Institute for Health and Consumer Protection

European Chemicals Bureau

Technical Guidance Document on Risk Assessment

in support of

Commission Directive 93/67/EEC
on Risk Assessment for new notified substances

Commission Regulation (EC) No 1488/94
on Risk Assessment for existing substances

Directive 98/8/EC
of the European Parliament and of the Council
concerning the placing of biocidal products on the market

Part I

TGD

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EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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European Commission

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FOREWORD

I am pleased to present this Technical Guidance Document which is the result of in-depth co-operative work carried out by experts of the Member States, the Commission Services, Industry and public interest groups. This Technical Guidance Document (TGD) supports legislation on assessment of risks of chemical substances to human health and the environment. It is based on the Technical Guidance Document in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and the Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances, published in 1996. This guidance was refined taking into account the experience gained when using it for risk assessments of about 100 existing substances and hundreds of new substances. Furthermore, it has been extended to address some of the needs of the Biocidal Products Directive (Directive 98/8/EC of the European Parliament and of the Council).

Concerning Chapter 2 on Risk assessment for human health, the Exposure assessment (Assessment of workplace exposure and Consumer exposure assessment) as well as the Effects assessment were improved and refined. However, for the following sections the revision process is not yet finalised and thus, the current TGD version uses the previous text: section 2.4 on Assessment of indirect exposure via the environment and section 4 on Risk characterisation. These sections are expected to be available by the end of 2003.

With respect to Chapter 3 on Environmental risk assessment, the Environmental exposure assessment and the Effects assessment underwent major improvements. A new chapter on Marine risk assessment was added.

Concerning Chapter 7, five out of eight available Emission scenario documents (ESDs) were revised (IC-3 Chemical industry: Chemicals used in synthesis, IC-7 Leather processing industry; IC-8 Metal extraction industry, refining and processing industry; IC-10 Photographic industry; IC-13 Textiles processing industry). Furthermore, a document on Rubber industry (IC-15) and a number of ESDs for the Biocidal Product Types or parts thereof were added. Some of the Emission scenario documents are still subject to on-going consultation in the OECD and thus, may need to be revised at a later stage. In addition, ESDs to cover all 23 Biocidal Product Types are under development. Consequently, it is anticipated that the set of Emission scenario documents will be continuously expanding in the future.

The White Paper outlining a future chemicals policy was adopted in February 2001 by the Commission. This TGD is therefore to be used in support of the current legislative instruments as described above until they are revoked and replaced by the future legislation implementing the White Paper.

I hope you will agree that this TGD makes a valuable contribution to the development and harmonisation of risk assessment methodologies not only within the Community but also worldwide in the context of the activities of the Organisation of Economic Co-operation and Development and the WHO/ILO International Programme on Chemical Safety.

Ispra, April 2003

Kees van Leeuwen
Director
Institute for Health and Consumer Protection

OVERVIEW

This Technical Guidance Document is presented in four separate, easily manageable parts.

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Chapter 1

GENERAL INTRODUCTION

CONTENTS

1 GENERAL INFORMATION

1.1 LEGISLATIVE BACKGROUND

Council Directive 67/548/EEC (as amended for the seventh time by Directive 92/32/EEC) on the approximation of the laws, regulations, administrative provisions relating to the classification, packaging and labelling of dangerous substances requires the manufacturer or importer of a new substance, before they place it on the market, to notify it to the competent authority of the Member State in which it is manufactured or into which it will be imported. Having received the notification, the competent authority is required to carry out an assessment of the risks of the substance to man and the environment in accordance with the principles set out in Commission Directive 93/67/EEC on risk assessment for new notified substances.

The Council Regulation (EEC) No. 793/93 on the evaluation and control of existing substances requires under Article 10 the real or potential risk for man and environment of priority substances to be assessed using principles which have been laid down in the Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances. The risk assessments are carried out by competent authorities designated by the responsible Member States to act as rapporteurs.

Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market requires that a biocidal product is only authorised if, besides other requirements, the active substances used in the product are listed in Annex I or Annex IA of the Directive. Active substances may enter Annex I (List of active substances with requirements agreed at community level for inclusion in biocidal products), Annex IA (List of active substances with requirements agreed at community level for inclusion in low-risk biocidal products), or Annex IB (list of basic substances with requirements agreed at community level) after the risk assessment if they fulfil the requirements of the directive. For existing biocidal active substances, which were on the market before 14th May 2000 a 10-year-review programme according to Art 16 (2) of Directive 98/8 was initiated with Regulation 1896/2000. This review programme will be the main task under the Biocidal Products Directive for the next years.

This guidance supporting the risk assessment legislation, is based on the Technical Guidance Document (TGD) in support of the Directive 93/67 and the Regulation 1488/94, published in 1996. This guidance was subject to further refinement taking into account the experience gained and is extended to the needs of biocides. Facing the fact that in several cases the processes and use of biocides may be very specific, additional scenarios describing emissions of biocides from those processes are still being developed. Such scenarios allow for quantitative emission estimation, which is an important first step in the exposure assessment of biocides, and generally has a significant influence on the outcome of risk assessments.

The environmental risk assessment (exposure, effects and risk characterisation) and the human health effects assessment of the biocidal active substance will follow the methodologies as described in the present TGD. The exposure assessment for humans via the biocidal products is being elaborated in a separate guidance (document expected on 2002) and the guidance for risk characterisation for humans shall be given in the TNsG for Annex I inclusion.

A separate document provides guidance on the preparation of the recommendations for risk reduction measures for existing substances (Guidance Document on Risk Reduction Strategy and Risk Benefit Analysis).

1.2 WHOM THE GUIDANCE IS FOR

This set of technical guidance documents is intended for use by the competent authorities appointed by Member States under the provisions of Directive 67/548, Regulation 793/93 and Directive 98/8. It is issued by the European Commission (DG JRC) to help competent authorities to carry out the risk assessments on new notified substances, existing substances and on biocidal active substances or a substance of concern present in a biocidal product.

This guidance is also intended to be useful for notifiers of new substances as well as for applicants of a risk assessment of a biocidal active substance and for those manufacturers and importers who are obliged under the provisions of Regulation 793/93 to submit all relevant information for the risk assessments and to fulfil any request for further information or testing as a consequence of a risk assessment. It should help them to understand how the risk assessments are conducted and how decisions on their conclusions/results are taken. In particular, there are clear indications of the circumstances which may give rise to a request for further testing/further information.

This guidance may also be useful for other bodies interested in risk assessment inside and outside the European Union, i.e. non-governmental organisations which may be involved in the risk assessment or international fora with programmes on chemicals safety like OECD or IPCS.

1.3 WHY THE GUIDANCE IS NEEDED

The general principles for the risk assessment of new substances, existing substances and biocidal active substances or substances of concern present in a biocidal product as laid down in Directive 93/67, Regulation 1488/94 and Directive 98/8, respectively, do not include extensive technical detail for conducting hazard identification, dose (concentration) - response (effect) assessment, exposure assessment and risk characterisation in relation to human health and the environment. This guidance which has been produced with the assistance and endorsement of Member States, provides supplementary technical detail. The guidance is not legally binding, and the competent authorities may use other methods or approaches if they are more appropriate, provided that they are scientifically justified and compatible with the general principles laid down in Directive 93/67, Regulation 1488/94 or Directive 98/8. When other methods are used, the methods, including any assumptions, uncertainties and calculations, should be clearly described and justified.

The technical procedures relevant to the different aspects of risk assessment which are described in this guidance may, where appropriate, be subject to further refinement and development in the future.

2 GENERAL PRINCIPLES OF RISK ASSESSMENT

The risk assessment process, in relation to both human health and the environment, entails a sequence of actions which is outlined below.

(1) Assessment of effects, comprising

   (a) hazard identification: identification of the adverse effects which a substance has an inherent capacity to cause; and

   (b) dose (concentration) - response (effects) assessment: estimation of the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect, where appropriate.

(2) Exposure assessment: estimation of the concentrations/doses to which human populations (i.e. workers, consumers and man exposed indirectly via the environment) or environmental compartments (aquatic environment, terrestrial environment and air) are or may be exposed.

(3) Risk characterisation: estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a substance, and may include "risk estimation", i.e. the quantification of that likelihood.

While a risk assessment containing all steps must be carried out for all priority existing substances and biocidal active substances, the risk assessment process in relation to a particular effect or property can be stopped for notified new substances when the hazard identification related to that effect/property did not lead to classification in accordance with Directive 67/548 and if there are no other reasonable grounds for concern. Where investigations of an effect have not yet been conducted, for new substances the risk assessment does not need to consider this effect unless there is cause for concern.

Having followed the required steps, assessors will come to conclusions separately for human health and the environment which will, in a subsequent step, be reviewed and integrated in relation to the totality of risks posed by the substance. These overall conclusions/results will include one or more of the following conclusions/results:

Possible conclusions of the risk assessment for notified new substances (according to Article 3 of Directive 93/67):

(i) The substance is of no immediate concern and need not be considered again until further information is made available in accordance with Article 7(2), 8(3), 8(4) or 14(1) of Directive 67/548.

(ii) The substance is of concern and the competent authority shall decide what further information is required for revision of the assessment, but shall defer a request for that information until the quantity placed on the market reaches the next tonnage threshold as indicated in Article 7(2), 8(3) or 8(4) of Directive 67/548.

(iii) The substance is of concern and further information should be requested immediately.

(iv) The substance is of concern and the competent authority should immediately make recommendations for risk reduction.

Possible results of the risk assessment for existing substances (according to Article 10 of Regulation 793/93 and as extracted from Annex V of Regulation 1488/94):

(i) There is need for further information and/or testing.

(ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

(iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Possible results of the risk assessment for active biocidal substances (according to Article 11 of Directive 98/8):

- Recommendation of an inclusion of the active substance in Annex I, IA or IB (the inclusion shall, where appropriate, be subject to certain requirements).
- Recommendation of a non-inclusion of the active substance in Annex I, IA or IB.

Figure 1 illustrates schematically the principles described above.

Risk assessment is an iterative process for new and existing substances. For new substances an initial assessment of risk is made at the time of the first notification and the assessment is re-addressed and may be revised in the light of any further information on the properties of the substance and/or on exposure, whenever such information becomes available. Further information may be supplied in response to requests of the competent authorities as an outcome of the risk assessment; or it may be supplied when the next tonnage threshold is reached, other relevant changes occur or new relevant knowledge becomes available in response to requirements under Articles 7(2), 8(3), 8(4) or 14(1) of Directive 67/548.

Also for existing substances a risk assessment needs to be reviewed and, where necessary, be revised when new information is submitted by the manufacturer(s) and importer(s) following the request for further data as an outcome of the risk assessment according to Article 10 (2) of Regulation 793/93.

If risk reduction measures are appropriate, any recommendations for risk reduction of new substances shall, according to Article 3(4) and Annex V of Directive 93/67, be part of the risk assessment. However, for existing substances any risk reduction strategy will in compliance with Article 10(3) of Regulation 793/93 have to be submitted separately. Where, on the basis of the risk assessment and recommendations for risk reduction, particular control measures need to be applied, these shall be proposed under the appropriate EU legislation.

Directive 98/8 states in Article 10 that in the light of current scientific and technical knowledge, a biocidal active substance shall be included in Annex I, Annex IA or Annex IB for an initial period not exceeding 10 years. The inclusion of an active substance may be renewed on one or more occasions for periods not exceeding 10 years.

The Technical Guidance Document does not invalidate existing protection goals realised in other legislation or conventions. The test and assessment strategies are based on the current scientific knowledge and the experience of the competent authorities of the Member States. The scheme is intended to assess the risk to humans and the environment, posed by individual chemical substances and active substances and substances of concern present in a biocidal product, as required by Directive 92/32, Regulation 793/93 and Directive 98/8. Therefore additive or synergistic effects which may be caused by a combined action of several substances are not considered. The risk assessment procedures cover the whole life cycle of the substances under consideration, their effects on all human populations as well as fate and effects in all

environmental compartments. They should contribute to setting of specific quality criteria, as for specific environmental compartments or for human populations.

For priority existing substances Article 8(2) of Regulation 793/93 states that: "A substance subject to evaluation under other Community legislation should be placed on a priority list only if that evaluation fails to cover risk to the environment or risk to man, including workers and consumers, or if those risks have not yet been adequately evaluated. An equivalent evaluation carried out under other Community legislation should not be repeated under this Regulation." The practical implementation of this article has been discussed and the Competent Authorities provided the following advice:

- If other Community legislation covers certain uses of a Priority Chemical, then the Commission (or a Member State volunteer) should carry out a comparison of the protection goals of the other Community legislation and Regulation 793/93, as defined in Regulation 1488/94 and implemented in the Technical Guidance Document;
- Where the other Community legislation covers the same protection goals as Regulation 793/93 and where the assessment is seen as being equivalent to the evaluation carried out under Regulation 793/93, then the use should not be re-assessed under Regulation 793/93;
- Where the other Community legislation does not cover all the protection goals of Regulation 793/93 or where the assessment is seen as not being equivalent to the evaluation carried out under Regulation 793/93, the use could be covered by Regulation 793/93, if considered relevant. The risk assessment under Regulation 793/93 should though not reach formal conclusions related to those protection goals covered by the other Community legislation.

Clearly, if a substance falls under any of these categories the reasons for including or excluding (certain parts of) the assessment should be highlighted in the risk assessment report with the appropriate argumentation.

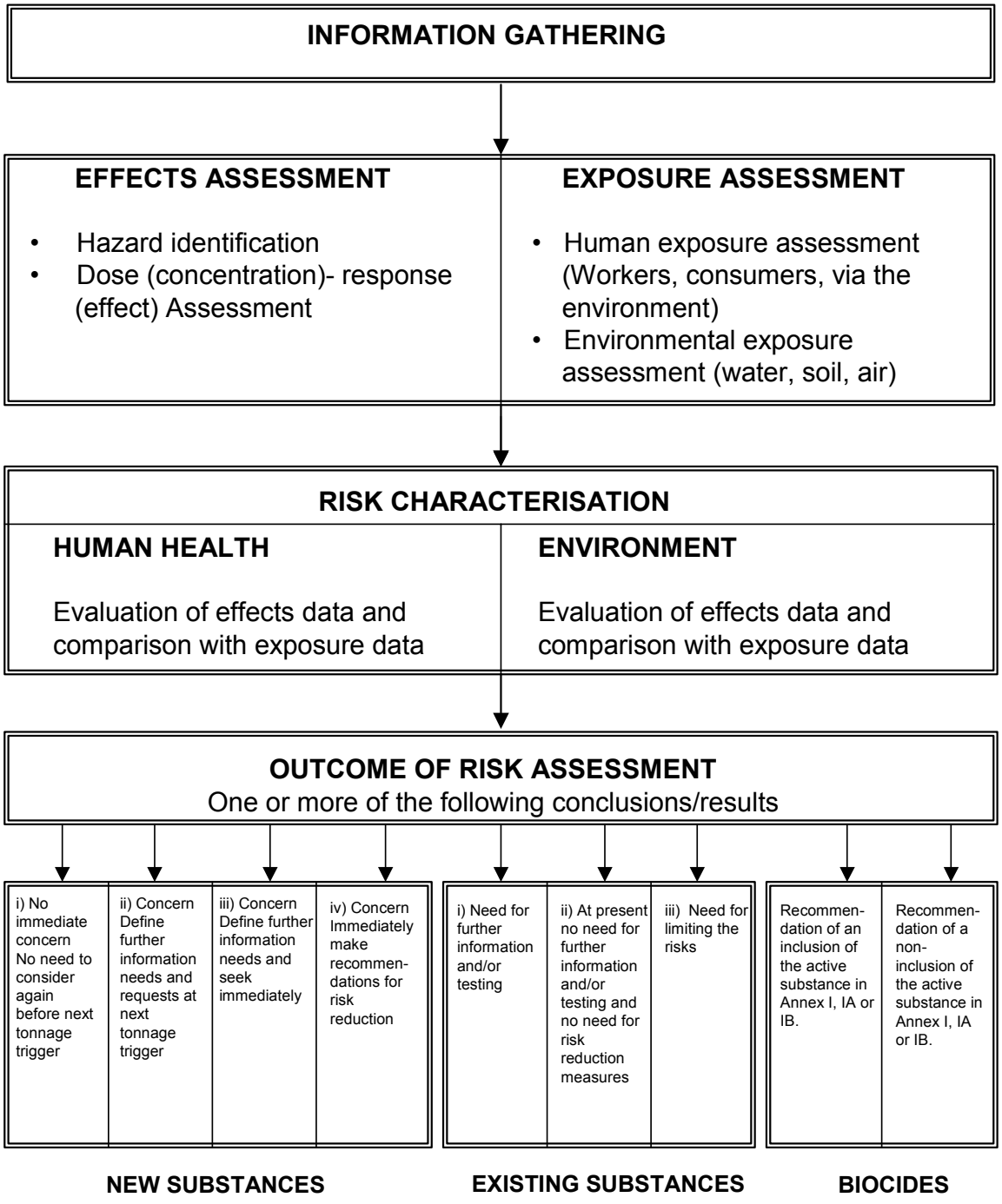

Figure 1 Risk assessment of new substances, existing substances and biocidal active substances and substances of concern present in a biocidal product: general principles.

3 PROCEDURES FOR PREPARING THE RISK ASSESSMENTS

3.1 NOTIFIED NEW SUBSTANCES

The risk assessments for notified new substances are circulated to the competent authorities of all Member States via the Commission together with or shortly after the circulation of the summary notification dossier. Where there are different views between Member States on the risk assessments or proposed testing strategies, agreement will normally be reached through a dialogue between Member States. If this is not possible, the decision may be referred to the committee of Member States according to Article 29 of Directive 92/32. Where measures for risk reduction are appropriate, these proposals may also be put to this committee for consideration and positive adoption.

3.1.1 Data used for risk assessment

The risk assessment of new notified substances is based on the data submitted by the notifiers in accordance with Articles 7, 8, 14(1) and 16 of Directive 67/548. The Directive lays down a scheme of step-wise, tonnage-related data requirements where the amount of available tests is dependent on the supply level. For use in the risk assessment, testing strategies have been developed to supplement the guidance given in Directive 67/548 and its Annexes (see Chapters 2 and 3). The tests for new substances need to be carried out in accordance with the EU test guidelines as laid down in Annex V to Directive 67/548 or, if no EU guidelines are available or they are not applicable, following internationally recognised guidelines, preferably those of the OECD. The tests must also be conducted in compliance with the principles of good laboratory practice as set out in Council Directive 87/18/EEC. These provisions are intended to assure the adequacy of data.

3.1.2 Risk assessment report

A common format for the risk assessment reports to be submitted to the Commission for circulation to all Member States has been established and is described in Chapter 6. This chapter also presents detailed guidance on how to prepare the risk assessment report.

3.1.3 Consultations

According to Article 3(5) of the Risk Assessment Directive for new substances (Directive 93/67) the notifier may be consulted by the competent authorities when the risk assessment indicates that the substance is "of concern" and further information or risk reduction measures may be necessary (conclusions (ii), (iii) or (iv)). Any relevant information obtained by the competent authorities shall be used to revise the risk assessment before sending it to the Commission.

3.2 PRIORITY EXISTING SUBSTANCES

The rapporteurs should aim to prepare the risk assessments within 6 months of receiving all relevant information and full test reports on the priority substances from the companies concerned, i.e. normally 12 months after publication of the priority list. On the basis of the risk assessment and, where necessary, a risk reduction strategy proposed by the responsible Member State, the Commission will prepare a proposal for discussion and adoption by the voting committee of Member States according to Article 15 of Regulation 793/93. At least one technical meeting of experts from Member States, industry and other bodies involved will precede this meeting of the voting committee to prepare the final Commission proposal. A summary risk assessment will after its adoption by the voting committee be published in the Official Journal.

If a need for risk reduction measures for one effect/population/compartment (result iii) is identified as well as the need for further information/tests for others, the risk assessment including these results shall be adopted and published immediately without awaiting the test results. When the information/test results are available the risk assessment will be revised with regard to the effects/populations/compartments affected and the revised assessment and result be adopted and published. This pattern may need to be run through several times, until for each effect/population/compartment either the result (ii) or (iii) applies.

3.2.1 Data used for risk assessment

3.2.1.1 Data base

The risk assessment of priority existing substances shall be based on the information on the substance submitted by the manufacturers and importers in accordance with Articles 3, 4, 7(1) and (2), 9(1) and (2) and 10 (2) of Regulation 793/93 and on other available information gained by the rapporteur. Normally for priority substances, when starting a risk assessment, the summary information submitted on HEDSET under the requirements of Article 3 which are stored in the IUCLID data base will be available to the rapporteur.

Additionally, the detailed information required under Article 9, i.e. in particular the complete test reports and detailed information on exposure has to be provided.

According to Article 9(2), the data to be made available to the rapporteur shall at least include the data set required under Annex VII A of Directive 67/548, i.e. the so-called base-set. See Appendix 1. Any gaps in the base-set data should be filled, unless the manufacturers or importers can justify not providing it according to Article 9(3).

Rapporteurs can agree exemptions from additional testing to complete the base-set, if the information is either unnecessary for risk assessment or impossible to obtain. An example would be when an Annex VIII test, e.g. a prolonged toxicity study with daphnia, is available, while the base-set test is missing.

In order to ensure that all relevant data for a risk assessment are available, the rapporteurs should conduct their own literature research. However, if the companies concerned provide a copy of the results of their literature research and the research profile used, unnecessary duplication of work could be avoided.

The rapporteurs should also use non-published data for the risk assessment, where relevant. Sources of this type of data include: (national) product registers, data banks containing exposure

data collected in the context of legislation on consumer products, data banks of poison control centres, results of governmental monitoring programmes or governmental testing programmes.

Data not publicly available may also exist in Member States other that of the rapporteur. Therefore all Member States should submit their unpublished information on the priority substances to the rapporteurs as early as possible.

3.2.1.2 Evaluation of data

The rapporteurs should during the whole process of risk assessment consider the evaluation of the adequacy and completeness of the data available.

Any tests carried out for the purpose of risk assessment under Regulation 793/93 should be conducted according to the methods laid down in Annex V to Directive 67/548 following good laboratory practices as set out in Directive 87/18. However, it is possible that test data already exist which have been generated by following other test guidelines or not applying GLP standards. The quality of such data and the need to conduct new tests according to Annex V to Directive 67/548 must be decided on a case-by-case basis taking into account among other factors, the need to minimise testing on vertebrate animals.

Particular account should be taken of tests carried out according to internationally agreed test guidelines and applying appropriate laboratory quality standards. When evaluating the adequacy of the data already available a general rule should be that a non standard test can be accepted if it provides the same information necessary for the risk assessment as the standard test according to Directive 67/548. Detailed guidance on the evaluation of data is given in sections 3 of Chapters 2 and 3.

3.2.1.3 Risk assessment report

A risk assessment report shall comprise:

- A comprehensive risk assessment report;
- A summary thereof; and
- The definitive data set including all relevant data for the risk assessment according to Article 6 and Annex 5 of the Risk Assessment Regulation 1488/94 on HEDSET or IUCLID.

Detailed explanations on the three different elements are presented in Chapter 6.

A common format for the risk assessment reports to be submitted to the Commission has been established and is described in Chapter 6. This chapter also presents detailed guidance on how to prepare the risk assessment report and the definitive data set.

3.2.2 Consultations

Regulation 793/93 lays down in Article 10(1) that the rapporteurs shall consult industry when it has been identified that further information/testing is necessary. While further contacts are not formally foreseen by the Regulation, it is recommended that consultations of industry, social partners and other interested bodies take place during the whole process of the risk assessment.

3.2.2.1 Consultation with industry

In order to facilitate the contacts between the rapporteurs and the companies concerned, i.e. manufacturers and importers who had submitted HEDSETs on the priority substances, industry may nominate a contact point for each substance on the priority list in order to co-ordinate the data collection and data submission and should inform the rapporteur who this is. While these contact points could be either a company or an industry organisation, actual submission of that data shall formally be made by the companies or one company acting on behalf of the others.

It is the task of the rapporteur to inform each company concerned of the other companies who have to submit data on the priority substances and to invite them to nominate a contact point, while the co-ordination of the submission of the data and carrying out of missing tests within the given time frames is in the responsibility of these companies.

Consultation with industry should normally take place via the contact points. However, with regard to specific questions, in particular to the exposure assessment of the substance, it will often be more appropriate for the rapporteur to consult the company concerned direct.

It is recommended that the rapporteurs inform the companies concerned of the outcome of the risk assessment, either directly or via the contact point.

If for a given effect/population/environmental compartment the risk characterisation indicates that the substance is "of concern" but a refinement of the risk assessment is possible, industry should be consulted as early as possible, i.e. the rapporteurs should not wait until the risk assessment has been finalised for all effects/populations/compartments. It may be that additional existing data are readily obtainable via industry, e.g. additional data on exposure and/or toxicity may be available in companies which are customers of manufacturers or importers which would allow this refinement.

Efforts should be made to obtain exposure data also from companies which are (currently) not obliged to submit data according to Regulation 793/93, particularly companies which are customers of manufacturers and importers or manufacturers and importers of volumes below 1000 t/a (which have to submit data under Regulation 793/93 in a later phase).

If data allowing a refinement of the risk assessment are not obtainable in the consultation phase within a reasonable time (not causing a delay in the procedure), the rapporteurs should continue the risk assessment and present the request for further testing/information and its result to the Commission as described above.

3.2.2.2 Consultation with other interested bodies

The consultation with other interested bodies in the risk assessment, e.g. trade unions or consumer associations, should take place in the margins of the technical meetings preparing the vote on the risk assessments described under section 1. The consideration of the scientific input in the stage of the preparation of the rapporteur's proposal of the risk assessments may, on practical reasons, sometimes only be possible on national level. The rapporteur should then ensure that these consultations consider a European risk assessment and do not cause a delay of their presentation at the Commission.

3.3 BIOCIDAL ACTIVE SUBSTANCES

The receiving competent authority shall, within 12 months of accepting the dossiers, carry out an evaluation. (A copy of the evaluation shall be sent to the Commission and the other Member States and to the applicant, together with a recommendation for the inclusion, or otherwise, of the active substance in Annex I, IA or IB). A decision on this recommendation will have to be taken at community level.

3.3.1 Data used for Risk Assessment

The data requirements for the active substance are laid down in Annex IIA and Annex IIIA of Directive 98/8/EC. Furthermore "Technical Notes for Guidance in Support of Directive 98/8/EC of the European Parliament and the Council Concerning the Placing of Biocidal Products on the Market. Guidance on data requirements for active substances and biocidal products" has been developed and are available on the ECB homepage http://ecb.jrc.it.

3.3.2 Risk Assessment Report

A common format for biocidal active substances has been proposed[1], but is still under discussion.

The Biocides Directive, 98/8, has a number of associated Technical Notes for Guidance (TNsG) to ensure a harmonised implementation of the Directive and to explain information expectations and data use. The relevant TNsGs for the risk assessment of the biocidal active substances are the present TGD and in addition the documents listed below. The documents are available on the ECB homepage http://ecb.jrc.it.

List of TNsGs for biocides

Doc. No.	SHORT TITLE	FULL TITLE
1	TNsG on Data Requirements	Technical Notes for Guidance in support of the Directive 98/8/EC concerning the Placing of Biocidal Products on the Market - Guidance on Data Requirements for Active Substances and Biocidal Products
2	TNsG on Annex I inclusion	Technical Notes for Guidance in support of Directive 98/8/EC of the European Parliament and the Council concerning the Placing of Biocidal Products on the Market - Principles and Practical Procedures for the Inclusion of Active Substances in Annexes I, IA and IB
3	TNsG on Human exposure	Assessment of Human Exposures to Biocides (first report, 1998) Second report in preparation, target date Sep. 2002

[1] EU-Project E2/ETU/980078: Preparation of Guideline for the Practical Implementation of Directive 98/8, concerning the placing of Biocidal Products on the Market (Short title: Practicalities Guidelines) Final Proposal from consultants 28 February 2000

3.3.3 Consultation

A copy of the evaluation shall be sent to the Commission and the other Member States and to the applicant, together with a recommendation for the inclusion, or non-inclusion, of the active substance in Annex I, IA or IB.

On receipt of the evaluation, the Commission shall prepare a proposal without undue delay for a decision to be taken at community level. The decision shall be taken at latest 12 months after the receipt by the Commission of the request (cf. Art 11(4) Directive 98/8)

APPENDIX I BASE SET

ANNEX VII. A
DIRECTIVE 67/548/EEC
INFORMATION REQUIRED FOR THE TECHNICAL DOSSIER ("BASE SET") REFFERED TO IN ARTICLE 7 (1)

3. PHYSICO-CHEMICAL PROPERTIES OF THE SUBSTANCE

3.0 State of the substance at 20°C and 101,3 kPa

3.1 Melting-point

3.2 Boiling-point

3.3 Relative density

3.4 Vapour pressure

3.5 Surface tension

3.6 Water solubility

3.8 Partition coefficient n/octanol/water

3.9 Flash-point

3.10 Flammability

3.11 Explosive properties

3.12 Self-ignition temperature

3.13 Oxidizing properties

3.14 Granulometry:

For those substances which may be marketed in a form which gives rise to the danger of exposure by the inhalatory route, a test should be conducted to determine the particle size distribution of the substance as it will be marketed.

4. TOXICOLOGICAL STUDIES

4.1 Acute toxicity

For tests 4.1.1 to 4.1.3, substances other than gases shall be administered via at least two routes, one of which should be the oral route. The choice of the second route will depend on the nature of the substance and the likely route of human exposure. Gases and volatile liquid should be administered by the inhalation route.

4.1.1 Administered orally

4.1.2 Administered by inhalation

4.1.3 Administered cutaneously

4.1.5 Skin irritation

4.1.6 Eye irritation

4.1.7 Skin sensitisation

4.2 Repeated dose

The route administration should be the most appropriate having regard to the likely route of human exposure, the acute toxicity and the nature of the substance. In the absence of contra indications the oral route is usually the preferred one.

4.2.1 Repeated dose toxicity (28 days)

4.3 Other effects

4.3.1 Mutagenicity

The substance shall be examined in two tests. One shall be a bacteriological (reverse mutation) test, with and without metabolic activation. The second shall be a non-bacteriological test to detect chromosome aberrations or damage. In the absence of contra-indications, this test should normally be conducted in vitro, both with and without metabolic activation. In the event of a positive result in either test, further testing according to the strategy described in Annex V should be carried out.

4.3.2 Screening for toxicity related to reproduction (for the record)

4.3.3 Assessment of the toxicokinetic behaviour of a substance to the extent that can be derived from base set data and other relevant information.

5. ECOTOXICOLIGICAL STUDIES

5.1 Effects on organisms

5.1.1 Acute toxicity for fish

5.1.2 Acute toxicity for daphnia

5.1.3 Growth-inhibition test on algae

5.1.6 Bacterial inhibitor

In those cases where biodegradation may be affected by the inhibitory effect of a substance on the bacteria, a test for bacterial inhibition should be carried out prior to undertaking the biodegradation.

5.2 Degradation

- biotic
- abiotic

If the substance is not readily biodegradable then consideration should be given to the need to carry out the following tests: hydrolysis as a function of pH.

5.3 Adsorption / desorption screening tests

APPENDIX II ABBREVIATIONS

AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area under the blood/plasma concentration vs. time curve, representing the total amount of substance reaching the plasma
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMF	Biomagnification Factor
bw	body weight
CA	Competent Authority
CAS	Chemical Abstract Services
CEN	European Standards Organisation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
dfi	daily food intake
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Communities
EN	European Norm
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]

FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
ISO/DIS	International Organisation for Standardisation/Draft International Standard
IUCLID	International Uniform Chemical Information Database (existing substances)
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOQ	Limit Of Quantitation
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOS	Margin of Safety
MW	Molecular Weight
NAEL	No Adverse Effect Level
NF	Norme Française
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
OECD	Organisation for Economic Cooperation and Development
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic

P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PCDD	PolyChlorinated Dibenzo Dioxin
PCDF	PolyChlorinated Dibenzo Furan
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SCE	Sister Chromatic Exchange
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
TGD	Technical Guidance Document [1]
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
vB	very Bioaccumulative
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
WHO	World Health Organization

Chapter 2

RISK ASSESSMENT FOR HUMAN HEALTH

CONTENTS

1 GENERAL INTRODUCTION TO RISK ASSESSMENT FOR HUMAN HEALTH

1.1 BACKGROUND

The carrying out of a human health risk assessment is required on new substances (Commission Directive 93/67), priority existing substances (Commission Regulation (EC) No. 1488/94) and biocidal active substances (Directive 98/8). This risk assessment should proceed in the following sequence:

- hazard identification;
- dose (concentration) - response (effect) assessment;
- exposure assessment;
- risk characterisation.

The risk assessment for human health shall address the following potential toxic effects and human populations, considering each population's exposure by the inhalation, oral and dermal routes:

Effects

- acute toxicity;
- irritation;
- corrosivity;
- sensitisation;
- repeated dose toxicity;
- mutagenicity;
- carcinogenicity;
- toxicity for reproduction.

Human population

- workers;
- consumers;
- humans exposed indirectly via the environment.

This document is intended to assist those carrying out the risk assessment for human health of new and existing substances and biocides. It includes advice on the following issues:

- how to establish the exposure levels and dose-response-relationships (Sections 2 and 3, respectively) and, where no quantification is possible or necessary, how to make qualitative assessments of exposure and effects;
- how to judge which of the possible administrative decisions on the risk assessment according to Article 3(4) of Directive 93/67 or Article 10 of Regulation 793/93 and Annex V of Regulation 1488/94 or Article 11 of Directive 98/8 need to be taken (Section 4);
- how to decide on the testing strategy, if further tests need to be carried out (Section 3).

The assessment of exposure to humans from biocidal products and the risk assessment for human health have been elaborated in separate guidance documents, Technical Notes for Guidance in support of Directive 98/8/EC (TNsG on Exposure Estimation, 2002; TNsG on Annex I Inclusion, 2001). The human health effect assessment of the active biocidal substance will follow the present Technical Guidance Document (TGD).

According to Article 9(2) of Regulation 793/93, the minimum data set that must be submitted for existing substances is the "base-set" testing package required for new substances which is defined in Annex VIIA of Council Directive 67/548/EEC. This ensures that for both new and existing substances results from studies are available on all effects listed above, except for carcinogenicity and toxicity for reproduction, and also information on toxicokinetic behaviour. For a new substance further data are foreseen at level 1 and level 2 (Annex VIII of Directive 67/548). For existing substances information beyond the base-set may be available of which the amount and quality of data are expected to vary widely. For the effects assessment there may be several data available on a single endpoint which give dissimilar results. Furthermore, there may be studies, in particular older studies, which have not been conducted according to current test guidelines and quality standards. Expert judgement will be needed to evaluate the adequacy of these data.

The data requirements for the active biocidal substances are laid down in Annex IIA and Annex IIIA of Directive 98/8. Furthermore, Technical Notes for Guidance on data requirements for active substances and biocidal products have been developed (TNsG on Data Requirements, 2000).

The human exposure assessment is based on representative monitoring data and/or on model calculations. If appropriate, available information on substances with analogous use and exposure patterns or analogous properties is taken into account. The availability of representative and reliable monitoring data and/or the amount and detail of the information necessary to derive realistic exposure levels by modelling, in particular at later stages in the life cycle of a substance (e.g. during and after use in preparations and articles), will also vary. Again, expert judgement is needed.

The risk assessment should be carried out on the basis of all data available, applying the methods described in the following sections of the document. As a general rule for the risk assessment the best and most realistic information available should be given preference. However, it may often be useful to conduct initially a risk assessment using exposure estimates based on worst-case assumptions. If the outcome of such an assessment is that the substance is of "no concern", the risk assessment for that human population can be stopped. If, in contrast, the outcome is that a substance is "of concern", the assessment must, if possible, be refined.

The guidance has been developed mainly from experience gained on individual substances. This implies that the risk assessment procedures described cannot always be applied without modifications to certain mixtures of substances. In particular the methodologies that may be applied to assess the risks of petroleum substances are specifically addressed in Appendix VII.

The risk assessments that have to be carried out according to Regulations 793/93 and 1488/94 for existing substances and Directives 67/548 and 93/67 for new substances, and for active substances in a biocidal product under Directive 98/8, respectively, are in principle valid for all countries in the European Union. Therefore in this document generic scenarios are applied to the exposure assessment. For biocides additional generic scenarios are given elsewhere, see http://ecb.jrc.it. It is recognised, however, that the exposure situation in different countries may vary according to working practices, consumer uses or environmental conditions. The last are

dealt with in Chapter 3. Where specific information is available, it may be used to refine the generic assessment.

A human health risk assessment containing all steps must be carried out for all existing substances and for those new substances which have been classified on the basis of their toxicological properties, or on the basis of certain physico-chemical properties (explosivity, flammability or oxidising potential), and to which human exposure is possible.

Similarly, this will also be necessary for substances which are not classified if there are other reasonable grounds for concern for human health. For example, the following situations would indicate a need for a full risk assessment:

- positive results from base-set mutagenicity tests;
- a clear indication of a toxic effect in a 28-study which is not sufficiently serious to result in classification of the substance, but which may become more serious in a longer or more specific study;
- indications of a particular potential adverse effect, for which the appropriate test has not yet been conducted, from structure activity relationships or elsewhere (e.g. the results of another toxicity test, as in the example above);
- other unclear or equivocal results from tests related to human health;
- concerns arising from the expected human exposure pattern/level, such as widespread exposure via a consumer product or significant human exposure via a route of exposure which has not been used in the toxicity tests already conducted.

New substances which are neither classified, nor give rise to concern for other reasons are considered of no immediate concern and may usually be set aside until further information is made available within the context of the notification scheme.

1.2 GENERAL PRINCIPLES

In essence, the procedure for the risk assessment for human health of a substance consists of comparing the exposure level(s) to which the population(s) are exposed or are likely to be exposed with the exposure level(s) at which no toxic effects are expected to occur.

Where possible, a risk assessment is conducted by comparing the exposure level, the outcome of the exposure assessment, with the No Observed Adverse Effect Level (NOAEL), the outcome of the dose-response assessment. Where it is not possible to establish a NOAEL but a Lowest Observed Adverse Effect Level (LOAEL) can be derived, the latter is compared with the exposure level.

The exposure levels can be derived based on available monitoring data and/or model calculations. The N(L)OAEL values are determined on the basis of results from animal testing, or on the basis of available human data. For some effects N(L)OAEL values are not usually available. For genotoxic substances and sensitisers it is considered prudent to assume that a threshold exposure level cannot be identified.

Also, for substances which are corrosive or skin/eye irritants, N(L)OAEL values are often not available.

The derivation and use of dose-response relationships for each of the effects to be considered are discussed in detail in Section 3.4.

For both the exposure assessment and the effects assessment, data on physico-chemical properties including chemical reactivity may be needed. The physico-chemical properties are required, for example, to estimate emissions and the human exposure scenarios, to assess the design of toxicity tests, and may also provide indications about the absorption of the substance for various routes of exposure. The chemical reactivity may also be of importance, e.g. in the estimation of the exposure of the substance, and also has an impact on its toxicokinetics and metabolism.

Dependent on the exposure level/N(L)OAEL ratio the decision whether a substance presents a risk to human health is taken. If it is not possible to identify a N(L)OAEL, a qualitative evaluation is carried out of the likelihood that an adverse effect may occur.

The comparison of the exposure with the potential effects is done separately for each human population exposed, or likely to be exposed, to the substance, and for each effect. It should be noted that, in any particular human population, sub-populations may be identified (e.g. with different exposure scenarios and/or different susceptibility) which may need to be considered individually during risk characterisation. Thus, exposure levels are derived separately for each relevant population/sub-population, and different N(L)OAELs, where appropriate, are identified for the different endpoints, and respective exposure level/N(L)OAEL values are established.

The risk assessment process depends heavily upon expert judgement in the interpretation of exposure and effects. The risk assessor should focus the assessment on those effects of toxicological relevance to humans which may be expected at the predicted levels of exposure.

Requirements for further information on effects and on exposure are inter-related, and are to a large extent addressed in the toxicity testing strategies in this document. However, when all the effects and all the expected human exposure patterns are considered, there may be indications for several tests, possibly using more than one route of exposure. Particularly when early and/or extensive further testing is being considered, it is important to ensure that either high quality and relevant measured exposure levels, or the best possible estimates of human exposure, are obtained so that the decision to test or not to test can be justified. In addition, it should be considered whether toxicokinetic, metabolic or mechanistic data/information, if obtainable, may be useful for defining which tests and which routes of exposure should be used, or such data may be useful in themselves in the assessment of the risks to human health. At any particular stage, integrated requirements for further testing must be developed, using professional judgement, so that the necessary information is obtained using the least amount of testing in animals.

2 EXPOSURE ASSESSMENT

2.1 INTRODUCTION

2.1.1 Core principles of human exposure assessments

Humans may be exposed to substances in the workplace (occupational exposure), from use of consumer products (consumer exposure) and indirectly via the environment. In this chapter guidance is given on how to perform an exposure assessment for each of these human populations. This guidance pertains to the general principles that apply, the data evaluation that needs to be performed and to the way the actual quantitative assessment, based on either measured or modelled data, should be performed.

In a first **screening** step of the exposure assessment, the likelihood of an exposure of the three populations to the substance under consideration has to be evaluated. If in the screening step it is indicated that exposure to one or more of the human populations does not occur or when the expected exposure is so low that it can be neglected further in the risk characterisation phase, no further assessment is needed and the conclusion can be mentioned in the risk assessment report. If actual or potential exposure has been identified a **quantitative** exposure assessment is necessary. Exposure levels/concentrations for each population potentially exposed need to be derived from the available measured data and/or from modelling. A range of exposure values to characterise different sub-populations and scenarios may result. These results are taken forward to the risk characterisation where they are combined with the results of the effects assessment in order to decide whether or not there is concern for the human population exposed to the substance. In some cases all three types of exposure estimates may contribute to an overall exposure value (combined exposure) which should be considered in the risk characterisation.

It may often be useful to initially conduct an exposure assessment based on “worst-case” assumptions, and to use default values when model calculations are applied. Such an approach can also be used in the absence of sufficiently detailed data. If the outcome of the risk characterisation based on worst-case exposure assumptions is that the substance is “not of concern”, the risk assessment for that substance can be stopped with regard to the effect/population considered. If, in contrast, the outcome is that a substance is “of concern”, the assessment must, if possible, be refined using a more realistic exposure prediction in order to come to a definitive conclusion.

The following core principles relate to human exposure assessments that need to be carried out for new substances, existing substances and biocides:

- exposure assessments should be based upon sound scientific methodologies. The basis for conclusions and assumptions should be made clear and be supportable and any arguments developed in a transparent manner;
- the exposure assessment should describe the exposure scenarios of key populations undertaking defined activities. Such scenarios that are representative of the exposure of a particular (sub)population should, where possible, be described using both reasonable worst-case and typical exposures. The reasonable worst-case prediction should also consider upper estimates of the extreme use and reasonably foreseeable other uses. However, the exposure estimate should not be grossly exaggerated as a result of using maximum values that are

correlated with each other. Exposure as a result of accidents or from abuse shall not be addressed;
- actual exposure measurements, provided they are reliable and representative for the scenario under scrutiny, are preferred to estimates of exposure derived from either analogous data or from the use of exposure models;
- exposure estimates should be developed by collecting all necessary information (including that obtained from analogous situations or from models); evaluating the information (in terms of its quality, reliability, etc.); thus enabling reasoned estimates of exposure to be derived. These estimates should preferably be supported by a description of any uncertainties relevant to the estimate;
- in carrying out the exposure assessment the risk reduction/control measures that are already in place should be taken into account. Consideration should be given to the possibility that, for one or more of the defined populations, risk reduction/control measures which are required or appropriate in one use scenario may not be required or appropriate in another (i.e. there might be sub-populations legitimately using different patterns of control which could lead to different exposure levels).

Exposure should normally be understood as external exposure which can be defined as the amount of substance ingested, the total amount in contact with the skin (which can be calculated from exposure estimates expressed as $mg \cdot cm^{-2}$ or $mg \cdot cm^{-3}$) or either the amount inhaled or the concentration of the substance in the atmosphere, as appropriate. In cases where a comparison needs to be made with systemic effects data (e.g. when inhalation or dermal toxicity values are lacking or when exposures due to more than one route need to be combined) the total body burden has to be estimated. Since the assessment of the amount that is absorbed after ingestion, by inhalation or by the skin is usually done in the effects assessment (section on toxicokinetics) this calculation of the total body burden is often placed in the section on risk characterisation.

Exposure is considered as single events, or series of repeated events, or as continuous exposure. The duration and frequency of exposure, the routes of exposure, human habits and practices as well as the technological processes need to be considered. Furthermore, the spatial scale of the exposure (e.g. personal/local/regional level) has to be taken into account.

2.1.2 Combined exposure

The exposure assessments that are described in the following sections provide quantitative exposure estimates for each of the human populations (workers, consumers and humans exposed via the environment). In some cases it may also be relevant to assess the combined exposure of humans via two or more routes. Workers may for instance be exposed in their private life to consumer products that contain the same substance as the products they are exposed to professionally. In addition, consumers may be exposed to substances via food packaging material and at the same time be exposed to water and/or air that contain the substance as a result of (diffuse) environmental emissions. In calculating the actual combined exposure value care should be taken of the time scales at which the exposures occur. In general, combined exposure can be of particular relevance when long-term exposure to substances with wide spread use and emissions occurs.

General guidance on when these situations become relevant cannot be easily given. On a case-by-case basis the assessor needs to decide whether the combined exposure of one or more populations leads to different or additional conclusions regarding the risks of substance.

2.2 ASSESSMENT OF WORKPLACE EXPOSURE

2.2.1 Introduction

In order to carry out a comprehensive review of exposure to a substance in the workplace, a lot of detailed information is needed. Unfortunately, in many cases this is not realistically achievable, and exposure assessment must be made using less data than is desirable. However, although in many cases data may be sparse, it is also possible to estimate exposure by various techniques. The purpose of this section is to provide an overview of workplace exposure assessment and to give detailed information on:

- the information needed by the assessor from industry to carry out the workplace exposure assessment;
- data collection;
- data analysis;
- the use of modelling techniques;
- assessment of exposure when personal protective equipment (PPE) is used, and
- which exposure values to take forward to risk characterization.

These are all developing areas, and will probably change quite regularly. However, the basic principles will remain the same. Therefore in preparing exposures assessments, the most up-to date information should be used. This may mean that advice not included in this document is used (e.g. a later version of the EASE model). There is no problem with this, as long as there is a general consensus among Competent Authorities that what is proposed is acceptable, as reflecting the state of knowledge at the time the assessment is prepared. The most up-to-date version of this document can be accessed via the ECB website http://ecb.jrc.it.

An overview of the process steps involved in the assessment of workplace exposure, as described in this section of the TGD, is provided in Appendix I. The summary provides an overview of the steps involved in carrying out an exposure assessment, as described in this section of the TGD. It does not provide all of the details needed for an assessor to actually perform the assessment. It should be used as an *aide-memoir* and should **not** be seen as a comprehensive description of the process.

2.2.2 General principles of workplace assessment

Substances in the workplace may enter the body by being breathed in (inhalation), by passing through the skin (dermal), or swallowing (ingestion). Exposure to a particular substance should normally be understood as external exposure. This can be defined as the amount of the substance ingested, the amount in contact with the skin and/or the amount inhaled, which is represented by the airborne concentration of the substance in the breathing zone of a worker. It does not usually refer to concentrations within the body, which are consistent with some measure of absorbed dose. The text should clearly indicate whether the exposures under discussion are external or internal.

Exposure can be considered as a single event or as a series of repeated events or as continuous exposure. As well as an estimation of the levels of exposure, either from measured or modelled data, the assessor needs to address other parameters such as duration and frequency of exposure and the size of the exposed workforce. It may also be appropriate to consider task-based exposures, particularly for acute effects. Exposure to substances causing local effects may also be of interest and should be described where appropriate.

2.2.2.1 Inhalation exposure

Exposure by inhalation is expressed as the concentration of the substance in the breathing zone atmosphere, and, for full shift (nominally 8 hours) is normally presented as an average concentration over a reference period. If the substance of concern has acute health effects or if exposure is of intermittent short duration there may also be interest in exposure over shorter periods. One convention in these circumstances is to assess exposure as a time-weighted average over 15 minutes. The assessment can also be based on exposure during specific tasks which may be carried out over varying time periods. Information on peak exposures may be important for assessing acute effects, however, measurement of this type of exposures can be difficult and there are rarely any data available.

2.2.2.2 Dermal exposure

For many substances the main route of exposure is by inhalation; however, substances may have the ability to penetrate intact skin and become absorbed into the body. Two terms can be used to describe dermal exposure:

- *potential dermal exposure* is an estimate of the amount of contaminant landing on the outside of work wear and on the exposed surfaces of the skin. It is the sum of the exposure estimates for the various body parts, including hands and feet;
- *actual dermal exposure* is an estimate of the amount of contamination actually reaching the skin. It is mediated by the efficiency and effectiveness of clothing assemblages and programmes to minimise transfer of contamination from work wear to the skin.

Potential dermal exposure is the most frequently used indicator.

Absorption through the skin can result from localised contamination, e.g. from a splash on the skin or clothing or in some cases from exposure to high air concentrations of vapour. Dermal exposure can be influenced by the amount and concentration of the substance, the area of skin exposure and the duration and frequency of exposure.

Hand transfer of contamination to other parts of the body is an important source of skin exposure. Contaminated clothing can also be a source of skin exposure particularly to the hands when removing contaminated PPE. Dermal exposure is expressed in terms of the mass of contaminant per unit surface area of the skin exposed. At present, there is no consensus view as to how dermal exposure is best assessed, although the model predictions, which are described later, make some suggestions.

2.2.2.3 Ingestion exposure

There are no accepted methods for quantifying exposure by ingestion. It is usually controlled by straightforward good hygiene practices such as segregating working and eating facilities and adequate washing prior to eating. These matters are normally dealt with as general welfare provisions in national health and safety legislation. Ingestion exposure is therefore not considered further in the assessment of workplace exposure. However, the potential for exposure via ingestion should be borne in mind when considering uncertainties in the exposure assessment as a whole.

2.2.2.4 Definition of scenario

The exposure assessment is carried out through an evaluation of different scenarios. An exposure scenario is the set of information and/or assumptions that tells us how the contact between the worker and the substance takes place. It is based on the most important characteristics of the substance in the view of occupational exposure e.g. the physical state, the vapour pressure as well as on its uses, processes, tasks (description, duration, frequency of exposure) and controls. Based on this information, relevant populations potentially exposed are identified and scenarios are established and evaluated. This process will be aided by a comprehensive description of the lifecycle of the substance given in Chapter 2 of the risk assessment report. Good cross-references between Chapter 2 and the scenario description should make the exposure assessment more transparent.

An exposure scenario describes a specific use of a substance with a set of specific parameters (process, activities (related to the process), duration and frequency, control measures, concentration of a substance in the formulation) and the exposure levels (inhalation and dermal) associated with the described situation. For each defined scenario, the most relevant moments of exposure should be described.

If the assessor judges that some exposure scenarios, or an exposure route are not relevant, reasons for this judgement should be given, e.g. the substance is no longer used in a certain industrial sector. Establishing the relevant exposure scenarios needs expert judgement and should be made in a transparent way. To aid the clarity of the document similar uses of the substance can be clustered into a single scenario.

Assessors should focus their attention on those scenarios for which a risk is anticipated or which are borderlines. The degree of detail required for an exposure scenario should be linked to the perceived magnitude of the risk. In this way, the problem of including excessive amounts of text for low-risk situations will be avoided, while at the same time giving serious risks the amount of attention they deserve. However, enough detail should be given to enable the reader to be confident that potentially important scenarios are not missed.

Some workers are exposed to higher concentrations than others because of differences in the pattern of use, exposure and control, and also because of differences in their tasks or individual work practices. It is sometimes unavoidable in the description of a scenario to also include assumptions, inferences and professional judgements in order to evaluate the information gathered and to fill the potential gaps in the information. These assumptions should be sufficiently described so that the reader can appreciate the limitations of the final estimation.

Similar exposure scenarios may occur both for workers and consumers. These may be scenarios where workers and consumers use the same products and technologies, e.g. use of cleaning agents, painting and adhesive use for carpet laying. There may however, be differences between the scenarios, e.g. duration of exposure, quantities used, which must be taken into consideration.

2.2.2.5 Information needs for workplace exposure assessment

In order to provide assessors with sufficient data to reliably and accurately estimate exposure via the different routes, there is a need for information which both describes the nature and degree of exposure and which, ideally, is supported by quantified data. In view of the uncertainties associated with assessing exposure in human populations, preference should always be given to obtaining representative measured exposure data. Where this is unavailable, analogous/surrogate

data should be referred to, provided there is sufficient information on duration and pattern of exposure, before default assumptions are used.

An effective assessment of occupational exposure to a hazardous substance will need to include information on the range of topics listed below:

- a description of the substance, its chemical and physical properties, exposure limits, etc.;
- an indication of how, where and in what quantities it is manufactured and used;
- a description of the circumstances of use, and the potential routes of exposure and numbers exposed;
- details of measured exposure information that is available, including any statistical parameters describing the information, with supporting core information and an indication of the comprehensiveness and reliability of the information;
- where appropriate, details of analogous/surrogate measured data which may be relevant;
- where appropriate, data from (computer) models which can estimate exposure for relevant process and circumstances;
- a comprehensive discussion (including a discussion of the uncertainties) of all the measured, modelled and analogous/surrogate data, to provide an overview of the situation.

More detailed information is given in Section 2.2.3 on the information needed by assessors in order for them to carry out a valid exposure assessment. Section 2.2.7 describes how this information relates to the process of risk characterisation.

Measured data

Measured exposure data and associated information describing these data should be available from workplace exposure assessments and routine monitoring regimes required as a consequence of the "so-called" Chemical Agents Directive (98/24/EC) and the Carcinogens Directive (90/394/EC). Such information may also be available from dedicated surveys or from work with analogous substances having close chemical and physical properties. Current information may be available from the relevant literature and should also be seen as a source of information. All data require careful evaluation before use.

Data should be accompanied by sufficient information to place the exposures in context with respect to the pattern of use, pattern of control and other relevant process parameters (see **Figure 1**, Section 2.2.3). Data should also be available that describe the frequency and duration of exposure with respect to these parameters. The data should have been collected following good occupational hygiene practice; preferably employing standardised procedures, particularly with respect to sampling strategy and measurement methods. Where possible, documents such as those from the European Standards Organisation, (CEN) or other relevant international standards, should be used as the basis both for the sampling strategy and associated measurement and analytical techniques (e.g. ENs 689, 481 and 482).

In some circumstances, analogous/surrogate measured data may be used instead of, or as well as, measurement data for the substance under assessment, e.g. when there are few measurement data for the specific substance. For the purposes of exposure assessment, analogous/surrogate data describes data from similar operations utilising the same substance or data for the same operation, but for similar substances. It is considered that most substances will have analogous/surrogate markers which, whilst not providing equivalent reliability in terms of their status in the data hierarchy, provide information which is more valuable than that obtained from modelled estimates.

