2.1 Quality and pertinence of the consulted data

The scientific data are critically reviewed and the reliability of each study is scored by using the method of Klimisch *et al* (1997). This makes it possible to retain the best technically performed studies and those most reliable scientifically. Only toxicity data and information, which are reliable for example obtained directly from a primary reference source, are used for the selection of 'key' and 'supporting' toxicological studies. Secondary sources may be used for non-toxicological data, such as physical and chemical properties and general information on the toxicity of a chemical not directly used in the derivation of AETL values. Secondary

sources on toxicological data may also be used for the derivation of AETLs if they come from documents reviewed by authoritative bodies (e.g. AEGL committee).

The selection of studies takes into account the reliability based on the approach of Klimisch *et al* (1997) (standardised methods, GLP, detailed description of the publication), the relevance, and the adequacy of the data for the purposes of evaluating the given hazard from acute exposure.

The following definitions are generally used:

- Reliability - The inherent quality of a test protocol and report or publication relating to standardised methodology and the way that the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings.
- Relevance - The extent to which data and/or tests are appropriate for a particular hazard identification or risk characterisation. Criteria used to evaluate relevance of the studies in the context of AETLs are described in the next paragraph.
- Adequacy - The usefulness of data for assessment purposes. When there is more than one set of data for each effect, the greatest weight is attached to the most reliable and relevant.

The evaluation of reliability is performed considering certain formal criteria using international standards as references. This classification should not exclude all unreliable data from further consideration by experts where these data are particularly pertinent to the evaluated endpoints. In general, data with lower reliability may be used as supporting data.

The reliability of each study is evaluated using the criteria for reliability categories adapted from Klimisch *et al* (1997) and Rosner (1994). Reliability is classified into 4 categories/codes as described below. In this scoring system, studies conducted and reported according to internationally accepted test guidelines and in compliance with GLP have the highest grade of reliability and should be used as reference standards.

The following scoring system should be used:

- Reliability score 1: Reliable without restriction
 - 1a Guideline study (OECD, EC, EPA, FDA, etc.) conducted in compliance with GLP;
 - 1b Comparable to guideline study;
 - 1c Test procedure in accordance with national standard methodologies (AFNOR, DIN, etc.);
 - 1d Test procedure in accordance with generally accepted scientific standards and described in sufficient detail.
- Reliability score 2: Reliable with limitations
 - 2a Guideline study without detailed documentation;
 - 2b Guideline study with limitations which do not impair the overall conclusion from the data;
 - 2c Comparable to guideline study with limitations which do not impair the overall conclusion from the data;
 - 2d Test procedure in accordance with national standard methodologies with limitations which do not impair the overall conclusion from the data;
 - 2e Study well documented, meets generally accepted scientific principles, acceptable for assessment;

- 2f Acceptable calculation method (for physico-chemical information only);
- 2g Data from handbook or collection of data (for physico-chemical information only);
- 2h Data lacking for full assessment but considered as acceptable by expert judgement.
- Reliability score 3: Not reliable
 - 3a Documentation insufficient for assessment;
 - 3b Significant methodological deficiencies;
 - 3c Unsuitable test system.
- Reliability score 4: Not assignable
 - 4a Abstract;
 - 4b Secondary literature;
 - 4c Original reference not available;
 - 4d Original reference not translated (e.g. Russian);
 - 4e Documentation insufficient for assessment.

Additional guidance is provided on the reliability scoring of human studies because there are no standardised guidelines for such studies (except for odour threshold determination) and these are not usually conducted according to GLP.

- Reliability score 1c or 1d is assigned to well-designed and comprehensively reported studies. The report should include detailed information on exposure levels (concentration and duration), subject selection and eligibility criteria, health investigations conducted and the methodology, the results and statistical analyses conducted. The study should be designed and conducted in a way that minimises the influences of bias and confounding factors. Exposure levels should be measured using robust air sampling and analytical techniques. Health investigations should use established methods (for example, spirometry should be conducted according to American Thoracic Society, or equivalent, recommendations). Information on subjective symptoms should be collected using a validated questionnaire.
- Reliability score 2e can be assigned when the study appears to have been well designed but there are reporting limitations such that the robustness of all aspects of the study cannot be fully assessed.
- Reliability score 3a, 3b or 3c applies when there are significant study design and/or reporting weaknesses. For example, the quality of the study cannot be adequately assessed due to poor reporting; the exposure conditions have not been clearly defined or exposure concentrations have not been verified by analysis; health endpoints have been assessed using non-standard or unreliable methods; the extent of bias and confounding factors cannot be assessed.
- Reliability score 4 applies to reports from secondary literature or short abstracts, which do not give sufficient experimental details.

A reliability score is not assigned to case reports.

The best studies are those that give a precise description of the nature of the toxic effect, the number of subjects or the percentage of animals affected by the observed effects and the exposure conditions (atmosphere generation, duration and concentration). The pertinence of the data should be determined in describing the endpoint being measured or estimated.

Because of different toxic endpoints for each AETL level and the use of different data or studies for each tier, the adequacy should be addressed separately for AETL-1, -2 and -3 values. The selection of the 'key study' and the background of the supporting studies should

be scientifically justified and discussed in the TSD. For each study, the reliability score should be given in brackets and explained or justified if necessary. The discussion should consider and specify consistency of data relating to the classification of Klimisch *et al* (1997) and relevance to the particular AETL level. As evaluation of the reliability (Klimisch scoring criteria) could lead to poor scoring (e.g. 3 or 4) but data could contain a high level of relevance for the particular AETL level, expert judgement should be used to justify the choice of the ‘key study’ and supporting studies.

In any case, reliability score 3 studies (‘not reliable’) should never be used as ‘key studies’, only as supportive ones.

Note that sensory irritation reports from unsubstantiated secondary sources should not be used, as the reliability of such information cannot be assessed. Review articles and evaluations by competent authorities may be used as supporting data if assigned an appropriate reliability score.

4.3.2.2 Selection of ‘key study’ and supporting data

The ACUTEX project group has developed guidelines for the selection and evaluation of ‘key studies’ for the derivation of AETL values. The proposed guidelines are intended to provide an exhaustive list of elements to help evaluate studies and a methodology for selecting ‘key studies’ for the derivation of AETL values.

During the process of deriving AETL values, the evaluation and qualification of each data set is necessary in order to prove its scientific validity, using logical scientific thinking and competent professional judgement. If a study uses scientifically valid methods, contains adequate and reliable data, and presents defensible conclusions, it may be used in the process of derivation of AETL values, even as ‘key study’.

The guidelines for study selection and evaluation should be based on scientific methods but not be so restrictive that they preclude competent professional judgement. Current OECD guidelines provide a basis for selection of a robust list of study elements that, together with professional experience, are used to qualify the data, which support the AETLs. Consequently, the EU TGD (EU, 2005) and the OECD Guidelines for the Testing of Chemicals (OECD, 2000) serve as a basis for study selection.

The EU TGD (EU, 2005) describes general considerations for the use of toxicological data in risk assessment. Regarding systemic effects, differences in kinetics can be observed from different routes of exposure. Therefore, a ‘key study’ used for the derivation of AETL values should have used the inhalation route of exposure. Toxicity data from studies using routes other than inhalation should not be included in the Technical Support Documents (TSDs) unless these data are important for the assessment of kinetics and dynamics. In the absence of inhalation data to derive an AETL value, toxicity data from the oral exposure route may be used if there is adequate information to perform a scientifically credible route-to-route extrapolation. In addition, data on possible carcinogenic, mutagenic or reprotoxic hazards will often be not available from inhalation data.

A ‘key study’ is that human or animal study which is used for the derivation of an AETL value. ‘Supporting studies’ are those, which support the toxicological findings and calculations, resulting from the ‘key study’. The selection of these two types of studies is performed using the list below (elements for the selection and evaluation of key and supporting data and studies) on a weight-of-evidence basis of scientific credibility. While all these elements are considered when evaluating a study, only those relevant for the derivation of the AETL values should be discussed in the TSD. In evaluating a study, a variety of

endpoints are preferred. However, a study measuring just one endpoint may be selected for development of an AETL if other studies have shown other endpoints to be less sensitive.

In some situations studies may exist which are confidential in nature or contain confidential data. In this case permission must be obtained from the relevant body to include these data in TSDs. If permission is not obtained, non-confidential data contained within such reports can be included in TSDs. However, due to limitations in assessing the quality of such data, they should receive an appropriate reliability score, and the data should be viewed in line with that score.

The derivation of AETL values is dependent upon existing clinical, epidemiological, and case report studies (including those from chemical accidents) published in the literature for data on humans. Many of these studies do not necessarily follow current guidelines on ethical standards that require effective, documented, informed consent from participating human subjects. If these older studies contain human data relevant for the derivation of AETL values, they should be used. The exception is where the data are known being obtained through force or coercion. Only human data, documents, and records should be used that are derived from publicly available sources. The information has to be recorded in such a manner that subjects cannot be identified either directly or indirectly.

Elements for the selection and evaluation of key and supporting data and studies

1. **Only toxicity data and information obtained directly from a primary reference source** may be used as the basis for ‘key’ toxicological studies. The exception being where a study is summarised as a robust summary, which has been peer-reviewed by a certified authority and is assigned an appropriate Klimisch score. All other studies important to the derivation of an AETL value or that serve as a weight-of-evidence rationale are obtained from a primary source. Secondary references may be used for non-toxicological data, such as physical and chemical properties, production locations, quantities, and background information on the toxicity of a chemical, provided the information is not directly used in the derivation of AETL values.
2. **Humans are the most relevant species studied.** Rats, mice, rabbits, guinea pigs, ferrets, dogs, or monkeys are acceptable. Other species require evaluation on a case-by-case basis. It is important to use a species for which there are control data and relevance to humans.

Although for irritants mice and guinea pigs are the most sensitive species, rat data are preferred to derive AETLs (for a further detailed discussion see section 4.5.2). Data from others species will be taken as supporting data. If they are used as the relevant species, the decision should be justified. For systemically acting substances, human data are preferred. If animal data have to be used, the species with a metabolic pathway closest to humans (or metabolism based on the same critical effect) should be chosen.

Guinea pig data will be used as reference for the evaluation of asthmatic effects if necessary to help the definition of a specific subpopulation.

3. **The number of subjects** is not rigid. As a general rule 5-10 rodents/sex/group is a valid number, but as few as 2-3 primates or dogs/sex/group may be acceptable. The acceptable number of subjects per group is influenced by the relationship between the within-group variability and the degree of change that is considered to be detrimental. Smaller numbers per group may be acceptable by increasing the number of treatment groups.
4. **The inhalation route of exposure is preferred.** The oral route can be used only “if there is adequate information to perform scientifically credible route-to-route

extrapolations". In addition, single exposure studies are preferred to repeated dose studies.

5. **Scientifically credible information is available:** on exposure concentration and exposure duration, and on the number of concentrations or doses used.
6. **Concentration or dose selection** establishes a clear dose-response relationship.
7. **Mode of exposure:** inhalation studies using nose only exposure are usually preferable to whole body exposure because there is a higher confidence in the capability of nose-only systems to deliver constant and measurable concentrations of the test substance to the breathing zone of the animal. However, whole body exposure may be preferable in the case of a substance for which there can be significant dermal uptake of the vapour/gaseous phase. When using the nose-only mode of exposure, past-flow and direct-flow exposure principles are preferred because the re-breathing of atmosphere is minimised. For whole-body exposures, to maximise the chances that all animals are exposed to the target concentration the exposure chamber should minimise crowding of the test animals and use a dynamic airflow design.
8. **Analytical procedures** are used to determine chamber concentration for inhalation exposure in controlled studies, and detailed, scientifically credible methods, procedures, and data are used to measure chemical concentration in epidemiological or anecdotal cases (accidental chemical releases). For oral exposure, dose may be determined from the amount of test chemical introduced into the subject.
9. **The observation period** is variable based on the time of onset of the toxic effect. If it is rapid (minutes to 2-3 hours) and associated with quick recovery, an observation period of 3-4 days may be sufficient. For effects that are slow in onset (2-3 days) and delayed in time, a minimum observation period of 14 days is recommended.
10. **Signs and symptoms** of toxicity are noted during and after exposure and reported separately by sex and concentration or dose.
11. **A concurrent control group is composed** of the same species as that in the treatment groups. For animal studies, the control subjects should be housed and cared for in the same manner as exposed animals, the body weights should be recorded throughout the study, the time of death should be recorded if applicable, and necropsy should be conducted with at least gross examination results noted.

To help the evaluation of the studies, points 1, 3, 5, 6, 7, 8, 10 and 11 should be considered as reliability issues and points 2, 4 and 9 as relevance issues.

4.4 Dose response modelling

4.4.1 Introduction

Planning of prevention and recovery measures in case of accidental chemical releases requires some assessment of the risk (i.e. the probability) of resulting effects. The characterisation and understanding of the key cellular and molecular changes that are responsible for adverse effects observed might improve the accuracy of health effects assessments. Because of the limitation of human data, adverse effects of toxicants are generally evaluated in laboratory animals at significantly different doses than those to which humans are usually exposed. This highlights the major drawback of the no-observed-adverse-effect-level method of setting 'safe' threshold. To overcome such issues, quantitative methods based on dose response modelling techniques such as the benchmark dose method have been developed. This method has the potential to determine reference concentrations that are scientifically more defensible

and often significantly different than the levels set to be safe by traditional approaches. The estimation of the benchmark dose involves fitting a dose-response model to the data.

To produce a dose-response model, a measure of toxicity must be defined. That is specifying what is meant by a response to an exposure (e.g. lethal effects (AETL-3) or potential lesions and their severity, reversibility (AETL-2 and -1)). Once a measure of toxicity is defined, the main goals of many dose-response models are to estimate the probability that an individual has an adverse response after an exposure to chemical substances and to set reference concentrations (AETL).

The concept is that for a given toxicant, an individual can handle with no adverse effect a certain dose, say μ . This dose is, *a priori*, unknown and subject specific. It has a variance σ and leads to a definition of toxicity that is the probability of the dose exceeding a certain threshold. More precisely:

$$P(\text{response}) = F\left(\frac{\text{dose} - \mu}{\sigma}\right) \quad (\text{Equation 1})$$

Where F is the cumulative distribution function (cdf) of the threshold dose in the population.

Equation 1 shows that to define a dose response model, one needs to specify F and more importantly perhaps, to define the dose. There are basically two groups of models: those with no link to the biology, which may be called empirical models, and those that incorporate biological data. In the next section, such models and also the benchmark dose approach are discussed.

4.4.2 Existing models

As mentioned above several dose response models exist. They are characterised by their cumulative distribution function F and by the definition of the dose used.

4.4.2.1 The Probit model: an example of classical dose-response model

Empirical models (no biological information) are generally based on the so-called Haber's Law or its generalisations (ten Berge *et al*, 1986) and use the following definition of the dose:

$$\text{dose} = a \log(C) + b \log(\tau) \quad (\text{Equation 2})$$

Where C is the mean exposure concentration in the environment, τ is the exposure duration, a and b are unknown real numbers to be estimated from the response data. Once the dose is defined, the next step is to specify the cumulative distribution function.

Usual models, for discrete endpoints are Probit, Log-Probit, Logistic, Log-Logistic, Weibull, Quantal Linear, Quantal Quadratic, Gamma, Multi-Hit and Multistage. The most popular model among these is the Log-Probit, which is obtained by substituting Φ , the cumulative distribution function of the normal distribution function for F in (Equation 1), that is:

$$P(\text{response}) = \Phi\left(\frac{a \log(C) + b \log(\tau) - \mu}{\sigma}\right) \quad (\text{Equation 3})$$

With Φ given by

$$\Phi(u) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^u \exp\left(-\frac{t^2}{2}\right) dt \quad (\text{Equation 4})$$

The only difference between the classical models results from the choice of the distribution of the threshold dose. Though, in most of the applications the Log-Probit link is used as a model for dose-response. However, there is no evidence that, in any situation, the Log-Probit model would perform better than the others would. Wherever, there is enough data, these different models would generally provide comparable estimates. However, there could be significant differences when extrapolating outside the range of experimental exposure (e.g. low dose extrapolation, inter-species transposition, etc.).

These models have been criticised for their limited abilities of transposing the conclusions from laboratory experiments to the expected conditions of exposures in the environment (see Diack and Bois, 2005, and references therein).

Biologically based-models accounting for the time- and dose-dependent changes in biological systems provide important capabilities for improving the reliability of extrapolations and transpositions across dose and species (Zeise *et al*, 2002). Such models have the potential to determine reference exposure concentrations that are scientifically more credible.