Modelled data

Where it is necessary to model exposure data, owing to inadequate/insufficient measured data, this may be derived from the use of suitable validated exposure models. These models are useful within the range and under the conditions in which they have been validated. Their utility outside this range will be limited and may be of questionable reliability. The overall quality of modelled exposure estimates will in part be dependent on the proportion of reliable and specific exposure information available as inputs to the model, together with considerations affecting the operation of the model itself. Provision of good quality core information as outlined in **Figure 1** (Section 2.2.3) is required to assist in the modelling process.

In general terms, two types of model can be considered. These are (a) empirical/knowledge based and (b) mathematical mechanistic models. In empirical/knowledge-based models, a body of knowledge is encapsulated in an expert system. The system uses this knowledge to assess information input by the user and predict the likely exposure, often in the form of a range for the conditions of interest. Mathematical mechanistic models, on the other hand, use numerical inputs to estimate exposure by calculation. Equations derived from either theoretical principles or empirical studies determine the output predictions. The output may be a single figure, a time history, or a range determined by a statistical procedure.

The two model types have different attributes, and are strictly applicable only within their defined circumstances of use. In general, mathematical mechanistic models are concerned with specific circumstances or processes (e.g. paint spraying, drum-filling) and cannot usually be used for more general application. They are likely to give a lot of detailed information on the exposure to be expected. Empirical/knowledge based models, on the other hand tend to be applicable to a wider range of circumstances, and are based on many years of accumulated experience. While they can give a broad idea of likely exposure, they are not very precise. However, they are able to predict exposure to new and existing substances and to assess likely exposure across a range of uses of a substance. Model estimates are more widely used for exposure assessment of new substances because the data available are often poor or non-existent.

For the purposes of assessments undertaken for new and existing substances by Competent Authorities using this guidance, any relevant model can be used and there will be circumstances where mathematical mechanistic models may be able to provide some useful predictions for exposure assessment. However, the practice has developed of using a generic model, which was specifically developed for the purpose. This is the EASE (Estimation and Assessment of Substance Exposure) model. EASE is subject to a policy of continuous development and is currently in its second Windows version. Full details of the current version of EASE can be found at the website. Information on the concept behind EASE and other information can be found in Appendix I A-F.

In summary, the following preferential hierarchy should be applied to exposure data:

1. measured data, including the quantification of key exposure determinants,
2. appropriate analogous/surrogate data, including the quantification of key exposure determinants,
3. modelled estimates.

2.2.2.6 Information gathering

In order for the assessor to produce a valid exposure assessment there needs to be a good interaction between the assessor and industry. The lead industry contact, usually from a major

producer of the chemical, needs to provide the information required to the assessor in a timely fashion so that the assessment is not delayed.

Information is usually relatively easily obtained from producers/importers, as they are required to supply it, but is more difficult to obtain from downstream users. Although the difficulty in obtaining exposure information from downstream users is recognised, every effort should be made by the lead company to obtain this information. This will lead to greater confidence in the exposure assessment and also the risk assessment. Where information is lacking decisions are usually made on a precautionary basis to avoid underestimating the risk. This in turn may lead to excessive risk reduction measures being put in place.

In order to facilitate the production of a valid and relevant exposure assessment there needs to be a good interaction between the assessor and industry throughout the risk assessment process.

2.2.2.7 Uncertainties

The uncertainties relating to the process of occupational exposure assessment can be categorised into four groups:

- measurement uncertainties (including those arising from the physical sampling process);
- selection of measurement results;
- uncertainties of model results and
- assessment uncertainties.

Sampling and measurement uncertainties

Uncertainties can arise if the sampling strategy was not designed to obtain representative measurements for the workplace. This depends inter alia on the reason why the measurements were taken; e.g. to show compliance with an occupational exposure limit. The measurements provided may not cover all relevant activities, which can lead to bias.

Uncertainties can also arise in measurement. For example; not all of a physical sample during the chemical analysis may be recovered, which may lead to underestimated exposures. Some of the measurements may be below the limit of detection of the method used and will therefore underestimate exposure if recorded as zero, or overestimate it if recorded as equal to the limit of detection. There may also be uncertainties in the reading of laboratory measuring devices and uncertainties as a result of some other aspect of laboratory process (e.g. sample preparation). All of these factors should be taken into account when assessing data for exposure assessment.

In order to minimise these uncertainties, sampling should be done according to recognised protocols (e.g. EN 689) and measurements should preferably be made following good laboratory practice.

Selection of data

Uncertainties can arise as a result of the method by which measurements are selected for inclusion in the data set, particularly if data are pooled before being sent to the assessor. The pooling of measurement data could, for example, lead to a non-homogeneous small data set relating to a highly exposed group of workers and a large data set relating to less exposed groups being amalgamated. When calculating a 90^{th} percentile of these pooled data, the highly exposed group might not be represented. The variation in the spread of data generated by such a selection

mechanism and by non-transparent pooling of data is likely to be greatly increased as compared with a random or stratified sampling strategy.

The uncertainty due to the selection of measurement data is reduced if complete data sets are submitted to the assessor, together with sufficient detailed information. In this way, the assessor can sort out data not representing normal but accidental situations. If measurement data are pooled, it should be done in a transparent way.

The data sets received by assessors (mainly from industry) are often small and from diverse sources. For small sets of data points the question of statistical sampling uncertainties only arises if the data are used to estimate properties (e.g. the median or the 90^{th} percentile) of some notional population of occupational hygiene measurements. The smaller the number of observations, the larger the uncertainties associated with any inferences that may be derived from them. However, aside from a few of the more common chemicals where many data are held, most of the data received are in small data sets, and less than 12 data points are not uncommon.

It is questionable as to whether or not the data received from industry should be viewed as being representative of the population of all possible hygiene measurements. It depends on the qualitative information provided by industry. This again will affect the interpretation of any inferences made from the sample data set. The most relevant question to ask is whether the data obtained are appropriate for the purposes of deriving inferences for exposure assessments. It is also important to ensure that the available data are not over-interpreted.

Uncertainties associated with modelled data

In general terms two types of models can be used to predict exposures in the workplace. These are empirical/knowledge-based models and mathematical mechanistic models. Both approaches lead, in general, to more or less uncertain results.

For mathematical mechanistic models, the sources of uncertainty can be classified into three groups (US EPA, 1997):

- input parameter uncertainty. Input parameters are uncertain for the following reasons: variability or errors in measurement or sampling of data;
- scenario uncertainty. Scenario uncertainty includes uncertainties resulting from false or incomplete information, such as description, aggregation, judgement or an incomplete analysis; and
- model uncertainty. Due to lack of knowledge or errors in modelling and integrated relationships the structure of the model (i.e. the model in respect of the mathematical expressions of its hypothetical relationships) can also be uncertain.

Even if the sources of uncertainty are identified the various uncertainty contributions need to be determined quantitatively. One way is to predict point estimates by varying a parameter of a certain exposure scenario ("what if" analysis). The deviations between different scenarios can then be characterised by orders of magnitudes. This approach is especially useful as a means to provide screening for uncertainties.

The second way to perform a quantitative analysis of uncertainties is based on probabilistic techniques (Fehrenbacher and Hummel, 1996). Using a probabilistic technique (e.g. Monte-Carlo simulation), simultaneous uncertainties in the model inputs can be propagated through the model to determine their combined effect on model outputs. This yields a quantitative insight into both, the possible range and the relative likelihood for model outputs. Another purpose of probabilistic analysis is to measure the potential importance of model inputs as contributors to

the variation in the model outputs. Such a sensitivity analysis can provide insight into whether a real world system is sensitive to perturbations of some of its components or processes, assuming that such relationships are adequately represented in the model. This allows a ranking of the input parameters concerning their contribution to the overall uncertainty.

While the uncertainties of the input of mathematical mechanistic models can be handled quantitatively in a relatively stringent way the situation for empirical/knowledge-based models is somewhat different. Firstly, it is important to note that parameter and scenario uncertainty do also play an important role in the uncertainty analysis of empirical models. The model structure of an empirical model is not in the form of equations. Therefore, errors in the equations that are important errors in mechanistic models will not occur. However, the model structure of an empirical model can also be flawed, e.g. when an important parameter is not considered in the model, or the influence of a parameter is substantially over- or underestimated. Empirical/knowledge-based models are based on analogy considerations, i.e. using input parameters that are precisely defined, which then allow real scenario data to be linked to model scenarios. Since input parameters can be both quantitative (e.g. vapour pressure) and/or qualitative (e.g. use pattern) the uncertainty of the model input cannot be evaluated quantitatively in a stringent way. The uncertainties of qualitative input parameters and of the logical structure of the model can in general only be discussed qualitatively by addressing the following questions:

- do the exposure scenarios to be assessed provide sufficient reliable information that can be used to apply the deciding factors of the model?
- what are the underlying assumptions if gaps of knowledge have to be bridged?

When discussing the questions outlined above the assessor will in general come to a decision whether the output of the model can be used for the exposure assessment or should be rejected due to uncertainties of the model input. It is important to note that the discussion so far has been aimed at the possible uncertainties of the input parameters of empirical models. Another aspect of uncertainty is related to the models outcome and can be addressed by the question:

- do the model predictions correspond to independent monitoring and experimental data?

The assessor is often not able to answer this question precisely but it should be considered if reliable monitoring data are available.

Assessment uncertainties

In the assessment of exposure, all uncertainties described in the sections above can contribute to the overall uncertainty of the level of exposure. The uncertainty is even higher if data relating to similar workplaces are aggregated together. Generally, the uncertainty is higher, the less data and information are available, for instance, if information is available only from one company. In such situations, requests for additional measurement data should be considered.

If any of the sources of uncertainty are ignored, or at least some indication of their likely impact on the final assessments are not given, this will lead to assessments which will have spurious precision and accuracy associated with them. All of these uncertainties and errors need to be considered alongside those uncertainties related to the interpretation of the toxicology data in the process of risk assessment.

Therefore, it can be concluded that the analysis of uncertainty is an essential part of any exposure assessment, because it provides an important insight into the results and may detect weaknesses of the models. This in turn should lead to a more informed interpretation of the results.

2.2.2.8 Exposure assessment for mixtures

For the purposes of the risk assessment of new and existing substances it needs to be recognised that it can be either single substances, or single substances that are part of mixtures, or single CAS numbers that are mixtures that are being assessed. Therefore it is vital that the assessor clearly describe what is being assessed in any individual scenario. Where measured data are reported it also needs to be made clear exactly what has been measured and how it relates to the substance/s under assessment.

2.2.2.9 Particular exposure scenarios

Cleaning and maintenance of whole premises "plant stop"

There are exposure scenarios that are not generally assessed within the framework of existing substances. These are the large scale cleaning and maintenance of chemical facilities (so-called "plant stops") and the cleaning and maintenance activities in enclosed spaces, such as silos, storage tanks, largely closed mixers, etc.

It is generally recognized that in these scenarios risks for workers will occur. There are also indications from limited studies that relatively high exposure levels can occur. Exposure reduction depends heavily on the proper use of adequate PPE. However, proper methods of assessing the exposure in these situations are lacking. Exposure depends to a large extent on the level of contamination of the installation and equipment after purging and flushing. This level of contamination is highly variable and at present unpredictable. Therefore, exposure modelling of these situations is not possible. Due to the lack of useful assessment methods and the general reliance on PPE an assessment of risks in these situations is hardly possible and therefore not very meaningful. Only if detailed information on the process or the substance under consideration or proper measured data are available, the scenario should be described and assessed. If not, it should be mentioned in the text but without a detailed description. The possible high exposure levels should be described qualitatively. This information should be transferred through the risk characterisation to the risk reduction strategy meeting. For several substances, e.g. those with acute effects and for carcinogenic, mutagenic and reprotoxic (CMR) substances, it might be concluded that there is concern for these situations.

Note that small-scale (daily) activities related to cleaning and maintenance of (chemical) production facilities or facilities using the chemical to produce products should be assessed under the relevant scenario. Such activities are e.g. changing of filters in systems, repair of pumps and flanges, work on enclosed spaces from the outside (without entering). These activities should generally be done by either maintenance personnel of the facility or by contractors that are regularly working at the site.

Closed system intermediates

The revised version of Council Directive 2001/59/EEC (Annex VII A, VIIB and VIII) will allow a supply-tonnage-related reduced test package for new chemicals that qualify the substances as closed system intermediates. Regarding the conditions under which a reduced test package

would be acceptable it is important to note that the conditions are not defined on the basis of quantitative exposure levels but in terms of the technical specifications of the closed system under consideration. That is, exposure of workers is regarded as negligible under routine conditions if the substance is solely handled as an intermediate in closed systems with strict technical specifications. In principle there are the following definitions and exposure criteria:

- the substance is an intermediate which is solely manufactured and used for chemical processing. Monomers are excluded. When processed, the substance is "fully" transformed into chemically different molecules, not being polymers;
- the substance is restricted to a maximum number of 3 sites. For example, it may be manufactured by one company and then transported to another for processing;
- the supply to the enterprise which uses the intermediate for further processing should be direct and should not be through a third party in the EU;
- the substance must be rigorously contained by technical means during it's whole lifecycle. This includes production, transportation, purification, cleaning and maintenance, sampling, analysis, loading and unloading equipment/vessels, waste disposal/purification and storage. In general, an appropriate process would have all functional elements of the plant, such as filling ports, emptying equipment etc., either of a closed construction type with assured leakproofness or of a closed construction type with integrated exhaust ventilation;
- in case of cleaning and maintenance works special procedures such as purging and washing must be applied before the system is opened or entered, and
- the notifier must monitor all users to ensure compliance with the conditions listed above.

In practice the notifier performs the assessment of a plant by means of a so-called assessment index as explained in Directive 2001/59 (28th ATP of Directive 67/548). Systems are regarded as closed if all functional elements correspond to the assessment index 0.5. To facilitate the classification of a system by giving examples the Annex VII B contains a collection of closed (indicated by 0.5) and non-closed (indicated by 1, 2, 4) functional elements. The assessment of a plant can easily be done by checking each functional element against the technical requirements as laid down in the collection of examples.

To enable the Competent Authority to make a decision as to whether rigorous containment is achieved or not, the notifier must supply a statement as for the effectiveness of the containment in terms of assessment indices. The notifier is not requested to provide details of the integrity of every seal or efficiency of integrated exhaust ventilation. However, whatever means are used to achieve rigorous containment of the process it is important that the information is available, if needed, to verify that the assertions made for achievement of control are true.

2.2.2.10 Vulnerable groups

In occupational exposure assessments special consideration is not usually given to vulnerable groups. The assumption is made that two generally recognised vulnerable groups, children and elderly people, will not form part of the workforce. Other potentially vulnerable groups may be women (e.g. for some reprotoxic effects) or workers with specific vulnerability to certain types of effects, e.g. asthmatics. It is expected that exposure in general will not be determined by the vulnerability of the workers. On the other hand, "healthy worker selection" may occur and therefore result in vulnerable workers being underrepresented in specific situations. Since the exposure is estimated on an "as-is" basis, the estimate should not depend on the presence or absence of vulnerable groups in the workforce. The potential bias caused by the "healthy worker effect" should be considered in the hazard assessment if human data are assessed. The potential

differences in risks for vulnerable groups or non-vulnerable groups should be discussed in the risk characterisation part of the Risk Assessment Report

2.2.3 Information requirements for workplace exposure assessment

To assist in the interpretation of measured data, or in the generation of modelled data, high quality information on the processes in which the substances are used is required. It will enable exposures to be characterised sufficiently in order that a best estimate of exposure via all routes is obtained. For this purpose, certain core information requirements have been defined in **Figure 1**. These should be sought and incorporated into any exposure assessment, regardless of whether or not there are supporting measured data available. In addition, guidance on requirements for the analysis and presentation of measured exposure data and in the use of modelled data is provided.

For new substances, measured exposure data will necessarily be few. Some may be available from pilot plants and other developmental work. Alternatively, as with existing substances, some data may also be available for analogous/surrogate substances. The assessor will need to place appropriate weighting on this information. Although it is unlikely that measured data will be available, assessors still need to have all of the descriptive data in order that exposure models can be used.

Figure 1, below, identifies the information requirements necessary for workplace exposure assessment, in order to ensure that the exposure information is sufficient for it to be reliably interpreted. This information is considered to comprise the minimum necessary to ensure it is both representative of the conditions it was collected in and of sufficient quality to ensure its reliability. Where incomplete, or no information is sent to the assessors, judgements will have to be made to assess the usefulness of the information received. This may lead to the information being excluded from the assessment because of uncertainty as to its validity. Other information, e.g. peak exposure measurements, may be useful and should be included in the risk assessment report where available.

This information forms the basis for the assessor to evaluate the data and to assess exposure for each relevant scenario. Information and data on exposure relating to downstream users, who are not obliged to provide information, are very limited. However, **Figure 1** would be useful if downstream users or associations of such users contribute to the exposure assessment of existing substances.

General – Each company to provide information on:

- an indication of the size of establishment(s) involved in the manufacture or processing of the substance, or use of products containing the substance;
- where is the substance used? (including description of processes, activities and products);
- the composition of mixtures, formulations and products (including approximate percentages);
- how the substance is used (including description of work activities leading to exposure, quantities used and approximate percentage in process materials and finished products);
- the form in which the substance is handled (e.g. powder, pellets, liquid);
- how many people are involved in the work activity involving potential exposure;
- the nature of exposure (including description of tasks, approximate frequency and duration of tasks, duration and frequency of exposures) and
- what control measure (technical/personal) are used when relevant exposure activities are carried out;
- information to show that any personal protective equipment (PPE) used is suitable, is used as a last resort and that appropriate management systems are in place to ensure that the PPE is used correctly.

Core information on measured data

Where quantitative exposure measurements are available, then these should be capable of being linked to the above core requirements. The information should include:

- raw data reflecting personal exposures (comprising single data points) listing: measured concentration; units of concentration; sample duration; duration and frequency of relevant exposures; sampling and analytical methods; where necessary, annotations explaining apparent anomalies. Data may be anonymous as information on individual or company names is not necessary. Data should cover personal exposures over the working shift and/or describe short-term and/or peak exposures where acute hazards exist and/or where major tasks are undertaken which could give rise to significant exposure. Data collected using static samplers should only be used in assessment if there is sufficient information provided to demonstrate that they reflect personal exposures. Samples should be taken at breathing zone height and in the immediate vicinity of workers. If there is a large quantity of data available pooled and statistically evaluated data may be sent provided that the methods used to do this are made clear. The raw data should be available for the assessor to see if needed.
- generally, at least 12 data points would be required to adequately describe the exposure of a work activity within one company. However, it should be noted that data from one company might not be representative of an industrial sector.
- quality assurance information providing evidence that data have been collected and analysed according to recognised protocols and methods. This might include satisfactory performance within appropriate inter-laboratory quality assurance schemes and a description of the sampling strategy.
- details that enable the reliability and representativeness of the data to be assessed. This includes considerations such as:
  - when and why it was obtained?
  - what were conditions at the time, e.g. normal or abnormal?
  - were the data collected according to defined sampling strategies e.g. EN 689
  - do the data reflect past or present practice within the industry?
  - do the data reflect conditions in one company or is it representative of the industry?

Figure 1 Core information requirements for exposure assessment

2.2.4 Inhalation exposure assessment

In an occupational context, exposure is usually assessed based on external exposure, i.e. the available dose. For inhalation exposure the amount inhaled is represented by the airborne concentration of the substance in the person's breathing zone. Exactly how much a person absorbs into the body will be affected by a number of factors including; the physical state of the substance, the health status of the individual and the rate at which they are working. This is considered at the risk characterisation stage. It is important to remember that the air monitoring data/model predictions used in these exposure assessments are for external dose and not the biologically available dose.

2.2.4.1 Measured data

Measured inhalation exposure data should be representative of exposure over the sampling period. In addition, the data should be capable of properly representing exposure throughout the whole of the time-weighted-average reference period (normally 8-hour). Ideally, in order that data can be viewed as being representative for the workplace/company, they should be collected using randomised sampling strategies. Information collected using non-random strategies e.g. worst-case sampling as part of a compliance programme, will be biased, for the purposes of this risk assessment. Whilst such data can be useful in describing some exposure scenarios, it should only be used if sufficient contextual information is available. The bias in the data should be acknowledged. Any significant bias within the data should be capable of identification, at least in qualitative terms, and dealt with where appropriate. Bias alone should not exclude data from consideration; e.g. the removal of high-end exposures due to leaks, spills, etc. It should be identified and acknowledged.

In all cases, data should have been obtained using suitable validated sampling and analytical methods.

Analysis of measured data

The quality of exposure information and its applicability to the assessment process requires careful evaluation before it is incorporated into an exposure assessment. This evaluation should always be carried out using the application of occupational hygiene expertise, rather than applying simple conventions or the rigid use of statistical methods. For example, account will normally need to be taken of the conditions under which the information has been collected, in order to establish how representative this information is, and hence the relevance and weight it will have within the exposure assessment process. Information collected when processes go wrong may not be truly representative of routine operations, even though the data may be used to draw other conclusions on a variety of conditions. Conversely, large quantities of information collected on a substance from the routine operation of process plant will almost certainly not represent many downstream uses of the same substance.

In any analysis of occupational exposure information, the following basic considerations should be applied:

- a clear description of task/activity or process that is being addressed should be given, together with the relevance that it has within the EU. This will include information that clearly indicates which source(s) of information have been used for which purposes and why they have been chosen (and, if applicable others excluded);

- workplace exposure information that is included for the purposes of exposure assessment should be of a suitable quality. It should be collected and analysed according to recognised European norms (e.g. EN 689) and provided in a form that enables it to be assessed, analysed and presented in a manner that ensures clarity and traceability. All data should be both reliable and representative of the circumstances it is intended to describe. It is important for the assessor to be able to distinguish between data from a single company and pooled data from a number of companies in order that the representativeness of the data can be established;
- individual sets of raw data supporting single data points are the preferred starting point for any exposure assessment. These data points should be supported by core information, i.e. as outlined in **Figure 1**;
- the information contained in **Figure 1** identifies the core elements that are likely to be required in order that modelled estimates of exposure can be effectively determined for any given sector/activity. In some instances, statistical information describing sets of measured exposure data may also be useful, both as an adjunct to, or in lieu of individual data points;
- measured exposure data should reflect the personal exposures of the workers and should seek to describe, as a minimum, time weighted exposure over the working shift. Data may also be representative of task specific sampling. Where possible, this information should be supported by exposure information and data that describe the exposures representative of short-term tasks/operations, particularly those where elevated exposures may be anticipated. This is usually achieved by personal sampling. Data collected using static samplers should only be used to assess exposure if the results make it possible to assess exposure to the worker. Appropriate static samples should be taken at breathing zone height and in the immediate vicinity of the workers;
- where statistical interpretation of available measured data is attempted, then at least 12 data points should be available for specific tasks that are representative of a specific job type, activity or sector, e.g. loading or discharging the substance for a particular defined set of control conditions. The choice of 12 data points as the minimum required for statistical interpretation was a pragmatic one based on knowledge of the likely amounts of data available and the judgement of the authors. As well as statistical evaluation of the data, expert judgement is needed to draw conclusions on the representativeness of the data provided. Where less than 12 data points are available the quantitative information may help characterise any uncertainties, although there will be a need to supplement this with modelled estimates of exposure;
- measured data, from analogous substances relating to similar exposure scenarios can be pooled with data relating to the substance under assessment if deemed appropriate by the assessor. The assumptions used and judgements made should be fully and clearly described;
- data at the extremes of any set of exposure measurements should be treated consistently and transparently. For example, in the case of “non-detectable” data points, these should be reported and statistically assessed using a value of half of the detection limit. It is acknowlegded that this simple substitution method has little theoretical basis but rather it should be seen as a pragmatic approach to this type of data. It often performs well and has the advantage of being simple to use. The detection limit and the sampling time should be stated. All high-end exposures should be included in reported data unless clearly stated reasons are given to the contrary;
- suitable and appropriate expert judgement should be applied when interpreting exposure information, including the statistical evaluation of quantitative exposure measurements. All judgements made should be clearly and concisely explained. This is likely to include the use of suitably qualified occupational hygiene personnel.

Measured data presentation

For each scenario that the exposure assessment is intended to describe, information should be provided which details the number of samples, the number of facilities in which exposure measurements were carried out, exposure range, and median and 90th percentile value. The information should be in a form that ensures any need for data confidentiality is respected. In order that data analysis is consistent with normal quality assurance principles, the basis for the choice behind underlying assumptions and calculations should be provided. Graphical interpretations of the data are not necessary, but can be helpful in its interpretation by those without specific expertise.

2.2.4.2 Modelled data

Interpretation of the EASE inhalation model

The EASE model generates its output as a range of concentrations for continuous exposure at the process under consideration. The initial use of full-shift exposure information to develop the model was modified somewhat by the application of expert judgement. Knowledge that is based on a broad range of experience cannot easily take into consideration the detail of work patterns. As experience with using EASE has grown, it seems that the model output indicates exposure that occurs at the task itself. Some defaults for EASE use for inhalation exposures in common industrial activities are given in Appendix I D.

EASE outputs can be used as an assessment outcome by themselves, or they can be used to construct time-weighted averages for any observed pattern of work. However, as this procedure is based on pragmatic interpretation of the model rather than scientific rigour, the results it produces should be regarded with caution. Work is currently under way to collect more information on short-term exposures. When available this information will be incorporated into any revision of EASE.

Where there is likely to be exposure to both dust and vapour, EASE can be used to predict exposures for each physical state and subsequently added together to give the overall exposure range.

As it currently stands EASE cannot be used to predict inhalation exposures during:

- spray painting;
- welding;
- soldering;
- processes which lead to the formation of mists and
- for substances released as decomposition products.

2.2.4.3 Mixtures

The measured data used to derive the ranges in the EASE model were collected in workplaces where the substance measured was often only one component of the materials in use. Some substances are in fact always measured as mixtures, for example, oil mist or foundry particulate. Nevertheless, it was assumed for the purposes of the model that measured data could be treated as if the source was the pure substance. If a substance is supplied as or used in a mixture and relevant data on how the mixture might become airborne are not available, a simple approach would be to reduce the estimated exposure by a factor equivalent to the concentration of the

substance in the mixture. Other approaches have been used, notably multiplying the vapour pressure of the substance by its proportion in the mixture, to produce a revised vapour pressure which is then input to EASE. Where appropriate, information on the composition of formulations is needed in order to calculate the vapour pressure

Although there is no rigorous scientific basis for any of these approaches, and they do not always give the same results, they have the benefit of simplicity, and can be regarded as first-order approximations. Appendix I G gives an assessment of partial vapour pressure for components in multi-substance preparations as well as a possible method for calculating exposure and a reference to a computer based tool for the calculation.

2.2.4.4 Assessment when respiratory protective equipment is used

Health and safety legislation requires that respiratory protective equipment (RPE) should be the last option for controlling inhalation exposure to hazardous substances. RPE can only protect the wearer, whilst other control measures, e.g. local exhaust ventilation (LEV), protect everyone by preventing the substance entering the atmosphere of the workplace. Also if RPE is used incorrectly or badly maintained the wearer may receive little or no protection. No form of RPE provides complete protection against exposure; there is always some actual or potential leakage of a substance through or around RPE and into the breathing zone of the wearer. All of these factors need to be taken into account when assessing inhalation exposure when RPE is used.

Several groups have presented “Assigned Protection Factors” (APF) for respiratory protective equipment, for example the British Standard BS 4275. The assigned protection factor is defined as the workplace level of respiratory protection that would be provided to 95% of wearers by properly functioning RPE when correctly fitted by adequately trained and supervised workers. It is derived from protection factors measured in real workplaces against real workplace contaminants.

Exposure should always be assessed on the first instance for the unprotected worker and, if appropriate, a second assessment, should be made taking RPE into account. The effects of using RPE can only be taken into account if there is sufficient knowledge on the use and appropriateness of RPE; i.e. the assessor is confident that for a particular scenario appropriate RPE is worn by the vast majority of people exposed via inhalation. If there is confidence regarding the use of appropriate RPE, it should be assumed that RPE is used consistently for the relevant scenario and that reasonable procedures for fitting the RPE and cleaning and maintenance of the equipment exist, unless clear evidence to the contrary is available. Section 2.2.5.7 provides more detail on the criteria for establishing confidence in the appropriate PPE for skin exposure. Similar criteria should be used for RPE. The exposure reducing effect of the RPE in use can be made clear in the risk characterisation part of the risk assessment report. In those cases, the APF, as given by BS 4275 for the relevant type of RPE, should be used to calculate exposure with RPE from the exposure without RPE.

2.2.5 Dermal exposure assessment

Many of the factors which influence other forms of exposure, such as the way the job is done, environmental conditions, and the human factors introduced by the interface between workplace and operator, also influence the magnitude of potential dermal exposure. The variability in potential dermal exposures is very large indeed and the more variables present to modify exposure, the wider the distribution of results. Contamination will rarely be evenly distributed

over the body. In some cases it will occur on areas well protected by personal protective equipment (PPE), whereas in other cases the contamination may be directly onto the skin or even beneath protective clothing. Knowledge of the distribution of contamination on the body may lead to a more effective risk assessment. Ideally real representative exposure data should be used to assess the health risks from dermal exposure.

Data are currently being generated to complete exposure profiles for a number of occupational scenarios to enable a more acceptable and rigorous prediction of dermal exposure. This is being achieved via the RISKOFDERM project which is being funded by the EU. One of the aims of the project is to develop a validated predictive dermal exposure model, based on all relevant approaches in the literature and theoretical considerations, as well as the detailed results obtained in another part of the project.

The RISKOFDERM project started in February 2000 and will last for four years. Valuable information on determinants and data for potential and actual dermal exposure will become available throughout the life of the project. However, validated, predictive models will only become available nearer the end of the project, in 2004. Until more information on dermal exposure is available, an approach, including the use of analogy reasoning where possible and using EASE if real exposure data are not available, is suggested here for assessing dermal exposure. This approach is depicted in **Figure 2** below and described in detail in Appendix I E. Any new studies on dermal exposures, as well as knowledge gathered in the RISKOFDERM project will be incorporated into this approach when available.

All dermal exposure models or approaches require the user to make decisions on the categorisation of the exposure scenario in relation to dermal exposure. To make this type of decision, i.e. to use the analogy approach, the analogy of the scenario must be critically reviewed. Sufficient analogy is needed for the major potential exposure determinants:

- substance and product characteristics (e.g. dustiness, viscosity);
- percentage of substance in the product handled;
- tasks and activities; and
- frequency and duration of tasks and activities.

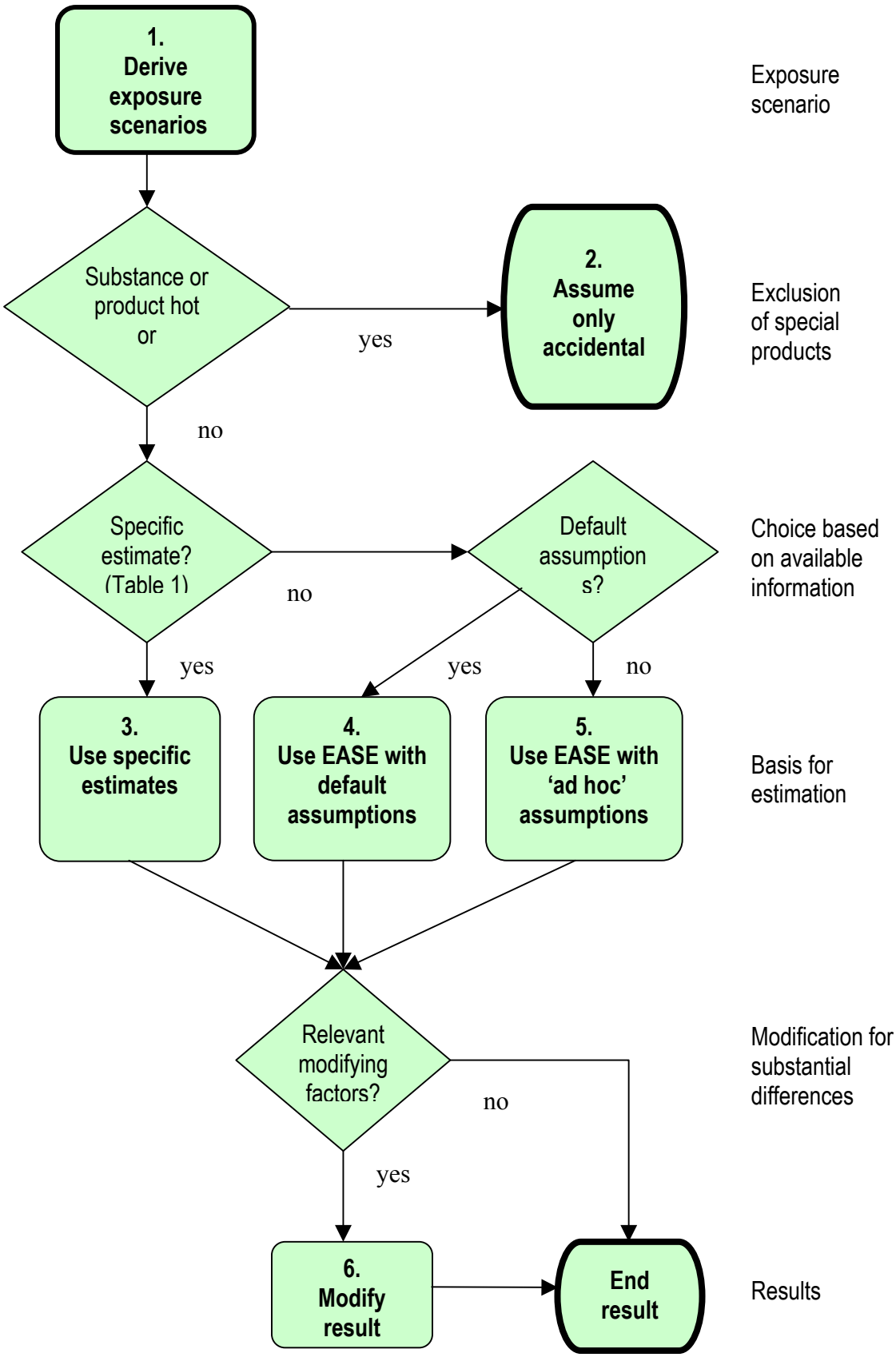

Figure 2 Dermal exposure assessment scheme

2.2.5.1 Measured data

The approach for dermal exposure is to use measured data for scenarios when they are available (including use of analogy reasoning) and to use EASE or other appropriate models if measured data on the scenario are not available. The general approach is given in **Figure 2** and the Table of Values for specific parameters in this approach are presented in Appendix I E. It should be emphasised that the presentation and use of measured data for dermal exposure of the specific substance in the specific scenario are much preferred to the use of any modelled data. The fact that some model approaches do exist to quantify dermal exposure should not detract from gathering better data, because all of the model approaches lead to highly uncertain results due to the limited overall knowledge on dermal exposure.

Measured dermal exposure data should include information on: surface area sampled (cm^2); mass of contaminant (mg); mass per unit area (mg/cm^2); duration of sampling/exposure (minutes); frequency of exposure (number of times per day that separate exposure situations occur, e.g. number of batches produced per day); sampling method and the composition of any mixtures. Supporting information should include details of workwear worn, including laundering and personal hygiene.

2.2.5.2 Modelled data

The dermal exposure model used in EASE is a very simple one and is related to the US EPA model. It is based on the available knowledge in 1993, when EASE was first developed, and some detailed discussions among occupational hygienists in Health and Safety Executive (UK). The model predictions are not derived from actual measured exposure data. Dermal exposure is assumed to be uniform and is assessed as a potential exposure rate predominantly to the hands and forearms (approximately 2,000 cm^2). It is also assumed that dermal exposure to gases and vapours is very low, that no personal protection of any sort is worn and that exposure depends only upon actual contact with a contaminated article of surface.

The model addresses neither the impact of personal hygiene (such as hand washing) nor evaporation or other types of loss from the skin (for example, through sweating or abrasion).

2.2.5.3 Corrosives

For the handling of corrosive substances and formulations, immediate dermal contacts occur only occasionally (repeated daily dermal exposure can be neglected). For properly labelled corrosives, therefore, it will not be necessary to assess the risk from dermal exposure. However, it should be noted that this applies to corrosive properties only, and effects due to other properties of the substance may need to be assessed. If, during the use of the corrosive substance or formulation diluting/mixing occurs which results in a substance or formulation which has no corrosive properties then dermal exposure should be taken into account, i.e. repeated dermal exposure cannot be neglected.

2.2.5.4 Irritants

Contrary to the situation for corrosives, it should not be assumed that exposure to irritants will be avoided as, although this may be the case for strong irritants, it may not hold for weak irritants. The situation will need to be judged on a case-by-case basis.

2.2.5.5 Evaporation rate

For highly volatile substances, dermal exposure is reduced because of the shortened retention time of the substance on the skin. For example, using the equation given in Appendix I F, for a substance with a vapour pressure of 21 kPa and a dermal exposure level of 1 mg/cm², the evaporation time is calculated to 4 seconds (parameters calculations and examples are given in Appendix I F).

This exposure reducing effect due to evaporation cannot be considered if workers have continuous direct contact with the substance, e.g. dipping hands into the liquid substance. Furthermore, for taking the fast evaporation of a substance into account, non-occlusive dermal exposure has to be the predominant exposure situation. However, there are scenarios (e.g. production and further processing in the chemical industry) for which the unhindered evaporation of substances from the skin (or the protective clothes) is probable.

2.2.5.6 High temperatures

Dermal exposure in scenarios which include the handling of hot products (>60^{o}C) does not need to be assessed as dermal contacts are only likely to occur occasionally for very short periods.

2.2.5.7 Assessment when personal protective equipment is used

This section describes, when and how the effect of PPE on reducing exposure should be considered in exposure assessment. The general lack of knowledge on quantitative and qualitative aspects of dermal exposure, as well as the little available information on the manner of use of gloves and other PPE and on their suitability, have led to repeated discussions on how to assess dermal exposure and also, how to consider the protection afforded by suitable or unsuitable gloves.

Exposure should always be assessed in the first instance for the unprotected worker and, if appropriate, a second assessment, should be made taking PPE into account. A second assessment taking PPE into account should only take place if:

- PPE is used regularly by a vast majority (90%) of workers in the vast majority (90%) of facilities making or using the chemical and,
- the PPE used is suitable for that situation.

In order for the assessor to make this judgement industry should provide comprehensive information:

- on the suitability of the PPE used (e.g. gloves: tested according to EN 374 for the substance under consideration),
- to show that other methods of control are not sufficient or not reasonably practicable to prevent exposure, i.e. that PPE is used as a “last resort”,
- to show that appropriate schemes are in place to manage the provision of suitable PPE,
- to show that the PPE is regularly maintained and cleaned and,
- to show that users of the PPE have been adequately trained in its use.

In the short term, default protection factors are proposed (see below). In the longer term, actual protection factors or probabilistic modelling based on available penetration distribution data may be necessary. It should be borne in mind, however, that human factors play a large part in

determining the extent of skin contamination beneath PPE. Impermeable materials can even promote skin exposure if adequate hygiene procedures are not followed.

European standards for protective clothing, including gloves, give information on the fundamental suitability of the glove material and only little indication of the levels of the protection that are afforded wearers in practice. Information on suitability for individual substances and, especially, for mixtures of substances, is less than comprehensive and of variable quality. Studies suggest that the spread of contaminants inside protective clothing, including gloves, is commonplace and significant.

Studies on low volatility liquids indicate that the quantity of contamination inside gloves appears to be independent of activity and the type of product used, but is dependent only on the time the glove is worn (related to the number of times the gloves are put on and taken off). This is probably because dermal contamination beneath gloves to low volatility liquids is not due to permeation through the fabric of the glove, but due to contamination getting around the protection, possibly from hands which are already contaminated. This may also be true for protective overalls. Wearing gloves, or overalls, already contaminated inside, can result in very high exposures. Practically no information is available for factors determining contamination inside gloves and overalls from volatile liquids.

Until further data are available for the effectiveness of PPE, a pragmatic solution needs to be adopted of selecting realistic default values for properly selected coveralls and gloves, say 90% protection (=10% penetration), which can be revised as further evidence becomes available. However, it is suggested that, in the longer term, at least for low volatility liquids, a default value based on the time the gloves are worn would give a better prediction of hand contamination. Also, studies suggest that, for protective coveralls, the default value for protection would depend on the extent of the challenge. However, this information is not normally available to assessors within the framework of the Existing Substances Regulation programme.

2.2.6 Biological monitoring

When available, biological monitoring data can be used within the exposure assessment. It can add value to the exposure assessment process by providing information that enables a better understanding of the nature and extent of exposure. Biological monitoring information serves as an additional data point that helps to both better characterise exposure and further reduce the uncertainty surrounding the effectiveness of control measures in the workplace. However, biological monitoring information requires careful interpretation by experienced practitioners.

Where biological monitoring information is available, it should be used to help describe the relevance of exposures in specific tasks or activities. This requires that the available data be appropriately pooled. To provide a reliable estimate of exposure for any defined scenario sufficient data points, to account for inter- and intra-individual variability, should be included within any workgroup for which biological measurement is undertaken. It is expected that this would contain at least 12-20 data points.

Sufficient information must be provided to show the relevance of the biological monitoring data to the substance, jobs and/or tasks. The half-lives of substances measured by biological monitoring can influence whether or not a measured result is representative of a day's exposure or a longer period. In some cases one sample at the end of the day is the appropriate sample, whilst in other cases a full day pooled sample (24 hours) should be used. The proper sampling methodology should also be used.

Biological monitoring information reflects actual exposure, i.e. it indicates that exposure has occurred and that absorption into the body has taken place. It often provides an important indication of the effectiveness of control measures, including PPE. However, biological monitoring information seldom indicates the primary route of exposure or the relative proportion that different exposure routes contribute to total dose.

Hence, biological monitoring information should be seen as equivalent (i.e. as having neither greater nor lesser importance) to other forms of exposure data e.g. airborne measurements. Biological monitoring data must meet all of the same quality requirements that relate to other forms of exposure information. That is, it must be of a high quality and representative of the circumstances it is intended to describe. For a number of compounds, biological monitoring is well established and described (in terms of methodology, analytical quality assurance and control parameters and pharmacokinetics). For the majority of chemicals however, methodology is still under development and essential features, such as quality control standards and programmes are lacking.

It should also be remembered that biological monitoring results reflect an individuals total exposure to that substance from any relevant route, i.e. from consumer products, and/or from the environment and not just occupational exposure.

2.2.7 Exposure levels taken forward to risk characterisation

The exposure assessment is carried out through an evaluation of different scenarios. Once the scenarios are described, a quantitative estimation of exposure should be provided. There are two endpoints of the scenario evaluation, the Reasonable Worst-Case (RWC) exposure level and the typical exposure level for each particular scenario and each relevant exposure route. These levels should be representative of the scenario described.

2.2.7.1 Uncertainty

In Section 2.2.2.5 the various uncertainties relating to occupational exposure assessment were discussed. These are:

- measurement uncertainties (including those arising from the physical sampling process);
- selection of measurement results;
- uncertainties of model results; and
- assessment uncertainties.

If any of the sources of uncertainty or variability are ignored or at least some indication of their likely impact on the final assessment are not given, this will lead to assessments which will have spurious precision and accuracy associated with them. All of these uncertainties and variabilities need to be considered alongside those uncertainties related to the interpretation of the toxicology data in the process of risk assessment.

2.2.7.2 Short-term sampling data

For some substances acute health effects will be of importance. In order to provide a relevant estimate of exposure the assessor should request short-term sampling data from industry. This should not be confused with measurement of exposure peaks, which is not often carried out and

therefore data reflecting this are hard to obtain. If such data are available they should be evaluated in the same way as described in Section 2.2.4. Where the data are of sufficient quality and reliability they should be used to provide a reasonable worst case and typical value for short-term exposure.

EASE outputs can be used as an assessment outcome by themselves, or they can be used to construct time-weighted averages for any observed pattern of work. However, as this procedure is based on pragmatic interpretation of the model rather than scientific rigour, the results it produces should be regarded with caution. Work is currently under way to collect more information on short-term exposures.

2.2.7.3 Particle size

Where exposure is to dusts, an indication of the particle size distribution should be provided, where available. This information is useful for the estimation of uptake through inhalation, because the uptake may depend on the deposition pattern in the airways. This deposition pattern in turn depends on the particle size distribution. The percentage of respirable particles is especially relevant and also the possible exposure to ultrafine particles (<0.1 μm). As a minimum the size selection characteristics of the sampling methods used should be provided, for measured data on dusts. It is vital to know whether inspirable dust or respirable dust is measured.

2.2.7.4 Type of air sampling data

Personal sampling data, taken by validated methods and using appropriate sampling strategies, is preferred for use in exposure assessment. However, static sampling data, taken using appropriate sampling strategies and thus reflecting personal exposure can also be used. Provided that there is sufficient information supplied which will enable the assessor to know how they relate to worker exposure. The static samples should be taken at breathing zone height in the immediate vicinity of the worker.

2.2.7.5 Biological monitoring data

For biological monitoring data a number of parameters should at least be mentioned. These include the exact parameter measured, the sampling strategy (e.g. spot sample at the end of the working day, or 24 hour sample), the biological half-time of the measured substance and any information that may help the interpretation of the data. Biological monitoring data should be presented with the same core information as data on inhalation or dermal exposure to enable proper interpretation of the outcome in relation to working conditions. Where available, established relations between biological monitoring levels and inhalation (or dermal) exposure levels should be presented. A clear discussion of the meaning of the biological monitoring data in relation to inhalation and dermal exposure levels, exposure duration and possible health outcomes should be provided.

2.2.7.6 Reasonable worst-case scenario

The reasonable worst case is regarded as the level of exposure which is exceeded in a small percentage of cases over the whole spectrum of likely circumstances of use for that particular scenario. It excludes extreme use or misuse but can include the upper end of normal use as it is

recognised that control of exposure may be poor or non-existent. Exposure which results from accidents, malfunction or deliberate misuse should not be addressed. Cleaning and maintenance, if carried out regularly and frequently, should be included in normal use.

Expert judgement is needed to define the circumstances that can be assumed as part of a reasonable worst-case scenario, in order to avoid the exposure estimate being grossly exaggerated. Measured or modelled data, and/or data from analogues can be used to derive a reasonable worst-case exposure level. In analysing measured and modelled exposure data, some degree of interpretation and expert judgement is needed.

To decide the reasonable worst case, all measured data sets and qualifying information gathered for a particular scenario should be considered. Since the aim is the identification of highly exposed workers, the aim is to try to approximate to the 90^{th} percentile values.

When the data set is small, values near the highest end of the concentration range should be used, to ensure that highly exposed workers are represented. In this case, critical evaluation of outliers, to determine whether or not they should be excluded, becomes essential to avoid an unrealistic estimation. For instance, an outlying result could have been the consequence of an error in the collection of samples. If this information is not known or not considered and the value is taken into account in the exposure assessment, the exposure would be seriously overestimated. In cases where the uncertainty in the estimation is high, the conclusion that there is a need for further information is fully justified.

If possible, it would be useful to also include a reasonable worst-case exposure level for short-term exposures.

2.2.7.7 Typical values

Typical exposure is an estimate of the approximate location of the median levels of exposure over the whole spectrum of likely circumstances of use for each scenario. It may be indicated by the central tendency of measured data which can be qualified using expert occupational hygiene opinion and expert knowledge of the process. If few good quality measured data are available expert knowledge and opinion will essentially determine the estimate of typical exposure.

The aim of providing typical values in the exposure assessment is to provide relevant information to aid risk managers in their decisions relating to the choice of appropriate risk reduction measures.

2.2.7.8 Criteria for determining reasonable worst case and typical values

The representativity and reliability of these data should be evaluated according to the principles established in Section 2.2. A combination of quality exposure data and professional judgement should go together in the derivation of the reasonable worst case and typical exposure levels. This estimation is best carried out by the application of occupational hygiene expertise rather than by a rigid application of statistical methods.

Measured data

In the evaluation of exposure data, and in order to decide on the reasonable worst case and typical exposure levels, the following should be taken into account:

- more weight should be given to the more complete and recent set of data but without omitting the rest. Old data sets should not be rejected without evaluation because they are also informative. This information is particularly important if the technological methods have not suffered an important change;
- outliers should not be eliminated from the analysis without a critical evaluation. When a data set includes an abnormally high result, an explanation of the reasons for such a high value should be provided. This clarification is necessary to know whether or not it should be considered in the decision of the reasonable worst-case exposure value;
- in general, data revealed as low quality data should not be used, but sometimes they are the only available measured data and in that case some information can be drawn from them;
- measurements are often sampled using a worst-case strategy. This type of sampling strategy only covers exposure episodes in the higher range. A similar situation occurs if the data where gathered for enforcement purposes, since they could represent an extreme situation. These facts should be taken into account in the assessment;
- it is possible that the measured data do not reflect the full range of activities within a scenario. For instance, when a closed system is being evaluated, it might happen that activities leading to highest exposures such as sampling, filling and maintenance would not be covered by a data set that only includes plant operators. Therefore, these measurements are not representative and cannot be used to derive the reasonable worst case and typical estimates.

When quality measured data are not available for a particular scenario, it may be possible to extrapolate from data from analogues. Due to the extrapolation process, the uncertainty in the estimation will increase. To make this uncertainty as low as possible, data from analogues could be used assuming the following requirements:

- quality analogous data are available,
- differences between the substance and the analogue are small and,
- the process from which data come from is similar to the assessed process.

Obviously, expert judgement becomes an important component in this extrapolation process.

Modelled data

Modelled exposure data are often obtained from the EASE model. Exposure depends on the physical properties of the substance, processes involved and the measures of control in place. Professional judgement and experience is needed both to introduce input values and to interpret outputs. For instance, different patterns of control (e.g. LEV and dilution ventilation) could be used in different companies that use the substance in the same way. In these cases, it should be assumed the least effective control (e.g. dilution ventilation) as an input parameter in order to approximate to the reasonable worst case.

EASE provides results as ranges of exposure. As stated before, in order to decide the reasonable worst case and typical exposure levels from the exposure range, information is needed about the process, how the exposure takes place, the duration and frequency of exposure. EASE predicts on the basis of exposure to the pure substance and allows for estimates to be corrected where the substance of interest is present only as a proportion of the formulation in use. Consequently, this information should be used in the derivation of the reasonable worst case and typical exposures.

EASE results are independent of the amount of substance used. It would be advisable to consider this aspect when making decisions, for instance, if a low quantity is involved the exposure level can pragmatically be assessed as being towards the bottom of the range. A high amount of substance would correspond with the highest part of the range.

Dermal exposure is assumed to be uniform and it is assessed by EASE as the potential dose rate predominantly to the hands and forearms (approximately 2,000 cm^2). Since it is unreasonable to assume that in some tasks, the whole hands and forearms surface would be covered of chemical, an estimate (in percentage terms) of the likely exposed surfaces should be provided in order to make a more meaningful estimation.

2.2.8 Workplace exposure assessment rating criteria

Table 1 shows a scheme for evaluating the usefulness and appropriateness of available exposure data and information in order to determine both reasonable worst case and typical exposure values. The aim of these criteria is to enhance the confidence with which data can be used. In **Table 1**, the conclusion that there is a need for more information is suggested if the basis for the exposure assessment is very poor. However, if industry is not able to provide any further information which would lead to clarification then the assessment should proceed as if no information is available.

Table 1 Workplace exposure assessment rating criteria

Data characteristics	Comments & interpretation
Actual data of high quality e.g. personal exposure data (including that obtained by biological monitoring) that are representative of the scenario being described; which have been collected and analysed according to recognised (e.g. CEN or equivalent) protocols; and that are available as sets of raw data supported by information of key exposure determinants.	This form of data is likely to enable a decision whether or not there is concern dependent on the MOS. Unless key activities are not covered by data of this type, a conclusion that there is a need for more information is unlikely to be necessary. Data confidence is high and this should impact the interpretation of the MOS at the RC stage of the risk assessment.
Analogous/surrogate data of a similar quality to the above and which describe exposures that derive either from: - Other substances having similar exposure characteristics (e.g. volatility, dustiness), or - Other comparable activities considered likely to provide a reliable estimate of exposure for the scenario in question. Actual data of intermediate quality e.g. data that have been consolidated and where only basic statistics are available to support them; where data have been obtained using non-standard protocols; where data cannot be described as being fully representative of the scenario; obtained from static sampling which can be shown to reasonably represent personal exposures, etc.	This form of data is likely to enable a decision whether or not there is concern dependent on the MOS. A conclusion that there is a need for more information may be more appropriate when the MOS is borderline. Data confidence is good and this should positively affect the interpretation of the MOS at the RC stage.
Predicted exposures derived from suitable models and using input criteria/values that are relevant for the scenario and are derived from accepted EU sources. Actual data of lesser quality e.g. where data are only available from compliance monitoring or static sampling; where limited information on key exposure determinants are available. Surrogate data of intermediate quality e.g. conforming to the definition for actual data contained in above, but where only basic statistics are available to support them or where data points may be insufficient to suggest representativeness.	To reflect the increased uncertainty of data, should yield the conclusion that there is no concern if associated MOS are correspondingly higher. The conclusion that there is concern may be appropriate when the MOS is low. Where moderate MOS are present, the conclusion that there is a need for more information is likely to be more appropriate. Data confidence remains acceptable, particularly when the exposure assessment is derived from an extensive range of sources. Exposure data derived from compliance monitoring are often biased towards reflecting high-end exposures. This in-built bias should be accounted for at the RC stage.
Exposure data arising from sources not addressed in any of the above classes. For example, this may include data obtained from non-appropriate static sampling; circumstances when input data for models are inadequately defined; some biological monitoring data which have been used to predict airborne exposure levels.	Cannot be used to reach the conclusion that there is no concern. The conclusion that there is a need for more information is the preferred default. The conclusion that there is concern may be indicated only in exceptional circumstances. Data confidence is questionable and these data alone cannot usefully be used to describe risk. However, such data can be useful in helping to interpret those scenarios where some exposure data may be deficient and in guiding decisions on the nature of gap filling.

MOS = margin of safety
RC = risk characterisation

2.3 CONSUMER EXPOSURE ASSESSMENT

2.3.1 Introduction

The objective of this section is to describe an efficient, step-wise and iterative procedure for the assessment of consumer exposure to both existing and new substances.

The consumer, i.e. a member of the general public who may be of any age, either sex, and in any stage of health, may be exposed to a new or existing substance by using consumer products. A consumer product is in general regarded as a product that can be purchased from retail outlets by members of the general public. It can be the substance itself, or a preparation, or an article containing the substance. Consideration of consumer exposure is of importance because the possible means of controlling the extent of exposure are extremely limited and cannot normally be monitored, or enforced beyond the point of sale of the products. For a new substance, use as or in a consumer product may be regarded as "other reasonable grounds for concern" requiring a risk assessment, even if the substance is not classified.

The assessment of the exposure of consumers should be conducted following a logical, iterative procedure, which starts with an initial "**screening**". This screening is needed to identify if the substance under investigation is actually used as or in consumer products or whether the expected consumer exposure is so low that it can be neglected further in the risk characterisation phase. If this is the case no further assessment is needed and the conclusion can be mentioned in the assessment report. If use as or in consumer products has been identified and the exposure is not considered to be negligible as described above, then a **quantitative** exposure assessment is desirable. The results of this quantitative assessment are taken forward to the risk characterisation where they are combined with the results of the effects assessment in order to decide whether or not there is concern for the consumers exposed to the substance.

In order to carry out a quantitative consumer exposure assessment to a substance, information on a lot of exposure parameters is typically required. Unfortunately, in most cases this is not realistically achievable, and exposure assessment must be made using the available data, expert judgement and reasonable assumptions. However, although in many cases data may be sparse, it is also possible to estimate exposure by various techniques. The purpose of this section is to provide an overview of the consumer exposure assessment and to give detailed information on:

- the scope of the consumer exposure assessment for new and existing substances;
- general background to the types of consumer exposure that need to be considered;
- types of the information needed to carry out a consumer exposure assessment;
- how to perform the initial screening;
- how to perform the quantitative exposure assessment using modelling techniques;
- how to use measured data in the assessment;
- how to improve the exposure assessment if the risk characterisation indicates the need to do so.

It is realised that these are all developing areas that will probably change quite regularly. However, the basic principles for performing the assessment will probably remain the same. Therefore in preparing exposures assessments, the most up-to-date information should be used. This may mean that advice not included in this document can still be used, as long as there is a general consensus among Competent Authorities that what is proposed is acceptable, as reflecting the state of knowledge at the time the assessment is prepared.

2.3.2 Scope of the consumer exposure assessment

2.3.2.1 Definitions

As indicated above, the assessment of consumer exposure in principle deals with consumer products that are can be purchased from retail outlets by members of the general public and may be the substance itself, a preparation, or an article containing the substance. Examples of human exposures to substances arising from the use of consumer products are:

- exposure to solvents from the use of glues/adhesives;
- exposures to substances released or leached from articles e.g. from use of baby bottles in child care;
- exposure to perfume/scent raw materials from the use of cosmetics.

Additionally, for the purpose of this TGD, other exposures of the consumer are included under Consumer Exposure despite the fact that the exposure does not arise from the use of consumer products. These additional exposures capture any other human exposure, which are neither considered as occupational nor as indirect exposure via the environment. Examples include:

- exposure to substances from contact with medical devices including implants etc;
- exposure to substances from handling/preparing food and drink including contact with cooking utensils, food packaging etc.;
- exposure to substances in the home after use of decorating or cleaning products by professionals;
- exposure to substances in indoor air (residential air: e.g. household, schools, nurseries) including the fraction adsorbed on dust particles arising from building materials;
- exposure to substances in public areas (e.g. swimming pools, recreational areas).

It is realised that in some cases no strict differentiation can be made between consumer exposure and indirect exposure of humans via the environment. An example of such a situation is the indoor air exposure arising from neighbouring industrial activities (e.g. through walls). In addition, the source of a substance in food may arise from the environment and/or food processing/packaging techniques making it difficult to separate the sources. In this TGD, indirect exposure of humans via the environment is defined as the exposure of humans via consumption of food and drinking water, inhalation of air and ingestion of soil which in turn are directly influenced by the releases of the substance into the environmental compartments air, water and soil. Therefore all other exposure situations of humans, although strictly speaking not always arising from the use of consumer products, should preferably be dealt with under the consumer exposure section of the risk assessment report.

2.3.2.2 Legislative considerations

Many consumer products are subject to other EU and/or national legislation (e.g. cosmetics, toys, food contact materials, pharmaceuticals).

For new substances, it should be noted that Article 1(2) of Directive 67/548 excludes from notification substances for which Community notification or approval procedures exist and for which requirements are equivalent to those of Directive 67/548.

Thus, for example, a substance will not be notified if it is only marketed as part of a cosmetic formulation and the substance will be included on one of the "permitted lists" of the Cosmetics Directive (76/768/EEC): Such substances need pre-marketing approval in accordance with Cosmetics Directive procedures. As notification takes place prior to marketing, the Competent Authorities will, in such cases, need to consider the potential risk to consumers via the proposed cosmetic formulation. Prompt communication with the agency concerned is strongly advised so that resources are used in the most efficient and appropriate manner.

For existing substances the relationship between the scope of the risk assessment performed under Regulation 793/93 and other Community legislation has been described in Chapter 1 (Section 2).

Clearly, the reasons for including or excluding the consumer exposure assessment should be highlighted in the risk assessment report with the appropriate argumentation.

2.3.2.3 Considerations regarding the assessment of reasonable worst-case situations

The consumer exposure assessment should normally address the intended uses of the products that contain the substances under investigation. However, since consumers may not accurately follow instructions for use of products or articles, a separate assessment of other reasonably foreseeable uses should be made. For example, consumers may over-dose (e.g. of dishwasher detergent in relation to the doses recommended on the product) or fail to take other recommended actions that are designed to minimize the potential for contamination (e.g. they may leave containers open after having used the product which can give rise to potential inhalation exposure to substances). However, consideration of deliberate abuse is not part of the exposure assessment process. It is recognised that the step from other foreseeable uses to abuse can in certain cases be small. In these situations the assessor should provide clear argumentation why a certain exposure situation is included or excluded in the assessment.

If a substance is used in more than one consumer product, or if more than one type of use is employed (e.g. brush painting and spraying), or if the product could reasonably be expected to be used in other ways (e.g. use of a washing machine detergent for washing by hand), it may be necessary to assess exposure for each case (see also Section 2.3.6.4 on aggregated exposure). If a substance is used in a consumer product that has different modes of use, the exposure assessment should be focused on those uses for which the highest exposure is expected to occur on a regular basis. Note that if a substance occurs in different consumer products the exposures should usually be aggregated in order to prevent underestimation of the potential exposure whereas, in case of different modes of use (of a given product), the highest exposure is taken forward the assessment. Data obtained for similar products with similar use patterns should be used as appropriate.

Certain exposed sub-populations may be exposed differently than others. If for instance exposure of young children is anticipated, their crawling behaviour and hand to mouth contact may bring them into contact with residues of products on the floor. In addition their small body size to surface area compared to that of adults may have a crucial effect on the exposure estimates. Therefore the exposure assessment needs to take into consideration factors specific to the exposed consumer sub-populations.

2.3.3 Types of consumer exposure

In general the way consumers are exposed to substances can be characterised by (1) the different routes of exposure, separately or in combination (2) identifying users and non-users and specific population sub-groups and (3) identifying the different phases of activity in handling the consumer product.

2.3.3.1 Routes of exposure

Substances may enter the body by being breathed in (inhalation), by passing through the skin (dermal), or by ingestion (oral) either separately or in combination. Exposure to a particular substance should normally be understood as external exposure. This can be defined as the amount of the substance ingested, the amount or concentration of the substance in contact with the skin and/or the amount or concentration of the substance inhaled, which is represented by the airborne concentration of the substance in the breathing zone of the consumer. The exposure estimates usually do not refer to concentrations within the body, which are related to some measure of the absorbed dose. However in some cases consumer exposure may be a direct exposure of internal parts of the body (e.g. exposure from medical devices, piercings, tattoos). In these cases internal exposure is assessed and the exposure estimates should be carried over to the risk characterisation.

Inhalation exposure

Substances can reach the air that is inhaled by consumers either during the actual use of the consumer product (for instance as the result of vaporizing solutions) or as a result of volatilisation after the product has been used (e.g. evaporation of solvents from paints). Exposure by inhalation is expressed as the concentration of the substance in the breathing zone atmosphere, and is normally presented as an average concentration over a reference time period (e.g. per day). If exposure is of intermittent short duration there may also be interest in exposure over shorter periods (e.g. per event). The assessment can also be based on exposure during specific tasks, which may be carried out over varying time periods.

Some consumer products are used as sprays in the form of aerosols. In this case the exposure to the substance is due to that of the droplets which needs to be considered specifically in the exposure scenario.

Dermal exposure

Substances may have the ability to penetrate the skin and be absorbed into the body. Dermal exposure is an estimate of the amount of substance contacting the exposed surfaces of the skin. It is the sum of the exposure estimates for the various parts of the exposed body surface. Dermal exposure can occur from splashes on the skin, from direct hand or body contact with the consumer product containing the substance, from deposition on exposed skin of particles or

aerosols from an airborne substance or from skin contact with residues of the substance after product use e.g. residues on clothing after laundering or dry cleaning. The amount and concentration of the substance, the area of skin exposure and the duration and frequency of exposure can influence the actual dermal exposure to a substance. Dermal exposure is expressed in terms of the amount of substance per unit surface area of the skin exposed.

Oral exposure

Substances occurring in products that can be ingested can cause oral exposure. The most noteworthy example is the exposure from the use of mouth and teeth care products. Oral exposure may also occur due to sucking, chewing or licking of products such as toys, children's books, kitchenware or textiles. This is of particular relevance to children due to their hand to mouth behaviour. In addition oral exposure can occur due to the ingestion of small amounts of food packaging materials, utensils or cosmetic products.

In some cases, also occasional and foreseeable exposures to chemicals in other products (e.g. detergents, glues, monomer residues and softeners in plastic and PVC-products) may need to be considered. A specific example of oral exposure is the uptake of dust and soil by children, provided that the loading of soil with substances is related to the use of consumer products.

Obviously, the exposure scenario needs to be realistic. The exposure to products and chemicals that are hardly ever accessible to children should not be considered. In case of risk of serious accidents caused by strong acids and alkaline chemicals or strong oxidants and other chemicals of high acute toxicity, the scenarios could be described in the risk assessment report, but then be left for other sectors to be dealt with.

Migration characteristics of the substance (packaging material), solubility (utensils) and amounts typically used (cosmetics) are important parameters to be considered. These parameters, together with concentration and contact parameters, are used to quantify the respective exposures. Oral exposure is expressed as the amount of substance ingested, and is normally presented as an average daily dose.

Other routes of exposure

Besides these three major routes of exposure, in special cases other routes of exposure must be considered, in particular the intradermal or intravenous routes. Intradermal exposure occurs when the integrity of the skin is disrupted by the use of consumer products (e.g. by earrings or tattoos). Intravenous exposure may occur during the use of medical devices (e.g. an infusion device from which migration of monomers or other substances takes place). In these cases, the exposure is expressed as the total amount of the migrating substance and normally presented as an average daily dose.

2.3.3.2 Primary and secondary exposure

Another way to characterise consumer exposure is by looking at the different (sub)-populations that are actually exposed to the products. Primary exposure to substances occurs to the individual who actively uses the products containing the substances, i.e. the user. Examples of primary exposure are wearing of textiles, use of glues or the use of paints or household cleaning products. Secondary exposure occurs to non-users or bystanders; these are individuals who do not actively use the products but are indirectly exposed to substances released during or after product use by another person (the user). Examples of secondary exposure of non-users include exposure to

paints, cleaners etc. during or after use by the user, and exposure to household articles and appliances (e.g. flame retardants in furniture, plasticisers in building materials) which have been treated with the substance and then placed in the home**.** Secondary exposure scenarios also include contact with the substance following the application of professional products in the home e.g. from paints after painting in the home by a professional or exposure of the farmer's family from agricultural products at a farm.

Note that according to this definition the user of a product may be subject to both primary **and** secondary exposure and, as a consequence, will often have the highest exposure, whereas the non-user or bystander has only secondary exposure. Such secondary exposures may be of less immediate concern than primary exposure unless this occurs to specific subgroups of the population that may experience higher exposures because of their specific behaviour (e.g. children crawling on the floor).

2.3.3.3 Phases of activity

A third way of categorising consumer exposure is by looking at the different phases of activity in which the products are actually used. There are up to four phases of activity that are relevant to consumer exposure:

1. preparation for use by the user, which includes tasks like handling and dilution of concentrates;
2. use or application by the user;
3. post-use or post-application leading to exposure of the user and/or the bystander;
4. removal/cleaning leading to exposure of the user who may be another individual than in the first phase. This includes activities such as emptying and cleaning equipment, stripping surface coatings, etc.

Each phase of activity may require a separate assessment, given that the first can reflect exposure to a concentrate, the second to a dilute solution, the third to a vapour or semi-dry residue and the fourth to "waste material". In practice however, the scenarios chosen for the different products may integrate some or all of these phases.

2.3.4 Data needs and sources

2.3.4.1 Data for the initial screening

As the initial screening specifically deals with the question whether or not the substance is actually used in consumer products, data are needed on the occurrence in products present on the European market. These data should normally be included in Chapter 2 "General Information on Exposure" of the risk assessment report. Also consultation of the occupational exposure chapter may indicate the occurrence of consumer products. In general, the most logical sources for obtaining this kind of information are:

- dossiers provided by the producing/importing company(ies);
- product registers that are available in some countries.

If these sources do not provide sufficient information other sources can be used such as:

- (inter)national trade associations;
- national consumer products inspectorates;
- poison control centres and case studies reported in the literature.

The diversity of consumer products does not allow for a single set of information sources, handbooks or databases to be consulted. Rather, it is necessary to explore which information sources apply to the substance of interest. A list of further valuable sources on exposure data is given in Appendix II (Section 6).

2.3.4.2 Data for a realistic quantitative exposure assessment

To assess the exposure to substances present in consumer products, information is needed on two sets of parameters: 1) contact parameters and 2) concentration parameters. The contact parameters denote by which route the exposure occurs (e.g. inhalation) and where, how long and how often contact with the consumer occurs. The concentration parameters are needed to estimate the concentration of a substance in a medium that might contact the body, the so-called potential exposure. This is not necessarily equal to the concentration of the substance in the product, because a product might be diluted, mixed, undergo evaporation etc. before the substance of interest actually reaches the human body.

By combining the contact parameters with the potential exposure, the actual exposure to a substance is obtained, that is, the dose or the concentration of the substance in a medium that is in contact with the body. A third component might be added to an exposure assessment, namely the amount of substance taken up into the systemic circulation. This latter component, however, needs to be considered in the risk characterisation phase, using information (on absorption) from the toxicokinetic section of the hazard assessment.

For a realistic assessment without the use of defaults, the following data would ideally be available:

Contact parameters:

- intended use of product;
- frequency of product use (contact);
- duration of product use (contact) per event;
- site of product use, including size of room and air exchange rate;
- physical form of product (aerosol, dry powder, large crystals, liquid, gas etc.);
- amount of product used per event;
- contact surface (if appropriate).

Concentration Parameters:

- weight fraction of substance in the product;
- concentration of substance in the product as used e.g. after dilution or evaporation has occurred (if available);
- amount of a substance in an article;
- amount of residual monomers;
- emission rate constant (if available);

Behaviour data:

- time spent indoors;
  - sleeping room,
  - living room,
  - bathroom,
  - kitchen,
  - in a vehicle (e.g. car, bus, train, aircraft),
  - office/workplace;
- time spent outdoors;
  - garden,
  - playground,
  - streets/traffic.

Information on actual product use by the consumer is not widely available. The instructions of the manufacturer provide information on the recommended use, not on the way products may be handled before or after actual use nor on reasonably foreseeable other uses. Information might also be available from Poison Control Centres and case studies reported in the literature. In some cases such data represent mostly the more extreme misuses of the product and might not be very informative about the normal range of uses. Some trade associations have provided data on product uses (amount, frequencies, use duration) for specific product categories that may be useful for estimating exposure. These data may be found in Appendix II (Section 5.3).

Specific information on use durations and contact frequencies for consumer products is often lacking. An estimate of these parameters can be derived from time budget data where available. Time budgets comprise information on the behaviour of a population during a day, week or year. Assuming that certain products are used for one or more recorded tasks, data on how long and how often those tasks are typically performed provide an estimation of product use durations and contact frequencies. The main disadvantage of time budget data is that the task categories are often non-specific such as “being indoors”, “driving a car” or “cleaning a bicycle”, etc. Therefore, a direct link between a time budget task and the use of a certain product might be problematic. Further, time budget data are gathered by a variety of groups but most of this information remains in internal publications. Because time budgets may vary geographically, it is useful to check if the national statistical agencies have gathered such data on a regional basis.

For some such consumer products (e.g. food contact materials) there is a legal requirement for the supply of measured exposure data to the regulatory agencies concerned. The assessors should use these data, where available and appropriate, when conducting the exposure assessment.

2.3.5 Initial screening

Initially, the likelihood that an exposure of consumers to the substance under consideration occurs is evaluated. If the result of the initial screening is that the substance is not used as or in a consumer product or if the resulting consumer exposure is judged to be low enough to be neglected further in the risk characterisation phase, it is not necessary to continue the assessment of the consumer exposure.

In order to be able to reach such a decision, the assessor should review all relevant information on the exposure and use pattern of the substance submitted by the notifier in the notification dossier (for a new substance) or by manufacturers and importers under Article 9 (2) of Regulation 793/93 (for an existing substance).

When the assessor is notified that a new substance is now intended for a new use as or in a consumer product, that substance will also then be subject to a consumer exposure assessment.

For existing substances it is often necessary to supplement the information received from industry by consulting additional sources of information as described above before taking such a decision. In practice the manufacturers and importers of existing substances can seldom provide the assessor with complete data on downstream uses. If there are indications of a potential exposure of consumers to the substance this should be pursued by consulting industry.

If the initial screening verifies that consumer exposure occurs or cannot be excluded as negligible, a list of the relevant categories of use should be established (see Appendix II, Section 2). For each category a quantitative assessment needs to be conducted to assess the potential exposure of consumers to these substances via inhalation, ingestion, and/or the dermal pathway.

It should be noted that under the current legislation for new and existing substances the producers and importers of products which are often referred to as the downstream users do not have any obligations towards providing information to the producers and importers of substances on the way these substances are used in their products. This implies that a definitive answer to the question whether or not substances are used in consumer products can often not be easily given. In most cases the assessor needs to apply a weight of evidence approach based on all information that is at hand.

2.3.6 Quantitative exposure assessment

2.3.6.1 Scenario building

A substance may be used in different types of consumer products and this will lead inevitably to different patterns of exposure. In order to systematically assess the exposure of consumers to (a range of) consumer products, so-called consumer exposure scenarios have to be defined. A consumer exposure scenario describes the circumstances of the exposure of a person to a substance. It links the information on substance amount or concentration in a medium or location with the activity patterns and other behavioural information for a consumer in contact with that substance. The description of a scenario can be divided into three parts:

- use of a substance;
- the pathway of the substance (release, distribution and elimination) in order to describe the contacting amount/concentration;
- behaviour of the exposed (sub)-population.

For each consumer exposure scenario the reasonable worst-case exposure should be estimated. This is the exposure that could be expected to occur under consideration of the manufacturer's maximum suggested usage frequency and application rates (the combination of the two should be checked for realism, see also Section 2.3.6.3). If data are not available, some information on default patterns of use data for various consumer product types can be found in Appendix II (Section 5.3). Worst-case exposure may also be caused by poor ventilation, small room volume, extensive skin contact, etc. (the maximum amount used and unfavourable use conditions may occur simultaneously).

The actual estimation of the exposure can be made by using simple models that describe the mode and extent of contact of the exposed consumer with the substance. The assessment of

duration and frequency of exposure requires an understanding of the substance and/or product/article use category and use scenario. Appendix II (Section 3.1) gives details of simple algorithms which are recommended for use in assessing consumer exposure for a number of common exposure scenarios. These should be selected and used with the appropriate care. Useful equations are given for the following estimates:

1. for inhalation exposure: an estimate of the concentration of the substance in surrounding air without consideration of time;
2. for dermal exposure: an estimate of the concentration of the substance in contact with the skin and the amount of substance on skin;
3. for oral exposure: the amount ingested that is calculated by multiplying the estimated concentration of the substance in media by the volume ingested.

If the assessor judges that some exposure scenarios, or an exposure route are not relevant, reasons for this judgement should be given. Typical questions that can be posed when developing a consumer exposure scenario are listed in **Figure 3**. Establishing the relevant exposure scenarios needs expert judgement and should be made in a transparent way. To aid the clarity of the document similar uses of the substance can be clustered into a single scenario.

Typical questions that can be posed when developing a consumer exposure scenario:

- What is the frequency of use?
- What is the duration of use?
- Who is using the product?
- What is the vapour pressure of the substance?
- What is the room volume?
- Where is the product used?
- Relevant paths of exposure?
  - If the vapour pressure is high: consider inhalation exposure
  - Should dust and/or soil be considered as an important path of exposure
- Are there exposed persons in addition to the user (bystanders)?
- Are children exposed – consider child's behaviour?
- Are there specific geographical or cultural differences that need to be taken into account?

Figure 3 Typical questions

Assessors should focus their attention on those scenarios where the exposure is expected to be the most significant. The degree of detail required for an exposure scenario should be linked to the perceived magnitude of the risk. In this way, the problem of including excessive amounts of text for low-risk situations will be avoided, while at the same time giving potentially serious risks the amount of attention they deserve. However, enough detail should be given to enable the reader to be confident that potentially important scenarios are not missed.

In some cases the consideration of exposure to substances via dust and/or soil is needed. Dust and soil often act as carriers for substances that have a low vapour pressure. Those substances are either part of dust themselves or they are adsorbed to dust and soil (e.g. metals can be transported via dust and soil). A distinction should be made between the airborne dust particles that lead to inhalation exposure and those particles that lay on the ground (house-dust). The latter particles are typically important for dermal and oral exposure of children.

For effects which may be expressed following a single exposure (e.g. irritation; acute toxicity) mean and maximum exposure levels per event will be needed for risk characterisation. For repeated-dose toxicity the daily exposure level averaged over an appropriate period of time (e.g. a year) will be required. Both upper estimates from reasonable worst-case scenarios as well as averages will be needed.

When feasible, measured or estimated values should be used for each of the numerical parameters. When this is not possible default values (e.g. for room volumes) may be derived from available data sources. Representative values for human physiological parameters relevant to the assessment of consumer exposure (e.g. surface areas of skin of hands, forearms, head, etc.; breathing rates and volumes) are widely available via the literature (see references for some sources). Some default and representative values are given in Appendix II (Section 5.2). Reasonable assumptions need to be made which should be compared to data where this is available. The assessment report should describe in a transparent way the values that are selected and used in the exposure calculations

Sometimes workers and consumers may have similar exposure scenarios e.g. use of cleaning agents, use of paints and adhesives for carpets. It may be possible to use consumer exposure models for workers or worker exposure models for consumers taking into account that consumers usually will use the product less frequently and possibly during a shorter time. On the other hand, in a consumer exposure scenario, room ventilation is often insufficient, less guidance and safety information is available and gloves are often not used.

2.3.6.2 Highly exposed or more vulnerable (sub-) populations

In order to avoid unrealistic exposure estimates and an overestimation of exposure, it is recommended to calculate a population average and ranges (e.g. minimum and maximum exposure values) which allows the recognition of the variation of input parameters. The combination of group extremes for deriving a final estimate of exposure should be avoided. It should be recognised however, that there may be cases where sub-populations are exposed and overall population averages are too low. Some consumers may be exposed to higher concentrations than others because of differences in the behaviour and physiological parameters. Young children may for instance be exposed to higher levels than adults due to their distinct (hand to mouth) behaviour. The scenarios need to be designed in a way that they take such factors specific to the exposed consumer sub-population into consideration. Factors that might lead to a different internal dose of children, such as their low body weight or their relatively high body surface area, are normally taken care of in the risk characterisation phase.

Some consumers may be more vulnerable than the average population (e.g. neonates, persons in poor health, the elderly, or consumers with specific vulnerability to certain types of effects, e.g. asthmatics). It is expected that exposure in general will not be determined by the vulnerability of the consumer. The potential difference in risks between more vulnerable groups and the general population needs to be discussed in the risk characterisation part of the Risk Assessment Report.

2.3.6.3 Check for realism

When using any equations or computer models, particularly if default or “reasonable worst-case” values are used, it is essential to conduct a check for realism. For example, it might be considered reasonable, for initial screening purposes, to accept that 10% of a substance contained in a particular quantity of toothpaste could be ingested by an adult in a single, normal event; but if the calculation indicated that, for instance, 50%, or 500%, of the total amount of substance in the whole pack is ingested, the input parameters should be checked and adjusted as appropriate. Refining the parameters to be more “realistic” will not be necessary if the judgement is already that consumer exposure is of “no concern” and the refinement can only lead to lower estimates of exposure.

Whilst a reasonably conservative estimate of consumer exposure is justifiable in the first instance, grossly excessive estimates should be avoided (e.g. cumulative worst-case estimates). Also, care should be taken to avoid under-estimating exposure. The derivation of sets of exposure range values, for key products used under specific conditions, is recommended so that estimated values can be checked against the expected range.

2.3.6.4 Aggregated consumer exposure

Consumer exposure to a substance can occur from multiple sources, because products contain many substances and a substance may be present in multiple products in many different forms. Hence, when the individual scenarios are assessed separately, the exposures to a single substance need to be aggregated thus providing the aggregated consumer exposure. This aggregation is not a simple summation of the individual estimates but needs to take into account questions such as to what extent co-occurrence of products with the same chemical in households takes place and what is the co-use of products with the same chemical.

The first question refers to the percentage of households having/using the product. Information can be derived from, e.g., marketing studies or questionnaires. As a rough approximation, extent of dissemination to households can be divided into “low” and “high” and co-use can be estimated from frequency of use (i.e. use is “seldom” or “often”). The second question refers to correlations in product use. In the case of co-use, it can be assumed that products that are often used have a high probability to be used in a short time span from each other. As a worst-case assumption it can be assumed that products from different subcategories are used next to each other in the same household and therefore need to be considered when the aggregated exposure is calculated.

The timescale of the exposure also needs be considered. On a chronic time scale, products with a high use frequency will dominate human exposure. Co-use of products will be less important, because on a sufficiently long time scale, all products are co-used. Aggregated chronic exposure is calculated by summing all consumer exposures in a sufficiently long time interval (e.g. a year to life time) and averaging over the time interval. A typical dose measure will be a year averaged or life-time averaged daily dose. For short-term exposures the averaging interval will typically be event average or day-of-use average.

2.3.6.5 Outcome of the quantitative exposure assessment

The quantitative consumer exposure assessment results in reasonable worst-case estimates for the external exposure via the inhalation, dermal and/or oral route that are taken forward to the risk characterisation for consumers. With regard to inhalation exposure an estimate of the air concentration (e.g. mg/m^3) in the breathing zone of the consumer is needed. For dermal exposure an estimate of the concentration of the substance in contact with the skin (e.g. % w/w) and the amount of substance on the skin (e.g. mg/cm^2) is needed. For oral exposure an estimate of the amount ingested (e.g. mg/kg bw/day) is needed.

Exposure estimates are needed for the general population or, if relevant, for a specific highly exposed sub-population or for a specific sub-population that is specifically vulnerable and for which the exposure estimates for the general population do not apply. These estimates are obtained for all relevant scenarios that have been identified taking into account all relevant available information. In the risk characterisation phase these estimates are combined with the results of the effects assessment and conclusions are drawn whether or not there is concern for any of the scenarios assessed.

2.3.7 Use of measured data in the exposure assessment

As indicated in the introduction to the section on human exposure, in general measured data are preferred over modelled data provided they are reliable and representative for the situation that needs to be assessed. For most consumer exposure scenarios identified for new and existing substances, measurements of the actual exposure of consumers will not be available. However, it may be possible that for one or more of the parameters used in the estimations, chemical, product or scenario specific data are available than can be used to override the default values.

There may be measurements of external exposure (i.e. concentrations in the environment in which the contact takes place) as well as measurements of internal exposure (e.g. in blood or tissues). Biomonitoring programmes are occasionally performed to study the body burden of chemicals and the results may be very valuable for exposure estimations. Furthermore, monitoring programmes of industry, particularly for occupational exposure may be useful for comparative evaluations with consumer exposure although their number, representativeness and quality will often vary within wide ranges. Thus, the measured data available should be evaluated carefully.

Measured data from surrogate substances or analogues may also be useful when estimating exposure levels. Extrapolations using surrogate substances as well as surrogate scenarios (e.g. chamber measurements) should be transparent. In cases where surrogate data are the only data available and it is not possible to obtain further information, it may be necessary to conduct an exposure assessment based on such data.

On the other hand, measurements of exposure factors (model variables) may exist, e.g. room volumes, air exchange rates, migration rate constants, ad- and desorption as well as absorption rates (e.g. skin permeation rates). These measured data can be used for model estimations.

In some cases, exposure measurements for a specific product may be available or such studies might be conducted. Exposure surveys need to be large enough, well enough reported, and representative of the population and scenario of interest to be convincing.

2.3.8 Influence of personal protective equipment

There are very limited circumstances for consideration of Personal Protective Equipment (PPE) in consumer exposure, because people will not normally use PPE unless it is convincingly recommended by the manufacturer and provided with the product. Clothing is the only kind of PPE for which it may be considered reasonable to include in the exposure assessment for consumers since the actual dermal exposure can be affected by the nature and extent of the clothing worn. Even normal clothing can offer some degree of protection against external contamination.

Due to the large uncertainties of the influence on reducing exposure of more advanced personal protective equipment and the even larger uncertainties of whether this equipment is actually used by the consumer, these should not be taken into account in the exposure assessment. The correct selection and use of eye and face protection or respiratory protective equipment requires a level of training which is unavailable to the consumer. The use of protective gloves may require some more discussion. Good practice and personal hygiene will sometimes indicate that household gloves are desirable (e.g. for products that are irritating/corrosive to the skin, such as strongly acidic, alkaline or oxidant household chemicals). Although it can be considered that in cases where skin irritation is the critical adverse health effect and gloves are supplied with the product or substance, these gloves will in most cases be used, the actual assessment needs also to consider the reasonable worst-case situation which indicates no use of gloves.

2.3.9 Improvement of the exposure assessment

If the risk characterisation for consumers concludes that further information is needed to refine the exposure assessment for one or more scenarios and it is expected that this information can be obtained within the timeframe given by the respective legislation, then the assessor needs to decide how the refinement can be achieved. In principle a range of possibilities exist such as:

- gathering more information on one or more parameters used in the original exposure scenarios;
- gathering more specific information on the actual products that are used by the consumer;
- if more detailed data are available, use of more sophisticated models to investigate the potential exposure situation with a higher degree of certainty;
- performance of actual exposure measurements.

Although the quantitative exposure assessment as described in Section 2.3.6 must in principle be based on all relevant data available, in practice the calculations will in many cases involve a number of assumptions and the use of defaults and/or non-specific information for the products assessed. The most likely way to improve the assessment is therefore to gather information on one or more of these parameters in order to make the assessment as specific as possible for the scenario under investigation. Usually such an exercise will be targeted towards one or a few of the scenarios evaluated and not to all exposure routes that were considered initially. Some sort of model sensitivity analysis tool or some reasonable judgement may be used to find those exposure factors that need only a small change to cause a relatively large change in predicted exposure. These exposure factors should preferably be described by more realistic data if possible. On the other hand, exposure factors that hardly cause changes in predicted exposure do not need precise quantification. In this step and depending on the amount and quality of data available, the use of probabilistic analysis may be helpful to further characterise exposure.

In contrast to the models that are described in the section on quantitative assessment (Section 2.3.6) more sophisticated models exist that contain an adequate description of the processes underlying the exposure scenario. These models are expected to be of increased specificity and complexity and will require the quantification of a relatively large number of exposure factors. They may include for example the consideration of time dependent processes of migration and release of the substance from a matrix, the deposition (adsorption) to other matrices (e.g. dust) and its release (desorption) as well as the disappearance from the medium (e.g. by decrease of room air concentrations due to ventilation). If needed, for consideration of variability and uncertainty percentiles of distributions (generally 95^{th} or 99^{th} percentiles, but sometimes also 50^{th} or 75^{th} percentiles) can be defined to replace the reasonable worst-case assumptions.

When the consumer exposure assessment is refined using these more sophisticated models, a very careful description of the scenario and the subsequent models used for calculations, including all assumptions, is required. Results of model estimations should be given in an adequate manner and should be discussed critically and transparently.

Several models are used in either computer programs and/or published in the scientific literature. Recommendations for the use of certain models for exposure assessment will not be given. However, a selection of scenarios and models that may be useful for consumer exposure assessment can be found in Appendix II (Sections 3.2 and 4).

2.4 EXPOSURE OF HUMAN VIA THE ENVIRONMENT

2.4.1 Introduction

Indirect exposure of humans via the environment may occur by consumption of food (fish, crops, meat and milk) and drinking water, inhalation of air and ingestion of soil. The different routes of exposure are illustrated in **Figure 4**.

(Exposure via soil ingestion and dermal contact is not addressed in this guidance because they represent significant exposure routes only for specific situations of soil pollution.). The indirect exposure is assessed by estimating the total daily intake of a substance based on the predicted environmental concentrations for (surface) water, groundwater, soil and air.

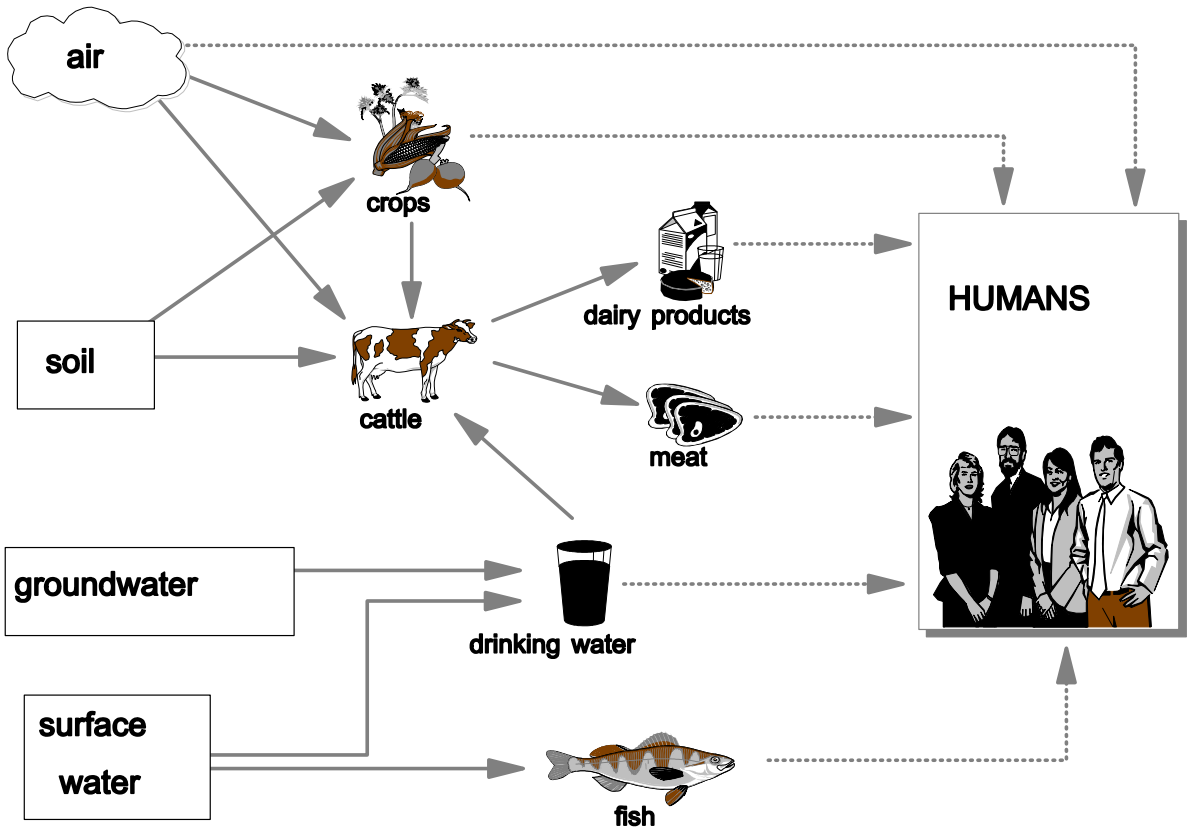

Figure 4 Schematic representation of the exposure routes considered in human exposure

The assessment of the indirect exposure via the environment is carried out following the sequence of the step-wise procedure:

- assessment of the concentrations in intake media (food, water, air and soil);
- assessment of the intake rate of each medium;
- combination of the concentrations in the media with the intake of each medium (and if necessary, using a factor for the bioavailability through the route of intake).

Because of their supply levels and the effects of distribution, new substances are not expected normally to attain environmental concentrations which could lead to significant indirect exposure levels for humans. Calculations of the total daily human intake of new substances on the basis of predicted environmental concentrations indicate that substances placed on the market in quantities below 1,000 tonnes per annum (tpa) do not normally attain indirect exposure levels which would cause concern in relation to the health of the public at large.

However, when there is a high level of concern about a substance because of its toxicity there is a greater possibility of a risk of adverse effects on human health arising from indirect exposure when supply levels reach 100 tpa. There will be a high level of concern if:

- the substance is classified "Toxic" with a risk phrase "R48"; or
- the substance is classified as a carcinogen or mutagen (of any category); or
- the substance is classified as toxic to reproduction (category 1 or 2).

Therefore, an assessment of indirect exposure, entailing calculation of total daily human intake, should normally be carried out for new substances placed on the market in quantities of 1,000 tpa or more. Assessment of indirect exposure at lower levels of supply should only be conducted if the substance has been shown to be of high concern because of its toxicity, as described above. Where an assessment of a new substance is required the approach given below for existing substances should be used.

The calculation methods described are simple, flexible and "state of the art" methods for predicting the indirect exposure to humans via the environment. They serve primarily for screening purposes. The concentrations in the environmental compartments which are required as input data in the models for the calculation of the total daily intake via the different exposure routes should be derived on the basis of monitoring data and/or modelling by applying the approaches described in Chapter 3 on Environmental Risk Assessment. The concentration of a substance in food is related to its concentration in water, soil and air and is also dependent on its bioaccumulation or biotransfer behaviour. The models for the estimation of daily intake allow the use of local or regional environmental concentrations, as appropriate. The methods require the use of a limited number of input parameters and can, if required, be adapted for specific human populations for which it may be necessary to assess the exposure separately. Standard default values for the input parameters are presented.

Human behaviour shows an appreciable amount of variation between the different EU countries. But also within countries, large deviations occur between individuals. As a consequence, indirect exposure will vary greatly over the population we seek to protect. The choice of the exposure scenario will have a major influence on the result of the assessment. This choice will always be a compromise as a scientifically sound solution is extremely difficult to obtain (this would involve elaborate statistical evaluation of human sourcing and mobility behaviour, as well as the distribution and intensity of all local sources).

Indirect exposure is principally assessed on two spatial scales: locally near a point source of the substance, and regionally using averaged concentrations over a larger area. In the local assessment, all food products are derived from the vicinity of one point source, in the regional assessment, all food products are taken from the regional model environment. It should be noted that the local and regional environments are not actual sites or regions, but standardised environments as defined in the Chapter 3 on Environmental Risk Assessment. Clearly, the local scale represents a worst-case situation. People do not consume 100% of their food products from the immediate vicinity of a point source.

Therefore, the local assessment represents a situation which does not exist in reality. However usually, one or two routes dominate the total exposure and local exposure through these routes may not be unrealistic. In contrast, the regional assessment represents a highly averaged exposure situation which cannot insure protection of individuals who consume food products from the vicinity of point sources. A regional assessment gives an indication of potential average exposure of the inhabitants of the region. In light of the above mentioned limitations, it is clear that a generic indirect exposure assessment, as required in this framework, can only be used to indicate potential problems. The assessment should be seen as a helpful tool for decision making and not as a prediction of human exposure actually occurring at some place or time.

For an indirect exposure assessment on EU-level, a standard consumption pattern needs to be defined. Food consumption rates and patterns differ between EU Member States so it is impossible to select an average or worst-case EU country. To account for the fact that intake rates vary between countries, for each food product, the highest country-average consumption rate of all member states will be used. This will of course lead to a total food basket which is an unrealistic, worst-case scenario. In practice however, usually only one or two routes form the bulk of the indirect exposure. The fact that in the exposure scenario worst-case intake through other routes also occurs is therefore negligible. This makes this scenario appropriate as a first approach to indicate possible concern. The outcome of this assessment is comparable to assessing all countries separately (using average intakes), and taking the highest exposure level of all countries.

It should be noted that extreme consumers of certain food products are not accounted for. Taking extreme consumption into account would lead to more severe worst-case local assessments since the entire food basket is already derived for 100% from the local standard environment.

In a case where the regional assessment indicates reason for concern, there is a clear need for refinement of the assessment. In cases where the local assessment does not indicate a potential risk, there is no reason for concern. The situation is less clear in the grey area where a regional assessment does not give reason for concern, but the local assessment does. It should be noted that there is no testing strategy triggered by the indirect exposure assessment. Instead, when there is reason for concern in the local assessment only, a further analysis of the major exposure routes is required to investigate the realism of the local exposure scenario. As the most important routes are indicated by the assessment, this provides a clear starting point for refinement.

Currently, the scenario for indirect human exposure cannot take into account exposure from aquatic organisms apart from fish, because to date an internationally validated bioaccumulation standard test is only available for fish and consumption data on aquatic organisms other than fish are scarce.

A general description of the different relevant exposure routes and guidance for the assessment of the resulting indirect exposure is given in the following sections. A detailed explanation including calculation procedures is given in Appendix III.

Table 2 Environmental concentrations used as input for indirect exposure calculations

Compartment	Local assessment	Regional assessment
surface water	annual average concentration after complete mixing of STP-effluent	steady-state concentration in surface water
air	annual average concentration at 100 m from source or STP (maximum)	steady-state concentration in air
agricultural soil	concentration averaged over 180 days after 10 years of sludge application and aerial deposition	steady-state concentration in agricultural soil
porewater	concentration in porewater of agricultural soil as defined above	steady-state concentration in porewater of agricultural soil
groundwater	concentration in porewater of agricultural soil as defined above	steady-state concentration in porewater of agricultural soil

Indirect exposure scenario	
Local	The entire food basket is sourced from the vicinity of the local point source as defined in the Table. The food basket consists of: fish, root crops, leaf crops, meat, dairy products, drinking water, and inhalation of air. For the standard assessment, the highest country-average intake rate for each food product is used.
Regional	The entire food basket is sourced from the region as defined in the Table. The food basket consists of: fish, root crops, leaf crops, meat, dairy products, drinking water, and inhalation of air. For the standard assessment, the highest country-average intake rate for each food product is used.

2.4.2 Exposure via the environmental compartments

2.4.2.1 Exposure via inhalation of air

This exposure route can contribute significantly to the total exposure for volatile compounds.

The concentration in the intake medium (air) can be calculated with distribution models of Chapter 3.

Only the intake scenario chosen has important consequences on exposure through this route. It is proposed to follow a worst case, but transparent, scenario: continuous, chronic exposure of humans to the air concentration (which is assumed constant). Exposure through inhalation will be summed with exposure through oral routes.

2.4.2.2 Exposure via soil ingestion and dermal contact

These exposure routes will not be handled in this context while exposure through these routes is usually very unlikely. Only in cases of extremely polluted soils (e.g. in dump sites or through calamities) can these routes provide significant contributions to the total exposure.

2.4.3 Exposure via drinking water

Drinking water can be prepared from surface water or from groundwater. Groundwater can be contaminated through leaching from the soil surface, surface water can be polluted through direct or indirect emission. Hrubec and Toet (1992) evaluated the predictability of the fate of organic substances during drinking water treatment. One of their conclusions was that groundwater treatment, which is generally not intended for removal of organic substances, can be neglected. The accuracy of the predicted removal efficiencies for surface water treatment is rather low. This is mainly due to uncertainties in the most effective treatment processes (such as activated carbon filtration).

2.4.4 Exposure via food consumption

Assessing concentrations in food products (in this context fish, leaf crops, root crops, meat and dairy products) in initial or intermediate screening stages usually involves calculation of bioconcentration (BCF) or biotransfer factors (BTF). These are defined as the external exposure (as a concentration or a dose) divided by the internal concentration in the organisms. The use of fixed factors implies that these factors describe a steady-state situation in which the exposure period is assumed long enough to reach a steady-state.

It should be noted that reliable (and relevant) experimental bioconcentration factors are always preferred above estimated factors.

2.4.4.1 Bioconcentration in fish

Fish, residing in contaminated surface water, are able to take up appreciable amounts of (especially lipophilic) substances through the gills or through their food. The concentration in fish may be orders of magnitude greater than the concentration in water. The bioconcentration factor in fish is found to be well correlated with the octanol-water partitioning coefficient (Kow), indicating that lipid or fat is the main dissolving medium. The estimation of fish-water bioconcentration is more specifically discussed in Chapter 4.

2.4.4.2 Biotransfer from soil and air to plants

Plant products form a major part of the food products for humans and cattle. Contamination of plants will therefore have significant influence on the exposure of humans. When trying to predict concentrations in plant tissues, one will immediately encounter several important conceptual problems:

- there are hundreds of different plant species forming the heterogenous group of food crops. Furthermore, varietal differences can also account for large differences;
- different tissues from plants are consumed (roots, tubers, fruit, leaves);
- crops differ in contaminant exposure, many crops are for instance grown in greenhouses;
- crops can be exposed through uptake from the soil, but also through gas uptake and aerial deposition.

From the above it may be clear that a modelling approach can only give a rough approximation of the concentrations in plants. To account for the predicted variety in plant products, it is proposed to distinguish between tuberous plants and leaf crops. Furthermore, the exposure of plants should include the soil route, as well as the air route.

Uptake from soil is, in general, a passive process governed by the transpiration stream of the plant (in case of accumulation in leaves) or physical sorption (in case of roots). Uptake into the leaves from the gaseous phase can be viewed as a passive process, in which the leaves components (air, water, lipids) equilibrate with the air concentration. A general form of steady state partitioning, coefficient) between these compartments is given by Riederer (1990). Kow and Kaw (the air-water partitioning coefficient) are used to assess the distribution between the air and the plant. It is proposed to use the modelling approach of Trapp and Matthies (1995) to estimate levels in leaves and roots due to uptake from soil and air.

2.4.4.3 Biotransfer to meat and milk

Lipophilic substances are known to accumulate in meat, and can be subsequently transferred to milk. Cattle can be exposed to substances in grass (or other feed) with adhering soil, drinking water, and through inhalation of air. Biotransfer factors can be defined as the steady-state concentration in meat, divided by the daily intake of the substance. Travis and Arms (1988) calculated biotransfer factors for cow's meat and milk by log-linear regression on a number of substances (28 for milk and 36 for beef).

Even though the theoretical background is limited, these factors provide a useful tool in risk assessment. It is proposed to use the same exposure estimates for air and crops which have been derived for human exposure for cattle, and the same soil concentration as for plants.

It should be noted that no distinction is made between different milk products like cheese or yoghurt. For all dairy products, the concentration in milk is used.

2.4.5 Total daily intake for humans

If concentrations in the intake media are calculated, the total daily intake of humans can be estimated from the daily intake rate of each medium by summing the contribution of each medium. This is elaborated in the indirect exposure scenario explained in Section 2.4.1.

3 EFFECTS ASSESSMENT

3.1 INTRODUCTION

The effects assessment comprises the following steps of the risk assessment procedure:

- hazard identification: the aim of the hazard identification is to identify the effects of concern and to review any current classification (including non-classification) of the substance (for existing substances and biocides) or determine the classification (for new substances and biocides).
- dose (concentration) - response (effect) assessment, which is the estimation of the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect. In this chapter it is referred to as "Dose-response". At this step the no observed adverse effect level (NOAEL), or, if this is not possible, the lowest observed adverse effect level (LOAEL), shall, where possible and appropriate, be determined for the observed effects. If appropriate, the shape of the dose-response curve should also be considered.

During both steps of the effects assessment it is of high importance to evaluate the data with regard to their adequacy and completeness. The evaluation of adequacy shall address the reliability and relevance of the data.

For the effects for which it is not possible to determine a N(L)OAEL, it is generally sufficient to evaluate whether the substance has an inherent capacity to cause such an effect. Where for such an effect it is possible to draw a relationship between the dose or concentration of the substance and the severity of an adverse effect, this relationship should be determined.

Generally human data will only be available for existing substances. If both animal data and human data are available, as a general rule, well reported relevant human data for any given endpoint is to be given preference for the risk assessment. Exemptions from this general rule are studies conducted with human volunteers. These studies are strongly discouraged as they are problematic from an ethical point of view. Results from such studies should be used only in justified cases (e.g. tests which were conducted for the authorisation of a medical product or when effects in already available human volunteer studies with existing substances have been observed to be more severe than deduced from prior animal testing). However, the potential differences in sensitivity of human studies and studies in animals should be taken into account in the risk assessment, on a case-by-case basis. In relation to hazard identification, the relative lack of sensitivity of human data may cause particular difficulty: negative data from studies in humans will not usually be used to override the classification of substances which have been classified on the basis of data from studies in animals in accordance with the criteria given in Directive 93/21 (Annex VI to Directive 67/548), unless the classification is based on an effect which clearly would not be expected to occur in humans.

In Sections 3.5 to 3.12 there is a common structure, which sets out logically the procedure for considering that effect.

The structure is as follows:

- definition of the effect;
- objectives of the guidance for that effect;
- data availability, divided into the information the assessor needs, and that information which is available;
- evaluation of available data on that effect;
- guidance on assessment for that effect.

For a number of effects, testing strategies which were designed to provide guidance on the systematic and stepwise gathering of information on new substances are presented in the following sections. For existing substances, these strategies should, in general, be used as a tool, in combination with expert judgement, to determine the need for further testing. The starting point should be the base-set. If the assessor decides further testing is needed, the tests which are required for new substances at each tonnage trigger should be considered in turn. The minimum amount of testing should be specified consistent with obtaining the necessary data. All end-points should be considered and an integrated test package agreed. Within these sections, further guidance on the applicability of each strategy to existing substances is given. There are additional problems in the risk assessment of petroleum substances as they are complex and variable mixtures. Appendix VII gives a suggested pragmatic approach to such assessments.

Core data requirements for biocides are specified in the Directive 98/8 in Annex IIA and further detailed in the Technical Notes for Guidance (TNsG on Data Requirements, 2000). In contrast to new substances, the data requirements for notification of biocides are independent of the annual production rate and entail the submission of a full data package (core data set possibly supplemented by product type-specific data), which is available from the very beginning of the effects assessment process. Therefore, the testing strategy for biocides is not based on the tonnage-triggger system, and the necessity of further testing solely depends on the level of concern that evolved from the previously conducted tests or in some cases from structure-activity considerations.

3.2 EVALUATION OF DATA

During both steps of the effects assessment it is very important to evaluate the data with regard to their adequacy and completeness. This is particularly important for well studied existing substances where there may be a number of test results available for each effect but where some or all of them have not been carried out to current standards. This section puts forward general guidelines on data evaluation. The term adequacy is used here to cover the reliability of the available data and the relevance of that data for human hazard and risk assessment.

3.2.1 Completeness of data

For new substances at supply levels in excess of 1 t per year data equivalent to those foreseen in Annex VIIA to Directive 67/548 (the "base set") will be available. For existing substances the quantity of data available will vary considerably and many substances may have information that goes beyond the base set. However, Regulation 793/93 requires that for existing substances all of the particulars listed in the base set must be available. This is intended to ensure that at least the information equivalent to the base set is provided before the risk assessment process begins. The reference to information equivalent to the base set recognises that there is some scope for reading information across from non-base set data. The scope for doing this depends upon expert judgement and must be considered on a case-by-case basis.

For active biocidal substances, Article 8 of the Directive 98/8 gives the dispositions on data requirements for authorisation. In Annexes IIA and IIB detailed core data requirements common to all active substances and biocidal products, respectively, are specified. Additional data requirements are given in Annexes IIIA and IIIB and depend on the use pattern and product type. The common core data requirements in Annex IIA together with the specific data requirements in Annex IIIA constitute the complete set of data on the basis of which an overall and adequate risk assessment can be carried out for the active biocidal substance. Due to the wide scope of the Biocidal Products Directive and the extensive variation of exposure and risks of different biocidal products detailed Guidance on Data Requirements (TNsG) have been published by the Commission and they are available in the web page http://ecb.jrc.it/biocides.

3.2.2 Adequacy of data

The adequacy of a test can be considered to be defined by two basic elements:

- reliability, covering the inherent quality of a test relating to test methodology and the way that the performance and results of the test are described;
- relevance, covering the extent to which a test is appropriate for a particular hazard or risk assessment.

Reliable, relevant data can be considered valid for use in the risk assessment. 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 test data with respect to reliability is outlined below. Additional sections consider issues specific to the reliability of human and *in vitro* data, relevance to humans and Quantitative Structure-Activity Relationships.

3.2.2.1 Reliability of test data

For new substances and active biocidal substances, tests conducted according to EU Annex V[1] methods and in compliance with the principles of GLP will be available, and consequently many of the issues addressed in this section will not be relevant. Any new tests carried out under Regulation 793/93 must also be carried out according to Annex V methods, or, where such a method is not available, in accordance with internationally recognised guidelines and to GLP.

For many existing substances and some existing biocidal substances, the test data available will have been generated prior to the requirements of GLP and the standardisation of testing methods. That data may still be used for risk assessment but the data and the methodology used must be evaluated in order to determine their reliability for assessment purposes. The evaluation needs expert judgement and must be transparent, so that the use made of a particular data set is clearly justified. The requirements of the appropriate standardised test method and GLP principles should be regarded as a reference when evaluating the available test data. That is, studies carried out according to current methods (e.g. EU Annex V, OECD or US EPA) appropriately reported, should be considered the most reliable for risk assessment.

When looking at a test report, the assessor should consider whether:

- purity/impurities and origin of the test substance are reported;
- a complete test report is available or the test has been described in sufficient detail and the test procedure described is in accordance with generally accepted scientific standards. The information in such a report should be considered to be reliable and should be used for risk assessment;
- the reliability of the data cannot be fully established or the test procedure described differs in some respects from the test guidelines and/or generally accepted scientific standards. The assessor must decide in that case whether the data will be taken into consideration in the risk assessment and how they will be used (e.g. as supporting information where a reliable study has already been identified) or whether they should be regarded as invalid;
- the following factors, among others, can be used to support the view that these data may be acceptable for use in a risk assessment:
  - there are other studies or calculations available on the substance, and the data under consideration are consistent with them,
  - other studies, for example on isomers with similar structure activity profile, homologues, relevant precursors, breakdown products or other chemical analogues, are available and the data under consideration are consistent with them,
  - an approximate value is sufficient for taking a decision on the result of the risk characterisation;
- if critical supporting information is not reported (e.g. species tested, substance identity, dosing procedure) the test data should be considered to be unreliable for risk assessment.

In principle, the same criteria apply to test data reported in the published literature. The amount of information presented will provide the basis to decide on the reliability of the data reported. In general, publications in peer-reviewed journals are preferable. High-quality reviews may be used as supporting information. Summaries or abstract publications may also supply supporting material.

1 The testing methods of Annex V to Dir 67/548 are currently spread in several directives (where Annex V methods are quoted here, they refer to Directive 67/548 as updated in the most recent ATP). An actualised list of available methods and references to the directives that lay them down is available for downloading at the European Chemicals Bureau website (ECB, http://ecb.jrc.it/testing-methods/index.htm).

General principles for data evaluation were discussed at the IPCS meeting on International Co-ordination of Criteria Document Production (the outcomes of the meeting are summarised in Annex 5 of the meeting report (IPCS, 1993)) and have also been described in relation to occupational exposure (EEC, 1992a).