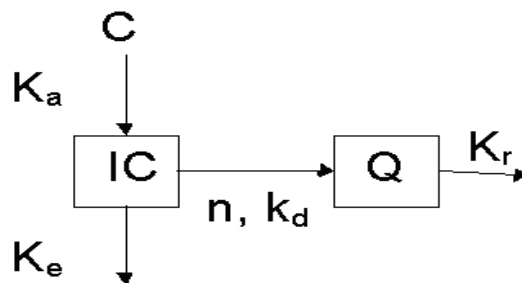
4.4.2.2 Empirical pharmacokinetic pharmacodynamic model (E.PK/PD)

Recall that

$$P(\text{response}) = F\left(\frac{\text{dose} - \mu}{\sigma}\right) \quad (\text{Equation 1})$$

The cumulative distribution function F could be any of the previously cited functions in the standard approach. In the sequel, F will be the cumulative distribution function of the normal distribution. The improvement proposed here is essentially based on the dose metric used. The biological effective dose that causally relates responses to an exposure can be expressed in a multitude of dose metrics including the tissue peak concentration (C_{\max}), the area under the curve of the concentration (AUC), the time spent above a certain level of the concentration, etc. (Diack and Bois, 2006). The ‘right’ measure of dose that best represents the effective dose probably depends on the mode of action of the chemical. In what follows, a measure of dose is used, which is termed the area under the effect-curve (AUEC). Its derivation is based on a compartment effect model (see Figure 4-5).

Figure 4-5: A compartment-effect model for dose response relationship



C is the concentration in the environment at a given time. Depending on how the chemical reacts in the body and which organ it affects, we define the parameter k_a to stand either for the pulmonary ventilation rate (in litres per minute), the blood flow or any relevant intake rate. k_e is the elimination rate expressed per min. IC represents the internal quantity of substance

reacting with the biological target (that we assume large enough to be considered constant with time) with the n^{th} order rate reaction to give internal damage dose, Q . Finally k_r is the repair rate and k_d represents the proportional factor between the internal quantity of substance and the internal damage dose. The parameter k_d is the damage rate. Then the following differential equations hold during exposure:

$$\frac{dIC}{dt} = k_a C - k_e IC - k_d IC^n \quad (\text{Equation 5})$$

$$\frac{dQ}{dt} = k_d IC^n - k_r Q \quad (\text{Equation 6})$$

Now, assume that the internal quantity of substance to internal dose reaction is rate limiting. That is, approximately, the following equality holds:

$$IC = \frac{k_a}{k_e} C \quad (\text{Equation 7})$$

From equations 5, 6 and 7, we get approximately:

$$\frac{dQ}{dt} = k C^n - k_r Q \quad (\text{Equation 8})$$

where the parameter k is given by $k = \left(\frac{k_a}{k_e}\right)^n k_d$.

The AUEC is defined here as the area under the curve of the internal damage dose Q defined by equation (Equation 8). Thus, from equation 1:

$$P(\text{response}) = F\left(\frac{\log(AUC) - \mu}{\sigma}\right) \quad (\text{Equation 9})$$

It is easy to show that classical dose-response models are included in the E.PK/PD approach (see Diack and Bois, 2005). Indeed, the model reduces to the standard log-probit if the exposure level is constant and the repair rate small. This compartment-effect model constitutes a bridge between standard empirical models and mechanistically based dose-response models, retaining the advantages of both. It fits a broad spectrum of data (as empirical dose-response models do) and makes links to the underlying biological mechanism that drives the response (the main feature of biological based dose-response models).

Note that to avoid identifying problems, k_a , k_e and k_d are not estimated. However, estimates for each of the following parameters n , k and k_r are derived.

4.4.2.3 Estimation of the parameters

There are several ways to estimate the parameters of the different models but for most of them, 'likelihood' plays a key role.

The log-likelihood is expressed as follows:

$$L = \ln(\text{likelihood}) = \sum \ln \binom{n}{r} + \sum r \ln P + \sum (n - r) \ln(1 - P)$$

(Equation 10)

$n = \text{number exposed}$ $r = \text{number responded}$
 $P = \text{estimated response according to model equation}$
summed for all separate exposures

The set of parameters that maximises L is adopted as estimates of the parameters as they are the most likely values for these unknown parameters.

There exist many methods for optimisation of the likelihood. Finney (1977) used the Taylor-McLaurin expansion (the first and second derivatives) of the likelihood function to estimate the parameters. This method is very similar to iterative non-linear regression analysis (ten Berge *et al*, 1986).

The ACUTEX software uses Bayesian statistics to estimate the parameters. The Bayesian approach requires, for any unknown quantity, a ‘prior’ probability distribution function P as input. This prior distribution termed ‘degrees of belief about the possible values of the parameter’ should reflect current knowledge as accurately as possible. When little knowledge is available on a particular parameter, a non-informative distribution (i.e. flat-shaped) can be used. If stronger prior knowledge is at hand, P should represent that information as accurately as possible. The set of prior distribution is updated using the observations (new evidence) to yield a posterior probability distribution. While the prior distribution may be specified independently from each other, the posterior is a joint distribution of all unknowns (i.e. correlations induced by the model fitting process are accounted for). It is proportional to the product of the priors by corresponding likelihood. The set of parameters with the highest product (= mode) are the parameters most likely values. The reliability of this method depends on the number of simulations.

So the maximum likelihood or, equivalently, the maximum posterior is the key to setting the parameters most close to the real values.

4.4.3 Model choice

Several models may fit the data and the problem is to find the ‘best’ one according to some criteria. This defines a decision rule. ‘Best’ depends on the purpose for which the model is to be used. If the main purpose of the modelling is to obtain a rule for predicting the future, as it seems to be the case for health risk assessment, then accuracy and robustness are the most important virtues of the model. There are many alternative concepts of the decision rule. Different decision rules serve some problems well and other problems badly. Therefore, there is no universal ‘best’ method. The method of maximum likelihood has proved to be successful in a great variety of situations and is playing a dominant role in the development of new tests and estimates. The maximum likelihood approach, as described in the book of Lehmann (1986) dedicated to testing statistical hypotheses, is designed to determine the correct value of a random parameter that is the value that produced the observations. This suggests considering for each possible value of the parameter how probable the observations would be if the parameter considered were the true value. The higher this probability, the more one is attracted to the explanation that the value in question produced the observations, and the more likely that value appears. Therefore, that probability as a function of the parameter is called the likelihood of the parameter. The same reasoning applies when one is concerned with an action problem involving a number of decisions. ACUTEX uses the

maximum likelihood method to compare different models. It has to be noted, however, as pointed out by Lehmann (1986), “the maximum likelihood principle is not based on any clearly defined optimum considerations... There exist examples for which the maximum likelihood procedure is worse than useless; where it is, in fact, so bad that one can do better without making any use of the observations.”

Another criterion that will be implemented in the ACUTEX software is the Akaike’s Information Criterion (AIC), which is a penalised log-likelihood measure. This enables it to balance the reduction of estimated error variance with the number of parameters being fit. ACUTEX software will also provide some other useful statistics, such as diagnostic plots, chi-square test, Student test, leave one-out-data.

Note that this package provides a set of statistical criteria that allow checking the goodness of fit of the model. These criteria will indicate whether the model used is internally consistent with data on hand. This, however, is not enough to say that the model is validated (Bois and Diack, 2005). Models can certainly be validated theoretically however, one must bear in mind that any model is wrong and it is a truism that for a sufficiently large sample size any model will ultimately be rejected. There is a difference between statistical significance and physical relevance!

The data required, the drawbacks and the advantages of each of the above two approaches to characterise the relationship between exposure and response are described below.

Log Probit models

- Advantages: These can fit a broad spectrum of data. They are easy to understand and use less parameter than the empirically driven PK/PD models.
- Drawbacks: Models are not biologically based. Follow up time is not considered. Responses depend on concentration (external) and exposure duration. Do not handle biological information. Methods on extrapolation to low doses or transposition from animals to humans are not robust. There are strong assumptions about the distribution of the threshold dose.
- Data requirements: Concentration, percentage of incidence in each category of severity, and exposure duration.

PK / PD models

The effective dose is given by the logarithm of the AUEC, the area under the effective-curve of the internal damage dose.

- Advantages: Models incorporate exposure duration and observation time. Can handle toxic endpoints categorised by grades. Incorporate time-varying concentrations. Some of the model parameters have a biological meaning. They bridge standard empirical models and one-compartment models. The body weight of the animals can easily be taken into account. They allow more robust methods for transposition from animals to humans. Models are for local and systemic effects.
- Drawbacks: Strong assumptions about the distribution of the threshold dose. Require more parameters, thus more complex than the log-probit models. The models do not handle active metabolites or eye irritation. They do not necessarily fit with concentration-related effects (Cmax). For example, extrapolation from experimental data over times greater than 1-2 hours may not be relevant for organic solvents. There may be some difficulties in estimating some of the parameters such as the repair rate, which does not

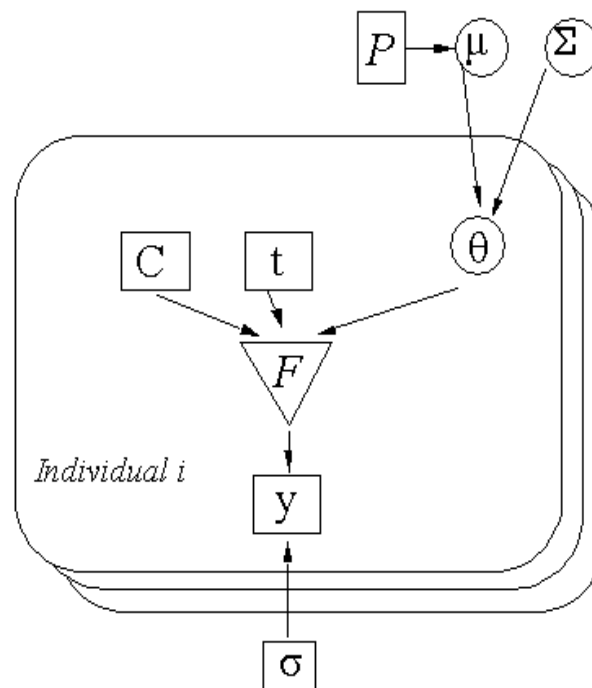
always correlate with the amount of damage. The estimates of the recovery rate seem not always consistent with biological observations. The domains in which the model weaknesses are most evident will hopefully be clarified with the help of the validation package of the ACUTEX software.

- Data requirements: Concentration, percentage of incidence in each category of severity, exposure duration and experiment duration.

4.4.4 Hierarchical analysis

The term ‘hierarchical data’ is used to describe data that consist of repeated measurements as in many cases in biostatistics. It refers broadly, to data in which the response of each experimental unit or subject is observed on multiple occasions or under multiple conditions. The presence of repeated observations on a subject requires a characterisation of the random variation in the data. There are two sources of variability, explicitly: random variation among measurements within a given subject and random variation among subjects. Sensible methods for analysing such data must appreciate both the relationships among the response variables and potential correlation among observations from the same subject or experimental unit. This is a useful exercise (especially when there is not enough data from single species) that consists of incorporating as much data as possible from multiple studies, which may differ in exposure duration and/or species. This may improve estimates and reduce uncertainty since it incorporates more information than a single study. This is what toxicologists call the ‘population approach’. The basic idea is that the same model can describe the data for each unit within a large population and that the model parameters differ randomly between units or between occasions within the same unit. This randomness characterises variability and is described by probability distribution. For example, individual ventilation rate will be assumed normally distributed around a ‘population’ mean with a ‘population’ variance. These population parameters are estimated by data fitting together with the model parameters for each unit (see Figure 4-6).

Figure 4-6: Graphical representation of a population model*



* Individual level with exposure C, duration t with individual characteristics represented by parameters θ and observations y. The relationship between C, t, θ and y is described by a model F. The individual parameters θ are randomly distributed in the population with population mean and variance given by μ and Σ^2 respectively. The distribution function is described by P. Measurement errors, modelling errors and intraindividual variability are lumped in a common variance term σ^2 .

4.4.5 Discussion of the benchmark approach

Benchmark dose methods are used to estimate reference (external) concentration in order to set standards for human health effects. For all the above methods, the concentration that would give an estimated incidence of x% can be obtained solving C from the estimated probability of incidence equal to x%. Examples of the calculation of such reference concentrations for 1 and 50% are shown below.

Standard Probit

It is convenient to re-parameterise the probability of an adverse response (see report on 'Modelling categorical toxicity data') as it follows:

$$p = \Phi \left(\frac{\log(C) + m \log(\tau) - \mu}{\sigma} \right) \quad (\text{Equation 11})$$

Where μ and σ represent respectively the average threshold dose of the population and a measure of inter-individual variability in threshold values.

Hence the effective (lethal in case of mortality data) concentrations at which 1% and 50% of the population is affected are respectively given by:

$$EC_{1\%} = \exp \left(-2.32 \sigma + \mu - m \log(\tau) \right) \quad (\text{Equation 12})$$

$$EC_{50\%} = \exp \left(\mu - m \log(\tau) \right) \quad (\text{Equation 13})$$

This results in: $n = 1/m$.

PK / PD model

$$EC_t 1\% = \left(\frac{k_e^2 \exp(\mu_t - 2.32\sigma)}{k_a(k_e \tau - \exp(-k_e(t - \tau)) + \exp(-k_e t))} \right)^{\frac{1}{n}} \quad (\text{Equation 14})$$

$$EC_t 50\% = \left(\frac{k_e^2 \exp(\mu_t)}{k_a(k_e \tau - \exp(-k_e(t - \tau)) + \exp(-k_e t))} \right)^{\frac{1}{n}} \quad (\text{Equation 15})$$

These are effective external concentrations evaluated at the end of the experiment (usually 14 days).

4.4.6 Time scaling to longer and shorter exposure

Duration plays an important role in acute inhalation toxicity. Mostly the exposure is so high that natural defence mechanisms of the organism cannot cope with the tissue damage caused by the acute high inhalation dose rate. The inhalation dose rate is directly related to the concentration and the pulmonary ventilation rate. The pulmonary ventilation rate per kg body weight is related to the body weight of the organism via an allometric scaling equation in the

same way as metabolic rate. When exploring inhalation toxicity, it is necessary to pay attention to the concentration in air and duration of exposure.

$$\begin{aligned}
 P_{robit} &= B_0 + B_1 * \ln(C) + B_2 * \ln(T) \\
 P_{robit} &= B_0 + B_2 * \ln(C^N T) \qquad N = \frac{B_1}{B_2} \\
 C &= \text{mg} / \text{m}^3 \quad [\text{exposure concentration}] \\
 T &= \text{minutes} \quad [\text{exposure duration}] \\
 \text{Percentage mortality} &= \frac{100}{\sqrt{2\pi}} * \int_{-\infty}^{Pr_{obit}} \exp(-\frac{1}{2} x^2) dx
 \end{aligned}
 \tag{Equation 16}$$

If acute inhalation studies are available with varying exposure durations within the expected accidental exposure times, the time extrapolation might be carried out on the basis of the concentration-time response relationship as observed for the experimental animal for the same toxicological endpoint. The term C^NT might be considered as the effective dose with a specific value for specific percentage response. So at a specific response, a series of concentrations and exposure durations can be estimated, being controlled by C^NT.

However, if acute inhalation toxicity effects and responses are available only for one specific exposure duration, it is not possible to extrapolate based on experimental observations. From the study of ten Berge *et al* (1986), it is clear that no general rule can be derived for N from the present acute inhalation toxicity observations (see Table 4-17).

Table 4-17: Values of N according to ten Berge *et al* (1986)

| Systemic Chemicals | Average N |
|-----------------------------|-----------|
| Hydrogen Sulfide | 2.2 |
| Hydrogen Cyanide | 2.7 |
| Methyl-t-butyl ether | 2.0 |
| Methylenechlorobromide | 1.6 |
| Ethylenedibromide | 1.2 |
| Tetrachloroethylene | 2.0 |
| Trichloroethylene | 0.8 |
| Carbontetrachloride | 2.8 |
| Acrylonitrile | 1.1 |
| Irritants | |
| Ammonia | 2.0 |
| Hydrogen Chloride | 1.0 |
| Chlorine pentafluoride | 2.0 |
| Nitrogen dioxide | 3.5 |
| Chlorine | 3.5 |
| Perfluoroisobutylene | 1.2 |
| Crotonaldehyde | 1.2 |
| Hydrogen Fluoride | 2.0 |
| Ethylene imine | 1.1 |
| Bromine | 2.2 |
| Dibutylhexamethylenediamine | 1.0 |

In the case of both systemically acting and locally acting chemicals the exponent N appears to be more or less specific for the chemical. From the acute inhalation toxicity studies, it has become clear that the exponent N is not species specific and is not affected by body weight. From some studies it was clear, that the exponent N depends on the selected range of exposure durations. This is possible in the case of two types of mode of action, narcosis in short-term high exposure and liver failure in the longer-term exposures, for instance with halogenated alkanes.