3.2.2.2 Human data

The evaluation of human data usually requires more elaborate and in-depth critical assessment of the reliability of the data than animal data (WHO, 1983). Epidemiological studies with negative results cannot prove the absence of an intrinsic hazardous property of a substance but well documented "negative" studies of good quality may be useful in the risk assessment. Four major types of human data may be submitted (1) analytical epidemiology studies on exposed populations, (2) descriptive or correlation epidemiology studies, (3) case reports and (4) in very rare, justified cases controlled studies in human volunteers.

Analytical epidemiology studies (1) are useful for identifying a relationship between human exposure and effects such as biological effect markers, early signs of chronic effects, disease occurrence, or mortality and may provide the best data for risk assessment. Study designs include:

- case-control (case-referent) studies, where a group of individuals with (cases) and without (controls/referents) a particular effect are identified and compared to determine differences in exposure;
- cohort studies, where a group of "exposed" and "non-exposed" individuals are identified and differences in effect occurrence are studied;
- cross-sectional studies, where a population (e.g. a workforce) is studied, so that morbidity at a given point in time can be assessed in relation to concurrent exposure.

The strength of the epidemiological evidence for specific health effects depends, among other things, on the type of analyses and on the magnitude and specificity of the response. Confidence in the findings is increased when comparable results are obtained in several independent studies on populations exposed to the same agent under different conditions and using different study designs.

Criteria for assessing the adequacy of epidemiology studies include the proper selection and characterisation of the exposed and control groups, adequate characterisation of exposure, sufficient length of follow-up for disease occurrence, valid ascertainment of effect, proper consideration of bias and confounding factors, and a reasonable statistical power to detect an effect.

Descriptive epidemiology studies (2) examine differences in disease rates among human populations in relation to age, gender, race, and differences in temporal or environmental conditions. These studies are useful for identifying areas for further research but are not very useful for risk assessment. Typically these studies can only identify patterns or trends in disease occurrence over time or in different geographical locations but cannot ascertain the causal agent or degree of human exposure.

Case reports (3) describe a particular effect in an individual or a group of individuals who were exposed to a substance. They may be particularly relevant when they demonstrate effects which cannot be observed in experimental animal studies.

When they are already available, well-conducted controlled human exposure studies (4) in volunteers, including low exposure toxicokinetics studies, can also be used in risk assessment in some rare cases. However, few human experimental toxicity studies are available due to the practical and ethical considerations involved in deliberate exposure of individuals. Such studies,

e.g. studies carried out for the authorization of a medical product, have to be conducted in line with the World Medical Association Declaration of Helsinki, which describes the general ethical principles for medical research involving human subjects (World Medical Association, 2000).

Experimental human toxicity studies must not be conducted specifically for the purpose of inclusion on to Annex I, IA or IB of the Biocidal Products Directive.

Criteria for a well-designed study include the use of a double-blind study design, inclusion of a matched control group, and an adequate number of subjects to detect an effect. The results from human experimental studies are often limited by a relatively small number of subjects, short duration of exposure, low dose levels resulting in poor sensitivity in detecting effects.

It is emphasised that testing with human volunteers is strongly discouraged, but when there are good quality data already available they should be used as appropriate, in well justified cases.

3.2.2.3 *In vitro* data

It can be expected that some of the available data for existing substances will have been derived from studies conducted *in vitro* - the basic (and perhaps additional) studies on genotoxicity, for example. There may also be data from *in vitro* studies on, for instance, metabolism and/or mechanisms of action (including studies in cell cultures from different species); dermal absorption (which may also be for different species) and various aspects of toxicity (e.g. tests for cytotoxicity in different types of cells; macromolecule binding studies; tests using embryo culture systems; sperm motility tests). For any of these studies, their usefulness will be influenced by their adequacy in the light of some of the general criteria already discussed, e.g. how well the study is reported, how well the test substance is characterised and to what extent the data requirements of the EU Annex V method have been met for the endpoint under consideration.

However, there are also some criteria which need particular attention when assessing the adequacy of *in vitro* studies, e.g.:

- the range of exposure levels used, taking account of the toxicity of the substance towards the bacteria/cells, its solubility and, as appropriate, its effect on the pH and osmolality of the culture medium;
- whether, for volatile substances, precautions have been taken to ensure the maintenance of effective concentrations of the substance in the test system;
- whether, when necessary, an appropriate exogenous metabolism mix (e.g. S9 from induced rat liver or from hamster liver) has been used;
- whether appropriate negative and positive controls were included as integral parts of the tests;
- whether an adequate number of replicates (within the tests and of the tests) was used.

3.2.2.4 Relevance of data

In order to evaluate the relevance of the available data, it is necessary to judge, inter alia, if an appropriate species has been studied, if the route of exposure is relevant for the population and exposure scenario under consideration and if the substance tested is representative of the substance as supplied. To be able to assess the latter it is necessary that the substance is properly identified and any significant impurities described.

Relevant human data of an adequate quality can, of course, sometimes be the best available data but, more frequently, the available human, animal and other data are considered together in order to reach a conclusion about the relevance to humans of effects observed in studies in animals.

The evaluation of the relevance for humans of data from studies in animals is aided by use of data on the toxicokinetics, including metabolism, of a substance in both humans and the animals species used in the toxicity tests, when they are available, even when they are relatively limited. Clear, well-documented evidence for a species-specific effect/response (e.g. light hydrocarbon-induced nephthropathy in the kidney of male rats) should be used as justification for the conclusion that a particular effect is not expected to occur in humans exposed to the substance.

In the absence of such information (on the substance itself or, if it can be scientifically justified, on a close structural analogue), "threshold" adverse effects observed in studies in animals will normally be assumed to be likely to occur also in humans exposed to the substance above a certain level of exposure.

In any case, the dose-response relationships in the animal studies (or the severity of the effect, when only a single dose was tested) are also assessed as a part of the risk assessment process. These assessments are taken into account at the risk characterisation stage when a judgement is made of the likelihood of occurrence of an adverse effect in humans at a particular level of exposure.

Interpretation of the relevance of data derived from tests conducted *in vitro* should be taken into account whether the results seen have been observed, or could be expected to occur (e.g. from a knowledge of the toxicokinetics of the substance) *in vivo*. According to the validation procedures established by the European Centre for the Validation of Alternative Methods (ECVAM), the relevance of an alternative (non-animal) test, such as an *in vitro* test, is assessed according to the scientific basis of the test system (scientific relevance) and to the predictive capacity (predictive relevance) of the prediction model, which is an algorithm for extrapolating from *in vitro* data to an *in vivo* endpoint (Worth and Balls, 2001).

In general, the results of *in vitro* tests provide supplementary information which may, for instance, be used to facilitate the interpretation of the relevance for humans of data from studies in animals, or to gain a better understanding of the mechanism of action of a substance.

Although *in vitro* data alone are rarely of direct relevance for humans, highly electrophilic substances which give positive results in genotoxicity tests conducted *in vitro* may be of concern with regard to their potential to be mutagenic in humans at the initial site of the contact (e.g. the skin or respiratory tract). The special case of interpretation of data from *in vitro* tests for genotoxicity is addressed in Section 3.10.

3.2.2.5 (Quantitative) structure-activity relationships ((Q)SARs)

When data do not exist for a given endpoint, or when data are limited, the use of Structure-Activity Relationships (SARs) may be considered. It should be noted that SAR techniques and methods, particularly for Quantitative Structure-Activity Relationships (QSARs) are not well developed in relation to mammalian toxicology. The SARs which are used for the risk assessment purpose are usually more of the "expert judgement" type.

SARs may be of value in indicating a potential hazard, toxicokinetic properties or the need for further testing. Additional comments are given in Chapter 4: Use of (Q)SARs. Validated QSARs may be available for some physico-chemical properties relevant for risk assessment (see Chapter 4).

3.3 EXPOSURE ROUTE AND DURATION

3.3.1 Introduction

The route, duration and frequency of human exposure to a substance during normal use (and, as appropriate, reasonably foreseeable other uses) need to be taken into account when evaluating the data on hazard identification: hazards which may not be expressed under one exposure scenario may become apparent under another.

When no reliable or adequate toxicity data are available for a relevant route of human exposure, but are available for another route, the possibility of using route-to-route extrapolation may be considered. Route-to-route extrapolation is defined as the prediction of an equivalent dose and dosing regime that produces the same toxic endpoint or response as that obtained for a given dose and dosing regime by another route (Pepelko and Withey, 1985). In general, route-to-route extrapolation is thought to be a poor substitute for toxicity data obtained using the appropriate route of exposure. Nevertheless, in the section below, a procedure for route-to-route extrapolation is described.

Similarly, the data available may only have been obtained from tests of short duration, not reflecting the long-term duration of human exposure. This issue is outlined very briefly below, and is discussed further in Section 3.9 on repeated dose toxicity, where a strategy for the assessment of the subacute, subchronic and chronic toxicity of substances is discussed.

3.3.2 Route of exposure

A strategy for selecting the appropriate route of exposure for toxicity testing is presented in Appendix V.

3.3.3 Route-to-route extrapolation

When route-to-route extrapolation is to be used, the following aspects should be carefully considered:

a. *nature of effect:* route-to-route extrapolation is only applicable for the evaluation of systemic effects. For the evaluation of local effects after repeated exposure, only results from toxicity studies performed with the route under consideration can be used.
b. *toxicokinetic data (absorption, distribution, metabolism and excretion):* The major factors responsible for differences in toxicity due to route of exposure include (see also Section 3.5):
   - differences in bioavailability (absorption)
   - differences in metabolism (a.o. first pass effects)
   - differences in internal exposure pattern (kinetics).

In practice, relevant data on kinetics and metabolism, especially after dermal and inhalation exposure, are frequently missing. As a consequence, corrections can only be made for differences in bioavailability.

There are some pragmatic approaches in order to calculate a NAEL (or LAEL) by extrapolation, when specific data are not already available. This No-Adverse-Effect-Level (NAEL) can be used to facilitate decision taking with regard to the potential need to ensure control of exposure, or to obtain further data, for a particular route of exposure. The methods described below are for

extrapolating from oral toxicity data since this is the route most often used for repeated dose toxicity studies in animals. A number of publications are available which provide guidance on route-to-route extrapolation (e.g. Pepelko and Withey, 1985; Pepelko, 1987; Sharratt, 1988; Vermeire et al., 1993; ECETOC, 1994a).

It should be noted that insight into the reliability of the current methodologies for route-to-route extrapolation has not been obtained yet (Wilschut et al., 1998).

3.3.3.1 Approximate dermal NAEL from oral NOAEL

Unless there are data that contraindicate route-to-route extrapolation (e.g. the oral LD_{50} is much greater than the dermal LD_{50}), it can be assumed that the NOAEL for repeated dose toxicity studies is the same for both routes on a $mg \cdot kg^{-1} \cdot day^{-1}$ basis. Dermal absorption is mostly less than, or no more than equal to, oral absorption and generally dermal absorption is slower than oral absorption (especially after gavage application). Extrapolation therefore errs on the side of caution. In case data on dermal absorption are available and/or in case data from dermal absorption studies exist, the available information should be used in the light of Annex B of Section 3.5, including the use of default values of 10 and 100% dermal absorption.

3.3.3.2 Approximate inhalation NAEL from oral NOAEL

One of the commonest problems in route-to-route extrapolation relates to inhalation exposure of humans when there is a lack of toxicity data for this route. For highly volatile substances or highly respirable substances (e.g. those with a high percentage of particles <5 μm), LC_{50} and oral LD_{50} values should be available from experimental studies. From the inhalation LC_{50} value (concentration inhaled) the equivalent inhalation LD_{50} (dose absorbed) value can be calculated by assuming a percentage value for absorption via the lungs (values of 75% to 100% are commonly used) and taking into account the respiration rate and body weight. If the inhalation absorption value is known, this should be used. The ratio of the calculated inhalation LC_{50} value to the measured oral LD_{50} value can then be used to estimate the inhalation NAEL from the oral NOAEL. The use of LC_{50} and LD_{50} values is only possible if the cause of death is comparable and even then uncertainties remain.

An alternative approach which could be used in the absence of an LC_{50} value is to convert an oral repeated NOAEL to an approximate inhalation NAEL using the physiological parameters above (see also Section 3.5). This method will gain relevance especially for new substances as the standard acute LD_{50}/LC_{50} tests probably will not be available in the future (see also Section 3.6).

3.3.4 Duration of exposure

Differences in duration of exposure between the exposed humans and the studies from which toxicological data are available may, in part, be addressed when considering the acceptability (for the situation of interest) of the exposure/NOAEL ratio: it is assumed that, often, the NOAEL will decrease as the duration of exposure in the toxicity study increases. In addition, it is necessary to take account of the possibility that a study of longer duration may reveal a target tissue/organ/system which was not affected in a relatively short-term study. Relevant toxicokinetic data (e.g. tissue distribution studies following exposure via the route of interest)

should be used to help in making decisions about the need for further testing. (Vermeire et al., 1993; ECETOC, 1994a).

3.4 DOSE-RESPONSE ASSESSMENT

It is generally agreed that many of the adverse effects of health caused by substances are not expressed until the substance, or an active metabolite, reaches a threshold concentration in the relevant organ. Whether or not this threshold concentration is reached is related to the level of exposure of the organism (human or test animal) to the substance: for a given route of exposure, there will be a threshold exposure level which must be attained before effects are induced. The threshold exposure dose or concentration may vary considerably for different routes of exposure, and for different species because of differences in toxicokinetics and possibly also in mechanisms of action. The observed threshold dose or effect level in a toxicity test will be influenced by the sensitivity of the test system and is a surrogate for the true NAEL. It is usually simply one of the doses or concentrations used in a repeated dose toxicity study identified as described in the following paragraphs.

Unless a threshold mechanism of action is clearly demonstrated, it is generally considered prudent to assume that thresholds cannot be identified in relation to mutagenicity, genotoxicity, and genotoxic carcinogenicity, although a dose-response relationship may be shown under experimental conditions.

The NOAEL identified in a particular test will be simply the highest dose level or concentration of the substance used in that test at which no statistically significant adverse effects were observed, i.e. it is an operational value derived from a limited test. For example if the dose levels of 200, 50, 10 and 5 $mg \cdot kg^{-1} \cdot day^{-1}$ of a substance have been used in a test and adverse effects were observed at 200 and 50 $mg \cdot kg^{-1} \cdot day^{-1}$ but not at 10 or 5 $mg \cdot kg^{-1} \cdot day^{-1}$, the derived NOAEL will be 10 $mg \cdot kg^{-1} \cdot day^{-1}$. Thus, the NOAEL and LOAEL (lowest observable adverse effect level) values for a given study will depend on the experimental study design, i.e. the selection of dose levels and the spacing between doses.

If there are several studies addressing the same effects from which different NOAELs could be derived, normally the lowest relevant value should be used in the risk characterisation. When it is not possible to identify the NOAEL in a repeated dose study, the "lowest observed adverse effect level" (LOAEL) should be used in the risk characterisation.

If a NOAEL becomes available subsequently, from another test, the risk characterisation should be re-addressed and revised, if necessary, in the light of the new information.

The sensitivity of a study, (which is related to the toxicological endpoint, the potency of the toxic substance, the exposure period and frequency, the variability within the species, the number of dose groups, the number of animals per dose group) may limit the extent to which it could be possible to derive a reliable NOAEL from a particular test. In these cases where it is impossible to derive a NOAEL, at least a LOAEL should be identified.

It is recognised that the NOAEL is not very accurate with respect to the degree to which it corresponds with the (unknown) true NAEL. Also, the data obtained at one dose (NOAEL) are used rather than the complete dose response data set (Woutersen et al., 1997). In case sufficient data are available, the shape of the dose response curve should be taken into account. In the case of a steep curve the derived NOAEL can be considered as more reliable (the greater the slope, the greater the reduction in response to reduced doses); in the case of a shallow curve, the uncertainty in the derived NOAEL may be higher and this has to be taken into account in the risk

characterisation stage. If a LOAEL has to be used in risk characterisation, then this value can only be considered reliable in the case of a very steep curve. In response to the general call for consideration of the dose response curve as a whole rather than to use only the data obtained at one dose (NOAEL) for risk characterisation, alternatives for dose-response assessment have been proposed such as the benchmark dose concept and categorial regression.

In categorial regression (Hertzberg, 1989) toxicological responses are translated into ordered categories of progressive effects: e.g. no effects, non-adverse effects, mild to moderate adverse effects, and severe or lethal effects. The output of such a model is an estimation of the probability of occurence of an effect worse than a given category, given a particular dose and duration.

One of the most promissing alternatives proposed is the Benchmark concept (Crump, 1984; Gaylor, 1988; US EPA, 1995; Slob and Pieters, 1998). In the Benchmark approach, a dose-response curve is fitted to the complete experimental data for each effect parameter. On the basis of the fitted curve, the lower confidence limit on the dose at which a predefined critical effect size is observed (i.e., the dose at which adverse effects start to arise) is defined as the Benchmark dose.

Advantages of this approach over the NOAEL are:

- the Benchmark dose is derived using all experimental data and reflects the dose-response pattern to a greater degree;
- the Benchmark dose is independent of predefined dose levels and spacing of dose levels;
- the Benchmark approach makes more reasonable use of sample size, with better designs resulting in higher Benchmark doses.

A disadvantage of this new method is the uncertainty with respect to the reliability of the approach in case results are obtained from toxicity studies performed according to the requirements defined in current guidelines (EU Annex V methods, OECD guidelines,). For the derivation of reliable dose-response relationships, the classical study design of three dose groups and a vehicle control group is far from ideal, especially if one considers the unfavourable possibility that in a particular experiment, adverse effects may be identified only at the highest dose level. An improved benchmark model fit would be possible by increasing the number of dose groups without changing the total number of animals in the test.

The concept of the Benchmark approach needs further development particularly in the following areas:

- optimisation of study design (Slob, 1999);
- predefinition of internationally accepted Critical Effect Sizes for each toxicological parameter (sufficiently based on biological, physiological and toxicological knowledge);
- development of specific dose response analyses for different types of experimental data (continuous, categorical and quantal) (Slob and Pieters, 1998).

However, such a change in study design would generally no longer allow a proper derivation of a NOAEL. Thus, in practice, the NOAEL and the benchmark concepts appear to be incompatible.

For the time being, determination of a NOAEL is mandatory for the risk assessment in the EU. Nevertheless, the benchmark dose method can be used parallel to derivation of a NOAEL (US EPA, 1995; Barnes et al., 1995; Slob, 1999; Vermeire et al., 1999). Especially in cases where a NOAEL cannot be established for the selected toxicological endpoint because only a LOAEL is available, benchmark modelling is considered to be preferable over LOAEL - NOAEL

extrapolation using more or less arbitrary assessment factors. Since generally accepted Critical Effect Sizes have not yet been established, one may consider postulating a default critical effect size (e.g. 5% over the background level for continuous endpoints). In any case, the chosen value should always be derived in a transparent way using the whole toxicological profile of the substance. Benchmark dose software (BMDS) is available from the US EPA Internet Site (www.epa.gov).

It is usual to derive a NOAEL on the basis of effects seen in sub-acute, sub-chronic, chronic and reproductive toxicity tests. However, for acute toxicity, irritation and skin sensitisation it is rarely possible to derive a NOAEL, particularly on the basis of EU Annex V methods, because of the design of the studies used to evaluate these effects.

For acute toxicity, substances which will be considered in the risk characterisation, will have been classified on the basis of an LD_{50} or LC_{50} value (or the discriminating dose if the Fixed Dose Procedure was used or the result of the Acute Toxic Class Method). These values give an indication of the relative lethal potency of the substances, and, where appropriate, the slopes of the dose-response curves indicate the extent to which reduction in exposure will reduce lethality: the steeper the slope, the greater the reduction in lethality for the same incremental reduction in exposure. It is not usually possible to derive a meaningful NOAEL from a test for acute toxicity.

The biocides legislation operates with a concept of acute reference dose. For detailed description see TNsG on Annex I Inclusion (2001).

Skin, eye and respiratory tract irritation are effects for which there are expected to be thresholds of substance concentration below which the effects will not be manifested. However, EU Annex V skin and eye irritation studies are conducted using a single amount of the undiluted substance (solids are moistened for the skin irritation study) so it is not possible to define N(L)OAELs on the basis of these. There is no Annex V method for respiratory irritation: if human experience is used to classify a substance as a respiratory irritant, it may be possible to derive a NOAEL from measured or estimated exposure levels.

The usual tests for skin sensitisation as carried out in the guinea pig employ only a single (maximised) concentration of the substance during the induction phase, and there is no EU Annex V method for respiratory sensitisation so definition of N(L)OAELs is not possible for skin or respiratory sensitisation. The local lymph node assay is carried out using multiple concentrations. Dose-response information, provided by the local lymph node assay may be useful in the risk characterisation for this end-point, in particular classification of sensitisers in categories of weak, moderate, or strong sensitisers (Van Och et al., 2000). It is currently not possible to identify an elicitation dose or concentration of a sensitising substance below which adverse effects are unlikely to occur in people already sensitised to a substance.

3.5 TOXICOKINETICS

3.5.1 Introduction

Data on the toxicokinetics of a substance can be very useful in the interpretation of toxicological findings and hence in the risk assessment process. Information on the fate of a substance in the organism is required to relate exposure to effects. Route-to-route or interspecies extrapolations may be possible on the basis of internal exposure data, which may replace the use of some default extrapolation factors. In addition, this may also enable sensitive sub-populations who may be at particular risk to be taken into account in the risk assessment by evaluating interindividual differences. In conjunction with information on the relationship between concentration/dose at the target site and the toxic effect, toxicokinetic information may be an important tool for extrapolation from high to low dose effects. Toxicokinetic data can be used to make informed decisions on further testing. In specific circumstances, valid toxicokinetic data may be used to support derogation statements. For example, proof that a substance is not systemically available may be considered as part of a justification for non-conduct of further testing, e.g. reproductive toxicity tests. Uses for toxicokinetic data in relation to mutagenicity testing are discussed in Section 3.10.

3.5.2 Definitions

The term toxicokinetics is used to describe the time-dependent fate of a substance within the body. This includes absorption, distribution, metabolism and/or excretion. The term toxicodynamics means the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects. The toxicodynamic effect is driven by the concentration at the effect site(s) directly or indirectly and may be reversed or modified by several factors (e.g. repair mechanisms for DNA damage, compensatory cell proliferation).

3.5.3 Objectives for investigating the toxicokinetics of a substance

Toxicokinetic studies are designed to obtain species-, dose-, and route-dependent data on the concentration-time course of parent compound and its metabolites, e.g. in blood, urine, faeces and exhaled air. From these data toxicokinetic parameters can be derived by appropriate techniques. The information which can be taken from *in vivo*/ex-vivo toxicokinetic studies is:

Primary information:

- the concentration-time profile of the substance/metabolites in blood (plasma), tissues and other biological fluids, such as urine, bile, exhaled air and the volume of the excreted fluids if appropriate;
- protein binding and binding to erythrocytes (if relevant) (*in vitro*/ex vivo studies).

Derived information:

- rate and extent of absorption and bioavailability;
- distribution of the substance in the body;
- biotransformation;
- rate and extent of presystemic (first pass) and systemic metabolism after oral and inhalation exposure;
- information on the formation of reactive metabolites and possible species differences;

- rate and extent of excretion in the urine, faeces, via exhalation, and, other biological fluids (e.g. milk, bile, sweat, etc);
- half-life and potential for accumulation under repeated or continuous exposure;
- information on enterohepatic circulation.

Enterohepatic circulation may pose particular problems for route-to-route extrapolation since the fraction of the compound undergoing enterohepatic recirculation after oral administration may be greater than after non-oral administration. This will result in an AUC (area under the blood/plasma concentration vs time curve, representing the total amount of substance reaching the plasma) which reflects both absorption/systemic availability of the compound and the extent of recirculation. As the relative extent of target organ exposure following different routes of exposure is often calculated from the ratio of AUCs, target organ exposure after oral exposure may be overestimated.

It is helpful to have information for the (expected) exposure route(s) in humans (oral, inhalation, dermal) at appropriate dosing level(s). From the plasma/blood concentration time profile and from the excretion over time it can be calculated whether the substance will accumulate when given repeatedly or continuously. However, it is only possible to make this extrapolation for substances that have linear kinetics. Hence, if information on the potential for a substance to accumulate is important for the risk assessment, it will be necessary to gather data from studies with repeated dosing regimes. Conducting toxicokinetics studies in more than one species will enable the presence or absence of interspecies differences to be assessed. In the absence of *in vivo* data some of the toxicokinetic data may be derived from *in vitro* experiments. These include parameters of metabolic steps, such as Vmax, Km, intrinsic metabolic clearance, as well as skin permeation rate and distribution coefficient. Physiologically based toxicokinetic modelling techniques may be used to simulate the concentration-time profile in blood and at the target site.

3.5.4 Data requirements

For new substances notified under Directive 67/548 at the 1 tonne per annum supply level the data requirement is for an “assessment of the toxicokinetic behaviour of a substance to the extent that can be derived from base set data and other relevant information”. Guidance on how to carry out such an assessment is provided in Annex A. When such substances reach the level 1 supply tonnage the requirement is for “basic toxicokinetic information”. At the level 2 supply tonnage the requirement is for “studies which cover biotransformation and toxicokinetics”. For existing substances, the basic data requirements specified in Article 9 (2) of Regulation 793/93 are for information on toxicokinetics as specified in Annex VII A of Directive 67/548.

For biocides the following requirements have to be fulfilled:

- irrespective of the supply tonnage a comprehensive toxicokinetics study including elucidation of the metabolism should be conducted;
- furthermore a study on dermal absorption is required;
- for existing substances relevant information that satisfies part or all of these data requirements may be obtained from pre-existing studies that may or may not have been conducted to current regulatory standards.

3.5.5 Types of studies to be used in risk assessment

3.5.5.1 Introduction

Certain tests for studying toxicokinetics of substances *in vivo* are described in Annex V to Directive 67/548. Other approaches could be considered or, for existing substances, may already be available including predictive and modelling approaches, data gathered from *in vitro* test systems and data gathered from studies *in vivo*, including humans. As stated in the OECD guideline 417 "Flexibility, taking into consideration the characteristics of the substance being investigated is needed in the design of toxicokinetic studies".

3.5.5.2 Predictive and modelling approaches (QSAR/SAR)

Preliminary predictions can be made by using physico-chemical data if no other information is available (See Appendix IV A for details). Also elaborate computer programs are available that make predictions about, for example, dermal penetration or metabolic pathways. However, these systems have often not been extensively validated against appropriate experimental data and it is not always certain if the results genuinely reflect the situation *in vivo*. On this basis, modelled data should only be used for risk assessment purposes where it is supported by other strands of evidence. For discussion of the available tools see: Ekins et al. (2000). Appendix IV B provides information on predictive systems for dermal penetration.

3.5.5.3 *In vitro* approaches

In recent years, several types of *in vitro* approaches have been developed to assess the absorption and metabolic pathways of substances.

There are several experimental *in vitro* tests available which are used for the prediction of

1) absorption
   a) extent of oral absorption: isolated organs, cell cultures in monolayer, e.g. CACO2 cells;
   b) extent of absorption through the alveoli of the lungs:- isolated organs, cultures of alveolar cells in monolayer;
   c) dermal absorption (TNO-reports V 97.528 "The applicability of *in vitro* test results for estimation of dermal absorption in humans for occupational risk assessment in registration", V 98.356 "Principles for study protocols addressing the dermal absorption of pesticides", V 98.1237 "Guidance document on the estimation of dermal absorption according to a tiered approach: an update").

2) metabolism
   a) isolated organs,
   b) tissue slices,
   c) primary and secondary cell cultures,
   d) cell fractions,
   e) purified enzymes,
   f) reconstituted systems,
   g) recombinant enzymes

Ex vivo systems derived from animals and from human organs have been used to investigate the *in vitro* metabolism of xenobiotics. Cell lines which are transfected to express species, specific metabolic enzymes, are an additional tool to be used to identify the enzymes involved in the metabolism of a specific substance. Blocking the metabolism by an enzyme specific substrate or by antibodies is helpful for the identification of the enzymes involved in the metabolism. Advantages and disadvantages of the different systems are under discussion (Tucker et al., 2001).

The *in vitro* approaches will give qualitative and under special circumstances, also, quantitative information. Information from *in vitro* experiments, in particular data on *in vitro* metabolism, has been used in PBTK models (Physiologicaly Based ToxicoKinetic models) (see Section 3.5.8). The quantitative use of data derived from in vitro test systems should be very carefully considered in the risk assessment process until such approaches have been appropriately validated. For validation procedures see Curren et al. (1995), Balls and Karcher (1995), OECD (1996).

3.5.5.4 Studies in animals

Toxicokinetic studies in animals should be performed and reported so that it is clear how the study was planned, executed, analysed and reported. It is acceptable to perform toxicokinetic studies as stand-alone studies or in the course of a repeated dose toxicity study albeit in additional groups of animals, so-called satellite groups in which no toxicity endpoint is investigated. For an assessment of kinetics, blood samples have to be collected at time intervals which allow for a description of the whole plasma concentration time course, including the absorption, distribution and elimination phase. Care has to be taken to cover the time period in the first hours after administration to cover the absorption phase. If measurements of parent compound and metabolite/s are made in this period this will allow the assessment of an extensive first pass effect. In order to obtain a reliable estimate for AUC after single administration it is required to take blood samples for 3 - 5 half-lives. For the assessment of kinetics at steady state which is reached after 4 to 6 half-lives, blood can be drawn at several occasions.

As an alternative to the toxicokinetic study, in which the whole concentration time profile is measured in the individual animal, it is recommended to consider sparse data sampling protocols, in particular in cases in which the constraints of the analytical method require high amounts of blood to be taken. Sparse data sampling will provide the relevant information when specific statistical techniques are used whereby a data analysis is performed in which the data from several animals are combined. In addition to the kinetic parameters which can be obtained (see Section 3.5.3), it is possible to search for relevant factors to explain intraspecies variability such as sex, age, strain, enzyme induction, nonlinearity etc. Sparse data sampling protocols combined with the adequate statistical analysis is given preference over the analysis of pooled data or the use of pooled blood.

Nonlinearity can be assessed by comparing the relevant parameters, e.g. AUC, after different doses or after single and repeated exposure. Whereas dose dependency may be indicative of saturation of enzymes involved in the metabolism of the compound, an increase of AUC after repeated exposure as compared to single exposure may be an indication for inhibition of metabolism and a decrease in AUC may be an indication for induction of metabolism. By using the sparse sampling approach, assessment of kinetics in the same animals used for toxicological testing may be considered, thus allowing for concomitant assessment of both toxicokinetic and toxicodynamics (Aarons et al., 1997; Ette et al., 1994; Meineke et al., 1998; Nedelman et al., 1993).

3.5.5.5 Studies in humans

For existing substances toxicokinetic data may be available from studies using human volunteers or might be derived from studies in working populations. When interpreting such data, care must be taken to ensure that exposures have been adequately characterised. Data on the substance and its metabolites in urine/plasma/blood can be used to identify metabolic pathways of the substance.

Data derived from the studies mentioned above are helpful to verify assumptions made in the toxicokinetic interspecies extrapolation as the basis for the extrapolation of toxicological findings from animals to humans. Because of the specific questions to be addressed no general recommendation can be given and the study design should be tailored to the specific needs. Toxicokinetic information from humans may also be used to derive appropriate biological monitoring strategies for risk management.

3.5.6 Assessment of the data

When analysing the results of *in vitro* or *in vivo* toxicokinetic studies the following points should be considered:

Analytical method

The analytical method is important for the interpretation of the data. It should be validated according to current standards (specificity, precision, lower limit of quantitation (LLQ), variability, recovery, and in the case of immunoassay, the cross reactivity of the parent compound and its metabolites). It is self evident that there is a need for a calibration curve, and internal or external standards. If the concentration has been measured with an unspecific method such as total radioactivity this limits the information that can be derived from the study. It is in particular critical to note that measurements of total radioactivity will include parent compound and those metabolites that retain the radiolabelled atom(s). Hence, strictly speaking, it is not possible to determine the plasma half-life for a substance based on the plasma/blood concentration time profile of total radioactivity. However, the information which can be derived from data on total radioactivity is a mixed half-life of the parent compound and the metabolite(s). Moreover, comparing AUCs after application by different routes may give a hint on the relative extent of exposure provided that the presystemic elimination is not different. The main information which can be derived from unspecific measurements is whether the substance and/or its metabolites is/are available to the systemic circulation after oral, dermal etc. administration and whether the substance or its metabolites is/are mainly excreted by urine or by bile. If the radiolabel is followed in the excreta, the information is helpful for information on the mass balance of a compound. In general, if not specifically used, the use of an immunoassay has the same drawbacks. In some cases, because of the constraints of the chemical methods, pooled plasma/blood from several animals is analysed for concentration. The resulting data are hard to interpret as they represent a mixture of concentrations in several animals. However, if different dose groups have been studied they may give some information, at least in studies with repeated administration at steady state, on dose-linearity.

Timing of blood samples (applies only for *in vivo* studies)

If after single dose administration the blood samples are not collected at time intervals which allow for a description of the whole plasma concentration time course, including the absorption, distribution and elimination phase, the information obtained is limited. In particular, data should

be available in the first hours after administration to cover the absorption phase. If measurements of parent compound and metabolite/s are made in this period this will allow assessment of an extensive first pass effect. In order to obtain a reliable estimate for AUC after single administration it is required to have blood samples for 3 - 5 half-lives. In some cases, blood has been drawn in the animals only at few, but different time points. This "sparse" data sampling may give relevant information if adequate statistical methods are applied to analyse the data (so-called population approach). Naive pooling of the data is an inadequate method of analysis.

From a single dose study, the relationship between elimination half-life and dosing interval can provide an indication for the potential of the substance to accumulate. For example, if the half-life is 12 hours and the dosing interval is 24 hours, the compound will accumulate only slightly because within the dosing interval of two half-lives 75% of the compound is excreted whereas 25% remains in the body. In contrast, for a compound with a half-life of 60 hours and a dosing interval of 24 hours accumulation will be high. In this case, under repeated or under continuous administration the concentration in blood and in the tissues, including the target site will increase until steady state is reached which is after 6 half-lives.

It is helpful when data are available to enable comparison of the plasma concentration time profile after single administration with that after repeated administration. This would enable determination of whether the substance has time dependent kinetics (due to induction of metabolism, inhibition of metabolism and/or accumulation and saturation of processes involved in distribution, metabolism and excretion). As kinetic studies with repeated administration are cumbersome to perform, it might be considered to extend the protocols for repeated dose toxicity testing by implementing blood sampling or by using satellite groups in order to address specific questions for individual chemicals, e.g. blood/tissue sampling to investigate enzyme induction.

Experimental conditions for *in vitro* systems

Presently, an official draft procedure exists for *in vitro* testing which is the guideline on transdermal absorption (OECD, 2000c). Formal validation procedures have been described in the ECVAM document on validation (Curren et al., 1995) and the corresponding OECD paper (1996). However, the only *in vitro* method to undergo such a validation process so far has been the above mentioned transdermal penetration method. However, in the field of absorption and of *in vitro* metabolism there are several systems in use for which extensive scientific information is available, thus providing reassurance that these systems may be used to give qualitative and quantitative information (see Pelkonen et al., 2001; Tucker et al., 2001).

3.5.7 Use of toxicokinetic data in risk assessment

General remarks

The primary endpoint of the kinetic studies is the concentration time profile of the substance in plasma/blood and other biological fluids as well as in tissues after single and/or repeated administration. The excretion rate over time and the amount of metabolites in urine and bile are further possible primary endpoints of kinetic studies, sometimes providing information on the mass balance of the compound.

From the primary data, clearance and half-life can be derived by several methods, such as model-dependent and model-independent methods e.g. compartmental, non-compartmental analysis. From the excretion rate over time and from cumulative urinary excretion data and plasma/blood

concentration measured during the sampling period renal clearance can be calculated. The same holds true for the biliary excretion.

Further refinements of methods require more elaborate models with explicit or implicit assumptions and defaults e.g. PBTK models which may also provide modelled information on the target concentrations/amounts.

As toxicological information is not always provided for all routes of possible exposure, toxicokinetic information may be helpful to bridge this gap. In most cases, toxicokinetic data are available in the species in which the toxicological studies have been performed. However, the toxicological risk assessment is done with the aim of predicting the toxicological effect of the compound under assessment in human beings. Toxicokinetic information in man, even if sparse, is helpful in the process of extrapolation. In this respect, *in vitro* data from human tissue/ primary and secondary cell cultures/cell fractions/purified enzymes/reconstituted systems/recombinant enzymes, are helpful tools, in particular if used as input into PBTK modelling.

Relative extent of absorption/systemic availability/internal exposure

Absolute systemic availability can only be calculated by comparing AUC after oral, inhalation, dermal etc administration with the AUC after direct administration into the systemic circulation, e.g. after intravenous administration. In addition, cumulative urinary excretion of the unchanged substance may be used to obtain the lowest estimate of the amount that must have been in the systemic circulation before being excreted by the kidneys. When radiolabeled substance has been used, the observation that 100% of the radioactivity given is excreted in the urine does not mean that 100% of the parent compound is systemically available. This is because a proportion of the parent compound may have undergone presystemic (first pass) metabolism. In most of the cases, it is not relevant for risk assessment to know the absolute systemic availability.

It may be useful for route-to-route extrapolation to estimate the relative systemic availability of a substance for different routes of exposure. Providing that the substance does not undergo first pass metabolism and there is no evidence of saturation of metabolic processes across the dose range of interest, relative systemic availability can be determined by comparing the AUCs for different routes of administration. Assuming that the amount of the toxicologically active moiety that is present at the effect site is proportional to the systemic availability of that substance, it is then possible to infer the dose-response relationship for an effect for one route of administration using data derived from another route. Note that it is not possible to make such inferences for substances that undergo extensive first pass metabolism by one or more routes of administration or that show dose-dependent kinetics.

In cases where the data provide sufficient proof that the substance is not bioavailable, e.g. no plasma/blood concentrations were measurable using a sensitive method and no parent compound or metabolites could be detected in urine, bile or exhaled air, it may be possible to consider non-conduct of certain extensive animal tests, for example reproductive toxicity or carcinogenicity studies.

Distribution/accumulation

Distribution, including accumulation of a substance will be the same irrespective of the route of administration. However, distribution and accumulation at the site of application (inhalation, oral, dermal) may depend on the route. For example, administration of a substance by inhalation may lead to deposition of a proportion of the dose in the upper respiratory tract and may cause local toxic effects, in particular after repeated/continuous administration. In such cases local

accumulation may occur and may be responsible for tissue damage and its consequences. In these cases, systemic toxicokinetic behaviour of the substance may be of limited relevance for the risk assessment.

It is generally not crucial for risk assessment to determine the precise tissue distribution profile for a substance. In certain special cases, however, specific tissue distribution studies may assist or even be essential for the interpretation of available toxicological data. For example, in some cases it may be of interest to know whether the substance will cross the blood/brain barrier or will accumulate in specific tissues.

Metabolism

Knowledge of the rate and extent of metabolism and the metabolic enzymes involved will support interspecies extrapolation. For example, the ability to compare data from animals with that from humans will enable data-derived factors instead of default values to be used for interspecies extrapolation. In addition, knowledge of intraspecies variations in metabolic capacity will help to assess margins of safety which is an important factor in decisions surrounding the need for risk reduction measures. Knowledge of the metabolic profile of a substance may also help to build up a mechanistic model of action or at least may allow a mode of action to be ascertained. Whereas the systemic metabolism is the same irrespective of the route of administration, presystemic and local metabolism at the site of administration differs and this may be relevant from a toxicological perspective.

Blocking the metabolism by an enzyme-specific inhibitor or using “knockout” animals which are deficient for a selected enzyme or increasing the metabolic capacity by the use of specific inducers will enable the role of metabolism in the toxicological behaviour of a substance to be investigated. Identification of key enzymes may be important for substances metabolised by enzymes for which there are functionally relevant genetic polymorphisms in humans (e.g. CYP2D6, CYP 2C9, N-acetyltransferase, S-Glutathione-Transferase) since this will enable susceptible subpopulations to be identified. This will also help to determine the effects of concomitant exposure to other substances that share metabolic pathways.

The identification of enzyme-specific kinetic parameters (i.e. k_m, V_{max} and intrinsic clearance) and their relative content in different organs will provide information on dose dependent metabolism. For example metabolites arising from high affinity enzymes will predominate at low doses whereas metabolites from low affinity enzymes will also be present when high doses are administered.

Some substances may undergo first pass metabolism to such an extent that the parent compound does not reach the systemic circulation. This finding could be used to support derogation statements against further testing of the parent compound where appropriate data are available for the relevant metabolites.

Excretion

The term route of excretion is understood to describe the way in which the parent compound and its metabolites leave the body. A clear distinction should be made between parent compound and metabolites because this will help to correlate the presence of the toxicologically active compound (which might be the parent compound and/or the metabolite(s)) with the toxicological effect. With the exception of those substances which leave the body by exhalation, the main routes of excretion for parent compound and metabolites in rodents are in the urine and bile. Metabolism, by for example the liver, is another quantitatively important way to clear the parent

compound from the body, although it cannot be considered an actual route of excretion. In humans biliary excretion is a minor route for most substances and their metabolites. Chemicals may also be transported into the gut lumen by specific transporters in the cells lining the lumen of the gastrointestinal tract. This may act to prevent systemic absorption in the case where a substance is taken into the cells from the lumen of the gut and then directly excreted back. Alternatively, substances and their metabolites may enter cells from the blood and then be excreted into the gastrointestinal lumen. Knowledge of the main route(s) of excretion will enable the identification of susceptible subpopulations such as subjects with impaired renal and/or liver function, e.g. the elderly. This information will help to determine whether the margin of safety is adequate or not. It may also enable substance-specific safety factors to be derived to replace default values.

Substances and their metabolites may also be excreted in biological fluids such as saliva, sweat and milk. Although the amounts excreted by these routes are relatively small, the presence of substance in these fluids, particularly breast milk, may be the underlying cause of toxic effects.

3.5.8 PBTK-Modelling

Data from PBTK modelling are often presented to bridge data gaps between animals and humans. Several models have been employed and may be useful. When assessing a PBTK model it has to be considered what part of the model is supported by experimental data, what assumptions have been made and where default values have been used. The criteria used to determine the goodness of fit between the model and any experimental data should be statistically valid and clear explanations should be provided to show how the model fits the available experimental data. It may be helpful to seek statistical advice when evaluating and assessing the information for risk assessment from a PBTK model. If PBTK models are used to extrapolate from animals to humans the proposed model should be validated by data from humans if this is available and extrapolations from the model should be within or close to the range of experimental measurements used to validate the model.

If there is no validation of the model by data from humans, the PBTK model may be used to support an interpretation of toxicodynamic data or toxicological findings rather than as a basis for decision on human NAELs. As physiological data, e.g. inhaled air per unit of time, blood perfusion etc. have a range of values modelling should preferentially be performed with across a range of values and not with point estimates to determine the distribution of possible outcomes. A safe level may then be defined as being e.g. the 95% CL of the estimate of a mean value or the 99% percentile of the whole distribution thus providing a basis for transparent decision making.

3.6 ACUTE TOXICITY

3.6.1 Introduction

3.6.1.1 Definition of acute toxicity

The term "acute toxicity" is used to describe the adverse effects on health, which may result from a single exposure to a substance, via the oral, dermal or inhalation route (see Directive 92/32 (7th amendment of Directive 67/548), Article 2(2) (f), (g) and (h)). "Acute toxicity" as used here excludes local irritant or corrosive effects arising from a single application of a substance to the skin or eye, which are addressed in the next section. However, experience gained through the notification of new substances has indicated a value in integrating the outputs of testing for skin irritation and acute toxicity. These aspects are considered more specifically under 3.6.2.1. Criteria for the classification of substances on the basis of lethal or irreversible effects after a single exposure, or on the basis of the discriminating dose if the fixed dose procedure has been used, are given in Directive 93/21.

3.6.1.2 Objectives of investigating the potential for substance-induced acute toxicity

Generally the objectives of investigating the acute toxicity are to find out:

- whether single exposures of humans to the substance of interest could be associated with adverse effects on health; and/or
- in studies in animals, the lethal potency of the substance based on the LD_{50}, the LC_{50}, the discriminating dose and/or the acute toxic class; and/or
- what toxic effects are induced following a single exposure to a substance, their time of onset, duration and severity (all to be related to dose); and
- when possible, the slope of the dose-response curve; and
- when possible, whether there are marked sex differences in response; and
- to obtain information necessary for the classification and labelling of the substance for acute toxicity.

In relation to the second bullet above, it should be mentioned, that there is a general objective to move away from the induction of lethality in animal tests.

3.6.1.3 Information which would be obtained from Annex V tests for acute toxicity

There are a number of methods available for investigation of acute toxicity in EU Annex V: the (oral) fixed dose procedure (B.1 bis); the oral acute toxic class method (B.1 tris); the acute inhalation toxicity test (B.2) and the acute dermal toxicity test (B.3). It should be noted that the standard acute oral ($LD_{50,}$ B.1) test has recently been deleted from Annex V and will be removed as an OECD guideline as well. Furthermore, the oral up-and-down method (OECD 425) may be added to Annex V as a further test for acute toxicity.

From any of these tests, data should be acquired on any adverse effects occurring within a given time (usually 14 days) after administration of single doses of the substance to the test animals.

For the "standard" acute oral and dermal tests the LD_{50} should be determined except when the substance causes no mortality at the limit dose (usually 2,000 $mg \cdot kg^{-1}$). Similarly, for an acute inhalation toxicity study the LC_{50} should be determined, unless no mortality is seen at the limit concentration (5 mg/l/4 hr for aerosols and particulates, 20 mg/l/4 hr for gases and vapours). In the fixed dose procedure, the discriminating dose (which is the highest of the pre-set dose levels which can be administered without causing mortality) should be determined. For the acute toxic class and the up-and-down methods the final dose used in the study should be determined following the testing protocol except when the substance causes no mortality at the limit dose.

Whichever approach is used in determining acute toxicity critical information needs to be derived from the data to be used in risk assessment. It is important to identify those dose levels with which toxic signs are observed, the relationship of the severity of these with dose and the level at which toxicity is not observed (i.e. the acute NOAEL). However, it should be noted that a NOAEL is not usually determined in acute studies, partly because of the limitations in study design.

3.6.2 Data to be used in the effects assessment

3.6.2.1 Minimum data requirements

New substances

The minimum data requirements for new substances as specified in Annex VII A to Directive 67/548, are that the acute toxicity should be known for at least two routes of exposure, one of which should be the oral route. Gases should be tested by the inhalation route. Any acute toxicity tests must be conducted using EU Annex V methods.

Guidance for the choice of the second route of exposure is given in Appendix V of this document. For volatile liquids (vapours) this would normally be via the inhalation route. The dermal route of exposure has often been the choice of the second route for acute toxicity testing for new substances. Considerable information has become available through the notification of new substances on the value of this test method for assessing acute toxicity. A recent analysis of the acute tests submitted for notification of new substances clearly indicated that little useful extra information for classification was derived from testing for acute toxicity via the dermal route (Indans et al., 1998). Of 438 new substances notified to the base set level (Annex VIIA), 90 were classified for acute oral toxicity. Of these only four were found to express acute toxicity via the dermal route and in three cases the classification was no more severe than via the oral route. In all cases the substances were also found to be corrosive. These data provide clear evidence of the limited value for the routine use of the acute dermal study. With respect to inhalation exposure, such studies are more demanding technically and may not be appropriate if the physico-chemical properties of the substance and its use pattern indicates otherwise. In the case of substances with irritant (or corrosive) properties, these may be of particular concern in relation to inhalation exposure because of their potential to induce local effects within the respiratory tract. Thus information on irritant potential would be useful prior to consideration of further acute toxicity testing over and above the oral study.

In considering all of these aspects together, the testing strategy shown in **Figure 5** should be followed in a stepwise manner in order to efficiently integrate the information gathered for acute toxicity testing with that obtained from the study of skin and eye irritation and skin sensitisation, together with information on physico-chemical and SAR properties and exposure profile for new

substances. The strategy is based on the progressive gathering of information and reflects the increasing data requirements from Annex VIIC to Annex VIIA. However, it is still possible to use this approach even for those substances which are first notified at, for example, the base set (VIIA) level.

The starting point for the strategy is the performance of the acute oral study (performed at VIIC). Consideration of the information available informing on the potential for the substance to be corrosive should also be made. It should be noted that if the substance is predicted to be corrosive then further consideration should be given as to whether or not an acute oral study can be justified, particularly in relation to animal welfare prior to the conduct of an acute oral test. Justification for not conducting a test for these reasons should be given. If the substance is considered likely to be corrosive, then a hazard assessment can be made and no further testing is required unless there is a need to identify a threshold for respiratory tract irritation or there is evidence that systemic toxicity might occur at non-corrosive concentrations (the testing of corrosive materials for acute inhalation toxicity should not normally be carried out). As the standard LC_{50} test is not applicable for these substances it is highly recommended to contact the Competent Authority to agree an appropriate test design. Should there be no indicators for corrosive potential then skin/eye irritation and skin sensitisation testing is performed (as would be required at VIIB) according to EU Annex V methods (see Section 3.7). These tests may provide useful information on the potential for systemic toxicity which can be considered as part of the acute testing strategy.

Consideration is then given to the need for testing for acute toxicity via the inhalation route (normally at VIIA). Using the criteria in Appendix V to this document (and reproduced in **Figure 5**) together with the information obtained from the acute oral study and the skin and eye irritation and skin sensitisation studies a decision can be reached for the need for acute inhalation testing. If, using these criteria inhalation exposure is likely to be an issue then testing via this route for acute toxicity is indicated, particularly if systemic toxicity is seen via the oral route and/or was observed in the skin/eye irritation and/or skin sensitisation studies. Where systemic effects seen in skin/eye irritation and/or skin sensitisation studies is the single driving factor then this needs to be considered on a case-by-case basis in terms of the severity of response. For example, reactions leading to classification may be seen as strong enough to warrant further testing whereas barely perceptible responses in one animal may be judged as not requiring inhalation testing. The situation where no systemic acute oral toxicity is seen also needs to be considered on a case-by-case basis. If there are reasons to believe that uptake of the test substance by the gastrointestinal tract following oral dosing is unlikely (thus giving low acute oral toxicity) but that uptake or deposition in the respiratory tract is likely this may indicate the need for an acute inhalation study. However, if it is clear that uptake following oral dosing is likely to have occurred then it may be reasonable to avoid acute inhalation testing.

Consideration then needs to be given for the need for testing for acute dermal toxicity. In some cases it may be possible to draw conclusion about the potential for acute dermal toxicity on the basis of the data already available (e.g. high acute oral toxicity and the potential for high dermal absorption may suggest the same level of acute dermal toxicity as that seen following oral dosing) without the need for further testing. Testing for acute dermal toxicity is indicated if:

- systemic toxicity is observed in the skin/eye irritation and/or skin sensitisation studies;
- **or** if there is evidence for the potential for high dermal absorption **and** death or systemic toxicity was seen in the acute oral study;
- **or** there is the potential for high dermal exposure.

Evidence for the potential for high dermal absorption should be considered on a case-by-case basis but key factors will include the log Kow, the water solubility and the molecular weight of the substance (see Section 3.5 for further information). Similarly, the evidence for high dermal exposure should be considered on a case-by-case basis with consideration given to the actual conditions of exposure (e.g. the use of PPE may be an influencing factor in some cases).

If inhalation and dermal testing are indicated, the acute dermal study should only be asked for if:

- LD_{50} oral <200 mg/kg;
- or LD_{50} oral <2,000 mg/kg and high dermal exposure.

Whatever acute testing is conducted for a specific substance, a rationale, using arguments based on this strategic approach, should be provided for the test package that is adopted clearly laying out the arguments as to why any tests were not performed.

It is recognised that following this strategy may lead to testing for acute toxicity by only one (oral) route or indeed by all three routes. However, this situation will arise because of clear scientific reasoning taking into account all the available information on hazardous properties, exposure potential and applying knowledge gained through experience of the new substance notification scheme. For any specific new substance, any change in circumstances (for example use pattern that may lead to a change in inhalation exposure potential) or increase in the level of supply will require a reappraisal and may lead to the need for more information on acute toxicity.

Existing substances

The minimum requirements for existing substances are identical to those for new substances at base set (i.e. 1 tpa). Existing information from a wide range of tests and reports can be used to determine the acute toxicity (see Section 3.6.2.2). If applicable, the testing strategy proposed for new substances could be followed for existing substances in those cases where data are lacking. Any testing that might be required in such cases should be conducted according to EU Annex V methods.

Biocides

The common core data requirements for biocide active substances and biocidal products as specified in the Technical Notes for Guidance (TNsG on Data Requirements, 2000) are that substances other than gases shall be administered via at least two routes, one of which shall be the oral route. The choice of the second route will depend upon the nature of the substance and the likely route of human exposure. In some cases it may be necessary to study acute toxicity via all three routes. Gases and volatile liquids should be administered by the inhalation route.

For substances/preparations with low acute oral toxicity a limit test with 2,000 mg/kg bw may be sufficient. However, need for testing of higher doses could be decided on a case-by-case basis. When planning new tests, the EU Annex V methods B.1.bis, B.1.tris and the OECD guideline 425 are recommended. The recently deleted Annex V B.1 (or the also deleted OECD 401) should not be used. However, existing results based on method B.1 (or OECD 401) are accepted.

Under the Biocidal Products Directive experimental human toxicity studies must not be conducted specifically for the purpose of Annex I, IA or IB inclusion.