Where duration extrapolation is necessary, a prudent approach is recommended. This recommendation considers two cases:

- Mortality is observed at a much shorter duration than what can be expected in accidental human exposure, e.g. at a short distance from the place emergency. In this specific case, it is recommended to set $N = B_1/B_2 = 3$ (see equation 16 above) for extrapolation to shorter duration than the duration for which the LC_{50} or EC_{50} was observed.
- Mortality is observed at a much longer duration than what can be expected in accidental human exposure of inhabitants at a longer distance from the site of emergency. In this case, it is recommended to set $N = B_1/B_2 = 1$ (see equation 16 above).

If the mode of action for AETL-2 and -1 endpoint is the same as in the lethality study, use the 'N' value estimated from the lethality study. Otherwise, we should use default 'N' values (3 to extrapolate to shorter time and 1 to extrapolate to longer time).

For AETL-1, sensory irritation endpoint, the effect may be considered independent of time and only depending on concentration. The same AETL-1 value should be used for all exposure time periods.

4.5 Derivation of AETLs: Points of Departure

4.5.1 NOEL versus LOEL

In more routine risk assessments, it is common to use No-Observed-Effect-Levels (NOELs) or No-Observed-Adverse-Effect-Levels (NOAELs) as the Point of Departure (POD) for setting 'safe' exposure levels in various media including food, water, and air. Generally, in toxicology studies, this is the exposure level below that producing a toxic effect, or Lowest-Observed-Effect-Level (LOEL). However, the definitions for AETLs imply that conceptually, the POD is the threshold for the various health endpoints of concern. These include life threatening health effects or death, irreversible or other serious health effects or escape impairment, as well as reversible adverse health effects. In other words, the desired POD is essentially the breakpoint between the NOEL and the LOEL for AETL-3, AETL-2, or AETL-1 effects.

Most toxicology studies report NOELs and LOELs, or in some studies, only NOELs or effect levels. However, toxicology studies do not routinely report the threshold level where certain effects are expected to occur. Therefore, to estimate the threshold level, both NOELs and LOELs should be considered. While a central value between the two could be chosen as the POD, such an approach would not provide a practical means for establishing PODs for purposes of AETL development. Therefore, the various approaches described above, which include selecting the highest level not causing the effect of concern, are suggested. Professional judgement must however be used in order to ensure that such an approach does not result in PODs that are far from the AETL definitions under consideration. For example, if the highest level not producing lethality in an acute animal lethality study is used as a POD

for AETL-3, and if clinical data in humans indicate that exposures at this level do not produce even minor toxic effects, the POD should be reconsidered.

In addition, the various factors influencing NOELs and LOELs should be considered. For example, a number of investigators have compared the ratios of LOELs to NOELs for a range of different chemicals and different study durations including subacute, subchronic, and chronic exposures (ECETOC, 2003). These studies generally indicate that the LOEL rarely exceeds the NOEL by more than about 5-6 fold and is typically closer to a value of 3. As described by Fowles *et al* (1999), for acute toxicity studies, the central ratio of the LC₅₀ and non-lethal LC₀ is in the order of 2-fold (ranging from 1.1 to 6.5).

Also, as noted by numerous investigators, the LOEL/NOEL ratio highly depends on the spacing between the doses (ECETOC, 2003). Since recent study designs generally use a dose spacing of 2-4, it is logical to conclude that the ratio data support a value of 3 as default for repeat dose studies. In contrast, for studies with higher dose spacing, a much higher LOEL to NOEL ratio is observed.

4.5.2 Considerations on the selection of animal species

Toxicity tests required for hazard identification consider rodent species and specifically young adult laboratory rats to be the test species of choice. In using this approach, including generally accepted and harmonised testing guidelines, the highest degree of inter-laboratory comparison can be achieved. Likewise, this standardisation allows a comparative relative ranking of hazards from chemicals, which serve as the basis for regulatory actions, such as classification and labelling. Under such provisions, relevance to humans is not necessarily the primary focus of testing. To the contrary, testing of pharmaceuticals require data from rodent (commonly rats) and non-rodent species (commonly dogs) using the clinically relevant route of administration in order to allow a better appreciation of species differences and what is initially safe to humans. Accordingly, the approaches used within the AETL process to derive tolerable levels for the general population has more similarities with the paradigms applied for the latter testing approach than with hazard identification alone. Notwithstanding this ultimate objective, due to animal welfare considerations and the availability of data, the commonly applied approach needs to be flexible and take into account the availability of data.

4.5.2.1 Uncertainty involved in the extrapolation across species

Several animal species have traditionally been used in inhalation studies. When using data for hazard identification based on experimental animals, adjustments have to be made in terms of differences in toxicokinetics and toxicodynamics. The greater these differences are, the higher is the uncertainty involved in the extrapolation across laboratory animals and humans, requiring uncertainty-specific adjustment factors. This issue is complicated even further in inhalation studies where both the total inhaled dose and the dosing rate highly depend on the species under consideration because of differences in respiratory patterns and the respiratory minute volume. Moreover, some rodent-species have developed species-specific defence mechanisms, e.g. the stimulation of irritant receptors within the respiratory tract may have a marked impact on the respiratory minute volume. In addition, the stimulation of nerve endings in the respiratory mucosa cause reflex-related changes in body temperature, which occur almost instantaneously in small-mass species, such as mice, but do not occur to any appreciable extent in larger species. Furthermore, these effects may depend either on dose or on concentration.

Experimental animals may experience irritant-related stress and ensuing physiological response following inhalation, which may be absent following other routes. As these potential confounding factors are known to attenuate or enhance the toxic potency of the xenobiotic under investigation, additional physiological endpoints evaluated in animal models need to be considered.

The heterogeneity of the respiratory tract and the localised deposition patterns of inhaled substances in nasal or oronasal breathing laboratory animals contribute further to the difficulties of inter-species extrapolation in inhalation toxicology. Anatomical differences may also play a role in species-specific susceptibilities.

Anatomical differences may also play a role in species-specific susceptibilities. The population densities of epithelial cells (basal, ciliated, mucous (Goblet), serous, Clara, and brush) vary with the airway region and species. Also the cartilage is highly variable among species. In large animals, the cartilage is massive with respect to the fraction of the total airway wall it occupies, and it also extends into the distal tracheobronchial tree, i.e. cartilage surrounds respiratory bronchioles. In contrast, small rodents, i.e. hamster, lack cartilage in segmental bronchi. Significant differences amongst mammals have been reported with regard to the number of cartilaginous airways and pattern of branching.

This means the various species used in inhalation toxicology studies do not receive identical doses per unit body-weight not in comparable respiratory tract regions when exposed to the same external particle or gas concentration. Therefore, the biological endpoint or health effect of concern may be more directly related to the quantitative pattern of mass deposited within the respiratory tract than to the external exposure concentration. Since dosimetric adjustments require an understanding of mechanistic determinants of disposition and pathomechanisms of most concern, risk assessment based on inhalation data involves the least uncertainty.

The selection of target species requires a balanced analysis whether the most critical mode of action identified in the selected species is qualitatively and quantitatively similar to humans.

Mouse

With regard to the uncertainties associated with inter-species extrapolations in inhalation toxicity studies those associated with mice are highest for the following reasons:

- The mouse has a substantially higher breathing frequency when compared to rats, dogs or humans (approximately 300 breaths/minute, *versus* 120 in rats or 15-25 breaths/minute in dogs and humans). In mice many anatomical / morphological features are through evolution optimised to accommodate such high breathing frequency.
- When exposed to irritants, they down-regulate the ventilation in a concentration-dependent and relatively sustained manner. However, rats show similar qualitative responses, which are commonly less pronounced and less sustained.
- Secondary physiological responses occur as a result of the sensory stimulation of trigeminal nerve endings and cause bradycardia and hypothermia. The reduction in ventilation, associated with hypothermia, in turn, may cause substantial changes in the acid-base status, which then may exacerbate or potentiate exposure to acid gases.
- Mice are very susceptible to experimental variables, such as stress. This makes it difficult to use data from very short exposures of mice because of stress-related increase in ventilation.
- Mice are usually more aggressive than other small laboratory rodent species. Therefore, the handling (placing into head-nose only exposure tubes or plethysmographs) of mice is more elaborate than that for rats.

- The various strains of mice often show relatively different susceptibilities for pulmonary diseases making them uniquely sensitive under certain conditions.

Other disadvantages are generally related to its small size, i.e. relative difficulty to perform lung function measurements. Also sampling of sufficient amounts of bronchoalveolar lavage fluid or blood for clinicopathological analyses are limited. Urinalysis is practically impossible.

Based on these considerations the mouse appears to show relatively specific physiological responses that are either difficult to extrapolate to larger mammals *per se* or do not occur at all in larger mammals.

Dog

In contrast, beagle dogs are often the species of choice as an experimental model for the study of pulmonary responses. Their lungs bear a reasonable resemblance to human lungs. Mice and rats are obligatory nasal breathers whereas the dog and humans are oronasally-breathing species.

Dogs can be exposed in large inhalation chambers, in head-only inhalation chambers as well as by facemask. The dog is being used as the non-rodent species of choice for research of pharmaceutical aerosol formulations and is used in asthma research.

The structural and functional characteristics of their respiratory system are well documented. Briefly, sub-mucosal glands are observed throughout the bronchial tree of beagles. In contrast to human and dog lungs, bronchial glands are not found in small rodents. At the level of the terminal bronchioles, the lining cells of beagle dogs are mainly non-ciliated (Clara cells) whereas ciliated cells are rare. In the acinus (composed of airways distal to the terminal bronchiole) of beagle lungs, several generations of alveolated bronchioles (i.e. respiratory bronchioles) exist; human acini have a similar morphology. However, the lungs of small rodents have either no respiratory bronchioles or, at most, one generation. Also the number of alveolar pores, important for collateral ventilation, is similar in a canine and a human alveolus, but smaller in a rat alveolus. Since the acinar region is the main target site for irritant agents causing inflammation of the lower airways, such as phosgene, these interspecies differences in acinar morphology are important and must be considered when comparing pulmonary responses across species.

Methods are available for the assessment of changes in the pulmonary function of the dog.

As with other large animals, inhalation exposure of dogs can create some technical problems, especially in inhalation studies. The clear disadvantage of the dog is its excitability or susceptibility to distress or other external stimuli, and this species can experience a marked change in respiratory minute volumes due to panting.

Although not advocated for standard testing approaches, many determinations made in dogs (e.g. repeated blood sampling during and after exposure, lung function, analysis of blood gases, cardiovascular measurements, clinical pathology and haematology) have the highest degree of similarity with the procedures used in humans. The dog is of sufficient size to serve as a useful experimental model for various pulmonary physiological and inhalation toxicity studies, which may require training, surgical intervention, repeated blood sampling, and other experimental techniques that cannot be performed on the commonly used small rodent.

A particular advantage of the dog is that arterial blood can be sampled relatively easily, even during exposure, without any surgical intervention. Therefore, kinetic profiles of the test compound in blood as a function of the duration of exposure can be determined without the

use of additional groups, since a sufficient amount of blood can be sampled before, during, and after cessation of exposure.

Studies on aerosol deposition related to particle size, lung morphology, respiratory physiology parameters, and blood gas characterisation also compare very favourably with those observed in humans. Due to the potential of reflexively induced changes in rodents, results from controlled and well-performed inhalation studies in dogs should be given particular attention when extrapolating results from smaller laboratory animals across species.

For substances exhibiting a mode of action that is more similar in dogs than in rodents the lower uncertainties associated with the extrapolation across species outweigh the disadvantage of using low numbers of experimental animals (relative to rodents). The dog is not inbred and, therefore, it might be difficult to pick up small changes in clinical and pathological parameters.

Rat

The most abundant data source available is commonly from rats.

From a default perspective, the young adult inbred rat is the species of choice because of the biological and toxicological background data available. The experimental procedures used for rats have been validated extensively across different laboratories, so that those findings can readily be compared and put into perspective.

Compared to the mouse the respective responses in rats are less pronounced or level off during the course of exposure.

With regard to statistical analysis, the number of rats used in experimental studies is commonly higher than used in non-rodent studies. However, due to the repeated sampling of blood possible in larger species, time-related incremental changes can be suitably analysed, whilst in rodents statistical analysis is generally restricted to the inter-comparison of treatment groups.

Inhaled materials that would travel down to the lower respiratory tract in humans are more likely to be trapped and absorbed in the nasal passages of the rat or mouse. Due to geometric differences, nasal passages of experimental animals, in particular those of rodents, filter the inhaled air to a greater extent than those of humans. Consequently for local acting chemicals the effects observed in the nose of the rat are exaggerated and no additional safety factor must be added for extrapolation from rodent to human. It is not necessary either for extrapolation from primate to human, as the effects will be similar in location and intensity.

Guinea pig

For various anatomical reasons, guinea pigs are very sensitive to chemical irritants, responding at levels closer to asthmatic humans than normal humans.

Primate

For various ethical reasons, one would probably not generate data in primates *de novo*. But where such data are available in the literature, they are of high value for setting AETLs.

Conclusion and recommendation

Due to the many variables involved in inhalation dosimetry, for hazard assessment purposes those species should be given preference that show the most human-like dosing pattern within the lung and the ensuing response. It appears to be unwise to think in terms of a single 'best' animal model. Although, for reasons described above, dogs and primates are the preferred

species for assessing the toxicity associated with acute inhalation exposure, the use of studies performed with rodents is the standard procedure due to the lack of data on dogs and primates.

In situations where studies using several rodent species are available, acute inhalation data in the rat is preferred rather than smaller species showing a lung physiology and regional dosing pattern more difficult to extrapolate to humans. Furthermore, typical, rodent-specific responses are well characterised in the rat and standardised exposure methodologies are available. The additional advantage of the rat is its higher ventilation per body-weight, i.e. its inhalation uptake is substantially higher when compared to humans, making this animal model implicitly conservative.

However, if the data available for a particular chemical indicate that a species other than the rat is more relevant to the effect and mode of action in man, serious consideration should be given to the use of data from that species.

As an exception, the use of non-rodent species (preferentially dogs) should aptly be considered in 'proof of principle' type of studies in order to arrive at more relevant inter-/intraspecies assessment factors for compounds exhibiting toxicodynamic and/or toxicokinetic/dosimetric patterns in dogs that appears to be less difficult to translate to humans when compared to data from rats.

4.5.3 Methodological aspects of inhalation studies

Inhalation studies using laboratory animals are carried out under controlled conditions to assess the toxicity of aerosols, gases, and vapours. Inhalation studies appear to best simulate potential exposure conditions of humans and do not require substantial dosimetric adjustments or error prone route-to-route extrapolation.

To date, most inhalation facilities use dynamic exposure systems where the airflow and introduction of agents into the system are continuous. A dynamic inhalation exposure system with a suitable control system is desirable to monitor the inhalation chamber atmospheres with respect to aerosol and/or vapour concentrations, particle size, airflow rates, temperature, and humidity.

A real-time, direct reading monitoring device (e.g. aerosol photometer for particulates or a total hydrocarbon analyser for volatile materials) may be useful to demonstrate that temporally stable exposure conditions prevailed, that the time required to reach the inhalation chamber equilibrium concentration is negligible in relation to the total duration of exposure, and most importantly, that inadvertent, short-term high-level excursions did not occur.

'Nominal concentrations' reflect the mass of test substance introduced into the inhalation system relative to the total volume of air available for dispersion. Many factors – including wall loss, losses on the skin and fur of animals, for the whole body mode of exposure, sedimentation and impaction especially of larger particulates, chemical reactivity – cause the 'analytical' or 'actual' concentration to be less than the nominal concentration. Therefore, the concentration should always be measured by an appropriate instrument rather than reporting the nominal concentration. Commonly, actual concentrations are based on samples taken in the breathing zone.

4.5.3.1 Nose-only versus whole-body exposure

International regulatory bodies define the protocols of the studies by inhalation performed on rodents based on two patterns of exposure: nose-only and whole-body. In the nose-only

exposure study the rodents are constrained in a device and exposed to the substance by the nose-only for the duration of the exposure. In the whole-body inhalation study, the rodents are exposed in cages in an inhalation tank.

In the nose-only inhalation study, only the nose and respiratory tract are exposed to the substance concentrating the effects on the respiratory tract, while in the whole-body exposure study, the substance is in contact with the skin of the animals. Skin absorption of the chemical may increase the body burden of the substance, leading to an increased toxicological effect, compared to the nose-only study. In the case of irritant/corrosive materials the local effects may also increase the severity of the toxicity of a substance to the animal.

During nose-only exposure, the animals are exposed to the test substance in exposure tubes. The design of the restraining tube as well as the flow dynamics should make it impossible for the animal to avoid inhalation exposure.

The operational principle of nose-only chambers has a number of advantages:

- Actual breathing zone concentrations are defined from samples obtained in the vicinity of the breathing zone area.
- Re-breathing of the atmosphere does not take place, which is particularly important for reactive, water-soluble chemicals.
- When using dry air, collected test material is better defined: This is because humidity and other constituents contained in the exhaled air do not interfere.

A major disadvantage of the whole-body exposure mode is that losses to chamber surfaces can be a severe problem especially when atmospheres are generated at elevated temperatures due to condensation. In whole-body chambers, the applied flow rate as well as the arrangement of the animals at specific locations within the chamber may result to differences in exposure concentrations:

- Animals are always contained in small wire-mesh cages (in groups or individually). The disadvantage is that animals may either huddle together or bury their noses into their own hair-coat and this minimises exposure.
- Depending on the flow rate, the time required to attain steady state will be within approximately 10-30 minutes.
- To have homogeneous atmospheres across the chamber mandates very high flow rates.
- Due to the high flow rate, efficient generators have to be used to produce the desired/targeted atmosphere through dilution.
- Phase changes (evaporation of aerosol or recommendation of vapour) may occur.

Proper analytical characterisation needs to be done at least 4 different locations in the whole-body chamber (top, bottom, periphery and centre) to be able to determine concentration gradients within the chamber. Agents showing a higher water solubility/reactivity may react with humidity at chamber walls. High-density substances (e.g. phosgene) might show concentration gradients across the chamber. Exhaled air or degradation product from faeces (ammonia) may cause analytical problems for reactive agents tested at low levels. Due to the problem of attaining high concentrations in larger whole body chambers test substances have been conveyed directly into the chamber, which may cause substantial gradients within a chamber.

As a consequence of these methodological variables, concentrations in whole-body exposures are biased to be lower than those from the vicinity of the breathing zone of animals. The reason is that sampling is often made at locations of convenience rather than from the breathing zone and substances losses occur due to long sampling tubes.

Commonly, LC₅₀ values are lower in nose-only chambers when compared to whole-body chambers as the animals, due to some restraint-related distress, have higher respiratory minute volume and have no means by which to reduce the exposure (e.g. by huddling). Restraint-related distress may especially occur in animals when using hermetically sealed tubes from which urine and faeces cannot escape or the rats' thermoregulation via the tail is compromised. The least stress occurs when using directed-flow or past-flow, i.e. positive pressure nose-only chamber systems and tubes that are not sealed and allow rats to thermoregulate via the tail.

However, for some systemically acting agents the opposite has been observed (e.g. organophosphates, aniline, dimethylformamide). This was likely to be due to the direct wetting of the animal in whole-body chambers by the spraying process or the settling of larger particles onto the surfaces of the skin potentially contributing to toxicity.

Cross-contamination of laboratories (via contaminated hair-coat of animals) or oral uptake by grooming is another major disadvantage of whole-body exposure.

Due to the longer clearing time of the inhalation chamber in the whole body exposure mode there is also a longer time period between the cessation of atmosphere generation and the ability to collect samples for specialised examinations (e.g. blood sampling). This may lead to results that are difficult to interpret.

In summary, the nose-only mode of exposure provides the highest level of confidence in terms of time and concentration of actual exposure. Heterogeneities of exposure atmospheres, which inevitably lead to a disparity between intended and actual exposure concentrations, are a general problem in whole-body inhalation studies. The ventilation rate of the exposed animal is commonly higher when compared to whole-body which renders this mode implicitly more conservative. Dermal uptake may contribute to the toxicity of an inhaled substance. However, the extent of dermal uptake is highly dependent upon experimental variables either difficult to reconstruct or difficult to translate into real-life human exposure scenarios. Further advantages of the nose-only exposure mode are that for the purpose of dosimetry (measurement of respiratory minute volume) or assessment of physiological responses volume displacement nose-only plethysmographs can be used during exposure. Therefore, as a rule in deriving AETLs, results from nose-only acute inhalation studies should be considered superior to whole-body studies.

4.5.3.2 Other routes of exposure

Another way to bypass the scrubbing of the substance (gas or aerosols or particles) by the upper respiratory tract in the rodents is to expose the animals by intubation of the trachea or by direct instillation of the substance into the trachea. These methods permit the evaluation of the direct action of the substance on the lower respiratory tract of the animals, and could allow a better transposition from rodent to human.

Precaution needs to be applied in the interpretation of results of studies using the mouse-breathing model. Meldrum (1999) points out that the presence of the endotracheal cannula leads to changes in breathing pattern and volume of gas inhaled and may also produce some local mechanical tissue damage. Particularly with respect to very water-soluble gases key target areas of the upper respiratory tract are not exposed to the test substance.

Intratracheal exposure studies in the animals, bypassing the upper respiratory tract, maximise the lung exposure, providing an artificial exposure scenario, which cannot be directly used for setting AETLs.

Most useful protocols are those for acute inhalation toxicity which combine the histopathology examinations usually conducted on a subchronic inhalation study, focused on the local tissue damage over the full depth of the respiratory tract. (OECD, 1981a, b).

4.5.3.3 Mechanistic determinants of dose - route to route extrapolation

A major issue of inhalation toxicity is that of dose. Inhaled dose is more difficult to determine than the dose from other routes of administration. With oral or parenteral routes, a discrete amount is given in a bolus. With inhalation, the delivered dose depends on the exposure concentration, particle size, and breathing pattern. Deposition patterns within the various regions of the respiratory tract also are important. Over the past few decades, the concept of dose as applied to toxicological studies has changed considerably. Initially, 'dose' simply meant the concentration in the atmosphere in inhalation studies (or the amount ingested or instilled into the gastrointestinal tract in oral dosing studies) multiplied by the duration of study (Andersen, 1995; Jarabek, 1995). Empirical correlations were then used to evaluate the relationship of dose and response with little appreciation of the detailed biological interactions of the test agent.

Route-to-route extrapolations from acute oral to acute inhalation have received attention to 'predict' the more elaborate LC_{50} (after converting the inhalation concentration to dose per unit body weight using Haber's law; 'inhaled dose' = concentration x duration of exposure). Combined assessment of data demonstrated that when the LD_{50} was approximately 100 mg/kg, the LC_{50} varied 57-fold and when the LD_{50} was around 1000 mg/kg the LC_{50} varied 133-fold (ECETOC, 1995). This wide variation suggests that extrapolation from one route to the other is subject to tremendous errors and caution is advised when doing so. Default values are therefore not recommended and conversion factors must be calculated for each individual situation, making appropriate assumptions about body weight, minute volume, percentage deposition, retention and absorption. Pulmonary and extrapulmonary pathomechanisms should also be taken into account.

Overall, route-to-route dose calculations without appreciation of the actual breathing patterns of the test species may lead to false assumptions.

4.5.4 Critical health effects and dose-response

This section includes general principles for the selection of health-effect endpoints and points of departure to be performed on a chemical-by-chemical basis. The three tiers of AETL values provide much more information than a single value because these tiers indicate the slope of the dose-response curve. Under ideal circumstances, the specific health effects would be identified that determine each of the AETL values. A search of the published data on the chemical would be performed, and AETL values would be generated from those data. However, data relating to exposure and effect do not always follow ideal correlations and may lead to apparent inconsistencies in the use of endpoints to set AETL values. Whereas the EU TGD (EU, 2005) can be applied to derive NOAELs, the NRC (1993) provides guidance on general principles for evaluating data and selecting appropriate health effects. Combined with professional judgement, this can be used to establish values for the three threshold levels, taking into consideration that the definitions of AEGs and AETLs are different. Since ideal data sets for individual chemicals are not available, extrapolation methods and scientific judgement are often used to establish threshold values. In the absence of adequate data, it may be decided not to establish an AETL value.

4.5.4.1 Threshold level 1

The Threshold level 1 is the maximum airborne concentration at which it is predicted the general population could be exposed up to a specified exposure time without experiencing more than mild and reversible adverse health effects. AETL-1 endpoints for adverse effects include those that are asymptomatic or non-sensory, such as measurable levels of methaemoglobin, elevated blood enzyme levels or other biological markers related to exposure to a specific chemical. AETL-1 endpoints are always reversible and never lead to an impairment of escape. Above the AETL-1 value, discomfort becomes increasingly likely.

The setting of the AETL-1 should ideally be based on full knowledge of the key target organs and dose response relationships for a single short-term inhalation exposure. Usually this will need to be performed primarily on the basis of animal data, which meet the required quality criteria. However, in some cases, human data of adequate reliability will be available and should be given preference over animal data. As mentioned in before, standard OECD Test Guidelines that provide a comprehensive investigation into non-lethal inhalation toxicity are not available. However, study designs that combine the requirements of OECD TG 403 (1981a) with the functional observation battery, clinical chemistry, haematology and histopathology investigations of OECD repeat dose toxicity studies (for example OECD TGs 407 (1995) and 408 (1998)) would provide a basis for setting the AETL-1. Data from repeated exposure inhalation studies, in particular sub-acute studies, may help to identify target organs and confirm no-effect levels. In the absence of inhalation data, extrapolation from the oral route (in the case of systemic toxicity) where justified, and the use of expert judgement that takes account of all the available toxicity information and knowledge of the dose response relationships, may be used to set AETL-1s.

Ideally, for substances causing sensory irritation (upper respiratory tract irritation), human volunteer studies are required. These should involve clearly defined and verified exposure conditions and validated questionnaires in which the threshold for sensory irritation is identified. In the absence of such studies, information may be available from reports in the occupational setting. These may be of value if they give descriptions of sensory irritation with associated measurements of airborne concentration based on personal sampling. However, such information should be treated with caution, as there may be tolerance to sensory irritation resulting from long-term repeated exposures.

The following additional criteria should also be used in setting the AETL-1 values.

Effect level for a response

In developing AETL-1 values, a NOAEL is estimated as point of departure (POD) for reversible adverse health effects of moderate severity. Section 4.2 provides various levels of toxicity associated with AETL-1 effects according to target organs. It must be emphasised that mild reversible health effects may be observed below the AETL-1 value. In situations where the experimental data describes multiple AETL-1 effects of moderate severity, the one with the lowest threshold value should be chosen as point of departure for the derivation on a weight of evidence basis. If mild asymptomatic or non-sensory effects, such as mild sensory irritation, methaemoglobin formation or transient and clinically insignificant, altered pulmonary function are observed at one level of exposure and moderate health effects at a higher exposure, the former is used to set the AETL-1 value. More detailed guidance in the selection of AETL-1 effects is described in section 4.2.

The toxicological endpoint of concern and why it was selected should be described in the TSD, as well as the species, the concentration, and the exposure time causing the effect.

For locally acting substances, the POD is divided by an assessment factor of 3, regardless of whether human or animal data were used for identifying the POD. For systemically acting substances, the POD is divided by an assessment factor of 3 if human data were used as the POD and by a factor of 10 if animal data were used.

AETL-1 close to or exceeding AETL-2

In some instances the derived AETL-1 and AETL-2 values may be similar. In this situation it is recommended that the AETL-1 value be presented in the TSD and that the issue of its proximity to the AETL-2 value should be clearly highlighted.

In some cases the derived AETL-1 values may be above those for AETL-2. In this situation it may be believed erroneously that people experiencing mild irritation are not at risk when in fact they have already been exposed to extremely hazardous or possibly lethal concentrations. For this reason, when the derived AETL-1 value is above the AETL-2, it is recommended that the AETL-1 value should not be included in the TSD. In this situation, the TSD should clearly state the reason for not presenting the AETL-1 value.

Insufficient data

In the case of insufficient data, no AETL-1 can be established. The basis of this decision should be clearly explained in the TSD.

4.5.4.2 Threshold level 2

AETL-2 is the maximum airborne concentration at which it is predicted the general population could be exposed, up to a specified exposure time, without experiencing or developing irreversible or other serious adverse health effects including symptoms that could lead to impairment to escape. Above the AETL-2 value, there is an increasing likelihood that people may become disabled or are increasingly likely to experience serious or irreversible health effects. The term ‘disability’ indicates the situation where persons will require assistance or where the effects of exposure will be more severe or prolonged without assistance.

The setting of the AETL-2 should ideally be based on full knowledge of the key target organs and dose response relationship for single short-term inhalation exposure. Usually this will need to be performed primarily on the basis of animal data, which meet the required quality criteria, as human data of adequate reliability will rarely be available. As mentioned before, standard OECD Test Guidelines that provide a comprehensive investigation into non-lethal inhalation toxicity are not available. However, study designs that combine the requirements of OECD TG 403 on acute inhalation toxicity (OECD, 1981a) with the functional observation battery, clinical chemistry, haematology and histopathology investigations of OECD repeat dose toxicity studies (for example OECD TGs 407 (OECD, 1995) and 408 (OECD, 1998)) would provide a basis for setting the AETL-2. Data from repeated exposure inhalation studies, in particular sub-acute studies, may help to identify target organs and confirm no-effect levels. In the absence of such data, extrapolation from the oral route may be used, where justified, in conjunction with the use of expert judgement that takes into account all the available toxicity information and knowledge of the dose response relationships.

Impairment to escape may be caused by local irritation of the eyes with associated extreme tearing (lacrimation) and is thus different from effects causing irreversible or other serious health effects.

The following criteria should be used to select endpoints for use in setting AETL-2 values.

Data on AETL-2 effects available

From the data available, the highest exposure not causing irreversible or other severe adverse health effects (e.g. kidney pathology, behavioural changes), or not causing a severity level leading to impairment to escape (e.g. in case of impaired pulmonary function, mild narcosis, and methaemoglobin formation) should be identified and used as the point of departure. Section 4.2 provides various levels of toxicity associated with AETL-2 effects according to target organs. It must be emphasised that clinical toxicity, which is less serious and is reversible, may be observed below the AETL-2 value in situations where the experimental data describe multiple AETL-2 effects. The one with the lowest threshold value should be chosen as point of departure for the derivation on a weight of evidence basis. If minor reversible effects are observed at one level of exposure and disabling effects at a higher exposure, the former is used to set the AETL-2. If the exposure associated with disabling effects cannot be determined from experimental data, then the highest level causing reversible effects and discomfort may be used to set the AETL-2 value. If the exposure associated with disabling effects cannot be determined from experimental data, then the highest level causing reversible effects and discomfort may be used to set the AETL-2 value.

The toxicological endpoint of concern, and why it was selected, should be described in the TSD, as well as the species, the concentration, and the exposure time causing the effect. If escape impairment was used to set the point of departure, this must be clearly highlighted in the TSD and all AETL-2 values must be accompanied by an (*).

For locally acting substances, the POD is divided by an assessment factor of 3, regardless of whether human or animal data were used to identify the POD. For systemically acting substances, the POD is divided by an assessment factor of 3 if human data were used as the POD and by a factor of 10 if animal data were used.

Data on AETL-2 effects not available

In the absence of specific data to determine an AETL-2 value, a fraction (e.g. one-third) of the AETL-3b value can be considered to establish the AETL-2 value. This approach can only be used if the data for AETL-1 and/or AETL-3 effects indicate a steep exposure-based relationship. The rationale for using this approach should be clearly stated. A justification of the divisor used should also be provided in the TSD.

AETL-2 close to or exceeding the AETL-3

In some instances the derived AETL-2 and AETL-3 values may be similar. In this situation it is recommended that the AETL-2 values be presented in the TSD and that the issue of their proximity to AETL-3 values should be clearly highlighted.

In some cases the derived AETL-2 values may be above those causing AETL-3 effects. In this situation it is recommended that AETL-2 value should not be included in the TSD. A justification for not presenting the AETL-2 value should be included in the TSD.

4.5.4.3 Threshold level 3b

The AETL-3b is the maximum airborne concentration at which it is predicted the general population could be exposed, up to a specified exposure time, without experiencing life threatening health effects or death. Above the AETL-3b, there is an increasing likelihood of

death or life-threatening effects occurring. It must be emphasised that severe health effects will be observed at levels exceeding the AETL-3b. In cases in which data to determine the highest exposure level that does not cause life-threatening effects are not available, levels that cause severe health effects without producing death may be used.

The setting of the AETL-3b will usually be based on animal data, which meet the required quality criteria. Appropriate, reliable human data are unlikely to be available. Ideally an acute inhalation toxicity study of lethality, which at least meets the requirements of OECD TG 403 on acute inhalation toxicity (OECD, 1981a), should be used to derive the AETL-3b values. In the absence of such data it may be possible, using expert judgement, to extrapolate from the exposure levels causing non-lethal reversible or serious non-lethal adverse health effects in single exposure inhalation studies, if such information is available.

The following criteria should be used to determine the point of departure for the derivation of AETL-3b values.

Data-rich situation for lethality including reported time intervals

If available dose-dependent data for lethality include information on the duration between application and death, a dose response model described in section 4.3 can be used to calculate dose-time-response relationships in animals. In that case, the calculations can be derived for different exposure durations. Any time scaling to longer or shorter exposure duration will implicitly be contained in the model.