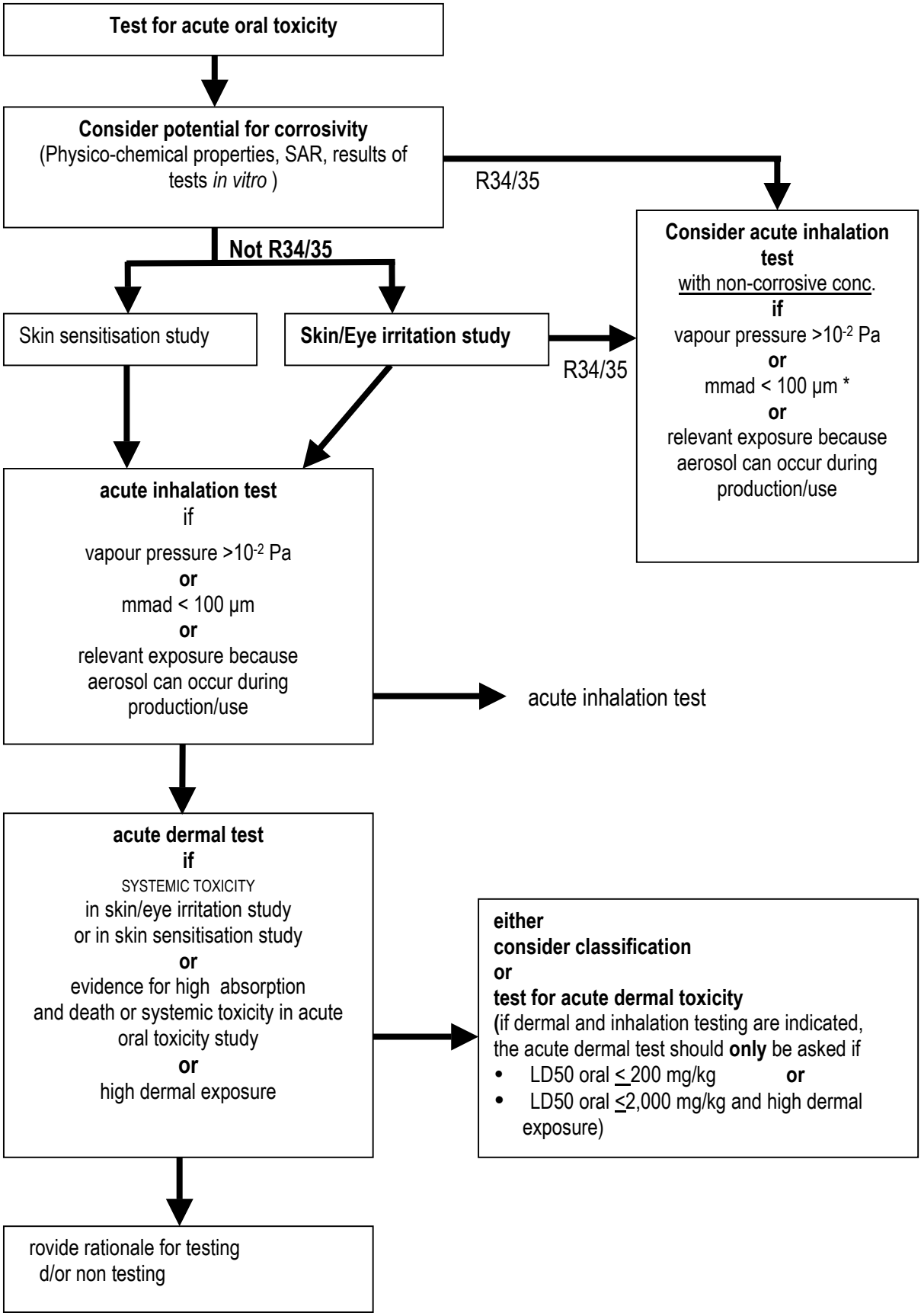

Figure 5 Testing strategy for acute toxicity
* mmad < 50 µm under Biocidal Products Directive

Dermal toxicity must be reported in an active substance/preparation, except for gases, using EU Annex V method B.3 (or corresponding OECD 402).

Inhalation toxicity must be reported where the active substance/preparation is:

- volatile (vapour pressure $>1 \cdot 10^{-2}$ Pa at 20°C),
- a powder containing a significant proportion (e.g. >1% on a weight basis) of particles with particle size mass median aerodynamic diameter (MMAD) <50 μm, or
- to be included in preparations which are powders or (for preparations applied in a manner which generates aerosols, particles or droplets in an inhalable size range (MMAD <50 μm).

Substances/preparations classified as corrosive to skin must not be studied for acute inhalation toxicity. EU Annex V method B.2 (or the corresponding OECD 403) should be used. A full study using three dose levels may not be necessary if a substance/preparation at an exposure concentration to the limit concentrations of the test guideline (limit test) or at the maximum attainable concentration produces no substance-related mortalities. For substances or preparations with low acute dermal toxicity a limit test at 2,000 mg/kg body weight may be sufficient. Substances/preparations which are classified as corrosive must not be tested.

3.6.2.2 Data which may already be available

Human data on the acute effects of substances may be available from (case) reports on the effects of accidents or abuse, from reports on effects following short-term exposures during use, in some cases from studies in volunteers and/or, for some substances, from experience gained from use of the substance as a medicinal agent. Human data may sometimes be reported in secondary sources (e.g. toxicology handbooks) simply as, for example, “minimum lethal doses”, without any reference to an original source. This may be useful as supporting evidence.

Data may be available from animal studies:

- acute toxicity studies using EU Annex V methods or corresponding OECD guidelines;
- studies using other acute toxicity test protocols (e.g. simple lethality studies; dermal or inhalation tests in which the periods of exposure are different from those specified in EU Annex V; tests to study effects on particular organs/systems such as the cardiovascular system);
- sighting studies conducted as preliminary/dose-ranging studies for e.g. repeated dose studies;
- single dose studies for mutagenicity (e.g. a micronucleus test);
- unreferenced data reported in secondary sources (e.g. toxicology handbooks).

Other studies

There are currently no validated *in vitro* methods for acute toxicity, but methods using “structural alerts” may provide useful information for assessment of the acute toxicity of certain substances (e.g. for highly water soluble salts of substances with well characterised toxic properties, the systemic toxicity can be expected to be similar).

3.6.3 Evaluation of the available data

3.6.3.1 New substances including new biocidal substances

In general, only tests conducted in accordance with EU Annex V (or corresponding OECD guidelines) are considered to be adequate to characterise the acute toxicity of new substances.

3.6.3.2 Existing substances including existing active biocidal substances

Human data

Well-documented human data can often supply very useful information, particularly on subjective effects of the type (e.g. nausea, headache) not observable in studies in common laboratory species, provided that such effects are clearly a specific consequence of exposure to the substance.

The usefulness of the information may be limited by relatively low exposures, poor reporting, lack of information on exposure levels, subjective or anecdotal reporting of effects, small numbers of subjects etc., but in some cases may enable derivation of an exposure level at which no overt acute effects were reported.

Poor quality of reporting often adversely affects the usefulness of reports of the effects arising from accidents or abuse, and may also be a problem in reports of the effects of short-term exposures in the workplace. Suspected subjective reporting of symptoms by the exposed people may complicate evaluation of a study. However, if there are several reports listing similar effects, this can be useful. Accidents, abuse and the use of the substance as or in a medicinal agent may involve exposure routes different from those of concern in normal use, and though the latter may have very good exposure data, possible differences in toxicokinetic parameters (see Section 3.5) will need to be taken into account. It is sometimes possible to derive a minimum lethal dose from reports of human accidents or abuse.

Animal data

Well-reported studies using EU Annex V methods, particularly if conducted in accordance with the principles of GLP, can be used to characterise the acute toxicity of substances following administration by a specified route of exposure. Frequently, there will be a number of acute toxicity studies already available for an existing substance, none of which are fully equivalent to the Annex V procedure. If the results from such a batch of studies are consistent, they may, together, provide sufficient information on the acute toxicity of the substance, for one or more routes of exposure. Even if there is quite a range of $LD(C)_{50}$ values, it is often possible to decide into which of the classification categories defined in Directive 93/21 the substance should be allocated. Reports of older "LD_{50}" tests often give little more than the LD_{50} value and usually no information is available on the onset and duration of clinical signs.

If the results from a batch of studies are not consistent, it will be necessary for the rapporteur to decide which are the most reliable studies based on the evaluation criteria outlined above.

Data from studies other than "acute toxicity studies" (i.e. single dose sighting studies, single dose studies for mutagenicity), if well reported (which they may not be in relation to the acute toxic effects) may provide useful information.

3.6.4 Assessment of the dose-response relationship

It may sometimes be possible to derive reliable NOAEL values for specific sub-populations from well-documented human data.

It is not usual to derive “acute NOAELS” for acute toxicity in animals. It is more usual that the only numerical value derived is the $LD(C)_{50}$ value. When reviewing classification, care should be taken when using $LD(C)_{50}$ values from dermal or inhalation acute toxicity tests in which the duration's of exposure were different from those specified in EU Annex V.

Where information is available from test reports or the literature on toxic signs and the dose levels at which they occur then this is useful information that can aid in the subsequent risk characterisation for acute toxicity. Equally, dose levels leading to no effect can provide useful information.

The slope of the dose-response curve is a particularly useful parameter as it indicates the extent to which reduction of exposure will reduce the response: the steeper the slope, the greater the reduction in response for a particular finite reduction in exposure.

3.6.5 Degree of uncertainty in studies of acute toxicity

Data from studies in animals will often give very good information on the acute toxicity of the substance in the test species, and, in general, it can be assumed that substances which are highly toxic to animals will be toxic to humans. However, while the reverse is generally true, it is not always true. There are subjective effects (e.g. nausea, central nervous system (CNS) depressant effects) which may be reported by humans exposed to substances but which cannot be observed in common laboratory species, so it is not certain whether such effects will be induced in humans by substances thought, from single exposure studies in animals, to be of low acute toxicity.

3.7 IRRITATION AND CORROSIVITY

3.7.1 Introduction

3.7.1.1 Definitions of irritation and corrosivity

Irrespective of whether a substance can become systemically available, changes at the site of first contact (skin, eye, mucous membrane/gastro-intestinal tract, or mucous membrane/respiratory tract) can be caused. These changes are considered local effects. A distinction in local effects can be made between those observed after single and after repeated exposure. For local effects after repeated exposure reference is made to Section 3.9. Local effects after single ocular, dermal or inhalation exposure are only dealt with in this section. Substances causing local effects after single exposure can be further distinguished in irritant or corrosive substances, depending on the (ir)reversibility of the effects observed.

Irritant substances are non-corrosive substances which, through immediate contact with the tissue under consideration, may cause inflammation. Corrosive substances are those which may destroy living tissues with which they come into contact.

Criteria for classification of irritant and corrosive substances are given in Annex VI to Directive 67/548.

The minimum data requirements are essentially the same for new and existing substances.

3.7.1.2 Objectives of investigating the potential for substance-induced irritation or corrosion

The general objectives are to find out:

- whether the substance is, or is likely to be, corrosive;
- whether, in studies in animals or *in vitro*, there is evidence of significant skin, eye or respiratory irritation;
- whether there are indications from human experience with the substance of skin, eye mucous membrane or respiratory irritation following exposure to the substance;
- the time of onset and the extent and severity of the responses and information on reversibility.

Taking into account the severity of the effect, in so far as it can be judged from the test data, the likelihood of occurrence of an acute corrosive or irritant response in humans using or otherwise exposed to the substance is assessed in a pragmatic manner in relation to the route, pattern and extent of the expected human exposure.

3.7.1.3 Information which would be obtained from Annex V testing methods for irritation

There are testing methods in Annex V to Directive 67/548 for skin irritation and eye irritation (EU Annex V B.4 and B.5 corresponding to the OECD 404 and 405). The testing strategy attached to these methods emphasises the need to evaluate all available information before attempting any *in vivo* testing. They both employ screening and interventional elements designed to avoid, as far as possible, *in vivo* testing of corrosive substances and to limit *in vivo* testing of severely irritating substances. In particular, it is recommended to test *in vitro*/ex vivo for skin corrosion (method B.40) before any attempts to assess skin or eye irritation/corrosion by animal testing and when no other information is available. There is not a EU Annex V method for respiratory irritation.

The main (*in vivo*) part of the EU Annex V skin and eye irritation tests will provide information on the local responses (erythema and/or oedema for skin; corneal opacity, iridal effects, conjunctival redness and/or swelling for the eye) in the rabbit following application of a single defined amount of the substance. The local responses are evaluated and graded for each exposed animal at specified intervals after application of the test substance. Information will also be obtained on the time taken to fully establish reversibility (or on the lack of reversibility), on any other local effects (e.g. pain, ocular discharge, necrosis, irreversible coloration of eyes) or any other toxic effects.

3.7.2 Data to be used in the effects assessment

3.7.2.1 Minimum data requirements

The minimum data requirements are that, in accordance with the requirements specified in Annex VII A to Directive 67/548, information should be available for substances on their potential for inducing skin irritation and eye irritation or causing corrosivity.

For new substances, the basic requirements are specified in Annex VIIA to Directive 67/548 and must be obtained using methods mentioned in Annex V to this Directive. For existing substances, the basic requirements are identical but there is more flexibility as to how data are obtained; use of methods other than those specified in Annex V (or corresponding OECD methods) may be accepted on a case-by-case basis.

3.7.2.2 Data which may already be available

Human data (e.g. from case reports or epidemiological studies) may be available.

Data may be available from animal studies:

- skin and/or eye irritation studies mentioned in Annex V to Directive 67/548;
- other toxicological studies in which local responses of skin, eye, mucous membranes and/or respiratory system were reported for animals exposed to the substance;
- other tests, not specified in Annex V to Directive 67/548 (e.g. the Alarie test for respiratory irritation (Alarie, 1973; 1981)).

Data from other studies (i.e. *in vitro* or physico-chemical tests) can provide information on the potential corrosivity or irritancy of substances.

3.7.3 Evaluation of the available data

3.7.3.1 New substances

In general, only tests conducted using methods mentioned in Annex V to Directive 67/548 are considered to be adequate to characterise the irritation and corrosivity potential of new substances after single exposure.

3.7.3.2 Existing substances

Human data

Well-documented human data can often provide very useful information on skin and/or respiratory irritation, sometimes for a range of exposure levels. Often, the only useful information on respiratory irritation, which can be a threshold effect in the workplace, is obtained from human experience. The usefulness of all human data on irritation will depend on the extent to which the effect, and its magnitude, can be reliably attributed to the substance of interest. Experience has shown that it is difficult to obtain useful data on substance-induced eye irritation, but data may be available on human ocular responses to certain types of preparations (e.g. Freeberg et al., 1986).

Animal data

Well-reported studies according to methods described in Annex V to Directive 67/548, particularly if conducted in accordance with principles of GLP, can be used to identify substances which would be considered to be, or not to be, corrosive or irritant to the skin or eye. There may be a number of skin or eye irritation studies already available for an existing substance, none of which are fully equivalent to the EU Annex V procedure. If the results from such a batch of studies are consistent, they may, together, provide sufficient information on the skin and/or eye irritation potential of the substance.

If the results from a batch of studies are not consistent, it will be necessary for the rapporteur to decide which are the most reliable studies based on the criteria for evaluation of the data given above.

Attention should be given to the occurrence of persisting irritating effects, even those which do not lead to classification. Effects such as erythema, oedema, fissuring, scaling, desquamation, hyperplasia and opacity which do not reverse within the test period may indicate that a substance will cause persistent damage to the human skin and eye.

Data from studies other than skin or eye irritation studies (e.g. other toxicological studies on the substance in which local responses of skin, eye mucous membranes and/or respiratory system were reported) may provide useful information.

However, they may not be well reported in relation to, for example, the basic requirements for information on skin and eye irritation. But it should be remembered that information from studies in animals on mucous membrane and/or respiratory system irritation can be very useful for risk assessment provided the irritation is clearly substance-induced, and particularly if it can be related to exposure levels.

There are no test methods for respiratory irritation specified in EU Annex V (e.g. the Alarie test, which may provide useful information on sensory irritation of the upper respiratory tract). It should be noted that use of data from the Alarie test to derive limit values has been criticised (e.g. large interlaboratory differences and inconsistent intra- and inter-species differences were noted) and care should be taken in the interpretation of the results of this test (Bos et al., 1992).

In vitro data

There is a wide range of *in vitro* test methods which give information on the potential irritancy of a substance. Some are designed specifically to address a particular type of irritation (e.g. eye irritation) while others are more general. If there are clear indications from these studies that a substance is likely to be irritant, this may be sufficient for hazard identification purposes (and can be judged only on the basis of the details of the principles and mechanisms of the particular test). Currently, however, data from *in vitro* studies alone would not be considered sufficient to define a substance as being non-irritating.

Other data

Physico-chemical data can be used to identify a substance as being corrosive, but not as being non-irritant. Such data (or practical experience) may also indicate that a substance has defatting properties. Defatting of exposed skin may cause irritation.

3.7.4 Assessment of the dose-response relationship

It may be possible to derive reliable non-irritating concentrations from human studies, though sometimes there is only simply the information that a substance is irritant or, often by inference only, that it is not.

For irritant substances it is not possible to derive non-irritating concentrations from studies for skin and eye irritation according to methods described in EU Annex, but values may be derivable from already available studies in which a range of substance concentrations was used. Non-irritating concentrations may be derivable from studies using inhalation exposure in which respiratory system irritation was observed. In such studies, the slope of the dose-response curve is a particularly useful parameter as it indicates the extent to which reduction of exposure will reduce the response: the steeper the slope, the greater the reduction in response for a particular finite reduction in exposure.

3.7.5 Degree of uncertainty in studies of irritation and corrosivity

Usually it is possible unequivocally to identify (or accept) a substance as being corrosive, whatever type of study provides the information.

There may be a significant level of uncertainty in human data on irritant effects (because of poor reporting, lack of specific information on exposure, subjective or anecdotal reporting of effects, small numbers of subjects, etc.).

Data from studies in animals according to methods described in EU Annex V will usually give very good information on the skin or eye irritancy of a substance in the test species, and, in general, it is assumed that substances which are irritant in Annex V studies in animals will be skin and/or eye irritants in humans, and those which are not irritant in Annex V studies will not

be irritant in humans. Good data, often clearly related to exposure levels, can be obtained on respiratory and mucous membrane irritation, from well-designed and well-reported inhalation studies in animals. Inconsistent results from a number of similar studies increases the uncertainty in deriving data from animal studies.

The data obtained from *in vitro* studies may include many dose levels and replicates: when such a study has a well-defined mechanistic basis and indicates that a substance is expected to be irritating, this may suffice for defined hazard identification purposes. However, the uncertainty in the state of the art for identification of substances as being non-irritant by testing *in vitro* is too high for definitive use in risk assessment.

3.8 SENSITISATION

3.8.1 Introduction

3.8.1.1 Scope of the guidance

A number of diseases are recognised as being, or presumed to be, allergic in nature. These include asthma, rhinitis, conjunctivitis, allergic contact dermatitis, urticaria and food allergies. In this Section (3.8), the endpoints discussed are those traditionally associated with occupational and consumer exposure. Photosensitisation is potentially important but its mechanism of action is poorly understood, so it has been considered but not discussed in detail.

3.8.1.2 Definitions of skin and respiratory sensitisation

A sensitiser is an agent that is able to cause an allergic response in susceptible individuals. The consequence of this is that following subsequent exposure via the skin or by inhalation the characteristic adverse health effects of allergic contact dermatitis or asthma (and related respiratory symptoms such as rhinitis), respectively, may be provoked. Although asthma and rhinitis are generally thought to be a result of an allergic reaction, the understanding, in recent years, that other, non-immunological, mechanisms may occur, makes it more appropriate to use a term based on disease rather than mechanism.

This wider understanding is reflected in the criteria for the classification of skin and respiratory sensitisers, which provide a useful tool against which the hazardous properties of a substance can be judged. These criteria are given in the 22nd Adaptation to Technical Progress to Directive 67/548 [Directive 96/54/EC, Official Journal L248; pp 227-229]; Annev VI has been recast in the 28th Adaptation to Technical Progress (ATP) (Directive 2001/59, Official Journal L225; pp 1- 333).

Respiratory hypersensitivity is a term that is used to describe asthma and other related respiratory conditions, irrespective of the mechanism by which they are caused. When directly considering human data in this document, the clinical diagnostic terms asthma, rhinitis and alveolitis have been retained.

In summary, in this guidance, the term skin sensitisation specifies an allergic mechanism of action, while respiratory hypersensitivity does not. For this reason, the two health hazards have on occasion been approached differently in this guidance.

Where EU Annex V methods are quoted here, they refer to Directive 67/548 as updated in the most recent ATP. The local lymph node assay is expected to be adopted in a subsequent ATP.

3.8.1.3 Objectives of investigating the potential to cause allergic contact dermatitis or respiratory hypersensitivity

The general objectives are to find out:

- whether there are indications from human experience of skin allergy or respiratory hypersensitivity following exposure to the agent;
- whether the agent has skin sensitisation potential based on tests in animals.

The likelihood that an agent will induce skin sensitisation or respiratory hypersensitivity in humans who are using or who are otherwise exposed to this agent is determined by several factors including the route, duration and magnitude of exposure and the potency of the substance.

3.8.1.4 Information which would be obtained from internationally acceptable tests

Skin sensitisation

There are two methods currently described in EU Annex V and OECD guidelines for skin sensitisation in animals; the guinea pig maximisation test (GPMT) and the Buehler test. The GPMT is an adjuvant type test in which the allergic state (sensitisation) is potentiated by the use of Freund's Complete Adjuvant (FCA). The Buehler test is a non-adjuvant method involving for the induction phase topical application rather than the intradermal injections used in the GPMT.

Both the GPMT and the Buehler test have demonstrated the ability to detect chemicals with moderate to strong sensitisation potential as well as those with relatively weak sensitisation potential. These guinea pig methods provide information on skin responses which are evaluated for each animal after several applications of the substance, and on the percentage of animals sensitised.

The murine local lymph node assay (LLNA) is another accepted method for measuring skin sensitisation potential. It has been validated internationally and has been shown to have clear animal welfare and scientific advantages compared with guinea pig tests. In June 2001, the OECD recommended that the LLNA should be adopted as a stand-alone test as an addition to the existing guinea pig test methods. The EU has already drafted a EU Annex V method (draft B.42).

ECETOC Monograph 29 (2000) contains a useful discussion of these tests.

At the time this guidance was written (2001) no acceptable methods had yet been developed to measure photosensitisation.

Respiratory hypersensitivity

There are currently no internationally recognised test methods to predict the ability of chemicals to cause respiratory hypersensitivity. Potentially useful test methods based on allergic mechanisms are the subject of research and development. However, there are currently no test methods under development which are designed specifically to identify chemicals that cause respiratory hypersensitivity by non-immunological mechanisms.

Once acceptable test methods become available, a testing strategy should be developed which takes account of structural alerts, the physical characteristics (whether inhalable), use pattern (likelihood of being inhaled) and known allergic properties (a skin sensitiser) of the compound under investigation.

3.8.2 Data to be used in the effects assessment

3.8.2.1 Minimum data requirements

Information should be available which allows correct assessment of the potential of an agent to cause skin sensitisation. For new substances, the minimum data requirements should conform to EU Annex V (or corresponding OECD guidelines). For existing substances, where data already exist, there is more flexibility and the use of Annex V methods or OECD guidelines is not obligatory: however, any new testing that is performed should comply with these guidelines.

The data requirements for biocidal actives and products are that, as a general principle, tests must be conducted in accordance with EU Annex V (or the corresponding OECD guideline). The test is not required if the active substance is classified as a sensitiser or where the preparation contains a substance(s) which is/are classified a sensitiser(s) according to Directive 67/548 or it is otherwise known that the substance(s) has/have sensitising properties, e.g. on the basis of epidemiological data.

There are no minimum data requirements for respiratory hypersensitivity.

3.8.2.2 Data which may already be available

Human data

Sometimes case studies or epidemiological data will be available from human exposure, particularly in the case of existing substances and biocidal products. Studies that report on cutaneous (allergic contact dermatitis, eczema) or respiratory (asthma, rhinitis, alveolitis) reactions should be of particular significance. Studies indicating negative results should also be evaluated.

Data from diagnostic clinical studies may also be available:

- patch tests may have been conducted in subjects with suspected allergic contact dermatitis;
- bronchial provocation tests, skin prick tests and measurements of specific IgE antibodies may have been conducted in cases of suspected asthma or rhinitis.

These data may help in confirming that the reported disease is caused by the agent under suspicion.

Animal studies

There are a number of different types of studies performed in animals. Some of the following may be available:

- skin sensitisation studies using EU Annex V or OECD standard guinea pig tests;
- the local lymph node assay (LLNA) (draft EU Annex V B.42, draft OECD 429);
- other guinea pig skin sensitisation test methods (such as the Draize test, Freund's complete adjuvant test, optimisation test, split adjuvant test, open epicutaneous test);
- additional tests (such as the mouse ear swelling test);
- investigative respiratory allergy tests (such as the IgE test, cytokine fingerprinting or guinea pig models);
- other information from, for example, repeated dose studies that show effects indicative of an allergic response.

Other data

Some information can be obtained from consideration of structure-activity relationships and comparison with structures of known sensitisers. Structural alerts alone do not normally constitute a reason for positive classification, nor for the adoption of risk assessment/management activity. Likewise, the absence of structural alerts is not a reason for discounting positive clinical or experimental evidence. However, certain well-known groups of chemicals such as isocyanates and acid anhydrides are currently considered to cause respiratory hypersensitivity unless proved otherwise.

Validated *in vitro* methods for sensitisation testing are not yet available. Knowledge of protein reactivity and skin penetration properties from *in vitro* studies may provide useful information.

3.8.3 Evaluation of the available data

3.8.3.1 Human data

When evaluating human data, attention should be paid to:

- the number of well-documented cases in relation to the size of the exposed population;
- the relevance of any described cases and the association between clinical symptoms and clinical test results and exposure;
- the type of exposure (including: adequate substance identification, frequency, duration and magnitude of exposure, the physical state of the substance or biocidal product and exposure to other structurally-related substances). Data from subjects where exposure was not to intact skin or from subjects with pre-existing asthma should be interpreted with caution;
- the quality of the epidemiological data.

Bronchial provocation tests may have been conducted for diagnostic purposes. The design of the test should be evaluated against the following criteria. It should ideally have been performed under blind conditions with a negative control and a clearly sub-irritant concentration of the agent being tested. The test should also take into account possible confounding factors such as medication, smoking or exposure to other substances. The response compared with the control challenge should be convincing. Although in many studies, not all these conditions will be met, the information that they provide may nevertheless be useful.

3.8.3.2 Predictive assays

Assays to predict skin sensitisation

Well reported studies using internationally acceptable protocols, particularly if conducted in accordance with the principles of GLP, can be used for hazard identification.

Other studies, not fully equivalent to EU Annex V or OECD test protocols can give useful information. Particular attention should be paid to the quality of these tests and the use of positive and negative controls.

Particular points to take into account when evaluating results include:

- the choice of vehicle;
- whether skin irritation is observed at the induction phase of guinea pig tests;
- whether the maximal non-irritating concentration is used at the challenge phase of guinea pig tests;
- whether there are signs of systemic toxicity.

Assessment of cutaneous reactions at the challenge phase of guinea pig tests should be conducted carefully to discriminate irritation from sensitisation. Key considerations are:

- numbers of test and control guinea pigs;
- number or percentage of test and control animals displaying skin reactions;
- results of rechallenge treatments if necessary;
- existence of structure-activity relationships;
- checking of strain sensitivity at regular intervals by using an appropriate control substance (as specified in EU Annex V methods or OECD guidelines). Currently (2000) the recommended interval is 6 months.

The investigation of doubtful reactions in guinea pig tests, particularly those associated with evidence of skin irritation following first challenge, may benefit from rechallenge of the test animals. In cases where reactions may have been masked by staining of the skin, other reliable procedures may be used to assist with interpretation; where such methods are used, the submitting laboratory should provide evidence of their value.

For conduct and interpretation of the local lymph node assay the following points should be considered:

- the vehicle in which the test material has been applied;
- the concentrations of test material which have been used;
- any evidence for local or systemic toxicity or local skin inflammation resulting from application of the test material;
- whether the data are consistent with a biological dose response;
- the submitting laboratory should be able to demonstrate its competency to conduct the LLNA.

Other animal tests may also provide valuable information and, in the case of positive results, the substance or biocidal product can be considered as a potential sensitiser.

The specificity and sensitivity of all animal tests should be monitored through the inclusion of appropriate positive and negative controls.

Assays to predict respiratory hypersensitivity

Some animal tests have been developed to identify those chemicals that are able to induce respiratory hypersensitivity by allergic mechanisms. However, none of these methods has yet been validated. Attempts have also been made also to develop structure-activity relationships for respiratory hypersensitivity induced by chemicals, but likewise these have not been validated. See Section 3.8.2.2 for a more detailed discussion of the use of structure-activity relationships.

Some information can be obtained from a comparison with the ability to induce skin sensitisation.

- most chemical allergens that have been shown to induce sensitisation of the respiratory tract in humans are positive in one or more animal (guinea pig and/or mouse) tests for skin sensitisation. The ability of these chemical respiratory allergens to cause skin sensitisation in humans is less clear;
- most chemicals that elicit positive responses in one or more animal skin sensitisation (guinea pig and/or mouse) do not cause respiratory allergy in humans.

Further discussion of this issue is included in Section 3.8.5.

There are currently no predictive test methods to identify chemicals that induce asthma through non-immunological mechanisms.

3.8.4 Assessment of the dose-response relationship

3.8.4.1 Measurement of dose-response

There is evidence that for both skin sensitisation and respiratory hypersensitivity dose-response relationships exist (although these are frequently less well defined in the case of respiratory hypersensitivity). The dose of agent required to induce sensitisation in a previously naïve subject or animal is usually greater than that required to elicit a reaction in a previously sensitised subject or animal; therefore the dose-response relationship for the two phases will differ. Little or nothing is known about dose-response relationships in the development of respiratory hypersensitivity by non-immunological mechanisms.

It is frequently difficult to obtain dose-response information from either existing human or guinea pig data where only a single concentration of the test material has been examined. With human data, exposure measurements may not have been taken at the same time as the disease was evaluated, adding to the difficulty of determining a dose response.

Dose-response data can, however, be generated using specially designed guinea pig test methods (the open epicutaneous test being the most appropriate) or from local lymph node assays.

3.8.4.2 Measurement of potency

Appropriate dose-response data can provide important information on the potency of the material being tested. This can facilitate the development of more accurate risk assessments.

This section refers to potency in the induction phase of sensitisation.

Neither the GPMT/Buehler nor the standard LLNA is specifically designed to evaluate the skin sensitising potency of test compounds, instead they are used to identify sensitisation potential for classification purposes. However, all could be used for some estimate of potency. The relative potency of compounds may be indicated by the percentage of positive animals in the guinea pig studies in relation to the concentrations tested. Likewise, in the LLNA, the EC3 value (the dose estimated to cause a 3-fold increase in local lymph node proliferative activity) can be used as a measure of relative potency (ECETOC, 2000). The dose-response data generated by the LLNA makes this test more informative than guinea pig assays for the assessment of skin sensitising potency.

3.8.5 Degree of uncertainty in studies of sensitisation

Well-conducted human studies can provide very valuable information on skin sensitisation. However, in some instances (due to lack of information on exposure, a small number of subjects, concomitant exposure to other substances, local or regional differences in patient referral etc) there may be a significant level of uncertainty associated with human data. Moreover, diagnostic tests are carried out to see if an individual is sensitised to a specific agent, and not to determine whether the agent can cause sensitisation.

Although human studies may provide some information on respiratory hypersensitivity, the data are frequently limited and subject to the same constraints as human skin sensitisation data.

Reliable data can be generated on skin sensitisation from well designed and well conducted studies in animals. However, guinea pig tests in particular may be difficult to interpret when irritancy or skin staining occurs as the result of challenge. The use of adjuvant in the GPMT may lower the threshold for irritation and so lead to false positive reactions, which can therefore complicate interpretation (running a pre-test with FCA-treated animals can provide helpful information). In international trials, the LLNA has been shown to be reliable, but like the guinea pig tests is dependent on the vehicle used, and it can occasionally give false positive results with irritants. Where tests (guinea pig/mouse) rely on topical exposure rather than intradermal injection, false negatives may occur where the substance fails to be absorbed into the skin as for example with some metal salts. Therefore, careful consideration should be given to the vehicle used and the type of test performed. In some circumstances inconsistent results from similar guinea pig studies, or between guinea pig and LLNA studies, might increase the uncertainty of making a correct interpretation.

Note that, in some instances sensitisation may be due to impurities rather than the test material itself.

Major uncertainties remain in our understanding of the factors that determine whether or not a substance is an allergen, and if so, what makes it a skin or a respiratory sensitiser? A comprehensive analysis of the association between skin and respiratory allergy is needed, but has not been carried out at the time of writing this document (2001).

3.9 REPEATED DOSE TOXICITY

3.9.1 Introduction

3.9.1.1 Definition of repeated dose toxicity

Repeated dose toxicity comprises the adverse general (i.e. excluding reproductive, genotoxic or carcinogenic effects) toxicological effects occurring as a result of repeated daily dosing with, or exposure to, a substance for a part of the expected lifespan (sub-acute or sub-chronic exposure) or for the major part of the lifespan, in the case of chronic exposure. Criteria for classification on the basis of repeated dose toxicity are given in Annex VI to Directive 67/548. What is meant by "adverse effects" is discussed in Section 3.9.4, below.

A substance can induce systemic and/or local effects. A local effect is an effect that is observed at the site of first contact, caused irrespective of whether a substance can become systemically available. When a substance has passed through the physiological barrier, i.e., the skin, mucous membrane of the gastro-intestinal tract or mucous membrane of the respiratory tract and becomes systemically available, it can cause a systemic effect. A systemic effect is defined as an effect that is normally observed distant from the site of first contact. However, toxic effects on surface epithelia may reflect indirect effects as a consequence of systemic toxicity (e.g. uraemic gastritis) or secondary to systemic distribution of the test substance or its active metabolite(s). Commonly, the underlying mode of action is not clarified by routine toxicity studies. The decision as to whether or not an effect should be considered local or systemic, is based on expert judgement.

3.9.1.2 Objectives of investigating the potential of substances to induce repeated dose toxicity

Repeated dose toxicity tests provide information on possible adverse effects likely to arise from repeated exposure of target organs, and on dose-response relationships.

The determination of the dose-response relationship should lead to the identification of the No Observed Adverse Effects Level, NOAEL (see Section 3.4). As part of the risk assessment process for substances, data on the adverse effects which a substance may cause, and the dose levels at which the effects occur, are evaluated in the light of the likely extent of human exposure to the substance so that the potential risk(s) to health may be ascertained.

3.9.2 Data to be used in the effects assessment

3.9.2.1 Minimum data requirements

The minimum requirements are laid down in the relevant legislation for new and existing substances and biocides, respectively. The tests should be conducted following the latest version of the appropriate EU Annex V method or OECD guideline. Especially for existing substances "older" studies might be submitted which do not fulfill all requirements. In these cases the parameters which have not been covered need to be highlighted in the assessment, e.g. neurotoxicity parameters which have not been assessed in a study carried out in accordance with

OECD 407. Arguments should be provided to support the rapporteur's decision whether it is necessary to ask for further testing to complete the assessment for these specific endpoints.

Within the context of the requirements of the EU Annex V method, the maximum possible amount of information should be derived from the repeated-dose toxicity study. At least the following data should be obtained from a repeated dose toxicity test: toxic and other responses by sex, exposure level and time of observation, food and body-weight data, the results of the haematological and clinical biochemical examinations, and the findings of the gross and histo-pathology. The test report should contain a valid statistical treatment of the results, where appropriate, and a conclusion regarding the target organ(s), effects(s) and the NOAEL.

New substances

Investigation of the repeated dose toxicity of new substances is not usually required until supply levels reach 1 tpa when, in the absence of an acceptable reason for not performing a test, a 28-day test repeated dose toxicity study according to EU Annex V (or corresponding OECD guideline) should normally be conducted.

Modified test requirements have been agreed for closed system intermediates (28th ATP to Directive 67/548, OJ L225, 21.08.2001, p. 0001-0333).

Existing substances

For existing substances, the information which would be provided by conducting an EU Annex V 28-day repeat dose toxicity test is required as a minimum. Where data are not available from a dedicated Annex V 28-day repeated dose toxicity study, the minimum repeated dose toxicity data requirement may in certain circumstances be met by results obtained from a study conforming to OECD 422 (combined repeated dose toxicity study with reproduction/developmental toxicity screen). However, due to technical and methodological differences between the standard 28-day repeat dose toxicity study and OECD 422, the use of such a study should be evaluated on a case-by-case basis to assess the adequacy of the information provided. Particularly, it should be noted that in OECD 422 repeated dose toxicity is studied in the pregnant population, and it is generally assumed that there are differences in sensitivity between pregnant and non-pregnant animals. Consequently, where OECD 422 is used, for transparency the rapporteur should clearly indicate the use of data obtained from such a study.

Biocides

For biocides, at least the common core data requirements have to be met for notification purposes. Therefore, a comprehensive data set for toxicological assessment of biocidal active substances and products is available from the very beginning. Detailed guidance on data requirements for biocides is given in the Technical Notes for Guidance (TNsG on Data Requirements, 2000). In summary, the primary required data for biocidal active substances encompass oral 90-day studies in a rodent (rat preferred) and in a non-rodent species (dog preferred) performed according to EU Annex V methods or the corresponding OECD guidelines. If there is evidence that the dog is significantly more sensitive than the rodent species, an oral 12-month oral study with dogs has to be conducted and reported. It is possible to replace a 90-day dog study by a one-year study.

The conduct of oral 28-day tests (EU Annex V B.7 or the corresponding OECD 407) is not required, but must be submitted if available.

The submission of dermal and/or inhalation studies instead of or in addition to oral studies may be required depending on the physico-chemical properties of the substance, the proposed or potential application of the substance/products, or the outcome of acute-toxicity tests.

3.9.2.2 Data which may already be available

Human data

Human data may include epidemiological studies and other human experience. This does not include the conduct of human volunteer studies, which is ethically undesirable and therefore strongly discouraged.

Animal data

The number of repeated dose toxicity studies available for substances is likely to be variable, ranging from none to the 28-day repeated dose toxicity study according to EU Annex V or the equivalent thereof as a minimum, to a series of Annex V and/or non-Annex V tests for some existing substances. There may also be studies employing different routes of exposure. Special toxicity studies, investigating further the nature, mechanism and/or dose-relationship of a critical effect in a target organ or tissue may have been performed. Data on structurally analogous substances may be available and add to the toxicity profile of the substance under investigation.

3.9.3 Evaluation of the available data

When reliable and relevant human data are available, they can be highly useful for hazard identification and even preferable over animal data. However, human data adequate to serve as the sole basis for the dose-response assessment are rare. In many human studies the circumstances of exposure and the exposure levels themselves are not well known, mixed exposure may have occurred, the incidence of effects is low, the number of exposed individuals is small and the latency period between exposure and disease may be long. In addition, the exposed populations may be heterogeneous with respect to age, sex, diet, environment, activity patterns, physical fitness and genetic constitution. Therefore these studies require careful interpretation.

In addition to what has been noted in Section 3.2, the following guidance can be given for the evaluation of the available repeated dose toxicity data:

- preference is given to tests using a species in which the toxicokinetics and toxicodynamics of the substance are most similar to those in man; in the absence of a species that is clearly the most relevant, tests on the most sensitive animal species are selected as the significant ones;
- preference is given to tests using an appropriate route, duration and frequency of exposure in relation to the expected route(s), frequency and duration of human exposure;
- tests enabling the identification of an NOAEL should be given preference;
- preference is given to reliable and sufficiently detailed tests of longer duration; e.g. for hazard assessment, a 90-day repeated dose toxicity test should be given greater weight than a 28-day repeated dose toxicity test in the determination of the most relevant NOAEL.

If sufficient information is available to identify the critical effect (with regard to the relevance for human beings, the dose-response and the consequences) and the target organ or tissue,

greater weight should be given to specific tests investigating this effect in the identification of the NOAEL. The critical effect can be a local as well as a systemic effect.

In the situation where necessary data are either not available or inadequate to enable calculation of a NOAEL or LOAEL in mg/kg/day (e.g. dietary data on substance provided in ppm, but no food consumption or body weight data available), for consistency and transparency between evaluations the same set of default values for biological parameters should be used. A standard set of default values are presented in Appendix VI of the TGD based on a review of the various default values used by different organisations and regulatory authorities (Paulussen et al., 1998).

The derived default values for the different parameters are specified for species, the route of exposure, and the duration of the toxicity study, whilst for food and water consumption rather than default values allometric equations are given to allow calculation of these parameters on a case-by-case basis. The assessor should however be aware that use of allometric equations may lead to default values which differ from those routinely used by other bodies (e.g WHO (JEFCA, JMPR) and RIVM).

Depending on the specific circumstances of the study other default values may be considered more appropriate in some instances.

For transparency the risk assessor should always indicate which methodology has been used, especially where default values different from those laid out in Appendix VI are to be applied. Their use should be clearly stated and justified, and a reference provided to indicate the origin of the values.

3.9.4 Assessment of the dose-response relationship

Crucial in the identification of the NOAEL (or LOAEL), is the definition of "adverse effects". In repeated dose toxicity testing, the values of selected parameters are compared to the average values in untreated concurrent control animals. Adverse effects cannot be defined in purely statistical terms as significant changes relative to control values.

In the identification of the NOAEL, other factors need to be considered such as the severity of the effect, the presence or absence of a dose- and time-effect relationship and/or a dose- and time-response relationship, the biological relevance of an effect, the reversibility of an effect, and the normal biological variation of an effect such as may be shown by representative historical control values (IPCS, 1990).

Correlations observed between changes in several parameters, e.g. between clinical or biochemical and (histo)pathological effects, will be helpful in the evaluation of the adversity of effects. Further guidance to this issue can be found in several publications of the International Programme on Chemical Safety (IPCS 1978; 1987; 1990; 1999).

The decision as to whether or not a local effect should be considered as a substance-related adverse effect or caused by treatment procedures (e.g. adverse effects in the upper gastro-intestinal tract, mediastinum and lungs following bolus application in oral gavage studies), should be based on expert judgement.

Alternative approaches to the derivation of the NOAEL, for example the benchmark dose concept, and the limitations of their applicability, are described in Section 3.4

If local effects are clearly identified after repeated dosing, a NOAEL or LOAEL should be established for these effects in addition to N(L)OAELs for systemic effects.

If local toxicity is not observed or is not investigated in the repeated dose toxicity study, this should be mentioned. Supportive evidence for the occurrence or absence of local effects after repeated dermal and inhalation exposure may be available from the total toxicity profile of the substance. Lack of evidence for local effects in any type of study, i.e. skin or eye irritation, sensitisation, repeated dose toxicity study by routes other than the route of interest, does not exclude the possible occurrence of local effects upon repeated respiratory or dermal exposure (Rennen et al., 1999).

Substances which are skin or eye irritating or corrosive after single exposure (see Section 3.7) should be suspected of inducing local effects upon repeated respiratory exposure to low level concentrations. In contrast, local effects reported from skin sensitisation studies as well as dermal repeated dose toxicity studies are not predictive of local effects on the respiratory tract (Rennen et al., 1999).

Observations from irritation and/or sensitisation studies as well as repeated dose respiratory toxicity studies, are not predictive of local effects on the skin upon repeated dermal exposure (Rennen et al., 1999).

3.9.5 Degree of uncertainty in studies on repeated dose toxicity

The general uncertainty in the use of NOAEL for human health risk assessment is influenced by various factors including intraspecies and interspecies differences in toxicokinetics and sensitivity and by uncertainty with regard to the precision of the NOAEL. The precision of the NOAEL is largely based on the variability in the database and in particular on the conditions of a particular experiment. As indicated above (Sections 3.4 and 3.9.4), no use is made of the shape of the dose-response curve in the determination of the NOAEL.

3.9.6 Testing strategy

The following sub-sections have originally been developed for new substances. They should, where possible and relevant, be used for existing substances and biocides when additional testing is thought to be appropriate. The strategy is based on the tonnage-related testing requirements for new substances, and should be used, as appropriate, in conjunction with the other testing strategies (e.g. the strategy for selection of exposure route as described in Section 3.3 and Appendix V, or carcinogenicity as described in Section 3.11).

3.9.6.1 Objective of this part of the guidance

The objective is to describe an efficient and scientifically defensible testing strategy for the investigation of the repeated dose toxicity of substances which ensures that testing in animals is reduced as far as possible. Use of the strategy should enable the assessor to obtain adequate information on the repeated dose toxicity of substances, for use in risk assessment at the appropriate time in relation to the potential for human exposure. Animal tests should only be conducted when their conduct can be justified in relation to the legal requirements and when their outcome can be expected to be useful for risk assessment.

New substances

The requirements for repeated dose toxicity testing of new substances, as given in Directive 67/548 and outlined in **Table 3** specify particular tests to be carried out dependent on the supply level which has been reached.

Table 3 Requirements for repeated dose toxicity testing of new substances as given in Directive 67/548

Supply level	Repeated dose studies required	Remarks
<1 tpa	None	Additional testing can be required if the substance gives cause for immediate concern (Article 3 (4) (iii) of Directive 93/67)
1 tpa "Base set"	28-day study; preferred species is rat; route of exposure is oral unless contra-indicated	
10/100 tpa (50/500t cumulative)	Sub-chronic and/or chronic, including special studies, if results of base-set test or other information (e.g. SAR) show need for further appropriate investigation	Studies may be required at 10 tpa, but shall be required at 100 tpa unless the notifier can give good reason, supported by evidence acceptable to the assessor, why a given test/study is not appropriate or an alternative scientific test/study would be preferable (Article 7(2) of Directive 67/548).
1,000 tpa	Chronic toxicity study; additional tests to investigate organ or system toxicity	At 1,000 tpa, the test programme outlined in Directive 67/548 needs to be followed unless "there are strong reasons to the contrary, supported by evidence, that it should not be followed" (Annex VIII to Directive 67/548).

Whereas testing will normally follow the tonnage trigger as described above, the risk assessment may indicate that further information is required for revision of the assessment (Article 3 (4) (ii) or (iii) of Directive 93/67). Depending on the degree of concern for human exposure the Competent Authority will decide whether further testing is immediately required or can be defered until the quantity placed on the market reaches the next tonnage threshold, e.g. (sub)chronic toxicity study at 10 tpa (50 t cumulative).

Reliable estimates or measurements of human exposure levels should be available, especially at higher tonnage levels, and the pattern of human exposure known in order to help the Competent Authority decide whether a longer study is required for better definition of the repeated dose toxicity.

Existing substances

The basic requirement is for the information which would be provided by an EU Annex V 28-day repeated dose toxicity test to be available, as a minimum. Existing information should be reviewed to ascertain whether this requirement is met. A risk assessment which takes into account all the available information on the relevant properties (e.g. toxicological, toxicokinetic and physico-chemical) together with supply levels and human exposure patterns may however indicate that more repeated dose toxicity data are needed.

Biocides

Core data and additional data requirements regarding repeated-dose toxicity studies with biocidal active substances are given in Directive 98/8 and outlined in **Table 4**. The required administration route is oral, unless it can be justified that an alternative route is more appropriate. All studies conducted must be submitted. Further guidance on data requirements and on waiving thereof is provided in the Technical Notes for Guidance (TNsG on Data Requirements, 2000).

Table 4 Requirements for repeated dose toxicity testing of biocidal active substances, as given in Directive 98/8

Repeated dose studies required	Remarks
28-day studies	Not required, useful as range-finding test
90-day studies in one rodent and one non-rodent species	Required, preferred species: rat and dog; waiving of the non-rodent study is possible
1-year study, dog	May be required in addition to 90-day study depending on expert judgement
Chronic studies in one rodent and in one other mammalian species	Required, minimum duration 12 months. Preferred rodent species: rat (should be tested first). Depending on the test results more testing in the other species may be necessary. The use of the combined chronic toxicity/carcinogenicity study protocol is recommended. If waiving is applicable, no studies have to be conducted.
Delayed neurotoxicity studies	Required if active substance is an organophosphorous compound. Test species: adult hen unless another test species is considered more appropriate
Studies of toxic effects on livestock and pets	Required depending on product type and on a case-by case basis on expert judgement
Studies related to the exposure of the active substance to humans	Required on a case-by case basis depending on expert judgement: Testing of degradation products, by-products, reaction products, non-mammalian metabolites (plants, soil, etc.), related to human exposure
Mechanistic studies	Required on a case-by case basis depending on expert judgement

3.9.6.2 General principles

The amount of information initially provided for the substance under investigation depends on the minimum data requirements laid down in the relevant legislation for new and existing substances and biocides, respectively (see Section 3.9.2.1). In addition all available toxicity data have to be submitted. The adequacy of existing studies needs to be assessed critically with consideration of the quality of data and the adequacy of the protocol design. Where it is considered that the existing data are inadequate to provide a clear assessment of the toxicological potential, the need for further testing should be considered in view of all available relevant information on the substance, including its use pattern, the potential for human exposure and chemical properties and structural alerts.

The potential for human exposure at levels giving cause for concern in the light of the toxicity of the substance may indicate a need for further testing: the decision on when, or whether, to investigate further a specific observed effect (i.e. to do more testing) will be influenced by the outcome of the exposure assessment as well as the nature of the effect.

In summary, the need to conduct further repeated dose toxicity test(s) may be identified as an outcome of a risk assessment, or may arise as the supply level of a new substance increases.

Exemptions from testing are foreseen for new and existing substances as well as for biocides (escape clause, derogation, waiving). The information required need not be provided if it is not technically possible or if it does not appear scientifically necessary to give information, and the reasons are clearly stated and accepted by the assessor (Annex VIIA, Annex VIII to Directive 67/548). The decision whether the escape clause or derogation can be accepted for a new or an existing chemical substance is made on a case-by-case basis, e.g. derogation/escape may be considered in cases where there are data on a closely related substance that could be read across to fill the particular requirement or where a substance undergoes immediate disintegration and

there are sufficient data on the cleavage products. Common criteria for waiving of biocides can be found in the Technical Notes for Guidance (TNsG) on Data Requirements (2000).

When there is clear evidence that a particular effect observed in an animal study is not of relevance to humans (e.g. male-rat-specific light hydrocarbon nephropathy), further investigation of that effect (e.g. to establish a "no-effect level" for that particular effect) is not necessary.

The basis of the strategy for testing substances for repeated dose toxicity is stepwise testing, with the 28-day study as the usual starting point for chemicals and the 90-day study for biocides. However, a flexible approach is encouraged whereby the data required are identified and obtained in an efficient manner consistent with the needs of animal welfare. The strategy addresses the question of when more detailed investigation of specific system or organ toxicity (e.g. neurotoxicity) may be thought necessary, and how such investigation may be performed. The strategy should be used, as appropriate, in conjunction with the testing strategies given in the other sections of this document.

It is strongly recommended that, when possible, the type, scope and timing of the repeated dose toxicity testing to be applied to a substance are discussed between the notifier or company and the Competent Authority.

3.9.6.3 Preliminary considerations

Regardless of which repeated dose toxicity test is to be conducted, the most appropriate route of exposure has to be selected. The criteria for selection of the route of exposure are described in Section 3.3 and Appendix V.

Any other relevant information should be taken into account: toxicokinetic data, other toxicity data on the substance, or SAR can be used when taking decisions about when or whether to conduct studies, or about study design (e.g. duration of study, which investigations should be made). Specific aspects of the application of SAR for human toxicological endpoints have been reviewed by Hulzebos et al. (1999; 2001).

Before any repeated dose studies are undertaken, at least the following parameters should be considered:

- the physical form of the substance;
- relevant physico-chemical characteristics (e.g. solubility in water; log Kow);
- the results of any previously conducted toxicity tests;
- the chemical structure of the substance and its similarity to that of other substances of known toxicity (i.e. SAR);
- impurities in the substance, and likely metabolites or breakdown products;
- other relevant criteria for selection of the route of exposure given in the inhalation toxicity testing strategy;
- any data available on the substance in the published literature.

It is important to ensure that all new studies are conducted according to protocols that are adequate, not only with respect to EU Annex V and GLP, but also in relation to the specific substance under investigation, the end-point(s) of interest and the human exposure pattern. When such considerations are accommodated at the study design stage, it should ensure that the occurrence of inconclusive or irrelevant studies, and hence unnecessary use of animals, is minimised.

3.9.6.4 Considerations for initial 28- or 90- day toxicity testing

The design of the repeat dose toxicity study should take account of the factors listed above under "Preliminary considerations". It is recommended that minor extensions of the study protocol, such as the inclusion of an extra tissue for morphology or an additional clinical chemistry or haematological parameter, should be made when the need for this is indicated by existing information or by observations made during the study.

For instance, if a substance has a clear structural similarity to a known thyroid toxicant, measurements of thyroid hormone levels could be included.

In the revised EU Annex V B.7 (equivalent to OECD 407 (1995)) and Annex V B.26 (equivalent to OECD 408 (1998)) for the oral 28-day and 90-day studies, respectively, more emphasis is given to clinical observation, clinical chemistry and pathology and the identification of substances which induce adverse neurological or immunological effects. Use of the updated Annex V methods and OECD guidelines may reduce the amount of supplementary testing required. It is recommended to apply the updated study design of Annex V B.7 and 26 for all new 28-day and 90-day studies including studies with dermal and inhalation exposure (OECD 410/411 or 412/413), although the time-points of neurological/behavioural examinations as recommended in Annex V B.7 and 26 (OECD 407 and 408) may have to be adapted in studies involving dermal and inhalation exposure routes.

As mentioned above, investigation of repeated dose toxicity is usually initiated by the performance of an oral 28-day study for chemicals and a 90-day study for biocides, which are usually conducted in rats. On a case-by-case basis, there can be scientifically justifiable reasons for using a test of another duration as the initial repeated dose toxicity test.

Acceptable reasons for the performance of a 90-day study at base set level for chemicals instead of a 28-day study include:

- the substance is expected to be of low systemic toxicity, but may cause lung fibrosis (e.g. substances which can be inhaled, sparingly soluble dusts);
- the substance is expected (e.g. from SAR) to have some toxicity, but is also supplied at or expected quickly to reach higher tonnage levels and there is sufficient information available to enable selection of appropriate and adequate dose levels in a 90-day study;
- the nature of expected effects is such that they may not be detected in a 28-day study;
- together with one of the above, the exposure pattern with respect to level and duration indicates that a longer term study is appropriate.

3.9.6.5 Immediate further testing

Immediate further testing for repeated dose toxicity may be indicated in some cases. Circumstances which may indicate such a requirement could include one or more of the following:

- failure to identify an NOAEL in the initial repeated dose study;
- toxicity of particular concern (e.g. serious/severe effects);
- indications of an effect (e.g. immunotoxicity, neurotoxicity) for which the available evidence is inadequate for toxicological and/or risk characterisation;
- the route of exposure used in the initial repeated dose study was inappropriate in relation to the expected route of human exposure and route-to-route extrapolation cannot be made;
- particular concern regarding exposure (e.g. use in consumer products leading to exposure levels which are high relative to the dose levels at which toxicity is observed);

- SAR has indicated an effect which was not detected in the initial study.

When immediate further testing is required, its nature and scope will be decided on a case-by-case basis. It may range from re-examination of some of the archived tissues to a further study (e.g. a study of specific organ toxicity or mechanistic studies; another repeated dose study via a different route of exposure or/and of longer duration).

For new substances, if some of the conditions which would lead to consideration of immediate post-base-set testing exist, but not to an extent that immediate testing is required, the conclusion given at Article 3 (4) (ii) of Directive 93/67 will normally apply and - depending on the degree of concern for human exposure - the decision may be that further testing should be conducted at 10 tpa (50 t cumulative).

3.9.6.6 (Sub)chronic toxicity studies

The duration of any post-28-day study needs careful consideration: the optimum duration of a study to ensure that qualitative identification of all target tissues and definition of the NOAEL are achieved is difficult to define on the basis of currently available data, and will be influenced by the expected exposure pattern. However, it is recommended that, unless there is good evidence that a chronic study should be conducted, and an adequate protocol can be defined (e.g. dose levels), the sub-chronic (90-day) study should be selected to follow a 28-day study when further testing is necessary (as it is for new substances when supply levels reach 100 tpa). It may prove difficult to set dose levels for a chronic study in the absence of a 90-day study.