Data-rich situation for lethality without reported time intervals

If available dose-dependent data for lethality do not include information on the duration between application and death, dose-response modelling will be performed using the available tools. From the dose-response curve the LC_{01} is calculated and used. In other cases, the LC_{01} from the AETL-3a derivation is taken. In both situations, the AETL-3b is derived from the LC_{01} divided by an assessment factor in order to arrive at a level, which is below the lethal effect. An assessment factor of 3 is used for locally acting substances and for systemically acting chemicals if the data indicates that no differences exist between rodents and humans in pharmacokinetics. In other situations, an assessment factor of 10 is recommended.

Data not sufficient for statistical treatment

Acute lethality study available

When experimental lethality data are insufficient to determine the LC_{01} statistically, the highest experimental exposure that did not cause lethality in an experiment in which death was observed can be used as point of departure to derive AETL-3b values. The species, the concentration, and the exposure duration should be described in the TSD.

Only LC_{50} data available

When experimental lethality data are insufficient to use the benchmark concentration (BMC) or maximum likelihood estimate (MLE) approach, and all exposure levels tested caused lethality, but an LC_{50} value was determined from experimental data, a fraction of the LC_{50} value may be used to estimate the threshold for lethality.

It is recognised that the shape of the exposure-response curve widely varies for individual chemicals. Therefore, additional uncertainty is associated with use of an adjusted LC_{50} value as a point of departure. For this reason, the quality of the study used to estimate the threshold

for lethality must be classified at least as Klimisch score 2. Details from the study must also be available to show how the study was performed. The information, which should be given, includes the species, the concentration and the exposure duration. Other data must be available to support the reported LC₅₀ value, either in the same publication in other species or in a separate publication.

If the data are available, the slope of the exposure-response curve should be used to derive a chemical specific divisor.

Fowles *et al* (1999) analysed 120 published inhalation animal lethality data sets using the BMC method. Their analyses of inhalation toxicity experiments revealed that, for many chemicals, the ratio between the LC₅₀ and the experimentally observed non-lethal level was on average a factor of approximately 2 (ranging from 1.1 to 6.5). The 90th percentile was 2.9, and the 95th percentile was 3.5. Therefore, a divisor of 3.5 is recommended as default for AETL setting.

The rationale for using this approach should be clearly stated. A clear justification of the divisor used should also be provided in the TSD.

Data on acute lethality not available

When data are insufficient to estimate the highest exposure that does not cause lethality, exposure levels that cause severe health effects in the absence of lethality may be used as lethal threshold and point of departure for the derivation of AETL-3b values. Examples of severe health effects are described in Section 4.2. In the TSD, the toxicological endpoint of concern should be described, as well as the species, the concentration, and the exposure duration causing the effect.

AETL-3b values and physico-chemical properties

In certain situations, derived AETL-3b values may be above critical physico-chemical thresholds, such as explosive limits or maximum vapour concentrations. In instances such as these the derived AETL-3b values should be presented in the TSD and their relationship to specific physico-chemical properties should be clearly highlighted.

4.5.4.4 Threshold level 3a

The AETL-3a is the airborne concentration at which it is predicted that, after a specified exposure time, a certain percentage of the general population will die.

The setting of the AETL-3a has similar data needs to those described above for the AETL-3b.

Depending on the available data, mathematical dose-response modelling may be used to calculate values for different percentages (likelihood to die), which can be used as the point of departure for the derivation of AETL-3a values for different durations of exposure. For locally acting substances the derived AETL-3a values are assumed to be the same for humans and rats. However, for systemically acting substances the derived AETL-3a values are divided by an assessment factor of 3 when the data indicate that differences exist between rodents and humans for pharmacokinetics, or when no data are available to assess these.

If the data are insufficient for a statistical treatment, reported, LC₅₀ data could be used as the point of departure for estimating an AETL-3a value at which it is predicted that 50% of the general population will die during a specific duration of exposure. If these data are not available, an AETL-3a value cannot be derived.

AETL-3a values and physico-chemical properties

In certain situations, derived AETL-3a values may be above critical physico-chemical thresholds, such as explosive limits or maximum vapour concentrations. In instances such as these the derived AETL-3a values should be presented in the TSD and their relationship to specific physico-chemical properties should be clearly highlighted.

4.5.4.5 Level of distinct sensory awareness (LDSA)

The most common sensory awareness in case of exposure to a chemical is odour. Other examples of sensory awareness are experiencing a specific taste, a change in vision (e.g. colour vision), a change in colour of the skin (pale, grey) (hypoxia) and detection of an atmospheric haze.

Exposure insufficient to cause discomfort or adverse health effects may nevertheless be perceived by means of smell, taste, or sensations (mild sensory irritation) that are not uncomfortable. The awareness of exposure may lead to concern and complaints towards authorities and constitutes what is termed 'detectability'.

In the derivation of AETLs, the Level of Distinct Sensory Awareness (LDSA) is considered as a separate intervention value for information of the affected population.

This level is independent from the health effect threshold levels. During airborne exposure to particular chemicals, a sensory awareness may be missing even at threshold level 2 or 3. In these cases, detectability by itself would indicate that a serious situation exists. Other chemicals may have the effect of an individual level of distinct awareness increasing during the exposure. It has to be considered that the variability of detectability within the human population may be very high, and because of this the LDSA cannot be precisely identified.

The aim of the derivation of this level is to give an additional tool in order to assess an emergency situation. If an accident occurs and people smell or otherwise 'detect' a chemical, this may provide, in conjunction with the knowledge of three health effect threshold levels, additional information to assess the danger of the exposure.

Consequences of selecting a relative high or low awareness threshold

If the awareness threshold is set relatively high e.g. perceived by 50% of the population, the risk manager will receive many complaints and he will start his communication activities in response too late, which at that time may create anxiety.

If the awareness threshold is set relatively low e.g. perceived by 5% of the population, the risk manager will have to respond after a few complaints. Lower values will require the risk manager to communicate to a larger region, where almost nobody has observed anything yet.

If the primary objective of setting an odour level is to provide proactive communication to the affected community, then it is of the utmost importance that this communication is timely and therefore the aim should be to set the odour level to the low side. If the objective of setting an odour level is to provide the emergency response manager with an understanding of the severity of the incident, then the value should rather be set to the high side.

The most common sensory awareness is odour. Odour is most likely too uncertain a factor to ensure an independent assessment of the severity of the situation. It can only be used as supporting evidence and then only with a relative low weight. For that reason the objective of setting an odour level should focus on communication purposes.

This has been taken into account in the procedure for setting an odour level. It should be set in such a way that only when a significant proportion of people become aware of the distinct presence of the odour, communication activities are started.

Point estimate versus range

Providing a range of awareness thresholds offers information relative to the variability of responses in a given (test) population. This may also provide a better perception of the relative accuracy of the established value. A point estimate will serve an emergency response manager better, as it provides clear break points in his scenarios and as such provides clear trigger levels. This clarity is often needed in crisis situations. For this reason it is proposed to develop point estimates accompanied by a range reflecting the variability of responses within the population.

Selection of the appropriate studies

The LDSA can only be set on the basis of human experience. Information may be obtained from a number of sources (standardised methods, information from non-standardised odour or sensory threshold studies or other human volunteer studies). Information from other sources (e.g. experiences of workplace exposures) is most likely insufficient to set a LDSA level. Note that odour or sensory thresholds should preferably not be obtained from unsubstantiated secondary sources, as the reliability of such information cannot be assessed. If it is believed that the secondary sources are sufficiently reliable, the LDSA may, exceptionally, be set and based on these sources. This is a deviation from the general approach that is used for the setting of AETLs and justified in section 4.2. The exception is based on the predictive nature of the LDSA versus the preventive nature of the other AETLs.

Determination of the value

In the past many techniques have been used to determine the awareness threshold of a compound. Ideally, the method of Ruijten *et al* (2004) could be used for the determination of the level of distinct sensory awareness, when sufficient information is available.

Odour

Step 1: Obtain the odour detection threshold

The classification system, as proposed by Ruijten *et al* (2004), can assist in the selection of the key study.

Step 1 a: Select the key study using the quality levels 1-3

Level 1: Threshold value of a compound determined according to EN13725 (CEN, 2003); AS/NZS-4323.3 (EPA, Australia / New Zealand, 2001); NVN 2820 (NNI, 1995) (or equivalent).

Because these standards require minimum performance criteria, it will be possible to determine a geometric mean value from the data of one or more laboratories. Additionally, it will be possible to derive the uncertainty of this mean value, based on the number of available test results and the stated uncertainty for the laboratories involved. Some laboratories may work under international accreditation to quality assurance standards such as ISO17025 (1999) or data may become available from inter-laboratory comparisons related to such accreditation. That type of independently verified data constitutes the most reliable source.

Level 2: Threshold values from sources that include a reported value for n-butanol.

If internal consistency of results (long-term repeatability) can be established for a laboratory, or if a compound together with a reference compound, for which a level 1 quality value is available, was evaluated in the same measurement session, using the same panel of assessors, a correction can be made for the sensitivity of the method used.

The ratio of the experimental threshold for n-butanol, determined in a given test panel, and the reference value of 40 ppb can be used to calculate a level 2 odour threshold.

The same procedure can be used if an odour threshold is available for any other chemical with an established level 1 odour threshold.

Level 3: Threshold values without any internal reference to an n-butanol odour threshold.

Such thresholds are often found in compilations such as AIHA's. These compilations criticise thresholds reported in literature. The main issues that cause limited applicability are:

- too low stimulus presentation flow (<10 litres/minute);
- no reference odour used in panel session to ascertain panel sensitivity;
- in these cases, the crude but most effective approach is to use the lowest reported value from all acceptable sources. The geometric mean would be misleading, because the bias introduced by inadequate testing methodology is almost without exception towards higher odour thresholds.

Step 1b: Select the odour threshold value

If there are multiple studies that can be considered key studies, the following approach is followed to select the threshold value:

For level 1 and 2 studies, the geometric mean is calculated from the selected studies.

For level 3 studies, the lowest threshold is selected.

Step 1c: Correct the odour threshold using a reference compound

If in the experimental study a reference compound has been included for which an odour level has been quantified by using a level 1 study, the obtained odour threshold is adjusted as shown in the example below.

For example, the threshold for styrene in a test panel was 30 ppb. With the same panel, the threshold for n-butanol was assessed as 100 ppb. In this case, the level 2 odour threshold for styrene was estimated at $30 \times 40/100 = 12$ ppb.

Step 2: Derive distinct odour level

The level of distinct odour detection can be calculated, based on the dose response curve, which is referred to as the Fechner coefficient. In practice the level expected to generate a distinct odour perception under laboratory conditions is approximately 11.8 times the odour detection threshold. In the very exceptional case when sufficient data concerning the intensity of the odour are available, the Fechner coefficient can be directly calculated as shown in the paper of Ruijten *et al* (2004).

Step 3: Adjust for field conditions

Outside the laboratory many additional factors can influence odour detection (e.g. gender, age, sleep, smoking, head cold, nasal allergy and distraction). For this purpose an additional correction factor of 4 would be required.

However, it should also be considered that concentrations will be fluctuating and that it could be very common that an average concentration level calculated by a gas dispersion model for a one-hour time frame, will result in peak concentrations during shorter periods of time (e.g. 5 seconds), which exceed the average value by a factor of 3 and which will be easily detected by the general population.

Considering both the variations in humans (gender, age, etc.) as well as the variation in concentration (peak/average concentration) will lead to a correction factor of 1.33. This results in a level of distinct to strong odour perception under field conditions, which is 16 times the odour detection threshold.

Determination of the range

The range is based on the spread of lower and higher odour levels reported in laboratory tests. This range is not multiplied by 10, as it is believed that more sensitive people will detect the odour level below the level of distinct detection.

Determination of the time frame

Odour is perceived in a very short time frame (as short as 5 seconds). As the odour threshold is provided to indicate the alert value, it is proposed not to include a time frame for the odour level, as it will apply across all time frames.

It is known that the appreciation of the odour will change over time due to the process of adaptation or other physiological responses. If this is a very clear phenomenon, it will be indicated in the TSD. Where the chemical affects the odour detection ability (e.g. H₂S), this will also be noted in the TSD.

Type of odour

Determination of the type of odour will be expressed in words, such as fruity, fishy, hay, nutty etc. The American Society for Testing of Materials (ASTM) developed a list of 146 descriptors and used it to characterise 160 compounds in a standardised manner.

The characteristic of an odour may change with the concentration level. For example, H₂S at concentrations of 20 ppm or above ceases to be perceived as a 'rotten egg' smell. At higher concentrations it is perceived as 'sweet' and at even higher concentrations it becomes odourless. It is recommended to include a description of the odour in the TSD.

LDSA higher than AETL

In situations where the derived LDSA is higher than any AETL value, then the value should preferably still be listed.

In situations where the odour level is above the AETL-1, and where this was set based on sensory irritation, the LDSA level can generally not be set above the AETL-1. Nevertheless, as distinct odour detection may still be of value to the emergency responder, it is suggested to

derive an LDSA for odour, which should be provided as a footnote to the table with AETL values.

Recommendation for setting the LDSA level

For any sensory awareness, it is proposed to pragmatically use the same approach as defined above for odour. The laboratory data based threshold level is multiplied by 16 in order to obtain the distinct level. The low and high responses are taken as a range.

4.5.5 Use of assessment factors

4.5.5.1 General comments on assessment factors

It is widely accepted that the extrapolation of animal toxicity data to humans involves a degree of uncertainty. This uncertainty is taken into account by applying numerical assessment factors to cover interspecies and intraspecies variations. In general, substance-specific information should be given preference for the establishment of assessment factors. However, when the available data do not allow the derivation of substance-specific factors, default factors should be applied. A justification should be provided whenever substance-specific or default assessment factors are used.

General observations on interspecies variations

Variations in the sensitivity of different species to specific toxins are generally considered to result from differences in toxicokinetics and toxicodynamics. Toxicokinetic variation can be due to anatomical, physiological and/or metabolic differences between species, such as whether biotransformation leads to detoxification or activation (IPCS, 1999).

When extrapolating acute inhalation toxicity data from rat to man, the following points of uncertainty should be considered:

- The probability that the same mode of action applies in experimental animals and man.
- The variability of effects and of responses between species, if several species have been studied.
- The specific sensitivity of the species compared to man.
- The bioavailability, metabolism, detoxification and elimination in the cases of systemic toxicants.

An interspecies assessment factor of 10 is commonly used when extrapolating experimental data from rodents to humans for setting 'safe' exposure levels in various media including food, water, and air (IPCS, 1999). For the rat, the factor of 10 is composed of a toxicokinetic assessment factor of 4 and a toxicodynamic assessment factor of 2.5 (EU, 2005). With respect to toxicokinetic variation, it is important to note that metabolic rate is strongly related to lung ventilation rate. Thus, the faster metabolic rate, which tends to make rodents less susceptible than humans to systemically acting toxins, is counter-balanced by an increased inhalation rate. This increases uptake and thus susceptibility in rodents (ECETOC, 2003). It is also widely accepted that detoxification plays only a minor role during acute inhalation toxicity because the massive dose received over a short duration overwhelms the normal defence capacity of the body. Furthermore, the normal defence capacity of smaller animals with a high lung ventilation rate per kg bw is overwhelmed faster than that in large animals with a lower lung ventilation rate per kg bw. Therefore, when extrapolating from an animal inhalation study to

man the interspecies assessment factor for toxicokinetics may be ignored (EU, 2005). Thus for inhalation studies the general interspecies assessment factor of 10 is lowered to 2.5. As 2.5 is an inconvenient figure, it is rounded off to the higher integer value of 3.

For reasons just described when the available data do not allow the derivation of substance-specific factors, a default interspecies assessment factor of 3 is recommended when extrapolating data obtained in rats for systemically acting substances, to humans.

The mode of action of corrosive and irritant chemicals appears to be simple and comparable in animals and man (Pauluhn and Mohr, 2000). However, some important differences should be noted. It is well established that rodents are obligate nose breathers and, as such, they are better protected against the irritant action of water-soluble corrosive gases than man. The anatomy of the upper respiratory tract in rodents is also different from that in man. The narrow and complex tube system of the nasal conchae in the rat absorbs a relatively higher amount of a substance than humans before it enters the more distal portions of the respiratory tract (Monticello *et al*, 1991). This process, which is often termed scrubbing, suggests exposure to a chemical may be higher in the upper respiratory tract in rodents compared to humans, while the reverse is true for lower parts of the respiratory tract (ECETOC, 2003). However, computational fluid dynamic modelling of deposition in the upper respiratory tract has revealed that deposition is quite comparable between rodents, monkey and humans. Furthermore, long-term studies with irritants indicate the severity of tissue damage in the respiratory tract of rodents and humans is comparable (Kalberlah *et al*, 2005).

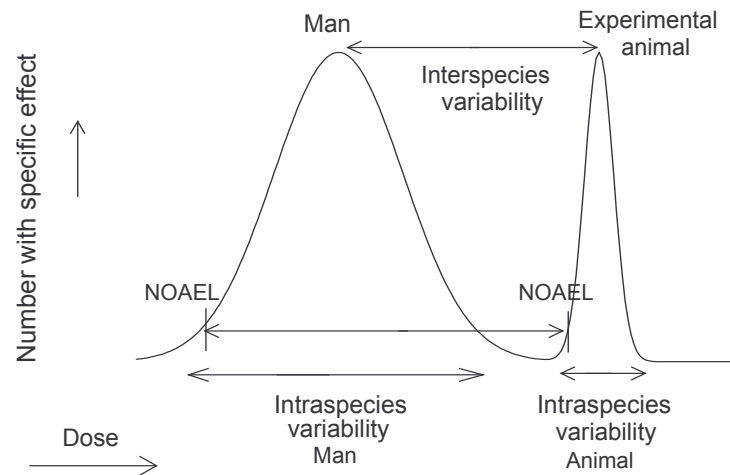
When evaluating airborne exposures to corrosive and irritant chemicals, special attention should be given to the physical state of the substance i.e. whether the exposure is to a gas/vapour or aerosol/particles. This is especially important for substances causing skin and eye irritation.

When the available data do not allow the derivation of substance-specific factors, a default interspecies assessment factor of 1 is recommended when extrapolating data obtained in rats for local acting substances, to humans.

4.5.5.2 Variability within humans

Intraspecies assessment factors are used to account for the variability in chemical sensitivity that exists in the human population. They are used to establish exposure levels that are protective of the majority of the population and not just the population average. During interspecies extrapolation the sensitivity of the average experimental animal is compared with that of the average human. In this regard laboratory animals utilised in most experimental studies are highly inbred and show considerably less variation than is expected in the human population. The intraspecies assessment factor takes into account the variability of sensitivity within both species. When using a NO(A)EL for a particular health effect as POD based on data in an experimental animal the intraspecies variability of the experimental animal has already been mainly accounted for. This means that the intraspecies assessment factor does not need to take into account the full variability of the human population but only the ratio of variability between man and the experimental animal. So the intraspecies assessment factor should be related to the ratio between the intraspecies variability of man and that of the experimental animal (see Figure 4-7). However, consideration should be given to the fact that NO(A)ELs are often based on comparisons of group means of a relatively small group of exposed animals with controls, and as such, are not based on the most sensitive animal in that group.

Figure 4-7: Interdependence of interspecies and intraspecies variability



The intraspecies variability is also reflected in dose response relationships. The ratio between the slope of the dose response relationships in rat and that in man can be used as a measure to estimate the intraspecies assessment factor. The reciprocal of the regression coefficient of the logarithm of the concentration in the log(dose) normal distribution response model is in fact the logarithm of the geometric standard deviation (GSD) of the sensitivity distribution. This provides the opportunity to get more feeling for the intraspecies variability in rat and man in inhalation toxicity studies.

ten Berge *et al* (1986) evaluated many inhalation studies in experimental animals. Based on the 18 rat studies in this paper a geometric mean of the GSD of rat intraspecies sensitivity of 1.56 for systemic effects and 1.57 for local effects was determined. The ratio between the LC₉₅ and LC₀₅ was found to be 4.35.

Concentration-mortality response information is generally not available in humans. However, human concentration-response studies for irritation of the respiratory tract are available, but are very few in number. Only a few inhalation studies with human volunteers are known. These were performed with the gases ammonia, chlorine, formaldehyde and sulphur dioxide (Anglen, 1981; Kulle, 1993; Verberk, 1977; Roger *et al*, 1985). The geometric mean of the GSD of the human intraspecies sensitivity was 2.48. The ratio between the EC₉₅ and EC₀₅ was 20.

Hattis *et al* (1999) have assembled a significant database on human variability in parameters representing a series of steps in the pathway from external exposure to the production of biological responses:

- Contact rate (breathing rates/body weight, fish consumption/body-weight).
- Uptake or absorption (mg/kg).
- General systemic availability net of first pass elimination and dilution.
- Systemic elimination or half-life.
- Active site availability/general systemic availability.
- Physiological parameter change/active site availability.

- Functional reserve capacity-change in baseline physiological parameter needed to pass a criterion of abnormal function or to exhibit a response.

On the basis of a weighted analysis, the summary aggregate GSD for human variability for different types of toxicants delivered in different ways was estimated. The GSD for human variability in case of inhaled systemic toxicants causing acute toxicity was estimated to be 2.83. The ratio between the EC₉₅ and EC₀₅ was 31. This is quite close to the 2.48 for local effects, estimated from human concentration response relationships for irritant gases.

The value of the GSD can be used to construct concentration response relationships. The EC₅₀ is set to 1 for rats and humans and the concentration levels with a lower response are estimated by means of the equation below for rat and man. The concentrations and the corresponding response are presented in Table 4-18 below (compare also with Figure 4-7 above).

The above-derived information supports the extrapolation rule for intraspecies extrapolation.

In case of AETL-3a and AETL-3b, a human concentration-duration mortality response relationship is derived in such a way that the human LC₀₁ is 1/10 of the human LC₅₀.

For these reasons when the available data do not allow the derivation of substance-specific factors, a default intraspecies assessment factor of 3 is recommended when deriving AETL-3b, -2 and -1 levels. Intraspecies assessment factors are not recommended when deriving AETL-3a levels.

Table 4-18: Default assessment factors for the derivation of AETL values

| | Type of effect | Interspecies factor* | Intraspecies factor | Total Assessment factor |
|---------|----------------|----------------------|---------------------|-------------------------|
| AETL-3a | Local | 1 | 1 | 1 |
| | Systemic | 3 | 1 | 3 |
| AETL-3b | Local | 1 | 3 | 3 |
| | Systemic | 3 | 3 | 10+ |
| AETL-2 | Local | 1 | 3 | 3 |
| | Systemic | 3 | 3 | 10+ |
| AETL-1 | Local | 1 | 3 | 3 |
| | Systemic | 3 | 3 | 10+ |

* Not to be applied if using human data.

† For simplicity, when using an intraspecies factor of 3 and an interspecies factor of 3, the total assessment factor is 10.

4.5.5.3 Modifying factors (MF)

In the procedures used to conduct human health risk assessments, many expert groups worldwide provide for the possible use, on a case-by case basis, of an additional modifying factor (MF) to account for residual uncertainty. Often, the MF is used to account for uncertainty related to use of a less than complete database or for use of poor quality data. This is a valid concern for development of AETLs. Also, for AETLs, an MF may be used to account for the use of a toxicological effect that is somewhat less serious than described in the AETL definitions.

In cases where the database is reasonably complete and the toxicological endpoint used meets the respective AETL definition, the default MF used is 1. In cases where there are serious deficiencies in the existing database or where the toxicology endpoint used is less serious than implied by the AETL definition, use of an MF of 2 or 3 may be appropriate. As such, appropriate use of the MF reflects a certain degree of professional judgment, including consideration of the magnitude of the overall uncertainty factor used to derive specific

AETLs. A justification should be provided whenever an MF is applied during AETL derivation.

4.5.5.4 Description of subpopulations and additional intraspecies assessment factors

The general statement on sensitive subpopulations and their consideration during the derivation of AETL values can be found in section 3.3.1 ‘Population of Concern’.

AETL values are derived from the population at risk being the general population. Hence, AETL-values do not take into consideration specific sensitive subpopulations. However, it has been decided to inform risk managers about possible sensitive subgroups. Thus, if information on susceptible subpopulations in relation to the substance of interest is available, possible sensitive subgroups are to be identified and described in the TSD. Where possible, the different sensitivity of each subpopulation in question should also be quantified. If quantification is not possible, a description of the subpopulation should be given, thus enabling risk managers to take decisions according to the local situation and the local needs. The decision to include a specific sensitive subpopulation or not in a risk assessment should be made by the end-user of AETL values.

4.5.5.4.1 Description of subpopulations

The following subpopulations may be more sensitive under specific exposure conditions:

Local effects (resulting from exposure to irritant and corrosive chemicals)

A considerable proportion of the chemicals considered for AETL development are irritant or corrosive compounds, which lead to local reactions of the tissues following direct contact. For the mucous membranes of the respiratory tract, these reactions depend on the chemical (physical state: gas/vapours, aerosols, particles; water solubility; reactivity) and on the conditions of the individual subject (anatomy of the respiratory tract; type of breathing: nose/mouth; ventilation rate/physical activity; pre-existing inflammation of the mucous membranes). Whereas local effects in the upper airways do not vary greatly among the general population and hence, no sensitive subpopulation can be identified, reactions in the lower airways can vary to a great extent. Individuals with pre-existing conditions (in particular those with pre-existing inflammation of the mucous membranes of the airways as well as the skin) are at higher risk.

The following subpopulations should be mentioned in the TSD.

Table 4-19: Response of subpopulations to irritant and corrosive chemicals

| Subpopulation | Effect of exposure |
|----------------------------------|--|
| Patients with: | |
| Pre-existing airway obstructions | Swelling of mucous membranes |
| Asthma | Swelling of mucous membranes, bronchoconstriction |
| Pneumonia, chronic lung diseases | Toxic lung oedema, acute inflammation, with permanently reduced lung function; bronchial shedding of epithelial lining, irreversible lung damage |

For the last group of patients with chronic lung diseases, the severity of the local effect might be the same as in healthy subjects, but the consequences of (further) impaired lung function with diminished oxygen supply might be much more severe. Besides these sub-groups with differences in dynamics, kinetic factors may also influence the severity of effects on the respiratory tract. For example, the higher ventilation rates of children may lead to a higher deposition of an inhaled chemical on the mucous membranes on a surface-basis, with higher local concentrations. The implications of this are difficult to evaluate because many factors are involved.

Systemic effects

Higher ventilation as well as kinetic and dynamic factors may lead to higher sensitivity regarding systemic effects. Depending on the conditions of exposure, the following sensitive subpopulations as listed in Table 4-20 have to be considered for the derivation of AETL values:

Table 4-20: Specific systemic effects in subpopulations

| Subpopulation | Effect of exposure |
|---|---|
| The unborn child | Teratogenic/embryotoxic/foetotoxic effects Danger from maternal toxicity (hypotension/shock, diminished oxygen supply) |
| The young child | Systemic effects due to the parent compound (under 1 year of age) (kinetic differences, depending on concentration and duration) Carcinogenicity/mutagenicity (under 2 years of age) Formation of methaemoglobin (under 4 months of age) Formation of toxic metabolites (from about 1 to 6 years of age) <i>If possible from short-term inhalation exposure:</i> Endocrine disruption, impairment of reproductive function (from birth to end of puberty) Neuro-developmental disorders (from birth to few years old) Impairment of the immune function (from birth to few years old) Impaired synthesis of haemoglobin (from birth to few years old) Joint disorders, discoloration of teeth (from birth to end of puberty) |
| Patients with pre-existing organ damage leading to higher susceptibility | |
| Heart disease (HD) | Diminished oxygen supply Hypotension/shock Unspecific: acute stress |
| Cyanotic heart defects | Diminished oxygen supply |
| Cardiac arrhythmia | Induction of cardiac arrhythmia |
| Cardiac insufficiency | Cardiotoxicity, peripheral vasodilatation |
| Hypertension | Peripheral vasoconstriction |
| Anaemia | Diminished oxygen supply Toxic damage of bone marrow |
| Renal insufficiency | Toxic metabolites, which are renally eliminated Nephrotoxicity |
| Polymorphism | In case of 2D6, 2C9/19 metabolism (unimportant for most VOCs) |
| Reduced resistance of red blood cells Diseases with haemolytic crises | Haemolysis |
| Respiratory insufficiency | Diminished oxygen supply (different causes, e.g. asthma, cold, emphysema, pneumonia, embolism, fibrosis) |

| Subpopulation | Effect of exposure |
|--|--|
| Certain immune deficiencies Thrombopoenia, ITP | Toxic damage of bone marrow |
| Current infections | Immune suppression |
| Auto-immune diseases | Irritation of the immune system |
| Allergies | Induction of allergies |
| Porphyria | Induction of porphyria |
| Gout | Increased uric acid |
| Cirrhosis, hepatitis | Hepatic toxicity |
| Gastroenteritis Colitis ulcerosa Crohn disease | Induction of diarrhoea |
| Subileus | Induction of paralysis of intestine |
| Polyneuropathia | Peripheral neurotoxicity |
| Epilepsy | Convulsions |
| Pre-existing brain damage | Central neurotoxicity Diminished oxygen supply Central inhibition of breathing |
| Insufficient cerebral perfusion | Diminished oxygen supply |
| Lifestyle: smokers, alcoholism | Higher concentrations of toxic metabolites |
| Drug treatment: Cytostatica | Toxic damage of bone marrow |

4.5.5.4.2 Additional intraspecies assessment factors for sensitive subpopulations

Ideally, the quantification of the degree of susceptibility of a sensitive subpopulation should be performed on a chemical-by-chemical basis, taking into account the physico-chemical properties and mechanism of action of the toxin and the physiology and/or pathophysiology of the health effect present in the subpopulation. If the estimated sensitivity of a subpopulation cannot be covered by the intraspecies assessment factor used when deriving an AETL value, then an additional intraspecies assessment factor should be applied. The Technical Support Document (TSD) should indicate which sensitive subpopulations should be considered for each AETL level, based on the critical health effect used to derive the AETL value, and what additional intraspecies factor should be applied to protect the subpopulation.

In general, the quantification of the degree of susceptibility that is expected in a specific subpopulation is extremely difficult. This is often due to the fact there are limited or no data assessing the degree of susceptibility. Furthermore, the degree of susceptibility within a specific subpopulation is rarely uniform. For these reasons the degree of susceptibility in a specific subpopulation can often only be estimated. When the available data do not allow the quantification of the degree of susceptibility of a sensitive subpopulation, default values as described in the following four scenarios are recommended.

Systemic effects due to parent compound

Due to higher ventilation rates (on a body weight basis), internal exposure is higher in children. This is especially pronounced in the case of immature metabolism. Therefore, newborns are expected to experience the highest internal exposure, and an additional intraspecies assessment factor of 3 to 5 has to be applied to allow for the differences in kinetics (default case: if no data are available). For children older than 4 weeks, a factor of 2

should be sufficient. This will especially apply to ‘Category 3’ gases (low water solubility and reactivity resulting in high alveolar absorption rates).

Systemic effects leading to diminished oxygen supply

Following different mechanisms, exposure to certain chemicals may result in a diminished oxygen supply due to the formation of methaemoglobin and carboxyhaemoglobin, and due to asphyxiants, haemolysis, and intracellular hypoxia (HCN, H₂S). This leads to a risk of ischemia which is increased in the following subgroups with pre-existing critical local or general oxygen supply: patients with anaemia, ischemic heart disease, insufficient cerebral perfusion, cyanotic heart defects, and respiratory insufficiency. An additional, intraspecies assessment factor of 3 has to be applied to allow for these differences in dynamics.

With respect to methaemoglobin formation, young children up to 4 months of age are more susceptible.

Irritant/corrosive effects on mucous membranes of the lower respiratory tract

Inhalation exposure to irritant/corrosive chemicals (e.g. sulphur dioxide, chlorine) may lead to severe symptoms such as bronchoconstriction and lung oedema.

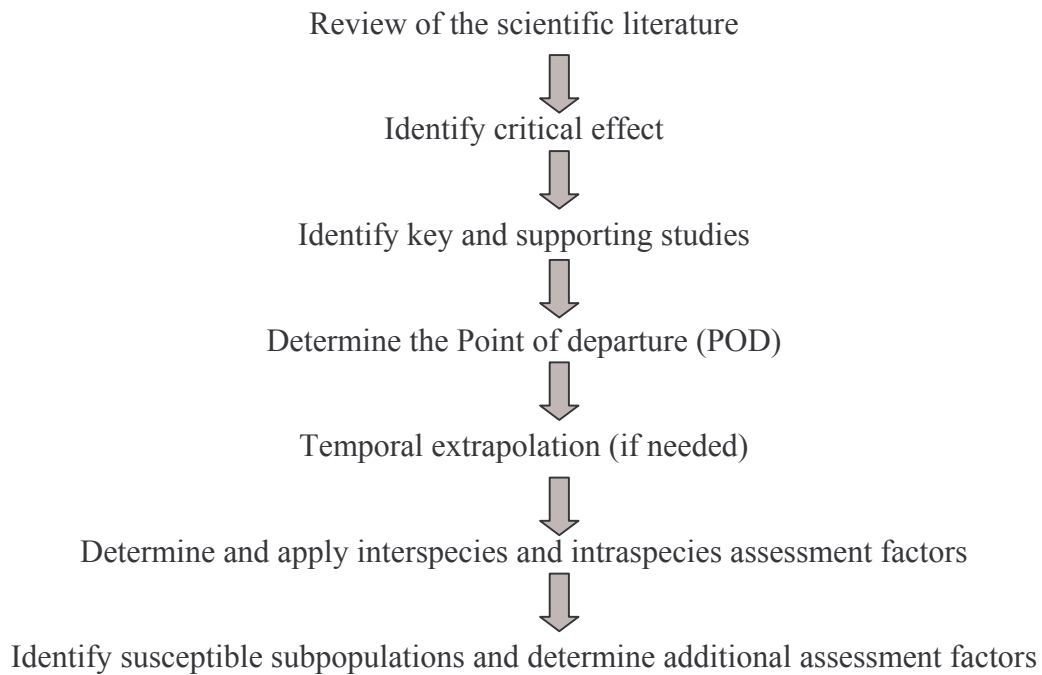
With regard to this exposure scenario, patients with asthma are at higher risk (more than patients with chronic obstructive pulmonary disease). Also all patients with respiratory insufficiency (e.g. severe stage of cystic fibrosis, pneumonia, emphysema, and rare chronic lung diseases like alveolitis, asbestosis, lung fibrosis or broncho-pulmonary dysplasia in former pre-term infants), as well as patients with other diseases leading to a pre-existing critical local or general oxygen supply are at higher risk. An additional intraspecies assessment factor of 5 has to be applied to allow for these differences in dynamics.