For biocides, two oral 90-day toxicity studies conducted in a rodent and in a non-rodent species represent the basic requirement of repeated-dose toxicity testing. However, waiving of the non-rodent study (usually performed with dogs) can be considered:

- if the 90-day rodent studies do not indicate substance-related effects at the limit dose level (corresponding to a substance intake of 1,000 mg/kg bw/day).
- if the mechanism of the toxicity is known, if it can be justified that the toxicological effect is not specific to the rodent species and if mechanism studies provide scientific evidence that the toxicological profile does not differ between the animal species.

In general, a 90-day non-rodent study does not have to be conducted if a 1-year non-rodent toxicity study is available. Waiving of the 90-day or 1-year non-rodent study is not possible if long-term toxicity studies in both species are waived under the conditions described further below.

The justification for not conducting a 90-day study would need to be substantial as the 90-day (sub-chronic study) is an important basis for classification, and may be used to set dose levels for other tests (e.g. reproductive toxicity studies and studies of organ/system toxicity). An example of the sort of substance for which sub-chronic/chronic testing might be considered superfluous would be a non-reactive, insoluble substance which cannot be inhaled and for which there is no evidence of absorption and no evidence of toxicity in a 28-day “limit test”, particularly if such a pattern is coupled with limited human exposure.

Chronic toxicity studies are normally required in two species for biocides (rat and second mammalian species) and in one species (normally the rat) for new chemicals at 1,000t/a. Chronic toxicity studies may also be required for new substances at lower supply levels and for existing substances depending on the available toxicity and exposure data; e.g. for substances to which

lifetime human exposure is possible (such as components of household/laundry cleaning products for manual use), a chronic toxicity study will normally be required.

Planning of the long-term studies should be made on the basis of previous short-term toxicity study results. In addition, toxicokinetic studies, if designed appropriately, may reveal accumulation of a substance or its metabolite(s) in certain tissues or organs which would possibly remain undetected in short-term toxicity tests but which are liable to result in adverse effects upon prolonged exposure. Thus, based on these results, a case-by case expert judgement may require the submission of a chronic toxicity study at lower annual production rate levels.

The minimum duration for a chronic toxicity study conducted in rats is 12 months, according to the EU Annex V method. Longer studies (e.g. "lifetime" - 2 years in rats) may be considered appropriate for adequate identification of the NOAEL. It is recommended that if a carcinogenicity study is to be conducted on a substance, an investigation of chronic toxicity should form part of the study protocol. It is now usual practice to investigate chronic toxicity in the same study as carcinogenicity and for this purpose to use sufficient satellite groups, which are treated for at least 12 months, to identify the NOAEL for systemic toxicity. This takes account of potential age-related changes in the animals that can confound interpretation of observed effects after lifetime exposure. In long-term studies, it is important to choose the species/strain of experimental animal in relation to its known geriatric effects and the toxic effects which are under investigation.

For biocides, waiving of both chronic toxicity studies may be considered if the subchronic studies in rodents and non-rodents do not indicate substance-related adverse effects at the limit-dose level.

3.9.6.7 Specific system/organ toxicity

General aspects

For some specific system/organ effects the testing methods of EU Annex V or the OECD may not provide for adequate characterisation of the toxicity. There may be indications of such effects in the standard studies for systemic toxicity, or from SAR. For adequate characterisation of the toxicity and, hence, the risk to human health, it may be necessary to conduct studies using other published test methods, "in-house" methods or specially designed tests. Some references are given in **Table 5**. When it is considered necessary to conduct a study to investigate specific organ/system toxicity, it is important that the study design is discussed by the contractor/laboratory and the assessor, paying particular attention to the protocol to be used, before initiating the study. The need for (and scope/size of) studies using live animals should be particularly carefully considered.

Some specific investigation of organ/systemic toxicity (e.g. hepatotoxicity and nephrotoxicity) is undertaken as part of the EU Annex V repeated dose toxicity tests. Reproductive toxicity is specifically examined using special methods (Annex V) and a strategy for addressing this concern is to be found at Section 3.12. Specific investigation (or further investigation) of any organ/system toxicity (e.g. kidney, cardiac, adrenal, thyroid) may sometimes be considered necessary and should be addressed on a case-by-case basis. The Competent Authorities have requested that guidance on specific investigation of neurotoxicity and immunotoxicity forms a part of this testing strategy. Also addressed herein, as a discrete issue, is lung overload and fibrosis.

Definition of neurotoxicity

Neurotoxicity is the induction by a chemical of adverse effects in the central or peripheral nervous system, or in sense organs. It is useful for the purpose of hazard and risk assessment to differentiate sense organ-specific effects from other effects which lie within the nervous system. A substance is considered "neurotoxic" if it induces a reproducible lesion in the nervous system or a reproducible pattern of neural dysfunction.

Substances are not classified specifically as neurotoxicants: neurotoxic substances will be classified as very toxic, toxic or harmful, in accordance with the criteria given in Directive 2001/59 (Annex VI to Directive 67/548).

Introduction

It is recommended that a hierarchical approach is taken in the investigation of the potential neurotoxicity of substances. The starting point for the testing strategy should be exposure considerations, *in vitro* data, SAR and should proceed via data already available from base set tests to more specific testing. Thus, any indications of specific or non-specific neurotoxicity in the acute and repeated dose toxicity tests should be carefully noted. The present EU and OECD oral 28-day and 90-day tests (EU Annex V B7, Annex V B26, OECD 407, 1995; OECD 408, 1998) examine a number of simple nervous system endpoints (e.g. clinical observations of motor and autonomous nervous system activity, histopathology of nerve tissue), which should be regarded as the starting point for evaluation of a substance potential to cause neurotoxicity. It should be recognised that the standard 28-/90-day tests measure only some aspects of nervous system structure and function, while other aspects, e.g. learning and memory and sensory function is not or only superficially tested. SAR considerations may prompt the introduction of additional parameters to be tested in standard toxicity tests or the immediate request of studies such as delayed neurotoxicity (EU Annex V B37 or B38, OECD 418 or 419; see below). Any indication of potential neurotoxicity of substances can also be a trigger for testing for developmental neurotoxicity. For detailed guidance see Section 3.12.6.7.

If there are no indications of neurotoxicity in humans, and no indications in adequately performed acute and repeated dose toxicity tests, and none from SAR, it will not be necessary to conduct any special tests for neurotoxicity.

Structure-activity considerations

Structural alerts are only used as a positive indication of neurotoxic potential. Structural alerts for neurotoxicity may be found in TNO (1999); including organic solvents (for chronic toxic encephalopathy); organophosphorus compounds (for delayed neurotoxicity), and carbamates (for cholinergic effects). Several estimation techniques are available, one of which is the rule-based DEREK (Deductive Estimation of Risk from Existing Knowledge) system developed in the UK. The rulebase comprises the following hazards and structural alerts: Organophosphate (for direct and indirect anticholinesterase activity); N-methyl or N,N-dimethyl carbamate (for direct anticholinesterase activity); gamma-diketones (for neurotoxicity)

Initial testing

Signs of neurotoxicity in standard acute or repeated dose toxicity tests may be secondary to other systemic toxicity or to discomfort from physical effects such as a distended or blocked gastro-intestinal tract. Nervous system effects seen at dose levels near or above those causing lethality should not be considered, in isolation, to be evidence of neurotoxicity. In acute toxicity studies

where high doses are administered, clinical signs are often observed which are suggestive of effects on the nervous system (e.g. observations of lethargy, postural or behavioural changes), and a distinction should be made between specific and non-specific signs of neurotoxicity.

Neurotoxicity may be indicated by the following signs: morphological (structural)changes in the central or peripheral nervous system or in special sense organs; neurophysiological changes (e.g. electroencephalographic changes); behavioural (functional) changes; neurochemical changes (e.g. neurotransmitter levels).

The type, severity, number and reversibility of the effect should be considered. Generally a pattern of related effects is more persuasive evidence of neurotoxicity than one or a few unrelated effects.

It is important to ascertain whether the nervous system is the primary target organ. The reversibility of neurotoxic effects should also be considered. The potential for such effects to occur in exposed humans (i.e. the exposure pattern and estimated level of exposure are "acute") should be considered in the risk characterisation. Reversible effects may be of high concern depending on the severity and nature of effect. In this context it should be kept in mind that effects observed in experimental animals that appear harmless might be of high concern in humans depending on the setting in which they occur (e.g. sleepiness in itself may not be harmful, but in relation to operation of machinery it is an effect of high concern). Furthermore the possibility that a permanent lesion has occurred cannot be excluded, even if the overt effect is transient. The nervous system possesses reserve capacity, which may compensate for the damage, but the resulting reduction in the reserve capacity should be regarded as an adverse effect. Compensation may be suspected if a neurotoxic effect slowly resolves during the lifespan. This could be the case for developmental neurotoxicants (see Section 3.12.6.7). Irreversible neurotoxic effects are of high concern and usually involve structural changes, though, at least in humans, lasting functional effects (e.g. depression, involuntary motor tremor) are suspected to occur as a result of neurotoxicant exposure, apparently without morphological abnormalities.

For the evaluation of organophosphate pesticides, Competent Authority experts agreed to use the WHO/FAO Joint Meeting of Experts on Pesticide Residues (JMPR) recommendations on "Interpretation of Cholinesterase Inhibition" (FAO, 1998; 1999). The applicability of these recommendations, outlined below, could also be extended to biocides and new/existing substances.

Recommendations from the WHO/FAO Joint Meeting of Experts on Pesticide Residues (JMPR)

The inhibition of brain acetylcholinesterase activity and clinical signs are considered to be the primary end-points of concern in toxicological studies on compounds that inhibit acetylcholinesterases. Inhibition of erythrocyte acetylcholinesterase is also considered to be an adverse effect, insofar as it is used as a surrogate for brain and peripheral nerve acetylcholinesterase inhibition, when data on the brain enzyme are not available. The use of erythrocyte acetylcholinesterase inhibition as a surrogate for peripheral effects is justified for acute exposures resulting in greater acetylcholinesterase inhibition in erythrocytes than in the brain. However, reliance on inhibition of erythrocytic enzyme in studies of repeated doses might result in an overestimate of inhibition on peripheral tissues, because of the lower rate of resynthesis of the enzyme in erythrocytes than in the nervous system. Plasma acetylcholinesterase inhibition is considered not relevant. Regarding brain and erythrocyte acetylcholinesterase inhibition, the experts defined that statistically significant inhibition by 20% or more represents a clear toxicological effect and any decision to dismiss such findings should be justified. JMPR also agreed on the convention that statistically significant inhibition of less than 20% or statistically

insignificant inhibition above 20% indicate that a more detailed analysis of the data should be undertaken. The toxicological significance of these findings should be determined on a case-by-case basis. One of the aspects to consider is the dose-response characteristic.

Certain substances and/or certain effects are best investigated in particular species. Pyridine derivatives are neurotoxic to humans and primates but not to rats. Among other neurotoxic compounds, organophosphorus compounds are a group with known delayed neurotoxic properties, which need to be assessed in a specified test for delayed neurotoxicity, to be performed preferentially in the adult laying hen according to EU Annex V B.37 or OECD 418 (Delayed neurotoxicity of organophosphorus substances following acute exposure) and Annex V B.38 or OECD 419 (Delayed neurotoxicity of organophosphorus substances: 28-day repeated dose study). Such studies are specifically required for biocidal substances of similar or related structures to those capable of inducing delayed neurotoxicity. If anticholineesterase activity is detected, a test for response to reactivating agent may be required.

Further neurotoxicity testing

If the data acquired from the standard systemic toxicity tests are inadequate or provide indications of neurotoxicity which are not adequate for risk characterisation, the nature of further investigation will need to be considered. If further standard 28- or 90-day studies are to be conducted, a number of nervous system endpoints will be examined. These endpoints should be included in the tests irrespective of the administration route. A standard study with additional parameters could be considered. In some cases, it may be necessary to conduct a specific study such as a neurotoxicity test using the OECD method 424 (or corresponding EU test method which will eventually be introduced into Annex V) with possible inclusion of a satellite group for assessment of reversibility of effects. The OECD 424 is intended for confirmation or further characterisation of potential neurotoxicity identified in previous studies. The OECD guideline allows for a flexible approach, in which the number of simple endpoints which duplicate those already examined during standard testing may be minimised, and where more effort is put into in-depth investigation of more specific endpoints by inclusion of more specialised tests. Adjustment of dose levels to avoid confounding by general toxicity should be considered. If additional studies are considered necessary the design of further studies should be discussed by the assessor and the contractor/laboratory before these tests are started.

If data from standard toxicity studies are clearly indicative of specific neurotoxicity, e.g. neurotoxicity occurring at lower dose levels than systemic toxicity, further specific neurotoxicity testing is required to confirm and extend the findings from the general toxicity studies and to establish an NOAEL for neurotoxicity. Again, the neurotoxicity test according to OECD 424 is considered appropriate for this situation.

Standard exposure conditions may not always be adequate for neurotoxicity studies. The duration of exposure needed to induce specific neurotoxic effects in an animal experiment will depend on the underlying mechanism of action. Short-term peak exposures can be important for certain types of substance/effect. When the test compound is administered as a bolus via the intravenous, subcutaneous or oral route it is essential to determine the time-effect course, and to perform measurements of neurotoxicity parameters preferentially at the time of peak effect.

For example, the neurotoxicity associated with short-term exposure to some volatile organic solvents has largely been identified following human exposure - particularly occupational exposure. Acute inhalation studies, using protocols designed to detect the expected effects, are ideal for such substances/effects. For some neurotoxic substances a long exposure period is necessary to elicit neurotoxicity.

The most appropriate methods for further investigation of neurotoxicity should be determined on a case-by-case basis, guided by the effects seen in the standard systemic toxicity tests and/or from SAR-based predictions. Extensive coverage of methods which may be used is given in OECD (2000), IPCS (1986) and ECETOC (1992), and some are summarised in **Table 5**, below.

Table 5 Methods for investigation of neurotoxicity

Effect	Methods available	References *
Morphological changes	Neuropathology. Gross anatomical techniques. Immunocytochemistry. Special stains.	Krinke, 1989; O'Donoghue, 1989; Mattsson et al., 1990
Physiological changes	Electrophysiology (e.g. nerve conduction velocity (NCV), Electroencephalogram (EEG), evoked potentials).	Fox et al., 1982; Rebert, 1983; Mattsson and Albee, 1988
Behavioural changes	Functional observations. Sensory function tests. Motor function tests (e.g. locomotor activity). Cognitive function tests.	Robbins, 1977; Tilson et al., 1980; Cabe and Eckerman, 1982; Pryor et al., 1983; Moser and McPhail, 1990; Moser, 1995
Biochemical changes	Neurotransmitter analyses. Enzyme/protein activity. Measures of cell integrity.	Dewar and Moffett, 1977; Damstra and Bondy, 1982; Cooper et al., 1986; Costa, 1998

* Given in full in ECETOC (1992), IPCS (1986) or Mitchell (1982) in the References.

Definition of immunotoxicity

"Immunotoxicity" is the ability of a substance to adversely affect the immune system: the immune response of affected individuals is altered. Immunotoxic responses may occur when the immune system is the target of the chemical insult; this in turn can result in either immunosuppression and a subsequent decreased resistance to infection and certain forms of neoplasia, or immune dysregulation which exacerbates allergy or autoimmunity. Alternatively, toxicity may arise when the immune system responds to an antigenic specificity of the chemical as part of a specific immune response (i.e. allergy or autoimmunity) (IPCS, 1996). Changes of immunological parameters may also be a secondary response to stress resulting from effects on other organ systems. Therefore, it must be recognized that in principle all chemical substances may be able to influence parameters of the immune system if administered at sufficiently high dosages. However an immunotoxic effect should not be disregarded until a thorough investigation has been performed.

Introduction

The toxicological significance of immune responses is currently under discussion by several scientific groups (e.g., ECETOC, IPCS). Immunotoxicity is of particular concern for test substances that induce toxicity on the immune system at dose levels below those which induce toxicity at other target sites. If the immunotoxicity is the critical effect, it is recommended to assess immune effects in the risk assessment process as for any other toxic effect (IPCS, 1996; Richter-Reichhelm et al., 2001). As the revised test methods (EU Annex V B.7 and B.26, OECD 407 and 408) become applied routinely, it is expected that the database on immunotoxic potential of substances will increase and experience on the evaluation of immune effects will improve. Primarily the test guidelines are intended as a screening for immunotoxicity, and depending on the results immediate further testing may be needed.

Hypersensitivity

Skin and respiratory sensitisation to substances are examples of hypersensitivity. For further discussion on this topic, see Section 3.8 on Sensitisation.

Immunosuppression

The basis of the recommended approach to assessment of the potential immunotoxicity of a new substance is that many immunotoxic substances can be identified via the standard tests for systemic toxicity, particularly if the relevant additional measures of the updated EU and the OECD 28-day and 90-day test guidelines (see below) are used. As these additional measures do not comprise functional tests, it should be noted that discussions are currently taking place in the OECD as to whether these revised guidelines should be further enhanced by the inclusion of a function test (i.e. antibody response to sheep erythrocytes). Special studies to characterise effects of concern for immunotoxicity are used only when necessary for adequate risk characterisation. The nature of special studies, and when they should be conducted, need to be decided on a case-by-case basis. In particular, the use of *in vivo* tests should not be undertaken without detailed consideration of the need for such studies. A tiered approach to the identification of immunotoxic hazard in routine toxicology is described in IPCS (1996) and Richter-Reichhelm et al. (2001).

The revised protocols of both the EU and the OECD 28-day and 90-day studies EU Annex V B.7 and B.26, equivalent to OECD 407 and TG 408, respectively) now include the measurement of thymus and spleen weights and histopathological examination of certain lymphoid tissues (i.e. thymus, draining and distant lymph nodes, Peyer's patches, bone marrow section) in addition to the total and differential white blood cell counts and spleen histopathology required in the previous Annex V method. These tissues all have immunological function and changes to them can be indicative of adverse effects on the immune system.

The additional histopathological examinations listed above should be conducted on all control and high-dose animals. The stipulated tissues from all animals in all dose groups should be preserved. If tissues from high-dose animals show treatment-related changes, those from lower dose groups should also be examined to try to establish the NOAEL. The documentation of histopathology findings on immune organs can be improved by using a diagnostic system as developed by international colloborative studies (ICICIS, 1998; Kuper et al., 2000; Richter-Reichhelm and Schulte, 1996). In this system the lymphoid tissue is divided into compartments and the effects are assessed by application of a semiquantitative grading system. If there are changes in the bone marrow section, a bone marrow smear may be useful to quantify the changes: for a substance suspected to be immunotoxic (e.g. from SAR) it would be useful to prepare bone marrow smears in anticipation of this need. For these substances the study design could be further enhanced by adding parameters such as identification of lymphocyte subpopulations (flowcytometric analysis) and/or determination of serum immunoglobuline concentrations. Satellite groups could be included to conduct functional tests, e.g. antibody response to sheep erythrocytes.

If there are no indications of immunotoxicity in the 28-day (or 90-day) toxicity test, and also none from SAR, no further specific investigation for immunotoxicity will normally be required. However, when further studies of systemic toxicity are conducted on such substances, investigations for potential immunotoxicity, as described above should also be undertaken.

The need for further testing to examine in more depth the immunotoxicity of a substance giving rise to concern for immunotoxicity in the base-set repeated dose test will be considered on a case-by-case basis. Substances with SAR indications of potential immunotoxicity, but no

indications from the repeated-dose test results, may also need to be considered for further testing for immunotoxicity. The timing of any further testing to investigate immunotoxicity will be influenced by the level of concern in relation to both the observed/expected effects and the potential for human exposure. The severity of the effect, its implications for human health and which human population(s) are exposed (e.g. workers and/or consumers) will be influencing factors.

Indications of immunotoxicity from standard repeated-dose studies include one or more of the following signs:

- morphological changes of lymphoid organs and tissues including bone marrow (e.g. altered cellularity/size of major compartments);
- weight changes of lymphoid organs;
- changes in haematology parameters (e.g. white blood cell number, differential cell counts of lymphocytic, monocytic and granulocytic cells);
- changes in clinical chemistry parameters (e.g. serum protein levels, immunoglobulin concentrations if determined).

Further testing to investigate immune function (e.g. a T-cell function test for substances which cause histopathological changes in the thymus, host resistance models) should be conducted only if the results of such studies can be interpreted in relation to the risk assessment for the substance. In many cases, the observation of the morphological changes or of changes of in haematology and of clinical chemistry parameters, together with an NOAEL for those changes, will be sufficient for screening. Functional assays may give valuable information to identify immunotoxic effects and, in some cases, they can be more sensitive than non-functional assays. However, it should be noted that the observation of the immunological changes discussed above may not necessarily reflect a primary immunotoxic effect but may be secondary to other effects.

Currently there are few methods for specific investigation of immuntoxic effects which are regarded as sufficiently validated for routine use (IPCS, 1996; Richter-Reichhelm et al., 2001). The plaque forming assay or the equivalent using the ELISA method (Enzyme-linked Immunosorbent Assay) are recommended to identify altered T-cell dependent humoral responses (Van Loveren et al., 1991; Temple et al., 1993). Of particular value for risk assessment are so called host resistance models, in which the clinical relevance of immunotoxicity can be evaluated (Van Loveren, 1995; IPCS, 1996). Other methods may also be of value to provide information on the mode of immunotoxic action (e.g., mitogen stimulation tests, leucocyte phenotyping). However, further work is needed on standardisation and validation of these test methods.

As no specific guideline for immunotoxicity testing has been developed, it is recommended that the assessor and the contractor/laboratory discuss the need for further investigation of immunotoxicity and, when testing is required, discuss the test to be used and the study design before testing is started.

Other immunomodulating effects

Autoimmune diseases are another important area of substance-induced immunotoxicity. At present there are no specific assays to assess substances for their potential to induce autoimmune reactions.

Effects on the endocrine system

The endocrine system consists of a set of glands such as the thyroid, gonads and the adrenal glands, and the hormones they produce such as thyroxine, oestrogen, testosterone and adrenaline, which help guide the development, growth, reproduction and behaviour of animals, including human beings (EC Commission Communication, 1999). Guidance relating to endocrine disrupters and reproduction is given in the appropriate chapter of the TGD (see 3.12.7.3).

The IPCS has, together with Japanese, USA, Canadian, OECD and European Union experts, agreed the following working definitions for endocrine disrupters:

1. a potential endocrine disrupter is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations;

2. an endocrine disrupter is an exogenous substance or mixture of substances that alters functions(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.

Endocrine disrupters are believed to interfere with the endocrine system in at least three possible ways:

- by mimicking the action of a naturally-produced hormone such as oestrogen or testosterone and thereby setting off similar chemical reactions in the body;
- by blocking the receptors in cells receiving the hormones (hormone receptors), thereby preventing the action of normal hormones;
- by affecting the synthesis, transport, metabolism and excretion of hormones, thus altering the concentration of normal hormones.

There are associations between exposure to those endocrine disrupting chemicals so far evaluated and human health disturbances such as testicular, breast and prostate cancers, thyroid dysfunction as well as intelligence and neurological problems, although a causative role has not been verified.

In relation to hazard identification, although OECD 407 for the 28-day study is currently being updated with more emphasis to be placed on detection of endocrine effects, there are currently no test strategies/methods available which specifically detect all effects which have been linked to the endocrine disruption mechanism. However, the OECD has set up a Working Group (Task force on Endocrine Disrupters Testing - EDTA) with the specific objective of developing a harmonised approach to the screening and testing of chemicals for this endpoint. An overview of the extent and nature of current OECD activities on endocrine disrupters can be found at http://www.oecd.org/ehs/ENDOCRIN.HTM.

Overload phenomena and pulmonary fibrosis

Substances which can be inhaled, sparingly soluble in water and fat, and of low systemic toxicity may cause adverse effects in the lung (irreversible impairment of lung clearance, lung fibrosis and lung tumour formation) which can be explained by “overload phenomena”.

The available data on insoluble dusts indicate that, in the workplace, overload-related effects can be avoided by maintaining the atmospheric concentration of the substance below the specific gravity (relative density) value of the substance expressed as $mg \cdot m^{-3}$ (i.e. the atmospheric concentration should be $<1.6\ mg \cdot m^{-3}$ for a substance with a specific gravity of 1.6).

The principle outlined in the paragraph above does not, however, apply to substances which are cytotoxic at concentrations below those leading to overload: Such substances may induce fibrosis at lower concentrations. Therefore, it is recommended that inhalable, sparingly soluble substances with low systemic toxicity are examined immediately after the initial repeated dose toxicity testing, using an appropriate test for cytotoxicity (e.g. using primary macrophage cultures or epithelial cell lines *in vitro*; or analysis of broncho-alveolar lavage fluid (see Henderson, 1989)). Positive (e.g. silica) and negative (e.g. TiO_2) control substances should be included in the test. If the cytotoxicity test is negative, no further testing in relation to pulmonary fibrosis is necessary.

If the substance is considered to be cytotoxic, a repeated dose inhalation study of sufficient duration to detect fibrotic changes may be necessary to establish the NOAEL. If a 28-day study has been conducted using the inhalation route of exposure, early indications of fibrotic change may have been detected, and a NOAEL identified. When inhalation testing for a longer period is required to establish the NOAEL for a new substance, its timing will be influenced by the potential for human exposure as well as the amount of information available on the dose-response relationship. If human exposure is not well controlled (e.g. the substance is used as a consumer product) and/or there is insufficient information on the inhalation concentration-response from toxicity test data already available, further testing may be required without further delay (e.g. immediately post-base-set).

The need for such repeated dose inhalation testing of an existing substance would have to be established on a case-by-case basis taking into account all the relevant information available on the substance and the criteria discussed above.

3.10 MUTAGENICITY

3.10.1 Introduction

In the risk assessment of substances it is necessary to address the potential effect of "mutagenicity". It can be expected that some of the available data will have been derived from tests conducted to investigate harmful effects on genetic material ("genotoxicity"). Hence, both the terms "mutagenicity" and "genotoxicity" are used in this document.

3.10.1.1 Definitions of mutagenicity and genotoxicity

The chemical and structural complexity of the chromosomal DNA and associated proteins of mammalian cells, and the multiplicity of ways in which changes to the genetic material can be effected make it difficult to give precise, discrete definitions.

Mutagenicity refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. These changes may involve a single gene or gene segment, a block of genes or whole chromosomes. Effects on whole chromosomes may be structural and/or numerical.

Genotoxicity is a broader term and refers to potentially harmful effects on genetic material which are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include tests which provide an indication of induced damage to DNA (but not direct evidence of mutation) via effects such as unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE), DNA strandbreaks, DNA adduct formation or mitotic recombination, as well as tests for mutagenicity.

3.10.1.2 Objectives of investigating the potential for substance-induced mutagenicity and genotoxicity

Deleterious changes to the genetic material of cells may occur spontaneously or be induced as a result of exposure to ionising or ultraviolet radiation, or genotoxic substances. In principle, human exposure to substances that are mutagens can be expected to result in increased frequencies of mutations above background.

Mutations in somatic cells may be lethal or may be transferred to daughter cells with deleterious consequences for the affected organism (e.g. when they occur in proto-oncogenes, tumour suppressor genes and/or repair genes) ranging from trivial to detrimental or lethal.

There is considerable evidence of a positive correlation between the mutagenicity of substances *in vivo* and their carcinogenicity in long-term studies with animals. Genotoxic carcinogens are chemicals for which the most plausible mechanism of carcinogenic action is a consequence of genotoxic events. Some known carcinogens do, however, appear to be active through a non-genotoxic mechanism (i.e. initial steps of carcinogenesis do not involve direct interaction of the substance itself, or its metabolites, with DNA). Their carcinogenic potential will not be indicated by genotoxicity tests.

Heritable damage to the offspring, and possibly to subsequent generations, of parents exposed to substances that are mutagens may follow if mutations are induced in parental germ cells. To date, all known germ cell mutagens are also mutagenic in somatic cells *in vivo*. Substances that

are mutagenic in somatic cells may produce heritable effects if they, or their active metabolites, reach the genetic material of germ cells.

The aims of testing for genotoxicity are, therefore, to assess the potential of substances to be genotoxic carcinogens or to cause heritable damage in humans. Genotoxicity data are used in risk characterisation and classification of substances.

3.10.2 Data to be used in the effects assessment

3.10.2.1 Minimum data requirements

Minimum data requirements are defined for new and existing chemicals and biocides. These should be followed as far as reasonably practicable.

New and existing chemicals

The minimum data requirement for industrial chemicals is that, as specified in Annex VII A to Directive 67/548, genotoxicity data should be available from at least two tests: A bacterial gene mutation test and a test capable of detecting chromosomal aberrations which in the absence of contra-indications should be conducted *in vitro*.

There are some exceptions to the above requirements. For new substances supplied at relatively low levels (e.g. >100 kg pa but <1 tpa) the basic requirement is for a gene mutation test in bacteria only. A positive result should normally be followed at least by a second test according to the strategy outlined below (Section 3.10.5.6). However, the timing of this, and any other further testing, will be influenced by the potential for human exposure: the greater the potential extent of exposure, the greater the need for further testing. Consequently, especially if there is also a clear structural alert for mutagenicity, additional *in vitro* tests may be requested immediately.

Biocides

The Biocidal Products Directive (98/8 and associated Technical Notes for Guidance (TNsG)) defines a “common core data-set” that is required for the endpoint of mutagenicity. This data set comprises of three tests: a bacterial gene mutation test, an *in vitro* mammalian cell cytogenics study and an *in vitro* mammalian cell gene mutation assay.

3.10.2.2 Data that may already be available

In the case of existing substances, a wealth of genotoxicity data may be available from studies conducted *in vitro* and/or *in vivo* but many of the tests may have been conducted using methods different from those in Annex V to Directive 67/548. There may be non-standard studies available in which “site of contact” tissues (i.e. skin, epithelium of the respiratory or gastro-intestinal tract) were examined. In addition data from plant, fungal or Drosophila systems may be available. Occasionally, studies of genotoxic effects in humans may also be available. The validity and usefulness of each of the data sets to the overall assessment of genotoxicity should be individually assessed, taking account of protocol design and current expert views on the value of the test systems.

Useful data for the effects assessment may also be obtained from studies on toxicokinetics (including metabolism); *in vitro* studies on macromolecule binding; from a knowledge of the reactivity and electrophilicity of the substance and from the presence or absence of "structural alerts" for genotoxicity.

3.10.3 Evaluation of the available data

Evaluation of genotoxicity test data should be made with care. Regarding "positive" findings, responses may be generated only at highly toxic/cytotoxic concentrations, and the presence or absence of a dose-response relationship should be considered.

Particular points to take into account when evaluating "negative" test results include:

- the doses or concentrations of test substance used (were they high enough?);
- the volatility of the test substance (were concentrations maintained in tests conducted *in vitro*?);
- for studies *in vitro*, the possibility of metabolism not active in the system including those in extrahepatic organs;
- is the substance reaching the target organ?
- the reactivity of the substance (e.g. rate of hydrolysis, electrophilicity, presence or absence of structural alerts).

Contradictory results between different test systems should be evaluated with respect to their individual significance. Examples of points to be considered are as follows:

- conflicting results obtained in non-mammalian systems and in mammalian cell tests may be addressed by considering possible differences in metabolism or in the organisation of genetic material. Additional data may be needed to resolve contradictions;
- if the results of indicator tests (e.g. DNA binding; SCE) are not supported by results obtained in tests for mutagenicity, the results of mutagenicity tests are generally of higher significance;
- if contradictory findings are obtained *in vitro* and *in vivo*, in general, the results of *in vivo* tests indicate a higher degree of reliability. However, for evaluation of "negative" results in *vivo*, it should be considered whether there is adequate evidence of target tissue exposure.

Conflicting results may be also available from the same test, performed by different laboratories or on different occasions. In this case, expert judgement should be used to reach an overall evaluation of the data. In particular, the quality of each of the studies and of the data provided should be evaluated, with consideration especially of the study design, reproducibility of data, dose-effect relationships, and biological plausibility of the findings. The purity of the test substance may also be a factor to take into account. Furthermore, studies compliant with GLP may be regarded as being of a higher quality.

Human data and their relevance have to be assessed carefully on a case-by-case basis due to limitations of the techniques available. In particular, attention should be paid to the adequacy of the exposure information, confounding factors, and to sources of bias in the study design. The statistical power of the test may also be considered.

3.10.4 Assessment of the dose-response relationship

The default assumption for genotoxic chemicals, in the absence of mechanistic evidence to the contrary, is that they have a linear dose-response relationship. However, both direct and indirect mechanisms of genotoxicity can be non-linear or thresholded, and sometimes this default assumption may be inappropriate. Considerations of the dose-response relationship and of possible mechanisms of action are important components of a risk assessment.

Examples of mechanisms of genotoxicity that that may be demonstrated to lead to non-linear or thresholded dose-response relationships include extremes of pH, ionic strength and osmolarity, inhibition of DNA synthesis, alterations in DNA repair, overloading of defence mechanisms (anti-oxidants or metal homeostasis), interaction with microtubule assembly leading to aneuploidy, topoisomerase inhibition, high cytotoxicity, metabolic overload and physiological perturbations (e.g. induction of erythropoeisis).

In general, several doses are tested in genotoxicity assays. Determination of experimental dose-effect relationships may be used to assess the genotoxic potential of a substance, as indicated below:

- a dose-related increase in genotoxicity is one of the relevant criteria for identification of positive findings. In practice, this will be most helpful for *in vitro* tests, but care is needed to check for cytotoxicity or cell cycle delay which may cause deviations from a dose-response related effect in some experimental systems;
- routine genotoxicity tests are not designed in order to derive no effect levels. However, the magnitude of the lowest dose with an observed effect (i.e. the Lowest Observed Effect Dose or LOED) may, on certain occasions, be a helpful tool in risk assessment. Specifically, it can give an indication of the mutagenic potency of the substance in the test at issue. Modified studies, with additional dose points and improved statistical power may be useful in this regard;
- unusual shapes of dose-response curves may contribute to the identification of specific mechanisms of genotoxicity. For example, extremely steep increases suggest an indirect mode-of-action or metabolic switching and this could be confirmed by further investigation.

3.10.5 Testing strategy

3.10.5.1 Objective of the testing strategy

This testing strategy describes a flexible, stepwise approach for hazard identification with regard to the mutagenic potential of substances, so that sufficient data may be obtained for adequate risk characterisation including classification and labelling. It serves to help minimise the use of animals and costs as far as is consistent with scientific rigour.

A summary of the testing strategy is given in **Table 6.**

A “base-level” of information is defined in the strategy that is considered sufficient to provide adequate reassurance about the potential mutagenicity of most substances. Additionally, it provides guidance on how to process complex data sets and the need for further testing.

The essential themes of the strategy apply equally to industrial chemicals (i.e. new and existing substances) and biocides. However, differences in the regulatory approaches to assessing the health risks posed by these different categories of chemical have to be recognised.

For some substances, relevant data from other sources/tests may also be available (e.g. physico-chemical, toxicokinetic, and toxicodynamic parameters and other toxicity data; data on well-investigated, structurally similar, chemicals). These data may indicate that either more or less studies are needed than defined at the base-level.

New substances

The testing strategy is aimed at gradually extending the genotoxicity database in a scientific manner until sufficient data according to the requirements of Directive 67/548 have been obtained. Directive 67/548 requires that the mutagenic potential of new substances is studied to an extent which permits adequate risk characterisation at each defined supply level, decision taking on the need for further testing and classification and labelling. This strategy can be applied to the tonnage-related testing requirements for New substances, with the base–level recommendation being applicable to substances at >1 tpa.

The initial screening tests may not give adequate information and further testing may be considered necessary in the light of all available relevant information on the substance, including its use pattern. Further testing will also be required in advance of the usual tonnage triggers for substances which give rise to positive results in any of the *in vitro* tests, and also, particularly, for substances for which there is significant potential for human exposure.

Existing substances

The testing strategy can be applied to existing substances by using the results of previous genotoxicity tests and human exposure pattern (route, level, duration, involving consumers) as primary influences, rather than supply tonnage. If negative results are available from an adequate evaluation of genotoxicity from the existing data, there may be no requirement to conduct additional genotoxicity tests.

Biocides

In contrast to the supply level- or exposure-pattern-linked approaches for new and existing chemicals, the core data requirement for biocides consists of a battery of three *in vitro* tests (see Section 3.10.2.1). The biocides testing strategy is independent of the annual production. However, the strategy is comparable to that for industrial chemicals when considering the need for further testing.

3.10.5.2 Preliminary considerations

For a comprehensive coverage of the potential mutagenicity of a substance, information on gene mutations, structural chromosome aberrations (clastogenicity) and numerical chromosome aberrations (aneugenicity) is required.

It is important that whatever is known of the physico-chemical properties of the test substance is taken into account before devising an appropriate testing strategy. The chemical structure of a substance can provide information for an initial assessment of mutagenic potential. The need for special testing requirements in relation to photomutagenicity may be indicated by the structure of a molecule, its light absorbing potential or its or its potential to be photoactivated. By using expert judgement, it may be possible to identify whether a substance, or a potential metabolite of a substance, shares structural characteristics with known mutagens or non-mutagens. This can be used to justify a higher or lower level of priority for the characterisation of the mutagenic

potential of a substance. Where the level of evidence for mutagenicity is particularly strong, it may be possible to make a conclusive hazard characterisation without additional testing on the basis of structure-activity relationships alone.

In vitro tests are particularly useful for gaining an understanding of the potential mutagenicity of a substance and they have a critical role in this testing strategy. Animal tests will, in general, be needed, however, for the clarification of positive findings and in case of specific metabolic pathways that cannot be simulated adequately *in vitro*.

The toxicokinetic and toxicodynamic properties of the test substance should be considered as far as possible before undertaking, or appraising, animal tests. Understanding these properties will enable appropriate test protocols to be developed, especially with respect to tissue(s) to be investigated, the route of substance administration and the highest dose tested. If little is understood about the systemic availability of a test substance at this stage, an experimental investigation may be necessary.

Certain substances may need special consideration, such as highly electrophilic substances which give positive results *in vitro*, particularly in the absence of metabolic activation. Although these substances may react with proteins and water *in vivo* and thus be rendered inactive towards many tissues, they may be able to express their mutagenic potential at the initial site of contact with the body. Consequently, the use of test methods that can be applied to the respiratory tract, upper gastrointestinal tract and skin may be appropriate. It is possible that special test methods will be applied in these circumstances, and these may not have recognised, internationally valid, test guidelines. The validity and utility of such tests and the selection of protocols should be assessed by experts from regulatory authorities and industry on a case-by-case basis.

The pH, solubility, volatility and stability of a substance in test vehicles can affect the performance of mutagenicity tests and therefore influence the design of test protocols. Intractably insoluble substances could be subjected to appropriate extraction procedures, and the extracts tested (as is done for medical devices).

A substance giving an equivocal test result should be reinvestigated immediately, normally using the same test method, perhaps varying the conditions. Wherever possible, clear results should be obtained for one step in the strategic procedure before going on to the next.

Tests need not be performed if it is not technically possible to do so, or if they are not considered necessary in the light of current scientific knowledge. It is preferred that the test methods of Annex V to Directive 67/548 are used where possible, but other methods may be used when necessary provided that they are scientifically justified. It is essential that all tests be conducted according to rigorous protocols in order to maximise the potential for detecting a mutagenic response, to ensure that negative results can be accepted with confidence and that results are comparable when tests are conducted in different laboratories. At the time of writing this TGD, regulatory guidelines are still to be established for some of the *in vivo* tests included in the testing strategy described below. If one of these tests is to be conducted, consultation on the protocol with an appropriate regulatory authority is advisable.

3.10.5.3 Base-level testing

Two tests, normally to be conducted *in vitro*, are required to provide a base level of information on the mutagenic potential of any substance (i.e. new and existing chemicals and biocides). The 2 tests normally required are a gene mutation test in bacteria and an *in vitro* mammalian cell test

capable of detecting chromosome aberrations. As explained in Section 3.10.2.1, the base-level for biocides additionally includes an *in vitro* mammalian cell gene mutation assay.

For new and existing substances with significant toxicity to bacteria, an *in vitro* mammalian cell gene mutation test can be used as an alternative first test.

There are various options for selection of the mammalian cell test at the base level. These are as follows:

1. an *in vitro* chromosome aberration test, i.e. a cytogenetic assay for structural chromosome aberrations using metaphase analysis. Preliminary information can be obtained on potential aneugenicity by recording the incidence of hyperdiploidy, polyploidy and/or modification of mitotic index (e.g. mitotic arrest);
2. a mouse lymphoma assay (L5178Y cells, TK locus), if used with adequate colony sizing so that not only gene mutations but also structural chromosome aberrations are detected. This test is not sufficiently sensitive for the detection of aneugens;
3. an *in vitro* micronucleus test, which is capable of detecting structural chromosome aberrations as well as aneuploidy.

Normally no *in vivo* tests are required to fulfil the base-level testing requirements. However, there may be rare occasions when it is appropriate to conduct base-level testing *in vivo*, for example when it is not possible to perform satisfactory tests *in vitro*.

Substances which, by virtue of, for example, their physico-chemical characteristics, chemical reactivity or toxicity cannot be tested in one or more of the *in vitro* tests are considered on a case-by-case basis.

3.10.5.4 Requirement for testing beyond the base level. Introductory comments

There are several reasons why mutagenicity testing beyond the base level may be required. For new and existing industrial chemicals and biocides, concerns raised by positive results from *in vitro* tests, or from potentially high or poorly controlled levels of human exposure, will justify further testing. The chemistry of the substance, data on analogous substances, toxicokinetic and toxicodynamic data, and other toxicity data will also influence the timing and pattern of further testing.

For new and existing industrial chemicals, further testing can be triggered by attainment of tonnage thresholds, as defined in Directive 67/548, or concerns in relation to modelled or actual exposure data, respectively.

Further testing is used to investigate, either *in vitro* or in animals, the potential for effects of the test substance on somatic cells. Positive results in somatic cells *in vivo* constitute the trigger for consideration of investigation of potential expression of genotoxicity in germ cells.

3.10.5.5 Substances which are negative in the base-level tests

In general, substances which are negative in the base-level tests are considered to be non-genotoxic. However, the combination of *in vitro* tests at the base-level will not detect a small proportion of substances with the potential for *in vivo* mutagenicity (e.g. some aneugens will not be detected by a combination of the bacterial test and the mouse lymphoma test). The timing and extent of further testing of substances negative in the base-level tests will depend largely on the intended use of the substance and the extent of human exposure likely to occur.

A flexible approach should be applied when considering the options for further testing. The preferred test(s) will depend on which tests were conducted at base level and whether these allow a satisfactory assessment for gene mutations, effects on chromosome structure and effects on chromosome number. Also, adequate provision should be made for metabolic activation. In some circumstances, knowledge about the metabolic profile of a substance may indicate that use of an alternative to rat liver S-9 mix is appropriate. In addition to the 3 assays with mammalian cells available for base-level testing (see Section 3.10.5.3), the rat primary hepatocyte UDS test or, in exceptional circumstances, an *in vivo* test may be considered

For new industrial chemicals, further testing will not usually be initiated before supply levels reach 100 tpa (500 t cumulative), but may be required earlier when there is significant concern about human exposure during normal use because of physico-chemical properties and/or use pattern. It is unlikely that quantitative exposure data will be available for new substances. The potential for human exposure thus has to be assessed in the light of the physico-chemical characteristics of the substance, and use pattern, which will include consumer exposure.

3.10.5.6 Substances for which an *in vitro* test is positive

Substances for which only a bacterial gene mutation test has been conducted and for which the result is positive should be studied further using any one of the *in vitro* mammalian cell tests recommended for base-level testing.

When the mammalian cell test is negative, it will be necessary to decide whether any further testing is needed at this stage on a case-by-case basis. This further testing could be either *in vitro* or *in vivo*. Suspicion that a positive response observed in the bacterial test was due to a specific bacterial metabolism of the test substance could be explored further by investigation *in vitro*. Alternatively, an *in vivo* test may be required (see below). Substances for which there is minimal concern for human exposure may not need to be tested further.

Following a positive result in an *in vitro* mammalian cell mutagenicity test, adequately conducted somatic cell *in vivo* testing is required to ascertain if this potential can be expressed *in vivo*. It is recommended that the first test *in vivo* should be initiated as soon as possible. In exceptional cases, where it can be sufficiently deduced that a positive *in vitro* finding is not relevant for *in vivo* situations, *in vivo* testing will not be necessary.

Before undertaking any *in vivo* testing, a review of the *in vitro* test results and all available information on the toxicokinetic and toxicodynamic profile of the test substance is needed. A particular *in vivo* test should be conducted only when it can be reasonably expected from all the properties of the test substance and the proposed test protocol that the specific target tissue will be adequately exposed to the test substance and/or its metabolites. If necessary, an investigation of toxicokinetics should be conducted before progressing to *in vivo* testing. In case of biocides, if one of the 3 *in-vitro* tests is positive, an *in-vivo* test from (1) below has to be performed.

For test substances with adequate systemic availability (i.e. evidence for adequate availability to the target cells) the *in vivo* tests recommended are:

1. a rodent bone marrow or mouse peripheral blood micronucleus test or a rodent bone marrow clastogenicity study. Potential species-specific effects may influence the choice of species and test method used.
2. a rat liver Unscheduled DNA synthesis test.

Either test may be conducted, but this has to be decided using expert judgement on a case-by-case basis. For example, if the test substance has shown evidence of clastogenicity *in vitro*, then it would probably be most appropriate to investigate chromosome damage. If a positive result was obtained in the *in vitro* micronucleus test, the *in vivo* test should be a rodent micronucleus test to address clastogenic and aneugenic potential. The rat liver UDS test may be the most appropriate for substances that appear preferentially to induce gene mutations.

For insoluble substances, the possibility of release of active molecules in the gastrointestinal tract may indicate that a test involving the oral route of administration is particularly appropriate.

For substances that are short-lived, reactive, *in vitro* mutagens, or for which no indications of systemic availability have been presented, an alternative strategy involving studies with tissues at initial sites of contact with the body should be considered. Additionally, when it may be more appropriate to evaluate genotoxicity in systemic tissues other than the bone marrow or liver, alternative tests can be selected. Expert judgement should be used on a case-by-case basis to decide which tests are the most appropriate. The main options are the *in vivo* modification of the Comet assay (single cell gel electrophoresis measuring DNA strand breaks), gene mutation tests with transgenic animals and *in vivo* DNA adduct studies. Expert judgement may indicate which of these tests are the most appropriate to be used for certain substances.

At the time of writing this TGD, regulatory guidelines are still to be established for some of the *in vivo* tests mentioned above.

If the first *in vivo* test is negative, the need for a further *in vivo* test should be considered. In this regard, attention should be paid to the quality and relevance of all the available data, the adequacy of target tissue exposure and the potential for human exposure.

3.10.5.7 Substances that give positive results in an *in vivo* test for genotoxic effects in somatic cells

The potential for substances that give positive results in tests for genotoxic effects in somatic cells *in vivo* to affect germ cells should always be considered. The first step is to make an appraisal of all the available toxicokinetic and toxicodynamic properties of the test substance. Expert judgement is needed at this stage to consider whether there is sufficient information to conclude that the substance poses a mutagenic hazard to germ cells. If this is the case, it can be concluded that the substance may cause heritable genetic damage and no further testing is justified.

If the appraisal of mutagenic potential in germ cells is inconclusive, additional investigation will be necessary. In the event that additional information about the toxicokinetics of the substance would resolve the problem, a toxicokinetic study is preferred. The additional investigation should not be delayed, unless the potential level and extent of human exposure is of minimal concern.

If germ cell testing is to be undertaken, expert judgement should be used to select the most appropriate test strategy. Internationally recognised guidelines are available for investigating clastogenicity in rodent spermatogonial cells and for the dominant lethal test. Dominant lethal mutations are believed to be primarily due to structural or numerical chromosome aberrations. Alternatively, other methods can be used if deemed appropriate by expert judgement. These may include the Comet assay, gene mutation tests with transgenic animals, or DNA adduct analysis.

Substances that have given positive results only in cytogenetic tests *in vitro* and in somatic cells *in vivo* can be studied further, to differentiate between a clastogenic or aneugenic mode of action if this has not been established adequately already.

In principle, it is the potential for effects that can be transmitted to the progeny that should be investigated, but tests specifically to investigate transmitted effects (the heritable translocation test and the specific locus test) use very large numbers of animals. They are rarely used and will not normally be required for industrial substances or biocides.

Table 6 Summary of the mutagenicity testing strategy

	Bact	**MCGT** (CAbvit or MLA, or MNTvit)	***in vivo* test**	**General follow-up procedure** [1] [for detailed guidance, incl. timing of the tests, see text]
1	neg			[3.10.5.3] completion of base-level testing by an MCGT capable of detecting chromosome aberrations (CAbvit or MLA, or MNTvit)
2	neg	neg		[3.10.5.5] base-level testing completed, consider further testing; select further systems in such a way that all tests together enable thorough assessment for gene mutations and effects on chromosome structure and number; possible test systems include, among others, CAbvit, MLA, MNTvit, UDSvit, or, in exceptional cases, an *in vivo* test. if MLA is available but was conducted without adequate colony-sizing, an *in vitro* mammalian cell test capable of detecting chromosome aberrations is needed.
3	pos			[3.10.5.6] completion of base-level testing by an MCGT capable of detecting chromosome aberrations (CAbvit, MLA, or MNTvit)
4	pos	neg		[3.10.5.6] base-level testing completed, consider further testing; select further systems on a case-by-case basis; possible test systems include, among others, CAbvit, MLA, MNTvit, UDSvit, *in vivo* test.
5	neg or pos	pos		[3.10.5.6] select adequate somatic cell *in vivo* test, primarily on the basis of systemic availability of the substance: 1. adequate systemic availability: - MNTviv (pref. for in-vitro clastogens and/or aneugens) - CAbviv (pref. for *in vitro*-clastogens) - UDSviv (pref. for inducers of gene mutations) 2. lack of adequate systemic availability: - studies with tissues at initial sites of contact, e.g. *in vivo* comet assay or gene mutation test with transgenic mice If systemic availability cannot be ascertained with acceptable reliability, it should be investigated before progressing to *in vivo* tests.
6	neg or pos	pos	neg	[3.10.5.6] further *in vivo* test may be necessary pending on the quality and relevance of available data
7	neg or pos	neg or pos	pos	[3.10.5.7] consider potential germ cell mutagenesis on the basis of all available data on test results and toxicokinetic and toxicodynamic properties. if no clear conclusion can be drawn, additional investigations, on toxicokinetics (preferentially) or germ cell tests, are needed. When the mode of action for inducers of micronuclei is not known, further investigations may be performed in order to differentiate between clastogenic or aneugenic mode of action.

[1] For biocides a "common core data-set" is required which comprises of three tests: a bacterial gene mutation test, an *in vitro* mammalian cell cytogenics study and an *in vitro* mammalian cell gene mutation assay. If any of these are positive an in-vivo test must be performed

Abbreviations used in the table: **Bact**, bacterial gene mutation test; **CAbvit**, *in vitro* chromosomal aberration test; **CAbviv**, *in vivo* chromosome aberration test (bone marrow); **MCGT**, *in vitro* mammalian cell genotoxicity test; **MLA**, mouse lymphoma assay; **MNTvit**, *in vitro* micronucleus test; **MNTviv**, *in vivo* micronucleus test (erythrocytes); **UDSvit**, *in vitro* UDS test with primary rodent hepatocytes (**UDS** =unscheduled DNA synthesis)

3.11 CARCINOGENICITY

3.11.1 Introduction

3.11.1.1 Definition of carcinogenicity

Substances are defined as carcinogenic if they induce tumours (benign or malignant) or increase its incidence, malignancy or shorten the time of tumour occurrence when they are inhaled, ingested dermally applied, or injected. This effect may be route-specific. Carcinogens may be identified either from epidemiological studies, from animal experiments and/or other relevant data/studies. Classification criteria are given in Directive 93/21.

The process of carcinogenesis is now recognised as resulting from the transition of normal cells into cancer cells via a sequence of stages. Altered growth and death rates, and (de)differentiation of the involved cells is recognised to play an important role in this process. Cancer is the result of genetic alterations which can be induced directly or indirectly. Substances which are carcinogens have conventionally been divided into two categories according to the presumed mode of action: genotoxic and non-genotoxic.

Genotoxic carcinogens are chemicals for which the most plausible mode of carcinogenic action includes the consequences of genotoxic events (adopted from Section 3.10.1.2; for definitions of genotoxicity and mutagenicity see Section 3.10.1.1).

Non-genotoxic carcinogens are believed to exert their carcinogenic effects through mechanisms other than genotoxicity. There are many different modes of action thought to be involved in non-genotoxic carcinogenicity. For example, some involve action through specific receptors, some appear to have a non-receptor mediated mode of action. Also, prolonged regenerative proliferation is considered a mode of action by which tumours can be induced. The induction of urinary bladder tumours, for example, may in certain cases to be due to formation of bladder stones followed by irritation/inflammation/erosion and regenerative hyperplasia of the urothelium.

It is generally assumed that the modes of actions of non-genotoxic carcinogens can be associated with threshold doses, and it may be possible to define no-effect levels for the underlying toxic effects of concern.

Genotoxic carcinogens may possess both genotoxic and non-genotoxic properties that contribute to the carcinogenic effect. Such substances are sometimes called complete carcinogens since they may act as “initiators” as well as “promoters” (enhancers) in two-step/multistep experimental models of carcinogenicity.

It is generally considered that an effect-threshold cannot be identified for genotoxic carcinogens, i.e. it is not possible to define a “no-effect level” for carcinogenicity. However, it is also recognized that for certain genotoxic carcinogens a threshold may exist for the underlying genotoxic effect.

3.11.1.2 Objectives of investigating the potential of substance-induced carcinogenicity

The objective of investigating the carcinogenicity of substances is to identify potential human carcinogens. Carcinogenicity testing is intended to differentiate carcinogens from non-carcinogens. Associated targeted and mechanistic studies derive information on their mode of action. Carcinogens acting with no threshold or lacking acceptable proof of thresholded mode of action are to be discriminated from those carcinogens for which the existence of a thresholded mode of action was plausibly and convincingly demonstrated to be of relevance for humans. All relevant data are evaluated in a weight of evidence approach and provide the basis for regulatory decisions.

Experimental data on carcinogenesis and, if available, human epidemiological data and related effective dose/exposure levels are evaluated considering the expected/known human exposure to the substance to determine the potential health risk. Carcinogenicity data are used for classifying substances for carcinogenic hazard and in risk characterisation.

3.11.2 Data to be used in the effects assessment

3.11.2.1 Minimum data requirements

New substances

For new substances Directive 67/548 requires that the performance of a carcinogenicity study should normally be considered when annual production reaches 1,000 t/a or 5,000 t cumulative (level 2 supply tonnage), and should be conducted at that level unless there are good reasons to the contrary.

Existing substances

Studies on carcinogenicity are not part of the minimum data requirements for existing substances according to Article 9(2) of Regulation 793/93. However all available information relevant to this endpoint has to be evaluated.

Biocides

For biocides Directive 98/8 requires the performance of carcinogenicity studies in the core data set of Annex IIA only for active substances. One rodent and one other mammalian species should be tested. The carcinogenicity testing of an active substance may not be required where a full justification demonstrates that these tests are not necessary.