Carcinogenic effects (mutagenic mode of action)

If carcinogenic effects are observed following a short-term exposure to chemicals with a mutagenic mode of action (see 4.2.8), an additional intraspecies assessment factor of 10 is recommended in order to protect children under 2 years of age, and a factor of 3 to protect children 2 to 15 years old. This is the conclusion contained within the US EPA, which evaluated the age-dependency of experimental results (US EPA, 2003b). The data used to draw this conclusion (experimental studies in animals) contain a large degree of variability, resulting in a high degree of uncertainty about these factors. The ongoing discussion on this topic should be followed.

4.6 Flow chart of the AETLs development

The different steps followed for the development of each of the AETLs are summarised and presented on the following flow chart:



The derivation of the different AETL levels is presented in more detail in Appendix V.

4.7 Format of documents and supporting documentation (need for further testing)

The Technical Support Document (TSD) presents the review of all relevant data and information obtained from data sources. The document mentions all the key studies and the most important supporting studies and references for human and experimental animal exposures. For all studies, the evaluation of their adequacy for the derivation of AETLs is presented. Additionally, the choice of the key studies has to be justified.

The TSD also addresses the methods used in the derivation of each AETL value for the studied chemical and the appropriate references to the scientific literature or sources of unpublished information.

5 ABBREVIATIONS

| | |
|-----------------|---|
| ACH | Acetylcholine |
| ACUTEX | ACUTe EXposure |
| AEGL | Acute exposure guideline level |
| AETL | Acute exposure threshold level |
| AGW | Alarmeringsgrenswaarde (Alarm boundary values) |
| AIHA | American Industrial Hygiene Association |
| ALT | Alanine-aminotransferase |
| AP | Alcaline phosphatase |
| ASTM | American Society for Testing Materials |
| ATP | Adenosine triphosphate |
| ATS | American Thoracic Society |
| AUC | Area under curve |
| BAL | Bronchoalveolar lavage |
| BALC | Bronchoalveolar lavage cells |
| BALF | Bronchoalveolar lavage fluid |
| BCME | Bis-(chloromethyl)ether |
| BMC | Benchmark concentration |
| BTPS | Body temperature and pressure saturated |
| CA | Competent authority |
| CAS | Chemical Abstracts Service |
| CEEL | Community emergency exposure levels |
| CEN | Comité Européen de Normalisation (EU Committee for Standardization) |
| CFC | Chlorofluorocarbon |
| CNS | Central nervous system |
| CO | Carbon monoxide |
| CO ₂ | Carbon dioxide |
| COHb | Carboxyhaemoglobin |
| CYP2E1 | Cytochrome P450 2E1 |
| DCM | Dichloromethane |
| DECOS | Dutch Expert Committee on Occupational Standards |
| DNA | Deoxyribonucleic acid |
| DOE | Department of Energy (US) |
| DRCF | Dose-rate correction factor |
| DTL | Dangerous toxic load |
| EI | Emergency exposure index |
| EPA | Environmental Protection Agency (US) |
| EPEL | Eenmalige populatie expositie limiet (one time population exposure limit) |
| ERPG | Emergency response planning guidelines |
| FED | Fractional effective dose |
| FEF | Forced expired flow |
| FEV | Forced expired volume |

| | |
|-------------------------------|--|
| FOB | Functional observation battery |
| FVC | Forced vital capacity |
| G-6-PD | Glucose-6-phosphatase dehydrogenase |
| GI | Gastrointestinal |
| GSD | Geometric standard deviation |
| GSH | Glutathione |
| GST | Glutathione-S-transferase |
| GSTT1 | Glutathione-S-transferase theta 1 |
| H ₂ S | Hydrogen sulphide |
| Hb | Haemoglobin |
| HCl | Hydrochloric acid |
| HCN | Hydrogen cyanide |
| HFC | Hydrofluorocarbon |
| HCFC | Hydrochlorofluorocarbon |
| HSE | Health and Safety Executive (UK) |
| HSPA | Hydrocarbon Solvents Producers Association |
| IDLH | Immediately dangerous to life and health |
| INERIS | Institut National de l'Environnement Industriel et des Risques |
| INRS | Institut National de Recherche et de Sécurité |
| IPCS | International Programme on Chemical Safety |
| IPSN | Institut de Protection et de Sûreté Nucléaire |
| JMPR | Joint FAO/WHO Meeting on Pesticide Residues |
| LBW | Levensbedreigende waarde (life threatening value) |
| LC | Lethal concentration |
| LOAEL | Lowest observed adverse effect level |
| LOEL | Lowest observed effect level |
| LDSA | Level of distinct sensory awareness |
| MAS | Maximum average score |
| MetHb | Methaemoglobin |
| MF | Modifying factors |
| MLE | Maximum likelihood estimate |
| MTD | Maximum tolerated dose |
| N ₂ | Nitrogen |
| NAC | US National Advisory Committee |
| NADH | Nicotinamide adenine dinucleotide |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NIOSH | National Institute for Occupational Safety and Health (US) |
| NLM | National Library of Medicine |
| N ₂ O | Nitrogen monoxide |
| N ₂ O ₂ | Nitrogen dioxide |
| NOAEL | No observed adverse effect level |
| NOEL | No observed effect level |
| NRC | National Research Council (US) |
| NTE | Neurotoxic esterase |

| | |
|------------------|---|
| OECD | Organisation for Economic Co-operation and Development |
| OEHHA | Office of Environmental Health Hazard Assessment |
| OEL | Occupational exposure limit |
| PAH | Polycyclic aromatic hydrocarbons |
| PBPK | Physiologically-based pharmacokinetic (models) |
| PCB | Polychlorinated biphenyl |
| PEF | Peak expiratory flow |
| P _{enh} | Enhanced pause |
| PIF | Peak inspiratory flow |
| PII | Primary irritation index |
| PLIP | Phospholipid inositol phosphatase |
| PMN | Polymorphonuclear neutrophils |
| POD | Point of departure |
| QRA | Quantitative risk assessment |
| RADS | Reactive airways dysfunction syndrome |
| RBC | Red blood cells |
| RD | Respiratory depression |
| RMV | Respiratory minute volume |
| RT | Relaxation time |
| RUDS | Reactive upper-airways dysfunction syndrome |
| SCHER | Scientific Committee on Health and Environmental Risks |
| SCN ⁻ | Thiocyanate |
| SCOEL | European Scientific Committee of Occupational Exposure Limits |
| SEI | Seuil des effets irréversibles (irreversible effects threshold) |
| SEL | Seuil des effets létaux (lethal effects threshold) |
| SER | Seuil des effets réversibles (reversible effects threshold) |
| SLOD | Significant likelihood of death |
| SLOT | Specified level of toxicity |
| SO ₂ | Sulphur dioxide |
| SOPs | Standing operating procedures |
| SP | Seuil de perception (perception threshold) |
| STT | Saccharin transport time |
| TCDD | 2,3,7,8-tetrachlorodibenzo-p-dioxin |
| Te | Expiratory time |
| TEEL | Temporary emergency exposure levels |
| TF | Task force |
| TGD | Technical guidance document |
| Ti | Inspiratory time |
| TSD | Technical support document |
| VC | Vital capacity |
| VOC | Volatile organic compounds |
| VRW | Voorlichtingsrichtwaarde (instruction guidance values) |
| VSD | Virtually safe dose |
| WHO | World Health Organization |

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Appendix I: Available methodologies

Table of Commonly Used Acute Exposure Values

| <i>Types of Exposure Values Applied in EU Competent Authorities for Seveso Implementation</i> |
|--|
| <p>Acute Exposure Guideline Levels (AEGL), developed by the US National Advisory Committee on AEGLs (NAC/AEGLs), managed by the US EPA</p> <p><u>AEGL-1</u>: the airborne concentration (ppm) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.</p> <p><u>AEGL-2</u>: the airborne concentration (ppm) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.</p> <p><u>AEGL-3</u>: the airborne concentration (ppm) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.</p> <p>Exposure duration periods: 10 minutes, 30 minutes, 1 hour, 4 hours, and 8 hours.</p> |
| <p>Emergency Response Planning Guidelines (ERPG), developed by the American Industrial Hygiene Association (AIHA)</p> <p><u>ERPG-1</u>: the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 hour without experiencing more than mild, transient adverse health effects or without perceiving a clearly defined objectionable odour.</p> <p><u>ERPG-2</u>: the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 hour without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protective action.</p> <p><u>ERPG-3</u>: the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 hour without experiencing or developing life-threatening health effects.</p> <p>Exposure duration period: 1 hour.</p> |
| <p>Emergency Exposure Indices (EEI), developed by ECETOC</p> <p><u>EEI-1</u>: the maximum airborne concentration below which the exposed population is not likely to suffer discomfort.</p> <p><u>EEI-2</u>: the maximum airborne concentration below which the exposed population is not likely to suffer irritation.</p> <p><u>EEI-3</u>: the airborne concentration below which the exposed population is not likely to be incapacitated.</p> <p>Exposure duration: 15, 30 and 60 minutes.</p> |
| <p>Temporary Emergency Exposure Levels (TEEL), developed by the U.S. Department of Energy</p> <p><u>TEEL-0</u>: the threshold concentration below which most people will experience no appreciable risk of health effects.</p> <p><u>TEEL-1</u>: the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing other than mild transient adverse health effects or perceiving a clearly defined objectionable odour.</p> <p><u>TEEL-2</u>: the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing irreversible or other serious health effects or symptoms that could impair their abilities to take protective action.</p> <p><u>TEEL-3</u>: the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing life-threatening health effects.</p> <p>Exposure duration period: 15 minutes (for concentration-dependent chemicals), 60 minutes (for dose-dependent chemicals).</p> |

SEL and SEI (Threshold of Lethal Effects and Threshold of Irreversible Effects), developed by the French Ministry of Environment, INERIS, INRS, IPSN, University Hospitals, and Industry

The 'Lethal Effects Threshold' (SEL) corresponds to a concentration for a given exposure period above which mortality can be observed in the exposed population.

The 'Irreversible Effects Threshold' (SEI) corresponds to a concentration for a given exposure period above which irreversible effects may appear in the exposed population.

The 'Reversible Effects Threshold' (SER) corresponds to a concentration for a given exposure period above which reversible effects may appear in the exposed population.

The 'Perception Threshold' (SP) corresponds to a concentration that leads to a sensorial detection of the chemical substance by the exposed population.

Exposure duration periods: 1, 10, 20, 30 and 60 minutes as well as 2, 4 and 8 hours.

Dutch Intervention Guidelines (1999)

Life threatening value (Levensbedreigende waarde - LBW): the concentration of a substance above which death or a life threatening condition may develop within a few days after an exposure of one hour.

Alarming threshold (Alarmeringsgrenswaarde - AGW): the concentration of a substance above which irreversible or other serious health impairment may occur as a result of acute toxic effects after an exposure of one hour.

Communication guideline value (Voorlichtingsrichtwaarde - VRW): the concentration of a substance at which with a high level of probability will be perceived by the majority of the exposed population as hindrance or above which minor, quickly reversible health effects may occur after an exposure of one hour. Often this is the concentration at which exposed people start to complain about the perceived exposure.

Exposure duration period: 1 hour.

Dutch Commissie Preventie van Rampen (CPR)

For use in quantitative risk assessment studies a LC₅₀ for humans is derived from animal studies for an exposure period of 30 minutes.

No exposure duration period, but rather a probit function for concentration-time-endpoint for a specified time range.

Dangerous Toxic Load (DTL), developed by the UK Health and Safety Executive

Specified Level of Toxicity (SLOT): the airborne concentration level at which almost everyone in the exposed area is likely to suffer severe distress, a substantial fraction of which will require medical attention, and some people will be seriously injured, requiring prolonged treatment. For highly susceptible people, the possibility exists that they will be killed.

Significant Likelihood of Death (SLOD): the airborne concentration level at which the mortality of 50% of an exposed population is predicted.

No exposure duration period, but rather a probit function for concentration-time-endpoint for a specified time range.

Appendix II: Glossary of Definitions

Adverse effect:

An adverse effect is an unfavourable effect that may negatively impact the health of the individual. For more details, see ECETOC report no. 85 (ECETOC, 2002).

‘At which’:

No fine line can be drawn to precisely differentiate between a ceiling level, which represents the highest exposure concentration at which an effect is unlikely to occur, and a threshold level which represents the lowest concentration for the onset of effects. AETLs should therefore be considered as threshold levels that represent an estimated point of transition between one defined set of symptoms or adverse health effects and another set of more serious symptoms or adverse health effects.

General population:

The general population includes all persons that may potentially be exposed.

Impaired ability to take action or escape:

Persons are disabled by the effects of the exposure and cannot take actions necessary to protect themselves or escape safely.

Irreversible adverse health effect:

An adverse health effect as a consequence of exposure that is considered to be permanent and is expected not to heal or return to normal function.

Life threatening health effect:

A health effect as a consequence of exposure that could lead to death.

Medical attention:

Needs expert judgement and observation.

Medical intervention:

Medical treatment that can significantly and positively influence the outcome and duration of the acquired illness or condition.

Mild adverse health effect:

An adverse health effect the severity of which will not require medical attention. Example: mild skin irritation.

Reversible adverse health effect:

An adverse health effect as a consequence of exposure that heals or returns to normal function over time with or without medical intervention.

Sensory awareness:

Effects that are noticed by the senses of the exposed person e.g. by means of smell, taste or other sensations.

Serious health effect:

Health effects that are not life threatening but more severe than mild effects. Examples are: serious effects on the liver, kidney, respiratory tract, and nervous system dysfunction and blood disorders.

Susceptible subpopulation:

There are groups in the general population that can be considered more susceptible than the 'normal' population representing the healthy middle-aged adults. Which subpopulation is more sensitive depends on the particular chemical (no subpopulation is more susceptible *per se*). For details see sections 2.5.2 and 4.5.5.4.

Appendix III: AETL definitions and comparison with existing definitions

AETL-3a: the airborne concentration at which it is predicted that, after a specified exposure time, a certain percentage of the general population* will die (*see definitions).

- SLOD DTL (UK): the airborne concentration level at which the mortality of 50% of an exposed population is predicted.
- (NL): for use in quantitative risk assessment studies an LC₅₀ for humans is derived from animal studies for an exposure period of 30 minutes.
- SEL: the 'lethal effects threshold' (1%) corresponds to a concentration for a given exposure period above which mortality can be observed in the exposed population.

AETL-3b: the maximum airborne concentration at which it is predicted the general population* could be exposed up to a specified exposure time without experiencing life threatening health effects or death (*see definitions).

- ERPG-3: the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 hour without experiencing or developing life-threatening health effects.
- AEGL-3: the airborne concentration (expressed as ppm or mg/m³) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.
- EEI-3: the airborne concentration for exposures lasting up to a specified exposure time (t₁) below which direct toxic effects are unlikely to lead to death/permanent incapacity in the exposed population (including susceptible but excluding hypersusceptible groups) and above which, as the concentration increases death/permanent incapacity would be increasing more.
- LBW: the 'Life threatening value' (levensbedreigende waarde - LBW) is the concentration of a substance above which death or a life threatening condition may develop within a few days after an exposure of one hour.

AETL-2: the maximum airborne concentration at which it is predicted the general population* could be exposed up to a specified exposure time without experiencing or developing irreversible or other serious adverse health effects including symptoms that could lead to impairment to escape (*see definitions).

- ERPG-2: the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 hour without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protective action.
- AEGL-2: the airborne concentration (ppm) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.
- EEI-2: the airborne concentration for exposures lasting up to a specified exposure time (t₁) below which direct toxic effects are unlikely to lead to disability in the exposed population (including susceptible but excluding hypersusceptible groups) and above

which, as the concentration increases disability would become increasingly more common.