3.11.2.2 Data which may already be available

Human data

Human data may provide direct information on the potential carcinogenicity of the substance. When human data of sufficient quality are available, they are preferable to animal data as no interspecies extrapolation is necessary and exposure scenarios are likely to be more realistic. Epidemiological data will not normally be available for new substances but may well be

available for existing substances which have been in use for many years. Relevant epidemiological study designs may include cohort, case-control and correlation studies. Most analytical epidemiological studies on cancer concern occupational populations, and less frequently the general population. Cluster investigations and case reports, while not constituting epidemiological studies in a strict sense, may provide useful supporting information in specific cases.

Besides the identification of carcinogenic substances, epidemiological studies may also provide information on actual exposures, associated dose-responses, and risk characterisation, i.e. inform on the tissue-specificity and potency of the compound under the exposure conditions observed.

Techniques in biomonitoring and molecular epidemiology are developing rapidly. They may provide information on markers of both exposure and effects at current exposure levels. These data may become useful, particularly when combined with classical epidemiological observations and/or animal data.

Animal and *in vitro* studies and other substance-related data

A wide variety of study categories may be available, which may provide direct or indirect information useful in assessing the carcinogenic potential of a substance. They include:

- carcinogenicity studies (conventional long-term or life-time studies in experimental animals);
- short and medium term carcinogenicity tests (e.g., rat liver foci model, $XPA^{-/-,}$ p53+/-, Tg.AC mouse models, neonatal mouse model);
- genotoxicity studies;
- cell transformation and intercellular gap junction communication assays;
- repeated dose toxicity tests;
- studies on the induction of sustained cell proliferation;
- studies on immunosuppressive activity;
- structural alerts relationships;
- studies on toxicokinetic;
- studies on mechanisms of action.

3.11.3 Evaluation of the available data

The evaluation of the carcinogenic potential of substances often requires the consideration of a large set of data. An important part of the assessment of the available data regards the evaluation of the mode of action underlying the carcinogenic activity, as this information also allows an evaluation of possible human relevance, existence of thresholds and comparability with structurally related carcinogens. Genotoxicity data play an important role for predicting carcinogenicity as well as in the assessment of the mode of action of the carcinogen. Expert judgement and a weight of evidence approach are required for the evaluation.

Human epidemiological data

Human data may potentially be used for hazard identification, exposure assessment, dose response analysis, and risk assessment. They may reveal the carcinogenic potential of a substance for which experiments in animals either do not exist or have failed to indicate the carcinogenic potential of the substance.

The degree of reliability for each study on the carcinogenic potential of a substance should be evaluated using generally accepted causality criteria, such as that of Bradford Hill (1965). Particular attention should be given to the exposure data in a study and to the choice of the control population. Often a significant level of uncertainty exists about identifying a substance unequivocally as being carcinogenic, because of inadequate reporting of exposure data: chance, bias and confounding factors can frequently not be ruled out. A clear identification of the substance, the presence or absence of concurrent exposures to other substances and the methods used for assessing the relevant dose levels, should explicitly be documented. A series of studies revealing similar excesses of the same tumour type, even if not statistically significant, may suggest a positive association, and an appropriate joint evaluation (meta-analysis) may be used in order to increase the sensitivity, provided the studies are sufficiently similar for such an evaluation. When the results of different studies are inconsistent, possible explanations should be sought and the various studies judged on the basis of the methods employed.

Epidemiological data are also valuable for informing on the relative sensitivity of humans as compared to animals.

The relatively low sensitivity of epidemiological studies implies that it is very difficult to demonstrate the non-carcinogenicity of a substance, unless exposure conditions are exceptional and well documented. However, the resolution power of epidemiological methods may be improved substantially when combined with data on established early stages or indicators of cancer risks. In any case, well performed epidemiological studies may be very useful in demonstrating an upper bound on the human cancer risk.

Animal data: Carcinogenicity studies

For the acceptance of toxicological studies the quality criteria described in Section 3.2 should apply. However if the report of a test does not include all the information indicated in the EU Annex V method, expert judgement should be used in order to decide if the experimental procedures are or are not acceptable and if essential information is lacking.

Evaluation criteria include also exposure and observation times, the time of tumour onset, correction for differences in survival and multiplicity of tumour sites, the occurrence of the same tumour in concurrent and/or historical controls (OECD, 2001).

The route and method of administration must be documented in order to allow a proper evaluation of the potential hazards associated with the intended use of the compound. Examples exist that indicate that the carcinogenic potential of a substance may be route-specific.

When data are available from several different studies, all of which are assessed as being of an adequate quality, the results should be analysed for their consistency. It is seldom problematic to reach a conclusion about the carcinogenic potential of a substance, where there are consistent results from a number of studies, particularly if the studies were conducted in more than one species, or where there is a treatment-related incidence of malignant tumours in a single study.

Positive carcinogenic findings in animals require careful evaluation to determine their relevance to humans. Of key importance is the mechanism of tumour induction. The IPCS has developed a conceptual framework to provide a structured and transparent approach for the assessment of the overall weight of evidence for a postulated mode of induction for each tumour type observed (Sonich-Mullin et al., 2001). This was based partly on the Bradford Hill criteria for causality as modified by Faustman (1997) for developmental toxicity. The framework promotes confidence in the conclusions reached by the use of a defined procedure which mandates clear and

consistent documentation of the reasoning used and inconsistencies and uncertainties in the available data.

In general, tumours induced by a genotoxic mechanism are considered to be relevant to humans even when observed in tissues with no direct human equivalent. Tumours induced by a non-genotoxic mechanism are in principle also considered relevant to humans. However, there is a scientific consensus that some tumours seen in rodents arising by specific non-genotoxic mechanisms are not relevant for humans. This consensus exists for some mechanisms of tumour-formation, for instance specific types of rodent kidney, thyroid, urinary bladder, forestomach and glandular stomach tumours. For some of these mechanisms, the International Agency for Research on Cancer (IARC) has provided detailed characterisation and has identified the key biochemical and histopathological events which should be observed in order to conclude that the tumours arose via one of these mechanisms and can therefore be dismissed as not relevant for humans (IARC, 1999, IARC, In press).

If a single adequate study demonstrates no carcinogenic effects expert judgement is needed to decide on whether a second assay is needed to further support the non-carcinogenicity of the substance, based on all available data in addition to the carcinogenicity study.

The experimental results may not unequivocally demonstrate the carcinogenic potential of the substance under consideration. Even when EU Annex V methods, or their equivalent, have been used to test a substance for carcinogenicity in animals, uncertainty may remain about reaching firm conclusions as, for instance, there may be an increase only in the incidence of benign tumours or of tumours which have a spontaneously high incidence. Although less convincing than an increase in malignant and rare tumours a detailed and substantiated rationale should be given before such positive findings can be dismissed as not relevant.

The above remarks especially hold when evaluating non-conventional carcinogenicity studies, i.e. the various available short- and medium-term carcinogenicity assays with neonatal or transgenic animals (see Section 3.11.2.2). These assays have not yet been fully validated and accepted as alternatives to the conventional lifetime carcinogenicity studies, but may be useful for screening purposes. An evaluation of such studies has recently been published (ILSI/HESI, 2001).

Animal data: Evidence from other experimental data

Experimental data not directly detecting carcinogenicity as an endpoint may be informative about the potential of a substance to induce cancer.

Genotoxicity studies may provide information on whether or not the substance is likely to be a genotoxic carcinogen. Also, positive results in cell transformation or intercellular gap junction communication tests should be taken as alerts for potential carcinogenicity.

Repeated dose toxicity studies may indicate that the substance is able to induce hyperplasia (either through such mechanisms as cytotoxicity and mitogenicity, or interference with cellular control mechanisms) and/or preneoplastic lesions giving cause for concern for potential carcinogenicity by non-genotoxic mechanisms (i.e. depending on the outcome of genotoxicity tests). Toxicity studies may also indicate a strong immunosuppressive activity of the substance, a condition favouring tumour development under conditions of chronic exposure.

The chemical structure of the substance may contain structural alerts for genotoxicity and/or carcinogenicity (based on clear evidence for carcinogenicity of structural analogues).

Toxicokinetic data may reveal the generation of metabolites with such structural alerts, and their possible species-specificity. It may also give important information as to the relevance of carcinogenicity and related data on one species to another, based upon differences in absorption, distribution, metabolism and or excretion of the substance either directly or by the application of toxicokinetic modelling.

Finally, there may be other experimental data, e.g. on mechanisms of toxicity that may be informative with respect to the potential carcinogenicity of the substance.

Summary

Clearly, the assessment of the carcinogenic potential of a substance requires the integrated evaluation of many different categories of data. The assessment should consider the whole set of information available, i.e. evidence in humans and animal species as well as the results of genotoxicity tests, structure activity analysis, the biological mechanisms and the metabolic processes identified, the toxicokinetic and physiological data for interspecies scaling of dose. Expert judgement is required on the weight of evidence analysis.

As indicated above, for many substances, it is likely that there will be no adequate long-term studies on carcinogenicity available, but that the initial assessment will be based on what can be derived from other data. Obviously, the degree of uncertainty will then be related to the amount of adequate and relevant data that are available. Guidance on how to proceed is provided in Section 3.11.5, 'Testing Strategy'.

3.11.4 Assessment of the dose-response relationship

The purpose of dose-response assessment is to provide the basis for evaluation of potential risks to humans at specified exposure levels. For this purpose carcinogens are divided into those for which a dose threshold can be substantiated, and those which, for various reasons, a threshold cannot be established. The threshold paradigm implies that some exposure can be tolerated by an organism with essentially no elicitation of a toxic response. In the case of non-threshold carcinogens it is assumed that there is no level of exposure that does not pose a small, but finite, probability of inducing cancer.

The observed dose-response curve in some cases represents a single rate-determining step, however, in many cases it may be more complex and represent a superposition of a number of dose-response curves for the various steps involved in the tumour formation. Because of the small number of doses tested experimentally, i.e. usually only 2 or 3, almost all data sets fit equally well various mathematical functions, and it is generally not possible to determine dose-response curves on the basis of mathematical modelling.

It has been commonly considered that for non-genotoxic carcinogens it is possible to identify a no-effect level for the underlying toxicity responsible for tumour formation. For some non-genotoxic substances the mechanisms underlying the tumour formation have been well characterised, and an apparent no-effect level can readily be determined. In other cases less information is available on the mechanisms, and it is more difficult to identify the underlying toxic effect. In many cases little information is available, although it is likely that a no-effect level for the underlying toxicity could have been identified. For genotoxic carcinogens, for which the most plausible mode of carcinogenic action is a consequence of genotoxic events, the prudent position is to assume non-threshold dose-response curves. However, several lines of

evidence indicate that even for some genotoxic carcinogens a threshold may exist for the underlying genotoxic effect.

For non-genotoxic agents dose-response assessment for the relevant tumour types are performed in a two step-process. The first step is an evaluation within the range of tumour observations. In the absence of adequate human data for dose-response analysis, animal data will generally be used. In addition to tumour-data analysis, attempts are also made to identify and determine the significance and dose-response for toxicological effects possibly underlying the tumour formation, i.e. their role in the induction and/or promotion of the carcinogenic process. If appropriate, the analyses of data on tumour incidence and on "precursor steps" may be combined, using precursor data to extend the dose response curve beyond that of the tumour data. For data on "precursor steps" to be useful in extending the dose-response curve for tumour induction below the level of observation, it is important that data come from *in vivo* studies where exposure is given over an extended period of time. Moreover, it is desirable to have data on the precursor event in the same target organ, sex, animal strain, and species as the tumour data. Since an agent may induce multiple tumour types, the dose-response assessment may include analysis of several types, followed by an overall synthesis which includes an analysis and comparison of the risk estimates across tumour types, the strength of the mode of action information of the tumour type considered, and the anticipated relevance of the tumour types to humans. Normally, the most sensitive tumour endpoint is used in the risk characterisation.

The second step of dose-response assessment is extrapolation to lower dose levels typifying human exposures. If supported by appropriate data this can be based on extension of a biologically based model. In practice, the mode of action of carcinogenesis for a given substance is often not well understood. Thus, neither a genotoxic nor a non-genotoxic mode of action – thresholded or not – can be derived with scientific certainty. Based on an evaluation of all available data, especially on genotoxicity, the substance may be treated as a threshold or a non-threshold carcinogen or both in the risk assessment (see Section 4.8).

3.11.5 Testing strategy

3.11.5.1 General principles

The testing strategy is an efficient and scientifically defensible approach for the investigation of the carcinogenic potential of substances. Use of the strategy enables an assessor to obtain and use information on the carcinogenic potential of substances for risk characterisation. It takes into account both existing data and the actual or intended use pattern(s) of the substance. The strategy provides the elements to determine whether or not a bioassay or any other further testing is required. Decisions must be taken on the requirement for a carcinogenicity test in light of the potential risk to health and with consideration of the actual or intended use pattern(s). Carcinogenicity bioassays take a considerable time to conduct and report and involve large numbers of animals and so it may be preferable, in terms of timeliness and animal welfare, if decisions can be taken without conducting such a test.

When assessing substances for potential carcinogenic hazard, all relevant data should be considered (as detailed in Section 3.11.3).

After evaluation of all the available data using a weight of evidence approach and consideration of the points detailed above, one of the following conclusions is possible:

- (further) carcinogenicity testing is required;
- a clear conclusion cannot be reached and further investigations may be required;
- carcinogenicity testing is not required because one or more of the following elements can be demonstrated to apply to the satisfaction of the assessor;
- risk reduction measures on the substance are already equivalent to those applied to carcinogenic substances of category 1 and 2. Further carcinogenicity testing will not lead to further regulation of the substance;
- there are sufficient indications of carcinogenic hazard, and risk characterisation is possible on the basis of an understanding of the presumed mode of action;
- on the basis of a comprehensive evaluation of all that is known about the substance, it can be assumed that the likelihood of it being carcinogenic in humans is negligible;
- the potential for human exposure must be shown to be negligible and is foreseen to be so also in the future.

The approaches outlined below may be used in the assessment of the potential carcinogenic risk of a substance to humans, and to help decide whether or not a carcinogenicity test will be required and, if so, when. A strategy is outlined in **Figure 6**.

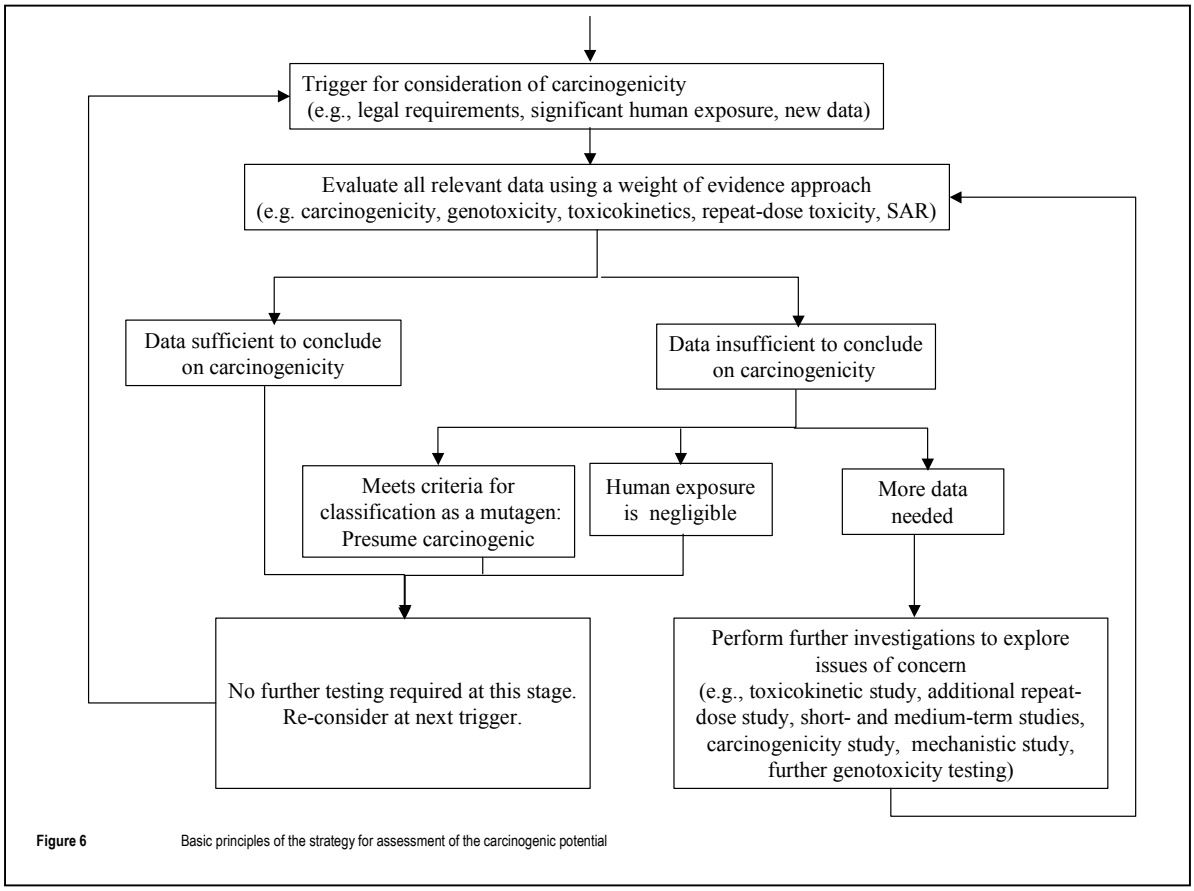

Figure 6 Basic principles of the strategy for assessment of the carcinogenic potential

3.11.5.2 Substances of no current concern for carcinogenicity

Substances without toxicological or SAR indicators of concern for carcinogenicity need not be considered further with regard to carcinogenic potential until additional information from toxicity studies or on exposure becomes available or when there is a significant increase in tonnage for industrial chemicals or for biocides, at the time of re-registration.

3.11.5.3 Substances with indications of concern for carcinogenicity

When effects or properties of concern in relation to carcinogenicity are detected, they should be investigated further. For a substance of concern, the type and timing of further testing will be decided on the basis of the type and strength of the indications for carcinogenicity, the potential mechanism of action and the type and level of human exposure.

Thus, the outcome of this procedure may be that further investigations can be deferred, e.g., if it can be demonstrated to the satisfaction of the assessor that the substance is used only in a closed system and that human exposures are negligible. A re-evaluation of the situation has to be performed when supply levels reach the next tonnage trigger or if there is notification of a change in the use pattern. In contrast, substances used in consumer products may be required to undergo additional testing without further delay, depending on the exposure levels, potential for absorption etc. If more data are needed, it may be considered useful to conduct toxicokinetic studies addressing the specific questions raised for the substance of concern.

Substances for which concern for carcinogenicity is solely based on genotoxicity data will, in a first step, be evaluated according to their genotoxicity/mutagenicity hazard according to the approach outlined in Section 3.10.

If no conclusion can be drawn regarding the potential genotoxicity/mutagenicity of the substance then, in general, further genotoxicity testing will be required.

When a substance is classified as a Category 1, 2 or 3 mutagen the default presumption should be that a genotoxic mechanism for carcinogenicity is likely. If there are sufficiently convincing reasons to believe that the substance, if tested, would fulfill the criteria for classification as a carcinogen, appropriate classification should be adopted. A carcinogenicity test will normally not be required. For substances not regarded as genotoxic/mutagens evaluation of all data in relation to carcinogenicity is necessary.

Subchronic and/or chronic studies are important to identify or exclude the presence and dose-relationships for toxic effects, hyperplastic or preneoplastic responses that are assumed to be related to tumour growth. The performance of short-term tests (e.g., on neoplastic transformation or cell proliferation) or medium tests (e.g., transgenic or neonatal model) may also provide useful data for use in the assessment process. As validated testing procedures are not yet available and published in the OECD test guideline programme, it is essential that the selection of a test system is based on careful consideration of the specific properties of the substance. Selection of the test system has to be justified.

Finally, if the questions on carcinogenic potential cannot sufficiently be answered by specific investigations then the conduct of a carcinogenicity bioassay should be considered.

For substances identified as non-genotoxic carcinogens mechanistic studies may be needed to clarify the underlying mode of action, and thus relevance of animal tumour findings for human health.

3.11.5.4 New substances

Testing for carcinogenicity must be considered when supply of the substance reaches "Level 2" (1,000 tpa or 5,000 t cumulative). At this stage, all of the data available on the substance should be evaluated with respect to their impact on the risk characterisation on carcinogenicity. As a consequence further testing in relation to carcinogenicity may be required.

Annex VII part B 1.2 of Directive, 97/63 states that, for new substances, risk characterisation is not normally needed for properties for which the hazard identification tests have not yet been conducted. The strategy for new substances requires the assessor to consider, at each tonnage level, whether hazard identification is possible on the basis of available data (particularly genotoxicity data) or whether the concern is such that hazard identification tests are required.

Further toxicity testing may be required immediately post-base-set and will be required (unless there are justifiable reasons for not conducting tests) at 10 and/or 100 tpa and at 1,000 tpa (or the equivalent cumulative tonnages) in accordance with the requirements of the Directive, the outcome of the risk assessment(s) and the recommendations of the other toxicity testing strategies.

Occasionally, on a case-by-case basis, expert judgement of the weight of evidence from the data obtained and used in the assessment processes outlined above may lead to a requirement for investigations on carcinogenicity before supply levels reach 1,000 tpa (5,000 t cumulative). This requirement would be a reflection of a particularly high level of concern, in relation to both potential carcinogenicity and human exposure.

3.11.5.5 Existing substances

There is a requirement to evaluate the information available from relevant epidemiological data, and also from animal and *in vitro* studies and other substance-related data. The assessor must then make a considered judgement as to whether enough information is available for an assessment on carcinogenicity to be made. As a conventional cancer bioassay is not part of the minimum requirements for existing substances, the need for specific investigations on carcinogenicity has to be considered during the risk assessment process.

If a decision is made to carry out further testing it is important to ensure that tests in animals are conducted only when they can be justified as necessary for providing adequate data for the purpose of making the assessment.

3.11.5.6 Biocides

As indicated in Section 3.11.2.1 on minimum data requirements for biocidal active substances, there should be at least two guideline-compliant, long-term carcinogenicity studies in two mammalian species (at least one in rodents) for assessing the potential for carcinogenic hazard. As common core toxicity data requirements, the applicant must submit these carcinogenicity bioassays unless he is able to provide a justification acceptable to the Competent Authority for waiving these data. Guidance on non-submission of data is given in the TNsG on Data Requirements (2000).

In exceptional cases, findings obtained in the carcinogenic bioassays may preclude a clear conclusion to be drawn on the carcinogenic hazard to human health and, therefore, further data, as outlined in **Figure 4**, may be required. In case further studies are needed, these should be designed to specifically address open points of concern. A dialogue-phase between applicant and Competent Authority may be useful to decide which type of information is needed for sufficient carcinogenic hazard assessment.

3.11.6 Carcinogenicity test

It is recommended that when a carcinogenicity bioassay is required, discussions are held between the assessor and industry on study design and the test protocol prior to starting the study.

Particular consideration, based on all the available data, should be given to the selection of the species and strain to be used in the carcinogenicity test and the route of exposure.

It is also recommended that when a carcinogenicity test is to be conducted, an investigation of chronic toxicity should, whenever possible, form part of the study protocol. It is usual practice not only to investigate chronic toxicity in the same study as carcinogenicity, but also to use sufficient satellite groups to identify the "no observed adverse effect level" for systemic toxicity (i.e. a more extensive study is conducted than that outlined in the EU Annex V method for a combined chronic toxicity/carcinogenicity study EU Annex V B.33). The duration of the chronic toxicity element of the study is discussed in Section 3.9.

3.12 REPRODUCTIVE TOXICITY

3.12.1 Introduction

3.12.1.1 Definition of reproductive toxicity

The term "reproductive toxicity" is used to describe the adverse effects induced (by a substance) on any aspect of mammalian reproduction. It is defined in Annex VI to Directive 67/548. It covers all phases of the reproductive cycle, including impairment of male or female reproductive function or capacity and the induction of non-heritable adverse effects in the progeny such as death, growth retardation, structural and functional effects. The definition of substances which would be considered as toxic to reproduction is given in Directive 92/32. Criteria for classification of substances on the basis of adverse effects on reproduction are given in Directive 93/21.

3.12.1.2 Objectives of investigating the potential for substance-induced reproductive toxicity

The general objectives of the testing are to establish:

- whether exposure of humans to the substance of has been associated with adverse effects on reproductive function or capacity; and/or
- whether, in studies in animals, administration of the substance to males and/or females prior to conception and during pregnancy and lactation, causes adverse effects on reproductive function or capacity; and/or
- whether, in studies in animals, administration of the substance during the period of pre- or post-natal development induces non-heritable adverse effects in the progeny;
- whether the pregnant female is potentially more susceptible to general toxicity;
- the dose-response relationship for any adverse effects on reproduction.

Substance-related adverse effects on reproduction are always of potential concern, but it is important, where possible, to distinguish between a specific effect on reproduction as a consequence of an intrinsic property of the substance and an adverse reproductive effect which is a non-specific consequence to general toxicity (e.g. reduced food or water intake, maternal stress). Hence, reproductive toxicity should be assessed alongside parental toxicity in the same study. Further guidance on the assessment of developmental toxicity in relation to maternal toxicity is presented in Section 3.12.7.1.

With respect to germ cell mutagens that meet the criteria for classification as Category 1 or 2 mutagens (according to Directive 93/21) and genotoxic carcinogens that meet the criteria for classification as both Category 3 mutagens and Category 1 or 2 carcinogens, the results of reproductive toxicity testing are unlikely to influence the outcome of the risk assessment. This is because the risk characterisation for such substances will be based on the assumption that a threshold exposure level for adverse health effects cannot be identified, which will normally lead to a recommendation for the most stringent risk management measures. Therefore, reproductive testing will not normally be required for germ cell mutagens and genotoxic carcinogens, unless there are case-specific reasons to indicate that the information gained from testing will be needed for the risk characterisation. Germ cell mutagens and genotoxic carcinogens not tested for reproductive toxicity should be regarded as potentially toxic to reproduction.

3.12.2 Data to be used in the effects assessment

3.12.2.1 Minimum data requirements

In order to fully assess the hazardous properties of a substance with respect to reproductive toxicity, the key data requirements for the new substances, existing substances and biocides programs are:

- a two-generation study (EU Annex V B.35 or OECD 416), and
- a prenatal developmental toxicity (teratogenicity) study in two species (EU Annex V B.31 or OECD 414).

However, these key data requirements can be modified, either as reduced testing or as a need for accelerated or extended testing, depending on the regulatory program, and influenced by factors such as structural relationships with a known reproductive toxicant, the results of other toxicity studies, concerns for endocrine disruption and anticipated use and human exposure patterns. This chapter provides strategic advice on a stepwise approach to hazard identification so that sufficient data may be obtained to meet the requirements of the new substances, existing substances and biocides programmes. The strategy seeks to ensure that data are obtained in the most effective and humane manner so that animal use and costs are minimised.

The two-generation study is a general test which allows evaluation of the effects of the test substance on the complete reproductive cycle including libido, fertility, development of the conceptus, parturition, post-natal effects in both dams (lactation) and offspring and the reproductive capacity of the offspring. The two-generation study is preferable to the one-generation study (EU Annex V B.34 or OECD 415) because the latter has a limitation in that post weaning development, maturation and the reproductive capacity of the offspring are not assessed, and consequently some adverse effects, for example oestrogenic- or antiandrogenic-mediated alterations in testicular development, may not be detected. The two-generation study provides a more extensive evaluation of the effects on reproduction because the exposure regime covers the entire reproductive cycle, permitting an evaluation of the reproductive capabilities of offspring that have been exposed from conception to sexual maturity. The prenatal developmental toxicity study provides a focussed evaluation of the potential effects on prenatal development.

<u>New substances</u>

For new substances reproductive toxicity data are gathered in a stepwise manner in relation to a supply tonnage trigger framework. Reproductive toxicity testing is not normally considered until supply levels reach Level 1 (10 tpa) and routinely commences at Level 1 (100 tpa). The requirement in Directive 67/548 to screen substances for toxicity related to reproduction at the base-set level (1 tpa) is “for the record” as there is no appropriate screening test/battery of tests in Annex V to Directive 67/548 or in the OECD guidelines. See Section 3.12.6.4 for further information on the testing of new substances.

<u>Existing substances</u>

The minimum data set for priority listed existing substances is EU Annex V method B.35 or OECD 416 and, in two species, Annex V method B.31 or OECD 414, with the need for the second species being dependent on the outcome of the study in the first species. Derogations from the minimum data set, based on exposure consideration or other information available on

the substance or related substances should be decided by the rapporteur on a case-by-case basis. See Section 3.12.6.5 for further information on the testing of existing substances.

Biocides

The core data requirements for biocidal active substances are EU Annex V method B.35 or OECD 416 and, in two species, Annex V method B.31 or OECD 414, as specified in the TNsG on Data Requirements (2000). The rat prenatal developmental toxicity (teratogenicity) study may unusually be waived on a case-by-case basis, as specified in the "Guidance on non-submission of data-version 4.3.3":

- if no effects are seen in the rabbit developmental toxicity study; and
- if no developmental or other reproductive effects are observed in the rat two-generation study (EU Annex V B.35 or OECD 416) at the limit dose level; and
- if certain human exposure conditions are met; and
- dependent on the toxicity profile of the biocidal active substance.

3.12.2.2 Data which may already be available

Human data

Human data on certain aspects of reproductive toxicity are sometimes available for existing substances and biocides. For example, data may be available from epidemiological studies on the incidence of menstrual disorders, on libido, fertility or pregnancy outcome, or on semen analysis, following exposure of humans to a substance; or there might be case reports of birth defects associated with certain exposures.

Data from studies in animals

Data may be available from a wide variety of animal studies, which give different amounts of direct or indirect information on the potential reproductive toxicity of a substance; e.g.:

- screening studies using OECD 421 or 422;
- one or two- (or multi-) generation studies of EU Annex V or OECD standard;
- developmental toxicity tests of EU Annex V or OECD standard;
- developmental neurotoxicity studies;
- peri-postnatal studies;
- male or female fertility studies;
- other short-term *in vivo* screening tests (e.g. Chernoff/Kavlock tests);
- repeated dose toxicity studies;
- toxicokinetic studies;
- studies in non-mammalian species.

Data from other studies

Other data may be available from the physico-chemical characteristics of the substance or from *in vitro* studies: inter alia, cell culture systems (e.g. embryonic cells, ova), embryo culture systems (using either pre- or post-implantation embryos), organ tissue cultures or semen analysis (sperm count, motility and/or morphology).

It may be possible to deduce from the physico-chemical characteristics of a substance whether it is likely to be absorbed following exposure by a particular route and, furthermore, whether it (or an active metabolite) is likely to cross the placental barrier or be secreted in milk.

3.12.3 Evaluation of the available data

When evaluating the whole set of data which is available for a particular substance in relation to the potential for that substance having adverse effects on reproduction, it must be remembered that the term "reproductive toxicity" covers a wide range of effects, not all of which may have been addressed. Thus, when evaluating studies, the rapporteur should consider whether any important parameters have not been covered, or may not have been sufficiently well investigated, in a particular study type.

Human data

It is often very difficult unequivocally to associate human exposure to a specific substance with adverse effects on reproduction unless the adverse effect is a rare birth defect and the exposure is very well characterised. This is partly because it is difficult to identify a cause-effect relationship for reproductive effects which have a high "natural" incidence (e.g. spontaneous abortion) and which may predispose to unreliable results due to recall bias. Also, some effects can be very subjective (e.g. effects on libido). As with much human data, there may be mixed exposures and/or lifestyle related confounding factors. Nevertheless, well designed and well reported human studies (such as epidemiological studies and workplace monitoring data) in which both reproductive and relevant non-reproductive effects are described will contribute to the weight of evidence for whether or not the substance is toxic to reproduction.

Animal data

Data from OECD 421 (reproductive/developmental toxicity screening test) may enable the rapporteur to identify a substance as being toxic to reproduction (i.e. the test gives a clear "positive" result). However, use of this method offers only limited means of detecting post-natal manifestations of pre-natal exposure or effects that may be induced during post-natal exposure. In addition, because of the study design (e.g. relatively small numbers of animals per dose level; relatively short study duration), the method will not provide evidence for definite claims of no effects. Similar criteria apply when evaluating data obtained using OECD 422 (combined repeated dose toxicity study with the reproductive/developmental toxicity screening test).

Clearly, well-reported two-generation or developmental toxicity studies of EU Annex V or OECD standard, particularly if conducted in accordance with the principles of GLP, can be used to identify substances as being specifically toxic to reproduction. These tests can also be used to identify substances as being of no concern in relation to the end-points that they address. Non-GLP studies and studies not using Annex V or OECD protocols may also be used in the same way to decide whether a substance is toxic to reproduction when sufficient animals of an appropriate species have been used and have survived (i.e. usually at least as many as specified in the Annex V methods or OECD guidelines, though expert opinion might be that a particular study with fewer animals is acceptable), when the dose levels used are sufficient in number and sufficiently high (as specified in Annex V methods or OECD guidelines) and the relevant observations have been made.

Data from repeated dose toxicity studies in which there are marked adverse effects on the reproductive organs (usually the testes) can also be used to identify a substance as being toxic to

reproduction. Data from such studies cannot be used to identify a substance as being of no concern in relation to reproduction.

If peri-postnatal tests, developmental neurotoxicity studies or specific male or female fertility studies are available they can be used to identify a substance as being toxic to reproduction. Data from such studies alone cannot be used to identify a substance as being of no concern in relation to reproduction.

Short-term *in vivo* studies (e.g. Chernoff/Kavlock tests), studies in non-mammalian species or *in vitro* studies will not, in the absence of more definitive data, provide a basis for a firm decision about the reproductive toxicity of a substance. "Positive" results from such studies indicate that there may be some concern in relation to the potential for reproductive toxicity, but they can be overridden by clearly negative data from well-conducted studies of EU Annex V or OECD standard, for reproductive toxicity. "Negative" data from these studies, if well conducted, may contribute to the weight of evidence.

3.12.4 Assessment of the dose-response relationship

Reproductive toxicity is usually considered to be an effect with an underlying dose threshold mechanism. Hence, when possible, a N(L)OAEL value for the adverse effects on reproduction should be identified for use in risk characterisation. The use of a benchmark dose as an alternative to the N(L)OAEL has been proposed for developmental toxicity studies; further information on this approach is given in Section 3.4.

If it has been possible to identify a NOAEL from well-reported and reliable human studies, this value may be used preferentially in the risk characterisation. However, it is expected that this will rarely be the case.

3.12.5 Degree of uncertainty in studies of effects on reproduction

Unless the effect is a very specific one of low "normal" incidence, there may be a high level of uncertainty in human studies of effects on reproduction (see above for a discussion of the evaluation criteria for human data).

It is obvious that there are limitations in many of the types of non-human studies relating to reproductive toxicity. Well-conducted tests of EU Annex V methods B.35/B.31 or OECD 416/414 standard can be used with confidence to identify substances as, or not as, being toxic to reproduction in relation to the endpoints addressed in the test. However, other studies, including tests conducted according to the OECD 421 and 422, may provide clear (in the case of the OECD methods) or indicative evidence of reproductive toxicity, but will not provide sufficient evidence for confidence about the absence of reproductive toxicity. The weight of evidence from other studies (including human data), toxicokinetic and/or mechanistic data, when available, can help in reducing this uncertainty.

3.12.6 Testing strategies

3.12.6.1 Objective of this part of the guidance

The objective is to give guidance on a stepwise approach to hazard identification with regard to reproductive toxicity. A principle of the strategy is that the results of one study are evaluated before another study is initiated. The strategy seeks to ensure that the data requirements are met in the most efficient and humane manner so that animal usage and costs are minimised.

3.12.6.2 General principles

Preliminary information

It is important that whatever is known of the physico-chemical, toxicokinetic and toxicodynamic properties of the test substance, as well as any available relevant information on chemical analogues (i.e. structure activity relationships, SAR) and the results of previously conducted toxicity studies, is taken into consideration when developing test programmes and selecting test protocols.

Test species

The choice of species for the reproductive toxicity tests must be carefully considered in the light of all the available information. However, because of the extent of background data and because this species is used for general toxicity tests, the rat is normally the species of first choice for the two-generation study. Developmental toxicity studies are usually conducted in the rat and rabbit, again because of the availability of background data. It is recommended that other species are used only when there are clear indications that they are more appropriate than the rat or rabbit.

Route of administration.

The route used should be the most appropriate in relation to the likely route(s) of human exposure, the nature and physico-chemical properties of the substance and its toxicity (systemic effects) by the routes of exposure under consideration, as well as the practical aspects of conducting tests for reproductive toxicity. Ideally, toxicokinetic and metabolism data should form part of the basis for a decision on the route of exposure used in the tests for reproductive toxicity. In practice, the oral route has been widely used and, unless there are strong indications that this route is likely to be inappropriate (e.g. from metabolism data for the substance itself or for a structurally related substance), oral dosing is generally considered to be the most practical when conducting a two-generation study. Although developmental toxicity studies can more readily utilise the dermal or inhalation routes of exposure if either is particularly indicated, the oral route using gavage is generally recommended. If the dermal route is chosen there may be stress related effects. It is recommended that neither intraperitoneal administration, nor those parenteral routes which may be used, for instance, when testing some pharmaceuticals (e.g. intravenous, intramuscular, subcutaneous), should be used when testing substances for reproductive toxicity, unless such use is justified, and agreed by the assessor. Further guidance on the selection of the appropriate route for toxicity testing is presented in Appendix V of the TGD.

Dose levels

Unless a "limit test" is to be used, with a single, high dose level (i.e. 1,000 mg/kg bodyweight per day in an oral gavage study), a test for reproductive toxicity should include a range of dose levels. The highest dose level should not normally exceed 1,000 mg/kg/day and should, where possible, give rise to some toxicity in the parent animals, but not severe toxicity, obvious suffering or lethality. The lower dose levels should be selected with the aim of establishing any dose-response relationship and the no-effect level for reproductive toxicity. (It should be noted that the EU Annex V method for developmental toxicity states that the highest dose "should ideally induce some overt maternal toxicity, such as slight weight loss, but not more than 10% maternal deaths". It is recommended that, while conforming with the requirement for induction of maternal toxicity, the highest dose used in a developmental toxicity test should not normally cause lethality.

3.12.6.3 Testing sequence

The first specific reproductive toxicity test to be conducted should usually be the two-generation study. Ideally, this test should be initiated after the rat 90-day sub-chronic repeated exposure study (if it is to be conducted) as this study can provide information necessary for selecting the appropriate dose levels for the two-generation study. Additionally, repeated exposure studies can provide toxicity information of relevance to reproduction that should be taken account of in the design of the two-generation study. For example, the observation of neurological effects can trigger the need for a developmental neurotoxicity evaluation (see Section 3.12.6.7 for further information on developmental neurotoxicity testing).

The first developmental toxicity study is normally conducted after completion of the two-generation study. The design of the developmental toxicity study should take account of any information derived from the repeat exposure and two-generation studies, in particular dose-response relationships and information on maternal toxicity. For new substances and existing substances the first developmental toxicity study is conducted in the rat. It is advantageous to conduct the rat study first because there is scope for a more reliable interpretation of the relationship between maternal and developmental toxicity in the rat due to the availability of toxicity data in pregnant animals from the two-generation study. The need for the second developmental toxicity study, in the rabbit, is dependent on the outcome of the first study. For biocides, the first developmental toxicity study may be conducted in the rabbit because the TNsG on Data Requirements (2000) allow a waiver of the rat developmental study in the event of a negative study in the rabbit and if certain other toxicity and human exposure conditions are met (see Section 3.12.2.1 for further information).

Further advice on testing strategies, specific to the new substances, existing substances and biocides programmes, is given below.

3.12.6.4 New substances

Two-generation study

Usually, the starting point for the reproductive toxicity testing programme is the two-generation study (EU Annex V B.35 or OECD 416), which is routinely conducted at Level 1 (at either 10 or 100 tpa) unless there is justification for delaying this test until the Level 2 (1,000 tpa) supply

tonnage trigger is reached. A number of factors will influence the decision on whether to conduct the two-generation study at 10, 100 or 1,000 tpa:

- when there are indications of potential reproductive toxicity already available (e.g. histopathological effects on the gonads in a repeated dose toxicity study; close structural likeness to a known reproductive toxicant), the two-generation test should be conducted when supply levels reach 10 tpa;
- the potential for human exposure will also influence whether testing is done at 10 tpa or deferred until 100 tpa. For example, when widespread human exposure which is difficult to control can be predicted, particularly consumer or indirect exposure, then testing will normally be required at 10 tpa;
- when there are no indications of concern with regard to potential reproductive toxicity (e.g. no histopathological effects on the gonads in a repeated dose toxicity study, no concern from SAR) and low concern in relation to human exposure, the two-generation study should normally be conducted when supply levels reach 100 tpa;
- for substances of low toxicological activity (i.e. no evidence of toxicity has been seen in any of the toxicity tests already available), and for which there is no evidence of significant absorption from toxicokinetic investigations, and there are low concerns in relation to exposure, consideration can be given to delaying the commencement of the reproductive toxicity testing until the level of supply reaches Level 2 (1,000 tpa) or, alternatively the conduct of the one-generation study may be considered at Level 1. For such low-concern substances, reproductive toxicity testing may not be required at Level 2 if it can be conclusively demonstrated by toxicokinetic data that there is no systemic absorption via relevant routes of exposure;
- in the rare event of the conduct of a one-generation study at Level 1, a two-generation study will normally be required at Level 2. If the one-generation test provides equivocal evidence of impaired fertility, further testing for clarification should be initiated without further delay. If practical considerations permit the extension of the original study to a second litter and/or a second generation this is preferable to the conduct of a separate, new study at this stage. A second litter from parental animals maintained on the original dose levels throughout the study may provide meaningful information on the fertility of the animals from which equivocal data were originally obtained.

The need for the inclusion of a developmental neurotoxicity evaluation must be considered at the planning stage of the two-generation study (see Section 3.12.6.7 for further information).

Developmental toxicity study

It is recommended that the first developmental toxicity test is normally conducted immediately after the two-generation study, except:

- if there are serious concerns about potential developmental toxicity (from SAR) and also about the potential for human exposure, it may be appropriate to conduct the first developmental toxicity test before, or at the same time as, the two-generation study;
- if the two-generation study was conducted at 10 tpa and there were no indications of likely developmental toxicity from this study or from SAR, the first developmental toxicity study can be conducted at 100 tpa;
- when the two-generation study conducted at 10 tpa or 100 tpa is well-conducted with one dose level of around 1,000 mg/kg bodyweight per day (oral) and shows no adverse effects on reproduction, and the notifier is able to satisfy the assessor that human exposure will be negligible (e.g. for an intermediate used in a closed system), and there are no other

indications of potential concern (e.g. from SAR), it is considered acceptable to defer testing for developmental toxicity until the level of supply reaches Level 2.

The requirement for the second developmental toxicity study, and its timing, will be influenced by the results of the first developmental toxicity study:

- substances shown to have clear adverse effects on developmental parameters, such that the criteria for classification as a Category 2 developmental toxicant are met (as defined in Section 4.2.3.3 of Directive 93/21) there will normally be no requirement for a second developmental toxicity study;
- substances giving rise to less severe manifestations of developmental toxicity (such as changes in the incidence of common structural variants, or a retardation of skeletal development), resulting in a "category 3" or no classification (see Section 4.2.3.3 of Directive 93/21), should be studied, without further delay, in a developmental toxicity test in another species so that the potential for induction of serious structural defects can be investigated further;
- when the first developmental toxicity study is clearly negative, a second study in another species should be conducted at Level 2 unless there are significant overriding reasons for conducting this study at Level 1 (100 tpa) such as toxicokinetic/metabolism data and/or potential widespread exposure of women of childbearing age via consumer products.

The need for the inclusion of a developmental neurotoxicity evaluation, if not previously conducted, must be considered at the planning stage of the developmental toxicity study (see Section 3.12.6.7 for further information).

Reproductive toxicity testing immediately after base set submission

Under specific circumstances, testing for reproductive toxicity may be required immediately after the base-set submission. Testing at this time will be required when there is particularly high concern for reproductive toxicity, based on the results of the base-set toxicity studies (the effects observed, their severity and the dose-response relationship) and/or on SAR considerations and when it is expected that reproductive toxicity may occur at doses close to known or estimated human exposure levels. Of greatest concern are situations in which the general public will be exposed to the substance. When testing for reproductive toxicity is required immediately after the base-set submission, the first test to be conducted, and the timing of subsequent reproductive toxicity tests, should be decided on a case-by-case basis, taking into account the nature of the anticipated reproductive effect and the exposed population. For example, if there is specific concern for prenatal developmental effects, then a developmental toxicity study (EU Annex V B.31 or OECD 414) should be conducted first; if there are concerns for effects on fertility or post natal development, then a two-generation study (Annex V B.35 or OECD 416) will be conducted first.

3.12.6.5 Existing substances

For priority existing substances not meeting the minimum data requirements, industry should liase immediately with the rapporteur to determine the most appropriate testing strategy. Usually, the testing sequence outlined in Section 3.12.6.3 should be followed. The need for the inclusion of a developmental neurotoxicity evaluation must be considered (see Section 3.12.6.7 for further information).

However, derogations from the minimum data set may be agreed by the rapporteur on a case-by-case basis, following the same principles described for new substances in Section 3.12.6.4. For existing substances shown to have clear adverse effects on developmental parameters in the first developmental toxicity study, such that the criteria for classification as a Category 2 developmental toxin are met (as defined in Section 4.2.3.3 of Directive 93/21) there will normally be no requirement for a second developmental toxicity study. Reduced testing requirements can be considered for substances of low biological activity (i.e. no evidence of toxicity has been seen in any of the toxicity tests already available), and for which there is evidence from toxicokinetic investigations that no significant absorption occurs, and when there are low concerns in relation to exposure (i.e. human exposure is low and consumer or indirect exposure is insignificant).

3.12.6.6 Biocides

The common core data requirements (see also Section 3.12.2.1) and waiver possibilities for active biocidal substances are specified in the TNsG on Data Requirements (2000). The applicant is responsible for the submission of a complete data set when applying for authorisation under Directive 98/8. However, in certain cases expert judgement by the applicant and by the Competent Authority may be necessary in order to assess, for example, whether an additional study is needed or can be waived, or the design of a specific test. The need for the inclusion of a developmental neurotoxicity evaluation (see Section 3.12.6.7) as an additional data requirement must be considered, based on the toxicity profile of the biocidal substance, on the product type and on the expected human exposure profile, taking into account both the proposed normal use and a possible realistic worst-case situation.

3.12.6.7 Developmental neurotoxicity study

Developmental neurotoxicity studies are designed to generate data, including dose-response characterisations, on the potential functional and morphological hazards to the nervous system that may arise in the offspring from exposure of the mother during pregnancy and lactation.

A standard test method is currently being developed, as OECD draft TG 426. The neurological evaluation consists of observations to detect gross neurological and behavioural abnormalities; the assessment of physical development, including sexual maturation, reflex ontogeny, motor activity, motor and sensory function, and learning and memory; and the evaluation of brain weights and neuropathology during postnatal development and adulthood.

A developmental neurotoxicity study can be conducted as a separate study or as an add-on study. It is generally recommended that developmental neurotoxicity testing be performed as an add-on to a two-generation study using offspring that would otherwise be discarded at weaning, since this will not involve the use of additional groups of animals.

Triggers for the developmental neurotoxicity study

In determining the necessity for this testing, a weight-of-evidence approach should be used. Data from all available toxicity studies, as well as potential human exposure information should be considered. Developmental neurotoxicity testing should be conducted to further characterise neurological effects observed in other studies, and should be considered if the substance has been shown to cause neurotoxicity or structural abnormalities of the CNS in other studies, or suspected of interfering with neurotransmission or neuroendocrine pathways (thyroid, pituitary,

or circulating sex hormones) at the CNS level. For example, neuroendocrine interference at the level of the hypothalamic-pituitary axis might be inferred from changes in the levels of circulating gonadotrophins or steroid sex hormones.

Results of the developmental neurotoxicity study and further action

Developmental neurotoxicity can be indicated by behavioural changes or morphological changes in the brain. The severity and nature of the effect should be considered. Generally, a pattern of effects (e.g. impaired learning during several consecutive trials) is more persuasive evidence of developmental neurotoxicity than one or a few unrelated changes. The reversibility of effects should be considered, too. Irreversible effects are clearly serious, while reversible effects may be of less concern. However, it is often not possible to determine whether an effect is truly reversible. The nervous system possesses reserve capacity, which may compensate for damage, but the resulting reduction in reserve capacity should be regarded as an adverse effect. If developmental neurotoxicity is observed only during some time of the lifespan then compensation should be suspected. Also, effects observed for example during the beginning of a learning task but not at the end should not be interpreted as reversible effects. Rather the results may indicate that the speed of learning is decreased.

The experience of offspring especially during infancy may affect their later behaviour. For example, frequent handling of rats during infancy may alter the physiological response to stress and the behaviour in tests for emotionality and learning. In order to control for environmental experiences, the conditions under which the offspring are reared should be standardised within experiments with respect to variables such as noise level, handling and cage cleaning. The performance of the animals during the behavioural testing may be influenced by e.g. the time of day, and the stress level of the animals. Therefore, the most reliable data are obtained in studies where control and treated animals are tested alternatively and environmental conditions are standardised.

Equivocal results may need to be followed up by further investigation. The most appropriate methods for further investigations should be determined on a case-by-case basis guided by the effects seen in the developmental neurotoxicity study, adult neurotoxicity studies and/or SAR-based predictions. Extensive coverage of methods is given in the OECD Guidance Document for Reproductive Toxicity Testing (in preparation) and the OECD Guidance Document for Neurotoxicity Testing (2000). No further testing will normally be required when the results of the developmental toxicity study are clearly positive. Also, no further testing will normally be required when the results are clearly negative. However, a second study in another species and/or examination of developmental neurotoxicity endpoints not covered yet may be considered when there are potential widespread exposure of women of childbearing age and indications of developmental neurotoxicity in humans.

3.12.7 Additional considerations

3.12.7.1 Relation between maternal toxicity and developmental toxicity

Developmental toxicity may be mediated by maternal toxicity, as experience has shown. However, in most studies evidence for a causal relationship is lacking. Developmental toxicity occurring in the presence of maternal effects does not itself imply a causal relationship between the two and therefore it is not appropriate to discount developmental toxicity that occurs only in

the presence of maternal toxicity. If a causal relationship can be established, it can be concluded that developmental toxicity does not occur at lower doses than the threshold for maternal toxicity, although the substance can still be considered as a developmental toxicant. In the absence of proven causality, the nature and severity of the developmental versus the maternal effects may well warrant the conclusion that a substance should be considered as a specific developmental toxicant when the effects are only observed in the presence of maternal toxicity. The assessment of the interrelationship between developmental toxicity and maternal toxicity and its influence on decisions regarding hazard classification must be conducted on a case-by-case by basis, using a weight of evidence approach, and with reference to the comments in the classification guidance in Directive 93/21. Further information on the interpretation of developmental toxicity occurring in the presence of maternal toxicity and the implications for risk characterisation is given in Section 4.9.

Because of possible differences in sensitivity between pregnant and non-pregnant animals, toxicity data from repeat dose studies have little use in the interpretation of maternal toxicity in reproductive studies. On the other hand, in reproductive toxicity studies, endpoints that were shown to be affected in repeated toxicity studies may be incorporated as maternal parameters. This may help to identify any differences in sensitivity to treatment between pregnant and non-pregnant animals due to pregnancy-induced changes in physiology.

3.12.7.2 Reproductive toxicity via lactation

Reproductive toxicity may occur through lactation in several ways. Substances may reach the milk and result in exposure of the newborn. On the other hand, the quality and quantity of the milk may be affected by maternal exposure to the substance, resulting in nutritional effects on the newborn. Three aspects are crucial in the risk assessment of lactational effects, as indicated below:

- the concentration of the substance transferred via the milk. Toxicokinetic aspects should be considered including the chemical-physical properties of the compound, the timing and duration of exposure, the bioavailability and the persistence of the substance. Fat-soluble chemicals that may be mobilized during lactation are of special concern;
- the sensitivity of the newborn as compared to the adult. A wide spectrum of toxic effects may occur in the newborn, ranging from general toxic effects which may present as reduced weight gain or delayed general development, to specific effects on the maturation of organs or physiological systems. The newborn may be more sensitive as compared to the adult, not only because of specific developmental endpoints, but also in view of a possibly higher intake of the substance per kg body weight and the immaturity of detoxification pathways and physiological barriers. Moreover, some effects may become apparent only later in life;
- effects on milk quality and/or quantity. These effects will usually be detected only through effects on the growth and development of the newborn. In addition, the underlying effect may be found in alterations in the anatomy and histology of the mammary gland which can be studied through histological analysis.

In general, the two-generation study (EU Annex V B.35 or OECD 416) is the best guideline-based study available to identify effects on or via lactation. In case of specific questions regarding lactation the protocol may have to be amended in view of any existing information on the substance under study, including physico-chemical, toxicokinetic and general toxic properties. Cross-fostering may establish whether toxicity to the offspring is the result of lactational effects or via uterine exposure.

3.12.7.3 Endocrine disruption

An endocrine disrupter is an exogenous substance that causes adverse health effects in an intact organism or its progeny through alterations in the function of the endocrine system. Thus, endocrine disruption is a mechanism rather than an adverse health effect.

The concern for endocrine disruption has resulted in the development of newly proposed test guidelines to specifically address effects on hormone homeostasis and on male and female reproductive organs. In addition, suggestions are being considered for new parameters to be incorporated into existing test guidelines. At the time when this TGD was being written several validation studies were still ongoing, precluding recommendations for the use of new test protocols: i.e. the enhanced TG 407, the rodent uterotrophic assay for the estrogenic effects and the Herschberger assay for the antiandrogenic effects. With respect to endocrine disruption, the two-generation study (EU Annex V B.35 or OECD 416) is currently the most complete study available. Both in this study and in the developmental toxicity study (Annex V B.31 or OECD 414), additional endocrine-sensitive parameters may be studied on a case-by-case basis when endocrine disruption is an issue of concern.

3.12.7.4 Additional testing

Further investigation of reproductive toxicity may be considered appropriate, in special cases, for substances which are of concern. Competent Authorities may consider asking for tests:

- to define better a no-effect level and/or the dose-response relationship;
- to investigate site/mode of action using toxicokinetic and/or mechanistic studies using *in vitro* and/or *in vivo* tests;
- to investigate the reversibility of effects on fertility;
- to investigate whether the effects recorded are species-specific.

4 RISK CHARACTERISATION

4.1 GENERAL ASPECTS

The assessor carries out the risk characterisation by comparing the quantitative and/or qualitative information on exposure for a human population to the N(L)OAEL or, where appropriate, a qualitative evaluation of the likelihood that an effect will occur at the given exposure. This is done separately for each population potentially exposed and for each effect. The assessor should focus the assessment on those effects of toxicological relevance to humans which may be expressed at the predicted levels of exposure.

Unless a N(L)OAEL value is available from human data, the N(L)OAEL values are those derived from the animal studies, without any modifications, as described below. When a human N(L)OAEL value is available, the approaches described below can still be used in principle but, obviously, some of the factors which need to be considered when using data from animal studies will not apply.