- SEI: the 'Irreversible Effects Threshold' corresponds to a concentration for a given exposure period above which irreversible effects may appear in the exposed population.
- Alarming Threshold (alarmeringsgrenswaarde - AGW): the concentration of a substance above which irreversible or other serious health impairment may occur as a result of acute toxic effects after an exposure of one hour.
- SLOT: the airborne concentration level at which almost everyone in the exposed area is likely to suffer severe distress, a substantial fraction of which will require medical attention, and some people will be seriously injured, requiring prolonged treatment. For highly susceptible people, the possibility exists that they will be killed.

AETL-1: the maximum airborne concentration at which it is predicted the general population* could be exposed up to a specified exposure time without experiencing more than mild and reversible adverse health effects (*see definitions).

- ERPG-1: the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 hour without experiencing more than mild, transient adverse health effects or without perceiving a clearly defined objectionable odour.
- AEGL-1: the airborne concentration (ppm) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.
- EEI-1: the airborne concentration for exposures lasting up to a specified exposure time (t_1) below which direct toxic effects are unlikely to lead to discomfort in the exposed population (including susceptible but excluding hypersusceptible groups) and above which, as the concentration increases discomfort would become increasingly more common.
- SER: the 'reversible effects threshold' corresponds to a concentration for a given exposure period above which reversible effects may appear in the exposed population.
- VRW: communication guideline value (voorlichtingsrichtwaarde): The concentration of a substance at which, with a high level of probability, will be perceived by the majority of the exposed population as hindrance or above which minor, quickly reversible health effects may occur after an exposure of one hour. Often this is the concentration at which exposed people start complaining about the perceived exposure.

Level of Distinct Sensory Awareness (LDSA): the airborne concentration at which it is predicted that a proportion of the general population* could experience sensory stimuli (e.g. odour) that may lead to public complaints, concerns or even panic (*see definitions).

- ERPG: this level currently does not exist.
- AEGL: in recent years, the US AEGL committee has been considering to add this level to their system. Within the AEGL methodology the odour level plays a key role. It is referred to as 'level of odour annoyance'. There is no mention of other stimuli that may trigger a level of awareness. The formal procedures to be used for setting this level still need to be finalised and have not yet been published.
- EEI: this level was not defined in this system.
- SP: the 'perception threshold' corresponds to a concentration that leads to a sensorial detection of the chemical substance by the exposed population.

Appendix IV: Systems for Grading for Eye Irritation Effects

Friendenwald *et al* (1944) published a “numerical estimation of the severity of lesions produced in the cornea of rabbit’s eyes by action of corrosive agents”. In addition to the factors listed in Table A-1, this scheme assigned grades, each with a maximum of 4, for the area of cornea affected and conjunctival discharge. It also rated four other parameters related to corneal effects. The maximum score (intensity of corneal opacity rating a maximum of 8, calculated by simple addition) was 40 with maximal corneal effects contributing 70% of the maximum.

Draize *et al* (1944) modified this scheme to make it more generally applicable by using a weighted scoring system involving multiplication factors as shown in Table A-2. The contribution of maximal corneal effects to the total score is 73%.

Table A-1: Grading scale for eye irritation effects according to EC and US Regulatory Agencies following OECD Guideline 405 (OECD, 2002b)

| I | CORNEA | Grade |
|-----|--|-------|
| | Opacity – degree of density (area most dense taken for reading): | |
| | No opacity | 0 |
| | Scattered or diffuse area, details of iris slightly obscured | 1 |
| | Easily discernible translucent areas, details of iris slightly obscured | 2 |
| | Opalescent areas, no details of iris visible, size of pupil barely discernible | 3 |
| | Opaque, iris not visible | 4 |
| II | IRIS | |
| | Values: | |
| | Normal | 0 |
| | Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combinations of any thereof), iris still reacting to light (sluggish reaction is positive) | 1 |
| | No reaction to light, haemorrhage, gross destruction (any or all of these) | 2 |
| III | CONJUNCTIVAE | |
| | <u>Redness</u> (refers to palpebral conjunctivae only): | |
| | Vessels normal | 0 |
| | Vessels definitely injected above normal | 1 |
| | More diffuse, deeper crimson red, individual vessels not easily discernible | 2 |
| | Diffuse beefy red | 3 |
| | <u>Chemosis</u> (Oedema): | |
| | No swelling | 0 |
| | Any swelling above normal (includes nictitating membrane) | 1 |
| | Obvious swelling with partial eversion of lids | 2 |
| | Swelling with lids about half closed | 3 |
| | Swelling with lids about half closed to completely closed | 4 |

Table A-2: Grading Scale and Multiplication Factors as used by Draize *et al*, 1944

| I | CORNEA | Grade |
|----|--|--------------------|
| | A Opacity - degree of density (area most dense taken for reading): | |
| | No opacity | 0 |
| | Scattered or diffuse area, details of iris slightly obscured | 1 |
| | Easily discernible translucent areas, details of iris slightly obscured | 2 |
| | Opalescent areas, no details of iris visible, size of pupil barely discernible | 3 |
| | Opaque, iris invisible | 4 |
| | B Area of cornea involved: | |
| | One quarter (or less) but not zero | 1 |
| | Greater than one quarter but less than half | 2 |
| | Greater than half but less than three quarters | 3 |
| | Greater than three quarters, up to whole area | 4 |
| | SCORE (AxB) x 5 | TOTAL MAXIMUM = 80 |
| II | IRIS | |
| | C Values: | |
| | Normal | 0 |
| | Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combinations of any thereof), iris still reacting to light (sluggish reaction is positive) | 1 |
| | No reaction to light, haemorrhage, gross destruction (any or all of these) | 2 |
| | SCORE C x 5 | TOTAL MAXIMUM = 10 |
| II | CONJUNCTIVAE | |
| | D Redness (refers to palpebral conjunctivae only): | |
| | Vessels normal | 0 |
| | Vessels definitely injected above normal | 1 |
| | More diffuse, deeper crimson red, individual vessels not easily discernible | 2 |
| | Diffuse beefy red | 3 |
| | E Chemosis (Oedema): | |
| | No swelling | 0 |
| | Any swelling above normal (includes nictitating membrane) | 1 |
| | Obvious swelling with partial eversion of lids | 2 |
| | Swelling with lids about half closed | 3 |
| | Swelling with lids about half closed to completely closed | 4 |
| | F Discharge: | |
| | No discharge | 0 |
| | Any amount of difference from normal (does not include small amounts observed in inner canthus of normal animals) | 1 |
| | Discharge with moistening of the lids and hairs just adjacent to lids | 2 |
| | Discharge with moistening of the lids and hairs, and considerable area around the eye | 3 |
| | SCORE (D + E + F) x 2 | TOTAL MAXIMUM = 20 |
| | MAXIMUM POSSIBLE SCORE = 80 + 10 + 20 = 110 | |

For the scoring, the eyes are examined at 1, 24, 48 and 72 hours. If there is no evidence of irritation at 72 hours, the study may be ended. Extended observation may be necessary if there is persistent corneal involvement or other ocular irritation in order to determine the progress of the lesions and their reversibility or irreversibility. The eye irritation potential of chemicals is often summarised as the 'Maximum Average Score' (MAS). The MAS is obtained by

averaging the individual animal weighted scores at each time of observation (e.g. 24 hours, 48 hours, etc.) and then selecting the highest (maximum) of these averages.

The two categories of classification for irritancy to eyes based on animal tests are:

Substances causing significant ocular lesions:

Substances and preparations which, when applied to the eye of the animal, cause significant ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours.

Ocular lesions are significant if the mean scores of the eye irritation test have any of the following values:

- Cornea opacity equal to or greater than 2 but less than 3;
- Iris lesion equal to or greater than 1 but not greater than 1.5;
- Redness of the conjunctivae equal to or greater than 2.5;
- Redness of the conjunctivae (chemosis) equal to or greater than 2;

or, the value should be equal to or greater than 2.5, when the test has been completed on three animals if the lesions on two or more animals are equivalent to any of the above values except that of the iris lesion.

Substances causing severe ocular lesions

Substances and preparations which, when applied to the eye of the animal, cause severe ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours.

Ocular lesions are severe if the mean values of the scores of the eye irritation test have any of the values:

- cornea opacity equal to or greater than 3;
- iris lesion greater than 1.5.

The same applies when the test has been completed using three animals if these lesions, on two or more animals, have any of the values:

- cornea opacity equal to or greater than 3;
- iris lesion equal to 2.

In this scoring system, ocular lesions are also considered severe when they are still present at the end of the observation time (21 days) and if the substance or preparation causes irreversible coloration of the eyes.

The following severe ocular responses, if seen in any animal and clearly treatment-related, warrant termination of a study and considering the substance as causing severe ocular lesions:

- corneal perforation or significant corneal ulceration including staphyloma;
- blood in the anterior chamber of the eye;
- corneal opacity (grade 4 as defined in the EU test method) which persists for 48 hours;
- absence of a light reflex (iridial response grade 2 as defined in the EU test method) which persists for 72 hours;

- ulceration of the conjunctival membrane;
- necrosis of the conjunctivae or nictitating membrane;
- sloughing.

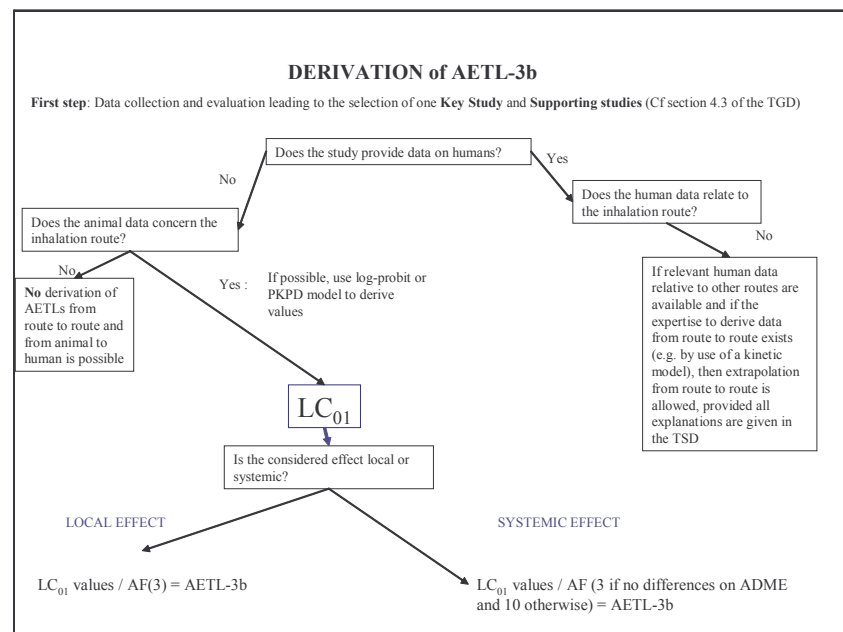
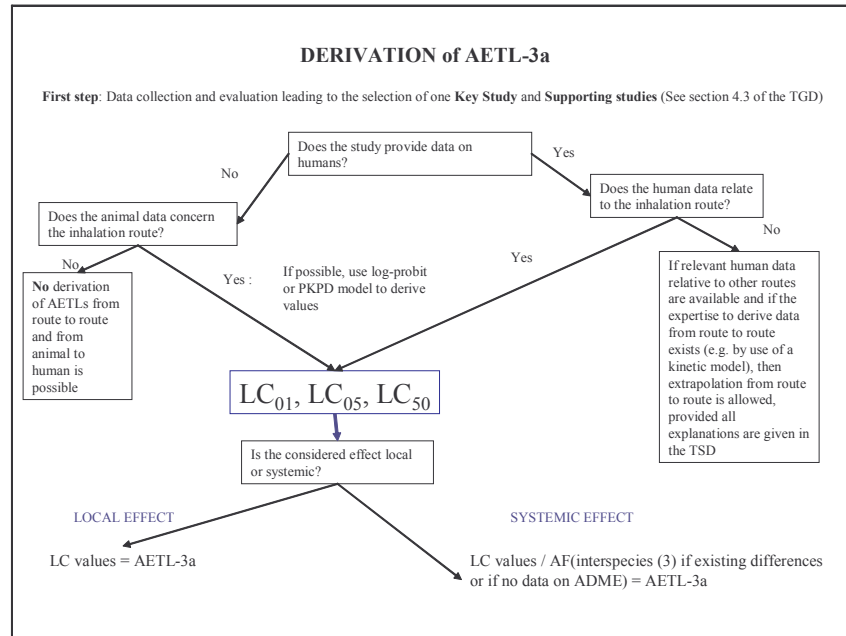
Reversibility of effects

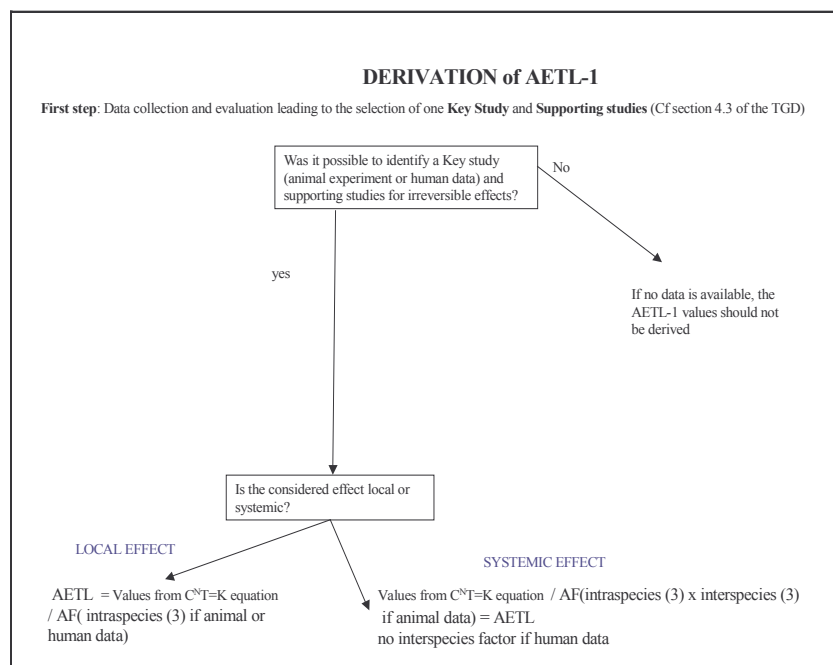
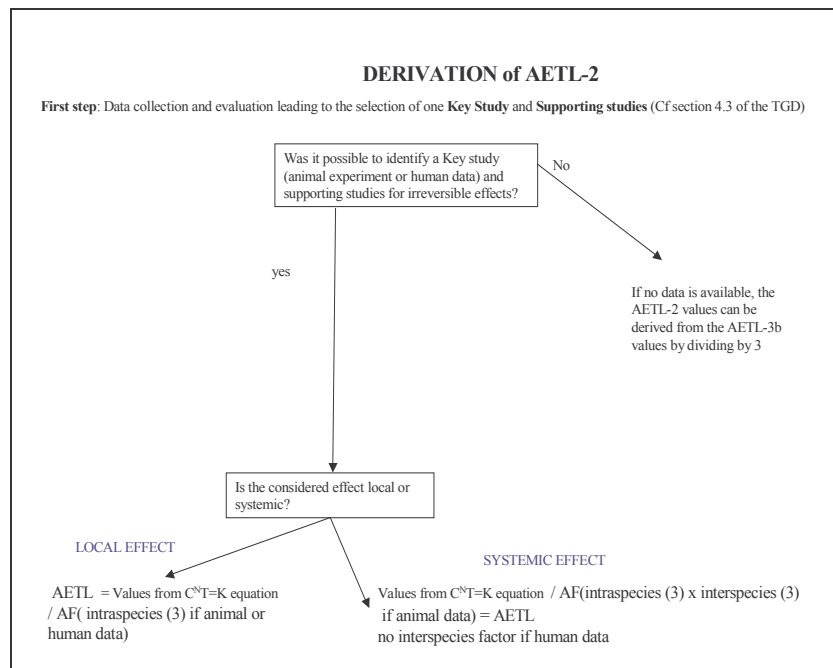
EU classification criteria stipulate no maximum duration for the observation period. In some situations, extension of the observation period beyond the typical 21-day observation is necessary to clarify the reversibility or irreversibility of a persistent lesion. For example:

- Persistence of a low grade corneal lesion (i.e. score 1) to 21 days after instillation, in the absence of other lesions such as neovascularisation;
- More marked corneal lesions (i.e. score >1) within 72 hours of exposure that show clear evidence of continuing recovery and do not disappear entirely by day 21.

It is suggested that a two-week extension of the typical 21-day observation period is sufficient to determine the reversibility of these effects. If full recovery is demonstrated in all animals by the end of that observation period, the effect should not be considered irreversible.

Appendix V: Derivation of AETLs





DERIVATION of LDSA

First step: Data collection and evaluation leading to the selection of one **Key Study** and **Supporting studies** (Cf section 4.3 of the TGD)