Where a N(L)OAEL has been identified for any of the effects set out in Regulation 1488/94 or Directive 93/67/EEC, it will be used to compare with the exposure estimate for the exposed human population. Where more than one N(L)OAEL has been identified for the specific effect, then the most relevant N(L)OAEL will be used.

Where it is not possible to determine a N(L)OAEL the likelihood that the effect will occur is evaluated on the basis of the quantitative and/or qualitative information on exposure relevant to the human populations under consideration. Where, despite a N(L)OAEL not having been determined, the test results nevertheless demonstrate a relationship between dose/concentration and the severity of an adverse effect or where, in connection with a test method which entails the use of only one dose or concentration, it is possible to evaluate the relative severity of the effect, such information shall also be taken into account in evaluating the likelihood of the effect occurring.

Where the exposure estimate is higher than or equal to the N(L)OAEL, this indicates that the substance is “of concern” with regard to the exposure of the human population considered. The assessor will need to decide whether additional data either on exposure or toxicity would allow a refinement of the exposure or N(L)OAEL values and, subsequently, a refinement of the comparison which would influence the risk characterisation result. For this decision it should also be considered whether such additional information could be predicted from the data set already available.

Where the exposure estimate is less than the N(L)OAEL, the risk assessor will need to decide which of the possible results applies. For this step, the magnitude by which the N(L)OAEL exceeds the estimated exposure (i.e. the “margin of safety”) needs to be considered taking account of the following parameters:

From Regulation 1488/94 and Directive 93/67/EEC:

- the uncertainty arising, among other factors, from the variability in the experimental data and intra- and interspecies variation;
- the nature and severity of the effect;
- the human population to which the quantitative and/or qualitative information on exposure applies.

Other factors:

- the differences in exposure (route, duration, frequency and pattern);
- the dose-response relationship observed;
- the overall confidence in the database.

Expert judgement is required to weigh these individual parameters on a case-by-case basis. The approach used should be transparent and a justification should be provided by the assessor for the conclusion reached. The above parameters and their use in risk characterisation are described in several publications (e.g. Dourson and Stara, 1983; EPA, 1987; Hart et al., 1988; Lewis et al., 1990; IPCS, 1994; Renwick, 1993; UK, 1993 and ECETOC, 1995).

For new substances, revision of the exposure assessment should always be considered by assessors before they require that, on the basis of the risk characterisation, *in vivo* toxicity tests are conducted in advance of the tonnage thresholds specified in Directive 67/548/EEC: such revision might indicate a lower exposure level than was initially predicted, thus reducing concern. Good evidence of negligible levels of human exposures may be used to defer testing, or even render it unnecessary, as described in the testing strategies.

Particularly for existing substances it may be possible that additional existing data are obtainable via industry, e.g. additional data on exposure and/or toxicity may be available in companies which are customers of manufacturers or importers. Therefore, consultation with industry should follow the procedures outlined in Chapter 1.

The procedure leading to a decision by the risk assessor to request the generation of additional data should be transparent and justified; the decision whether to request data to refine the exposure assessment or to refine the N(L)OAEL should be based on the principles of lowest cost and effort, highest gain of information and the avoidance of unnecessary testing on animals.

As stated earlier, the nature of the potentially exposed population should be considered in the risk characterisation step. Specifically, it is necessary to decide whether the exposed human population will include the very young, the elderly or the infirm e.g. exposure of the general public. In practice, lower “margins of safety” are normally seen for workers compared to those for consumers or humans indirectly exposed via the environment.

This is because it can be expected that the workforce does not include the more vulnerable humans (e.g. the very young, the sick, the elderly); workplace exposure levels and/or patterns of exposure can be controlled and populations or individuals potentially exposed in a workplace can be specifically protected and/or monitored. However, for certain types of effects (developmental toxicity, respiratory sensitisation) particular attention should be given to the magnitude of the “margin of safety” which may be necessary for the protection of sensitive sub-populations (pregnant women, individuals with high bronchial reactivity).

The extent to which better exposure data and/or toxicokinetic study results could influence requirements for further testing should be investigated. For example, if further repeated dose testing using the oral route (in respect of consumer exposure) needs to be balanced against a need for a repeated dose inhalation study (in respect of worker exposure), toxicokinetic data could be helpful in deciding whether a single test (a single route of exposure) would suffice for both purposes. The toxicokinetic studies which would clarify matters may, however, be very costly, take time and involve the use of animals. These factors need to be balanced against the other tests under consideration.

On a case-by-case basis, the further testing requirements at each stage of risk assessment should be considered in the light of all the available information, including the possibility of controlling exposure, so that justifiable and integrated recommendations for either or both of further testing and risk reduction can be made.

Dependent on the N(L)OAEL/exposure level ratio or the qualitative evaluation of the likelihood that an effect will occur at the given exposure, the risk assessor will decide which of the following possible results applies for each population potentially exposed and each effect:

New substances

(i) the substance is of no immediate concern and need not be considered again until further information is made available in accordance with the requirements of Directive 67/548/EEC;
(ii) the substance is of concern and the Competent Authority will define information required to refine the assessment and request that it is supplied when the quantity of the substance placed on the market reaches the next supply threshold;
(iii) the substance is of concern and the Competent Authority will request that defined information is supplied without further delay;
(iv) the substance is of concern and the Competent Authority will immediately make recommendations for risk reduction.

Existing substances

(i) there is need for further information and/or testing;
(ii) there is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already;
(iii) there is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

If further testing is necessary for one or more effect(s), the toxicological principles of the strategies for testing of new substances as outlined in this document should be applied. These testing strategies are, in the context of the risk assessment of existing substances, to be used only as a tool for the decision on the most appropriate test to clarify the concern with regard to the effect(s) under consideration. When it is considered that further information would be useful in order to refine the risk assessment, the information required should be clearly defined and justification given for requesting the data.

Where, even after refinement of the risk characterisation, the exposure estimate is still higher than or equal to the N(L)OAEL, the risk assessor will normally decide that there is a need for a recommendation that risk reduction measures are necessary taking into account (for existing substances) those measures which are already being applied.

4.2 ACUTE TOXICITY

In general, the occurrence of discrete exposures of humans to substances during normal use (such as maintenance operations or occasional household tasks) represents the human exposure pattern of concern in relation to acute toxicity. Foreseeable misuses may also need to be considered.

It is not usually possible to obtain reliable quantitative exposure levels for these discrete exposures, but it is often possible to derive values by calculation (e.g. from knowledge of the

amount of substance and the volume of space into which it may disperse) for the magnitude of exposures via the oral, dermal or inhalation routes, as appropriate, which are useable for the purpose.

When acute toxicity data from human studies are used in the risk characterisation, a pragmatic comparison should be made of the exposure levels associated with adverse effects in the studies and the human exposure level(s) under consideration.

Often, though, the acute toxicity data used in the risk characterisation will be from studies in animals. From these studies, data on the dose-response relationship should have been derived, but usually the only numerical values of any reliability will be the LD_{50} (or discriminating dose, if the Fixed Dose method was used) or LC_{50} values.

The human exposure values, including peak values, should be compared with the relevant doses or concentrations from the acute toxicity data.

Using judgement based on the doses or concentrations at which adverse effects were observed in the studies, the $LD(C)_{50}$ or discriminating dose values, the nature and severity of the adverse effects, the slope of the dose-response curve, the extent to which interspecies and/or route-to-route extrapolation is necessary and possible as well as the human population(s) under consideration, the assessor will decide which results apply.

When the assessor is satisfied as to the relevance and reliability of the available exposure and effects data, and it is judged that the difference between the human exposure level(s) and the relevant dose/concentrations level(s) in the acute toxicity study/studies is sufficient to ensure that acute toxic effects in humans are highly unlikely to occur, it can be concluded that no further information is required and that no risk reduction measures are necessary beyond those already in place. In order to justify this result the assessor should pay particular attention to:

- the slope of the dose-response curve, as the steeper the slope the greater the reduction in toxicity for a finite reduction in exposure;
- the population(s) exposed (generally, a population which includes children or the infirm, for example, is assumed to need a high degree of protection from potential harm);
- the volatility of the substance (very volatile substances may not be classified using the usual criteria for acute toxicity, but may be able to attain sufficiently high atmospheric concentrations to induce ill-health in exposed humans).

When the assessor is not able to conclude that no further information and/or testing is required and there is no need for risk reduction measures beyond those being applied already, the reasons should be sought. If it is clear that neither the exposure assessment nor the effects assessment could be substantially improved by the acquisition of defined better or more relevant data and that currently used risk reduction measures have been taken into account, then there will be a need for limiting the risks (i.e. for a particular use and population or sub- population).

However, the assessor may identify potentially better and/or more relevant information which could be acquired in order to refine the risk assessment. It is possible that better measured data could be obtained to refine the exposure assessment or physical or mathematical modelling may be useful. As far as further testing is concerned, this would normally be required only in the case that extrapolation or inference from the existing data is not possible in relation to a route of exposure for which an acute toxicity test is not already available and when there is concern about peak exposures of humans to the substance via that route of exposure. In those circumstances, an acute toxicity test would be required using the appropriate route of exposure.

4.3 IRRITATION

The assessor will need to establish whether, for any of the human populations, the exposure patterns (taking into account any risk reduction measures already in place) may lead to concern for potential skin, eye or respiratory irritation.

If non-irritating concentration values are already available, e.g. from human data, in relation to any of the irritant effects for substances with irritant properties, relevant quantitative assessments of exposure should be made when possible. Such assessments may range from simply ascertaining the weight fraction of substance in a preparation to derivation of atmospheric concentrations. The exposure level(s) should then be compared with the non- irritating concentration value(s). For skin and eye irritation, the assessor will usually be able to judge on the basis of the non-irritating concentration values and the exposure assessment which of the results apply.

For respiratory irritants, the non-irritating concentration value should be compared with the exposure estimate(s), and the assessor should judge which of the results apply.

When non-irritating concentration values are not available for any of these effects, the likelihood of occurrence of skin, eye or respiratory irritation in humans exposed to the substance should be assessed in a pragmatic manner in relation to the route, pattern and extent of the human exposure.

In all cases, the assessor will need to take into account, inter alia:

- whether there is any observed relationship between the dose/concentration of the substance and the severity of the irritant response;
- the severity of any irritant response when only a single concentration was tested;
- the reversibility (or irreversibility) of any irritant response;
- whether there is any potential for prolonged or repeated exposure during use of the substance;
- whether the substance is used as such or as a preparation;
- the relevant physico-chemical properties of the substance (e.g. its physical form; its hydrolysis products).

For skin and eye irritation, it should be very rarely, if ever, necessary to require further testing. For respiratory irritation, further testing to an appropriate study protocol may sometimes be thought necessary in order to identify a threshold exposure level. For all types of irritation, further information on exposure may be judged to be necessary, if possible, in order to refine the assessment

For substances which have been shown to have the potential to cause skin or eye irritation in humans, the assessor should consider whether respirable dusts, aerosols or vapours could be formed during use.

If it is predicted that this may occur, the assessor should evaluate any available data from human or animal studies in which exposure data was via inhalation and ascertain whether it is possible to conclude that respiratory irritation is unlikely. When it is thought that respiratory irritation may be caused by inhalation of the substance, it may be necessary to estimate exposure levels for comparison with the N(L)OAEL value for an analogue substance if possible. The risk characterisation procedure would then be as described above. It should be noted that there is only a limited possibility for obtaining further useful data on the hazard itself (i.e. respiratory

irritancy) but it may be considered necessary, if possible, to conduct further well-defined testing in order to identify a threshold exposure level.

4.4 CORROSIVITY

Normally, the pragmatic approaches outlined above for skin and eye irritation will be used for risk characterisation in relation to corrosivity. If the substance, or a preparation containing the substance, is respirable (or may become respirable in use) the approaches described above for the respiratory irritation should be used. It is not expected that further testing would ever be identified as necessary following risk characterisation in relation to corrosivity, but it may be expected that further information on exposure will sometimes be required.

4.5 SENSITISATION

For skin sensitisation, it will also be usual to use pragmatic approaches, as described for skin and eye irritation. When there is any useful information available on the severity of the effect or on the concentration-effect relationship this may be taken into account in the risk characterisation, as indicated above for skin and eye irritation. However, reliable information on the concentration-effect relationship will seldom be available and the primary aim must be to ensure that induction of skin sensitisation is prevented in humans using the substance.

As for corrosivity, it is expected that further testing would only rarely be required for skin sensitisation (e.g. if the available data gives an overall equivocal result), but further information on exposure may sometimes be required.

Respiratory sensitisation is a potentially life-threatening condition. However, the exposure conditions (dose/concentration, frequency and/or duration of exposure) which may induce human respiratory sensitisation to a substance are not clearly understood. Usually it is possible to identify a substance as a respiratory sensitiser only on the basis of positive human data, though the outcome of the skin sensitisation study and/or structure activity relationships and *in vitro* data (e.g. reaction with macromolecules) and the physico-chemical properties of the substances may be used to provide indicative evidence that the substance may be a respiratory sensitiser. (It should be noted that not all skin sensitisers are expected to be respiratory sensitisers.) Hence, there can be difficulty conducting risk characterisation for this endpoint.

When there is evidence from human experience that the substance is a respiratory sensitiser, the exposure conditions of the study which provided that evidence can be compared with the exposure conditions of the population(s) under consideration.

When it is necessary to use indicative evidence that the substance may be a respiratory sensitiser, the assessment will have to be more pragmatic, taking into account knowledge about the exposure conditions under which it is known that respiratory sensitisers are used without induction of adverse effects. In particular, well-documented and relevant negative data from human experience with the substance under consideration, if available, will be of assistance.

Because there are no widely recognised methods available, it is not expected that any *in vivo* testing could be identified as necessary following the risk characterisation, but *in vitro* testing (e.g. investigating the potential protein-binding capacity of the substance) and/or further information on exposure may sometimes be desired in order to refine the risk assessment.

4.6 REPEATED DOSE TOXICITY

The risk characterisation is carried out by comparison of the most relevant N(L)OAEL value(s) with the human exposure level(s). General guidance is given above on how to proceed to evaluate the ensuring exposure level/N(L)OAEL ratio(s) using a "margin of safety" approach. It may be possible to decide that no further information/testing or risk reduction measures are required for some or all of the human populations/sub-populations considered. If this is not the case, even when risk reduction measures which are already being applied have been taken into account, and it is clear to the assessor that neither further exposure assessment nor further information on effects would be useful to refine the assessments then risk reduction measures will be needed. A clear and justified explanation of the need for risk reduction measures should be included in the report of the risk assessment.

Typically, further information on effects may be required when, after using all the relevant available data (including in particular data from toxicokinetics studies and human experience), it is not possible to extrapolate in relation to the human route and/or duration of exposure. Occasionally, also, it may be thought necessary to conduct further testing in order to refine the NOAEL value on account of the severity of the effect of concern. Further investigation of specific system/organ toxicity may be required: guidance on the toxicological principles to be followed is given in the relevant parts of this document.

When further testing is required for repeated dose toxicity, the possibility of combining this testing with required further testing in relation to another effect should always be considered as it may be possible to reduce the overall amount of testing in animals and the cost of testing. For example, it is often possible to obtain further information on repeated dose toxicity when conducting a study to investigate reproductive toxicity (i.e. from a study of longer duration than 28 days).

It is important for all further testing, but particularly when combined tests are to be conducted, that the test protocol is very carefully elaborated, taking into account also the requirements of Annex V test methods.

In the early stages of the notification of a new substance, some of the necessary elements may not be available (e.g. estimates may be available for inhalation exposure levels of humans, which have to be compared with data from a 28-day oral toxicity study, requiring the conversion of the airborne concentration of the substance to the equivalent $mg \cdot kg^{-1}.day^{-1}$). Professional judgement will be required to decide whether further information is required, immediately or later, to revise the assessment.

4.7 MUTAGENICITY

Substances are of concern in relation to mutagenicity if they have given positive results in at least one test considered to be relevant and to have been adequately conducted (see Section 3.2). Those which give positive tests in studies *in vivo* (in somatic cells or germ cells) and those for which human data may indicate potential genotoxicity/mutagenicity, are of particular concern.

Unless a threshold mechanism of action has been clearly demonstrated, it is considered prudent to assume that a threshold cannot be identified in relation to mutagenicity. As stated by IPCS (1994) "there is no clear consensus on appropriate methodology for the risk assessment of chemicals, for which the critical effect may not have a threshold, such as genotoxic carcinogens and germ cell mutagens". Risk characterisation has therefore to be conducted on a pragmatic basis in which account is taken of the pattern and extent of human exposure, what is known or

can be deduced about the relevance for humans of the effect observed in the studies (e.g. from toxicokinetics data), the potency of the substance and the risk reduction measures already in place.

Following evaluation of all the available data, the assessor may conclude that there is a need for limiting the risks. In this case, the risk assessment report should contain an explanation of the need for risk reduction measures in addition to those already in place. It may, however, be concluded that further information can be identified which, if obtainable, could be used to refine the risk assessment. This may be further information on exposure. Further data on effects may be required (in accordance with the toxicological principles given this document) to establish firmly the nature of the effects, or the lack of effects, if such data were not fully elaborated at the hazard identification stage. Further information may be sought on the macromolecular binding and/or metabolism of the substance in order to refine the interpretation of the data with respect to relevance for humans.

Sometimes it may be possible to identify a threshold mechanism of action (e.g. for induction of aneuploidy). In such cases the dose-response assessment (in particular, a N(L)OAEL value, if obtainable), can be used in the risk characterisation, as described for repeated dose toxicity.

4.8 CARCINOGENICITY

When it is clear that a substance is known, or very strongly suspected, to be carcinogenic by a genotoxic mechanism or a non-genotoxic mechanism risk characterisation should be carried out according to the methods indicated in the mutagenicity section or the repeated dose toxicity section, above, for respectively non-threshold- and threshold-mediated effects.

If the conclusion is finally reached that further information is required on effects, very careful consideration should be given to deciding what sort of information can most economically (in terms of animal usage, cost and time) be provided which would facilitate an adequate and appropriate risk assessment. For example, further toxicokinetics data may enable a decision to be reached without the need for a lifetime bioassay. In the event that a bioassay is required, a test protocol should be developed which permits acquisition of the maximum amount of information on the repeated dose toxicity of the substance by the route of exposure used.

4.9 REPRODUCTIVE TOXICITY

Usually, the various aspects of reproductive toxicity are considered to be effects with underlying dose threshold mechanisms and a NOAEL or LOAEL value should normally be provided from the available data, though the threshold dose for specific aspects of reproductive toxicity is not always easy to identify. In the rare case that a NOAEL has been derived from well-reported and reliable human data, it should be used for risk characterisation, but generally a value from a study conducted in animals will be used.

The risk characterisation should be carried out as described for repeated dose toxicity. Particular attention should be given to the relationships between dose/concentration and both adverse effects on reproduction and other systemic toxicity. In general, the criteria leading to the various possible results will be similar, as will the subsequent actions, to those given in the section on repeated dose toxicity.

However, the possible requirement for further testing is specifically addressed here: it should be noted that, unless the available studies on reproductive toxicity were conducted using a route of

exposure which was clearly inappropriate or inadequate and nothing useful can be derived from the data, it is not usual to require that further reproductive toxicity tests are conducted using another route of exposure.

In certain cases, for substances which are toxic to reproduction, further objectives may be identified following risk characterisation and it may be considered that, for risk assessment, further testing to meet these objectives is justified.

When it is known that genotoxicity is the underlying mechanism for the reproductive toxicity of a substance, it is prudent to assume that a threshold dose/concentration cannot be identified. In such cases, risk characterisation should be carried out using the approaches described for genotoxic substances in the previous two sections.

4.10 OTHER CONSIDERATIONS

Substances which are of low systemic toxicity, are only sparingly soluble in water and fat and which are inhalable dusts, or which can form inhalable dust during use, may contribute to the pulmonary overload phenomenon. This may result in adverse effects such as impairment of pulmonary clearance, lung fibrosis or tumour formation.

The available data indicate that pulmonary overload can be avoided by maintaining the atmospheric concentration of the substance below the numerical value of its specific gravity expressed as $mg \cdot m^{-3}$ (Morrow et al., 1991; Morrow, 1992).

4.11 PHYSICO-CHEMICAL PROPERTIES

Risk characterisation with regard to human health must be carried out for new substances which have been classified on the basis of certain physico-chemical properties (explosivity, flammability or oxidising potential), or if there are other similar reasonable grounds for concern and to which human exposure is possible. Existing substances need an assessment related to their physico-chemical properties.

An assessment should be made of the likelihood that an adverse effect will be caused under the reasonably foreseeable conditions of use in the workplace or by consumers.

It is expected either that the substance will be considered of no immediate concern (e.g. in the workplace the conditions of use, storage etc. are clearly influenced by knowledge of the properties of the substance; for consumers, the dilution of the substance and/or limited pack sizes may indicate that a conclusion of no immediate concern is justified); or that recommendations for risk reduction are necessary.

4.12 OVERALL INTEGRATION OF HUMAN HEALTH RISK CHARACTERISATION

More than one of the possible conclusions may be reached for a particular substance in relation to different properties of the substance, the different uses of the substance and/or the different human populations involved. This does not necessarily cause difficulty as some conclusions can coexist: a conclusion requiring that risk reduction recommendations are made in respect of a particular effect may render a requirement for further investigation of another effect superfluous, for example.

However, there may be initial requirements for a number of further tests, some immediately and some later. For reasons of animal welfare and cost, it is necessary to integrate such requirements.

It should be remembered that some tests can take a long time to conduct and report: risk reduction measures may have to be recommended with regard to the effect of potential concern in the interim.

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Appendix I Occupational exposure assessment process

Appendix IA Overview of occupational exposure assessment process

This summary provides an overview of the steps involved in carrying out an exposure assessment, as described in this section of the TGD. It does not provide all of the details needed for an assessor to actually perform the assessment. It should be used as an aide-memoir and should **not** be seen as a comprehensive description of the process.

Preparation

1. Based on the information provided by industry and available from other sources industries handling the substances are identified.
2. Industry submits core exposure information (see **Figure 1** (main text) - core information requirements) including descriptive information on exposures and available measurement data relating to inhalation, dermal and biological monitoring. Where insufficient information is submitted for data interpretation or modelling, industry should be approached for further details.
3. Identify scenarios from industry data involving potential exposure. Consider reasonable worst-case and typical exposures (work done under permit as described in Section 2.2.2.9 can be excluded).
4. Apply the preferential hierarchy in the use of the exposure data, viz:
   measured data, including quantification of key exposure determinants,
   appropriate analogous/surrogate data,
   modelled data (use only when measured data are inadequate or insufficient).
5. Take account of modifying factors for mixtures.
6. Report uncertainties in the data (measured and modelled) and the impact on interpretation for risk characterisation (see **Table 1** (main text) - EU workplace exposure assessment rating scheme).

Implementation

1a. Inhalation - measured data analysis presentation

- For each scenario provide:
  - qualitative description of exposure for reasonable worst case (RWC) and,
  - if possible, typical exposures;
  - number of samples;
  - number of facilities sampled;
  - usual frequency and duration of exposure
  - range of exposure results (units);
  - median of results;
  - 90^{th} percentile of results;
  - an evaluation of the representativeness of the data, if available.
    - take account of exposure attenuation from use of RPE when appropriate (only when there is sufficient information that appropriate RPE is consistently used) - use Assigned Protection Factors (BS4275).
    - explain uncertainties.

1.b. Inhalation - modelled data analysis presentation

- Where there is insufficient measured data available, implement modelling.
- Detail input parameters for each scenario.
- Take account of usual exposure control measures for modelling typical exposures.
- For RWC consider exposures where usual controls not in place.
- Explain outputs.
- Explain uncertainties.

2. Dermal

- Follow the dermal exposure assessment scheme (Appendix I E).
- Take account of protection afforded by gloves where glove permeation data to EN374 is available (producers and intermediate operations only - i.e. not downstream users)
- Assign a default value of 10% penetration for properly selected overalls and gloves, as appropriate.
- Assign modifiers to take account of reduced exposure due to:
    - volatility;
    - dustiness;
    - percentage of substance in mixture;
    - duration of tasks involving exposure;
    - engineering controls minimising substance release and therefore skin contact;
    - PPE.
- Explain outputs.
- Explain uncertainties.
- Corrosive materials do not need assessment. This also applies to strong irritants which may be treated as corrosives and the handling of hot products (>60^{o}C)

3. Biological monitoring

- Where biological monitoring data are available, use this as an indicator of total absorbed dose and effectiveness of exposure control measures.

4. Exposure levels taken forward to risk characterisation

- Summarise exposures:
    - reasonable worst case;
    - typical;
    - short-term and long-term exposures (inhalation and skin contact).
- If dusts or mists - expected particle size.
- Personal v static monitoring results.
- Biological monitoring.
- Conclusion as to what to take forward to risk characterisation together with narrative on uncertainty. Describe the source of the uncertainty and, if possible, quantify the uncertainty.

Appendix IB Concept of EASE

EASE is essentially a series of decision trees. For any substance, the system asks a number of questions about the physical properties of the substance, the circumstances of its use and measures used to control exposure. For any one question (apart from those asking for physical data such as temperature and vapour pressure), the EASE user must select from a number of representative categories. Once all the questions have been answered, the exposure prediction is determined absolutely by the choices made.

EASE may be used to estimate inhalation and dermal exposure. For dermal exposure, the model is based on very limited knowledge and should be used with some caution. The model works best when used by occupational hygienists, as a degree of interpretation of its output, based on judgement and experience, is usually necessary. It is possible, however, for other users to obtain useful results from EASE, as long as they are aware of its background and limitations.

EASE was designed to assist exposure assessment for both new and existing substances, by providing a means for predicting exposure where little or no measured data exists. It was developed specifically as a simple device for modelling exposure across a wide variety of circumstances encountered in workplaces. The model is concerned with exposures resulting from normal use of substances – it does not deal with exposures which result from foreseeable spillages, accidental loss of containment or breakdown of normally reliable control measures. These conditions will have to be considered separately if they arise.

Appendix IC Other Models

Inhalation exposure

EASE is not the only possible model. Several other models exist that may be useful, especially for assessing exposure in specific scenarios. Where exposure prediction models have been chosen in preference to those contained within the TGD, then the reasons for the choice, including those relating to the exclusion of TGD preferred models, must be stated.

The US EPA has developed several deterministic models that combine equations on the emission of vapours from sources with equations to model the concentrations in the area around the source. These models generally model area concentrations and not personal exposure levels. They are considered to be most useful in situations that are relatively easy to model by deterministic approaches. Two examples are the modelling of concentrations of vapours due to sampling from closed systems and due to loading of volatiles into drums or tankers. The resulting models require knowledge of several parameters that are generally not included in information for new or existing substances in Europe. However, some defaults (based on USA practices) are also provided by the US EPA. The equations are suitable for inclusion of probabilistic techniques to account for uncertainty or variability in parameters (Fehrenbacher and Hummel, 1996). In a comparative study of literature data with modelling by EASE and the US EPA equations for loading, the EASE model was found to be as good as, or better than the US EPA model (Beijer et al., 1998).

The EASE model clearly is not very applicable to assess inhalation exposure to non volatile substances in spray applications. The options for handling of solids do not apply, since the spray application generally relates to liquid products. The option “aerosol formation = yes” for volatile substances always leads to very high results that are not reached in practice for non volatile substances. A specific model was based on literature data for non volatile substances by TNO (De Pater and Marquart, 1999). This model, that is an elaboration of the general analogy approach, is based on measured exposure levels to poly-isocyanates in spray coating and was found to give reasonably good results if compared to measured data on “total solids” in spray coating.

Dermal exposure

The dermal route is not well studied. Limited knowledge exists on dermal exposure levels and their relation with exposure determinants. Therefore, the validity of dermal exposure models is also very uncertain.

The dermal exposure part of EASE is a model that is partly based on experiments done with liquids in the USA and partly on expert judgement to fill the results of that model into the general knowledge on dermal exposure of the builders on EASE, as well as into the structure of EASE.

Based on the same experiments, the US EPA has constructed their own exposure model. Both the US EPA model and EASE use the same very general equation:

$$D = Q \cdot S \tag{1}$$

Explanation of symbols

where:	D	=	(total) dermal exposure (mg)
	Q	=	quantity of a product adhering to the skin due to a task (mg/cm^2)
	S	=	the surface area of the skin exposed (cm^2)

The results provided in EASE are for the parameter Q, while the surface is provided in a small number of categories.

The US EPA originally used other values for Q, for activities categorised in the exposure routes "immersion" and "contact", based solely on the experiments done on behalf of the US EPA. More recently workers from the US EPA have come up with new values for Q (and S) after studying available literature on measured dermal exposure levels. A US EPA author provided the Appendix on dermal exposure in the AIHA book on exposure assessment (Mulhausen and Damiano, 1998). A working group of the US EPA later decided on using another Table (US EPA - CEB, 2000).

Appendix ID EASE Defaults for inhalation exposure for common industrial activities

Table 1 EASE defaults for inhalation exposure for common industrial activities

Task / Activity	Typical Inhalation Exposure	RWC Inhalation Exposure	Comments
Production & processing of powders	No aerosol formation Dry manipulation LEV present	No aerosol formation Dry manipulation LEV absent	
Production of liquid formulations	No aerosol formation Non-dispersive use LEV present	No aerosol formation Non-dispersive use Dilution ventilation	
Production of solid formulations e.g. paints, adhesives	No aerosol formation Dry manipulation LEV present	No aerosol formation Dry manipulation LEV absent	
Manual use of paints, lacquers without spraying	No aerosol formation Wide dispersive use Direct handling with dilution ventilation	No aerosol formation Wide-dispersive use Direct handling without dilution ventilation	Apply partial pressure based on paint composition to EASE output
Printing	No aerosol formation Non dispersive use LEV present	No aerosol formation Non-dispersive use Dilution ventilation	Apply partial pressure based on composition in ink to EASE output
Manufacture of a chemical Sampling - Filling	No aerosol formation Non dispersive use LEV present	No aerosol formation Non-dispersive use LEV absent	
Use of a chemical intermediate Charging & unloading reactors	No aerosol formation Non dispersive use LEV present	No aerosol formation Non-dispersive use LEV absent	
Maintenance of photocopiers	No aerosol formation Low dust technique LEV absent	No aerosol formation Low dust technique LEV absent	Requires correction to reflect % substance in toner.
Manufacture of a solid (bagging & filling)	Low dust technique Direct handling LEV present	Dry manipulation Direct handling LEV absent	

Appendix IE An approach for dermal exposure assessment

Dermal exposure has not yet been studied in great detail for industrial chemicals. Only limited data are available. The EASE model contains a section on dermal exposure. This section is based on a (very limited) number of experiments with a few liquids, that did not really mimic true exposure situations and on experience and expert judgement. Because there are uncertainties relating to both the measured data and the EASE model an approach has been established to use all available information to the largest extent possible. This approach is shown diagrammatically in **Figure 2** (main text).

The elements of the approach are described briefly below. The numbering of the remarks refers to the numbers in **Figure 2**.

1. Relevant exposure scenarios are derived from the available information. Exposure is originally estimated for the product handled. In this approach "product" can be the pure substance, a formulation or a product containing the substance.

2 If the product handled is corrosive or hot (>60˚C), it is assumed that exposure will only occur very rarely by accidental contact.

3. If there are specific measured estimates for the relevant exposure scenario in **Table 2**, these are used as the basis for the estimation. Use EASE, if the exposure scenario to be assessed is not very similar to a scenario in **Table 2**. The data in **Table 2** can be used as an additional source of information if the measured exposure scenario has some similarity with the scenario to be assessed.

4. If possible, default assumptions are used with EASE. These assumptions are provided in **Table 3.**

5. If there are no relevant default values in **Table 3** for the scenario being assessed, use EASE with 'ad hoc' assumptions. The data and defaults in **Tables 2** and **3** may provide some guidance for the choice of the 'ad hoc' assumptions.

6. The results in **Table 2** and the default assumptions in **Table 3** are based on a scenario with specific values for important exposure determinants. If the relevant exposure determinants are substantially different in the situation to be assessed, a modification of the results of **Table 2** or **Table 3** is necessary. Factors that are considered very relevant exposure determinants are: amount of product handled; duration of the tasks; percentage of substance in product and volatility of the substance.

Substantially higher amounts, duration or percentage lead to higher exposure levels, while substantially lower amounts, duration or percentage lead to lower exposure levels. It should be realised that there are limits to the possible adherence of substance to the skin. These limits, according to a review made for the US EPA, are between 10 and 14 mg/cm^2 (SAIC, 1996).

Volatile substances will (partly) evaporate from the skin during the exposure process, thereby leading to lower than estimated exposure levels. A possible approach is given in Appendix IF.

Dustiness and particle size of powders also influence the dermal exposure levels. The size and direction of the influence of these parameters is not well-known and may vary from process to process. The values in **Table 2** are therefore of very low confidence for substances with very high dustiness or very low dustiness.

Engineering controls may also influence dermal exposure. A good local exhaust ventilation should remove aerosols before they reach the worker. The effect of this parameter on dermal exposure is not well-known. If the hands are very close to the source or between the source and the exhaust inlet, the effect will be low. However, data from situations with no local exhaust ventilation (e.g. painting of boats) may not be relevant for situations with good local exhaust ventilation (e.g. painting of small parts in a local exhaust cabinet).

Results in **Table 3** are provided as results for pure substances. If the product handled is not the pure substance assessed, the results for the product should be recalculated to results for the substance by multiplication with the fraction of substance in the product.

Dermal exposure is generally assessed for hands and forearms only (2,000 cm^2), with exposed skin surfaces ranging from 210 cm^2 (equivalent to half of one hand) to 2,000 cm^2. Hands and forearms are, by default, considered not to be covered by gloves or clothing. Exposure of other skin surface areas may be relevant. In these cases a clear reasoning should be given for the need to deviate from the standard approach. A possible example of this kind of situation is the use of a product by overhead spraying, in which case the face may be exposed. If a scenario leads to very substantial contamination of normal working clothes, assessment of other body parts than hands and forearms may also be warranted.

Although other body parts have been shown to be exposed in several studies, it is assumed that these parts will be covered and that possible contamination of the skin under the cover (clothing) is relatively minor compared to the contamination of hands and forearms. If hands and forearms are known to be covered by gloves or clothing, an additional assessment of dermal exposure underneath the cover can provide useful information. In such an additional assessment, expert judgement should be used to assess the appropriateness and the correct use of the cover, as well as the protecting effect. Very limited data exist on the protective effects of gloves. Reported practical reductions in dermal exposure by gloves vary widely from less than 75% to more than 99%. The exact way of using (and taking off and possibly re-using) of gloves probably is a very important determinant of their effectiveness.

In **Table 2**, estimates of dermal exposure to hands and forearms based on results of measurements are shown. Not all of the estimates are taken directly from measured data. To allow comparison of data sets, extrapolations have in some cases been made, e.g. extrapolation to a longer period of exposure or a lower amount of product handled. Values in the range of the RWC levels are probably related to relatively high amounts of product, duration of task or area treated within the measurement series, while values in the typical range are probably more related to the central tendencies of these exposure determinants. Measurement methods in the data sets include hand washing, glove sampling and fluorescent tracer measurements. These methods are not harmonised and their performance is not well studied. The data provide an indication of possible exposure levels in the type of activity with the type of substance and the type of amounts handled in the study. They cannot be considered fully representative for a specific scenario as they are generally based on one or two studies per scenario with only one or two substances. Most studies contain only limited numbers of measurements. However, the data are real for the scenario described and are should be considered together with results of the EASE model.

Concise descriptions of the data sets and situations are provided in the notes below **Table 2.** These descriptions should be compared to the situation to be assessed for evaluation of similarity. In **Table 2** the RWC values are based on (estimates) of the 90^{th} percentiles of the measured data and the typical values on the median or the mean.

A number of default assumptions for often-assessed exposure situations are provided in **Table 3**. If the scenario under discussion cannot be assessed based on **Table 2,** these defaults may be helpful. They are based on expert judgement. The main purpose of **Table 3** is to provide consistency in the assessments and to limit unnecessary discussion. If the situation to be assessed is not covered by either **Table 2** or **Table 3**, the use of the EASE model is preferred. 'Ad hoc' input parameters should be chosen and a transparent explanation should be given for the choices made.

Table 2 Summary of dermal exposure estimates for specific exposure scenarios before taking account of modifying factors

No.	Activity	Assumed exposed skin surface area [a] (cm^2)	Reasonable worst-case exposure		Typical case exposure		References
			Total [b] (mg)	Per surface area [c] (mg/cm^2)	Total [b] (mg)	Per surface area [c] (mg/cm^2)	
1	Spray painting (large areas)	840	10,000	12 [d]	2,500	3	Lansink et al., 1998; HSE, 1999
2	Low pressure spray application (e.g. of biocides and non-agricultural pesticides)	840	12,000	14 [d]	4,000	4.8	Preller and Schipper, 1999; HSE, 1999
3	Brushing and rolling of liquids	840	10,000	12 [d]	1,700	2	Guiver et al., 1997; Guiver and Foster, 1999; Roff, 1997
4	Lay up activities with pre-impregnated cloths in the aerospace industry	1,200	200	0.17	40	0.03	Boeninger et al., 1992
5	Handling bags in a formulating facility	1,600	1,100	0.7	500	0.3	Lansink et al., 1996
6	Dumping of powders (packaged in bags) in a formulation facility	1,600	3,000	1.9	900	0.6	Lansink et al., 1996
7	Removal of cake from a filter press	2,600	1,500	0.6	100	0.04	Marshall et al., 1992
8	Loading of trays of a tray dryer	2,600	160	0.06	85	0.03	Marshall et al., 1992

a) Skin surface area exposed is generally taken from the original papers and usually refers to the sampling area or to the area that the original authors consider their results applicable. True exposed skin surface area may be substantially lower, but in that case the exposure per surface area is substantially higher.

b) The total exposure to the full product generally is the result of recalculation of the measurement results to take account of the percentage of measured substance in the full product. In several cases the original authors presented exposure per skin surface area. In those cases the total exposure is calculated by multiplication with the exposed skin surface area as provided in the third column of Table 2. In other cases the original authors presented the total exposure.

c) The exposure per skin surface area to the full product generally is the result of recalculation of the measurement results to take account of the percentage of measured substance in the full product. In several cases the original authors presented total exposure. In those cases the exposure per skin surface area is calculated by dividing by the exposed skin surface area as provided in the third column of Table 2. In other cases the original authors presented the exposure per skin surface area.

d) These values are around the maximum adherence that is found to be possible, according to a review (SAIC, 1996), and should therefore be used with great care. Calculated adherence values for products higher than the maximum adherence can be reached. Values for products are usually extrapolated from measured values for ingredients of products. In the extrapolation, it is assumed that the whole product adheres to the skin. In practice, part of the product may evaporate (e.g. water, or an organic solvent). This part does not contribute to the *actual* total adhered amount. This *actual* adhered amount may thus still be far below the maximum adherence (if a large part of the product evaporates), when the *calculated* amount (including the evaporated part) is higher than the maximum adherence.

1 Application of paint by airless spraying to relatively large areas

Exposure is due to deposition of spray mist, back bouncing, contact with contaminated spray gun and possibly also with freshly painted surfaces. The estimates are based on an experimental study in 3 off-shore facilities where containers were painted (Lansink et al., 1998) and on studies by HSE and IOM on airless spray application of antifouling paint (HSE, 1999). The Lansink et al. (1998) study involved 12 painters, using 3 – 13 l of paint with a duration of 4 -21 minutes. A fluorescent tracer was added at 0.0074% (w/w). Exposure levels were presented based on the tracer and a linear extrapolation of exposure related to duration (3 hours, in which 150-200 l could have been applied) was done to compare the study with the other studies. The HSE compilation included a total of 70 exposure data provided by 18 separate surveys. The amounts of paint used during spray sessions in the HSE document ranged between 25 and 800 l and the spray session ranged from 40 to 360 minutes (median about 180 minutes). The 90^{th} percentile of the extrapolated results of Lansink et al. (1998) and the 95^{th} percentile of the HSE data were used as a basis for the RWC estimated.

2 Spray application of biocides and non-agricultural pesticides

Exposure is due to deposition of spray mist and contact with treated surfaces. Based on a study in the meat processing industry (Preller and Schipper, 1999) and on data of public hygiene pesticide spraying (HSE, 1999). This is low pressure spraying. In the HSE (1999) data some mixing and loading may be included. In the study by Preller and Schipper (1999) measurements were done in 10 companies on 15 workers. Data from nine workers who worked without gloves were used in this assessment, since the measurement method was hand washing. The amount of spray liquid used is not presented. The duration of spraying was between 7 and 108 minutes. Data from one worker who used an exceptionally low concentration of active ingredient (leading to an exceptionally high value for exposure to the product) were excluded. HSE (1999) included data of liquid spraying of 1 – 20l (n = 71) and insecticidal dusting using 60 - 2,200 g dusting powder (n = 15). Typically, the task took less than 2 hours per day. The 90^{th} percentile of the data by Preller and Schipper (1999) and the 95^{th} percentile of the data by HSE (extrapolated from the unit of mg/minute to two hours) were used as a basis for the estimate of the RWC. The medians were used as a basis for the typical value.

3 Application of liquids by brushing and rolling

Exposure due to contaminated tools and splashing of (small) droplets, as well as direct contact with freshly painted surfaces. Based on experimental study of consumers applying wood preservatives (Roff, 1997) and field study of consumers applying anti-fouling paints on their boats (Guiver et al., 1997; Guiver and Foster, 1999). Roff (1997) studied water-based fluids and spirit-based fluids and people working with and without gloves. The results of workers without gloves (n = 7) are relevant, because they represent exposure without PPE. Spirit-based fluids resulted in higher exposure levels. Environmental temperature had a marked influence on the results. Measurements were done with temperatures between 13 and 26.5^{o}C for 0.5 - 1 h with 1 - 2.5 l fluid. Based on regression analysis, Roff calculated a RWC value at 15˚C. For the purpose of this approach this value was recalculated from volume to mass, assuming a mass/volume ratio of 0.8 for the spirit based fluids. Guiver et al. (1997) and Guiver and Foster (1999) studied 10 amateurs applying antifouling paint by roller and brush. Their measurements were done by sampling gloves, underneath any protective gloves used by the amateurs. Only 2 of 10 amateurs did not use gloves, but one used previously used gloves. The amateurs applied 1- 2 coats in 1- 2 hours and used 1 - 7 l of paint. The

extrapolated result of Roff (at 15˚C) for workers without gloves was used as a basis for the RWC estimate. This value compared reasonably with the values measured by Guiver et al. (1997) for amateurs painting boats without gloves or with previously used gloves. The median value presented by Roff (1997) for spirit-based fluids and no gloves was used as the basis for the typical value.

4 Lay-up activities in the use of advanced composites in the aerospace industry

Fibre cloths are impregnated with MDA or pre-impregnated cloths are used. The impregnated cloths are sewn, cut (sometimes manually) and laid up manually. Exposure is due to direct contact with the impregnated cloths. Measurements were done in three facilities. The cloths were impregnated with formulations containing 12 – 17.5% MDA (w/w). In the extrapolation for **Table 2**, the values for MDA on one glove are multiplied by two and recalculated to total formulation assuming 12% of MDA in the formulation. A total number of 24 dermal exposure measurements (outside and inside of gloves) are included in the derivation of the estimate. A few more measurements were done but in these cases either one or both of the parameters was not reported. Duration of measurements was 0.5 - 6.3 hours. The amount of cloth handled is not reported (Boeniger et al., 1992). The approximate 90th percentile of the data is used as the basis for the RWC value, while the median is used as the basis for the typical value. These values are based on only one study (though in three facilities) and they may therefore be less representative for the scenario than the other values.

5 Handling bags in a formulating facility

Field study including gathering filled (closed) bags in the warehouse (n = 12) or discarding of emptied bags (n = 14) in ten paint producing facilities (Lansink et al., 1996). The bags were delivered to the facilities on pellets. Duration of the activities was 1 - 7 minutes and in that time 1 - 24 bags were handled. Exposure was due to contact with contaminated surfaces, mostly the contaminated outside of the bags. The measured substance was calcium carbonate in several grades. Calcium carbonate is a relatively dusty powder. Because of the similarity in exposure values the two activities are combined in this approach. The 90th percentile of the data is used as the basis for the RWC value and the median as the basis for the typical value. These values are based on only one study (though in ten facilities) and they may therefore be less representative for the scenario than the other values.

6 Dumping of powders (in bags)

Field study including manual dumping of calcium carbonate (several grades) from bags of into paint mixers in ten paint producing facilities (n = 19; Lansink et al., 1996). Calcium carbonate is a relatively dusty powder. The dumping lasted for 1 - 15 minutes and 2 - 24 bags, containing 10 - 1,000 kg calcium carbonate were dumped. Local exhaust ventilation was generally used during dumping. Bags were cut open using a knife and the powder was allowed to flow into the mixer. Exposure is due to direct contact with the flow of powder, deposition of the dust and contact with contaminated surfaces, including the outside of the bags. The 90th percentile of the data is used as the basis for the RWC value and the median as the basis for the typical value. These values are based on only one study (though in ten facilities) and they may therefore be less representative for the scenario than the other values.

7 Removal of cake from a filter press

Experimental study including removal of wet filter cake (calcium carbonate) from a filter press (Marshall et al., 1992). Batches of approximately 60 - 73 kg each (expressed as dry weight) were removed from the press per event in 0.9 to 1.2 hours. Exposure was due to direct contact with the dust when spilled in scooping from the filter and due to contact with contaminated surfaces. A total of 8 measurements were available. The original authors added the results of the two batches together for an estimate of "full shift" exposure. This is not done in **Table 2**: all results were seen as separate (independent) measurements. Because of the low number of measurements, the RWC value is based on the highest value. The typical value is based on the median (Marshall et al., 1992). These values are based on only one experimental study and they may therefore be less representative for the scenario than the other values.

8 Loading of trays of a tray dryer

Experimental study including loading of wet cake (calcium carbonate) onto the trays of a tray dryer (Marshall et al., 1992). Exposure was due to direct contact with the dust when scooping the cake onto the dryer and due to contact with contaminated surfaces. A total of 8 measurements were available. In 8 - 34 minutes, 75 - 132 kg of cake was loaded onto the trays. Because of the low number of measurements, the highest value is taken as a basis for the RWC case value. The median is taken as a basis for the typical value (Marshall et al., 1992). These values are based on only one experimental study and they may therefore be less representative for the scenario than the other values.

Table 3 Default assumptions of reasonable worst-case dermal exposure for a number of exposure scenarios to be assessed by EASE before taking account of modifying factors

Activity	Assumed exposed surface area (cm^2) [a]	Contact level [b]	Use pattern [c]	Estimated exposure level ($mg/cm^2/day$)	Estimated exposure (mg)
All activities with gases, vapours or non-dusty solids	n.r.	n.r.	n.r.	negligible	negligible
Manual addition of liquids	420	Incidental / Intermittent	non-dispersive	0.1 1	42 420
Coupling and decoupling of a transfer line	420	Incidental	non-dispersive	0.1	42
Quality control sampling of liquids	210	Incidental	non-dispersive	0.1	21
Drumming of liquids	210	Intermittent	non-dispersive	1	210
Automated weighing and addition	negligible	No direct contact	Closed system	negligible	negligible
Quality control sampling of solids	210	Incidental/ Intermittent	non-dispersive	1	210
Drumming or bagging of solids	420	Intermittent	non-dispersive	1	420
Powder coating	840	Intermittent	non-dispersive	1	840

a) The surface area exposed is generally assumed to be equivalent to half of one hand or one hand, except for powder coating, that may lead to larger areas exposed

b) Generally, less than 10 contacts per day are assumed, but in several situations more than one contact is to be expected; this factor may be changed, based on further information

c) The activities in Table 3 generally do not lead to wide spread emission of substance and are generally done by trained workers in the chemical industry. Therefore, non-dispersive use is chosen for most activities. Pattern of control is always assumed to be 'direct handling', except for automated weighing and addition.

Appendix IF Evaporation rate

For the purpose of determining the evaporation rate of a substance, an equation can be used which was derived within the framework of a research project (Weidlich and Gmehling 1986; Gmehling et al., 1989). This project was aimed at calculating airborne concentrations of substances when emitted from liquid mixtures taking into account the evaporation and the spreading of the substance at the workplace. To calculate the evaporation times of substances, an equation was derived based on the mass transfer at the interface between the liquid and the vapour (two-film-theory). Mass transfer during evaporation occurs until the equilibrium state is achieved. The main influence on evaporation is the transfer through the interface.

For pure substances, the following equation is used:

$$t_{(s)} = \frac{mRT}{M\beta pA} K \qquad (2)$$

Explanation of symbols

t:	time		[s]
m:	mass, EASE estimate,		[mg]
R:	gas constant:	8.314	[J · K^{-1} · mol^{-1}]
T:	skin temperature		[K]
M:	molar mass		[g/mol]
β:	coefficient of mass transfer in the vapour phase [m h^{-1}], for calculation: β = 8.7 m/h, see below		
p:	vapour pressure of the pure substance		[Pa]
A:	area, EASE:		1 cm^2
K:	conversion factor:		3.6 · 10^4

The skin temperature is normally 28 – 32°C (ambient temperature: 20 – 22°C). The reduction of the skin temperature and accordingly of the vapour pressure caused by the evaporation process is not considered in the equation. This could be done by choosing a lower mean temperature for the evaporation process. For calculating the evaporation time of the substance in contact with gloves, a temperature of 20 °C is chosen.

The coefficient of mass transfer β is described based on empirical studies:

Explanation of symbols

β =	(0.0111 · v$^{0.96}$ · D$_g$$^{0.19}$) / (v$^{0.15}$ · X$^{0.04}$)	
D$_g$:	coefficient of diffusion, gas phase	
v:	velocity of air	[m/h]
ν:	kinematic viscosity of air	[m²/h]
X:	length of the area of evaporation in the direction of the air stream	[m]

In the above given equation, the main influencing parameter is the velocity of the air (v). At workplaces v is often between 0.3 m/s and 0.6 m/s. Since the hands, from which a substance evaporates, are often in motion, the air velocity might be higher. For a conservative approach, the lower value (0.3 m/s) was chosen.

For different organic solvents, D_g is approx. 0.05 m^2/h. Using the range 0.03 – 0.06 m²/h, Dg$^{0.19}$ ranges between 0.51 and 0.58. A literature value was taken for the kinematic viscosity of air (5.4396 · 10^{-2} m²/h). The parameter X, represents the length of the area of evaporation in the

direction of the air stream [m] does not influence the outcome a lot because of its low exponent (0.04). For the calculation, a length of 10 cm can be used. Taking into account a rather low velocity of air (0.3 m/s), β is about 8.7 m/h. This value corresponds well with experimental values for similar substances: For ethyl acetate, β amounts to 8 m/h (air velocity 0.31 m/s) and for butyl acetate, a value of 9.2 m/h (air velocity 0.31) was obtained.

In **Table 4**, calculated evaporation times for different substances are given. The values should be regarded as representative of the order of magnitude, since it is not known in how far the interaction of the skin with the substance influences the evaporation time. The error caused by this interaction is regarded to be higher than the one caused by the uncertainty of the calculation of β. For different substances (7 substances were investigated) β differs about ±5%.

Table 4 Calculated evaporation times for T = 20°C (gloves) and T = 30°C (skin)

Substance	Molar mass	Temperature [°C]	Vapour pressure [Pa]	Time [s] (m = 1 mg) [1)]	Time [s] (m = 5 mg) [2)]
Ethyl benzene	106.2	20	930	102	511
		30	1,600	61	307
n-Propanol	60.1	20	1,930	87	435
		30	3,600	48	241
Toluene	92.1	20	2,780	39	197
		30	4,520	25	125
Benzene	78.1	20	9,970	13	65
		30	15,780	8	42
Cyclohexane	84.2	20	10,300	12	58
		30	16,200	8	38
Methyl acetate	74.1	20	22,580	6	30
		30	35,380	4	20

1) Upper value of EASE estimate: non dispersive use, contact level: intermittent
2) Upper value of EASE estimate: non dispersive use, contact level: extensive, or: wide dispersive use, intermittent

Appendix IG Assessment of partial vapour pressure for components in multi-substance preparations

If the substance under assessment is a component of a mixture, the corresponding partial vapour pressure may depend on the composition. Since the vapour pressure is decisive in the first steps of a quantitative exposure assessment, this may lead to inappropriate estimates, which may not be corrected later in the assessment process.

In order to clarify the influence of vapour pressure when components in multi-substance preparations evaporate from the liquid, investigations were carried out by Krafczyk et al. (2000). The results demonstrate that in many cases a simple prediction of volatility based on Raoult (2000)'s law would be incorrect. In addition, activity coefficients have to be included when partial vapour pressures are calculated.

The impact of omitting activity coefficients to calculate partial vapour pressures depends predominantly on the specific composition. Using typical mixtures from various areas the resulting error reached one order of magnitude (factor 10). However, the deviation from the correct result may be aggravated, if small amounts of dangerous substances in a solution were considered. In these cases activity coefficients may be very high. This can lead to totally different concentrations of substances in air as compared to the calculation assuming *ideal* circumstances as activity coefficients are neglected. For example, in benzene/water-mixtures the benzene vapour concentration can be 3,000 times higher compared against the ideal case. In general a calculated theoretical concentration simply based on molar fraction (Raoult's Law) and vapour pressure would underestimate the realistic evaporation. In addition, the use of weight percentage instead of molar fraction may also add errors.

If liquids form a non-ideal mixture a variable correction factor, γ (the activity coefficient) should be applied, so that:

$$Pn = y_n X_n P_n' \qquad (3)$$

Where P_n is the partial pressure of component n, γ_n the activity coefficient of component n, x_n the mole fraction of component n, P_n' the vapour pressure of the pure component n.

There are several methods of estimating the activity coefficient of which UNIFAC (Lyman et al., 1982; Lohman et al., 1998) is currently widely accepted. The basic idea of UNIFAC is that, whereas there are thousands of organic compounds, the number of the functional groups that constitute these compounds is much smaller. UNIFAC has the advantage of being able to form a very large number of molecules from a relatively small set of structural units. Calculation of activity coefficients from group contributions is quite laborious but can easily be automated by means of computer programs. A user-friendly UNIFAC activity coefficient calculator is available on the internet (Choy and Reible, 2001; http://www.hsrc.org/hsrc/html/ssw/ssw-downloads.html).

Appendix II Consumer exposure

1 INTRODUCTION

This appendix gives an overview on data and other useful information to be used for estimations of consumer exposure and the algorithms for model estimations. The appendix first gives a description how scenarios may be defined (Section 2). Subsequently the algorithms are given that can be used to perform a quantitative exposure assessment (Section 3). These algorithms are primarily applicable to the estimations described in Section 2.3.6 of the human health chapter. For more complex evaluations of exposure (c.f. Section 2.3.9), the assessor has to consider the complexity of the scenario and its respective model. Computer programs that include these algorithms are summarised in Section 4. Section 5 provides relevant anthropometric data and information on use and composition of certain product categories. Finally, in Section 6 a list of valuable sources for consumer exposure information is provided.

2 CONSUMER PRODUCT CATEGORIES

As pointed out in Section 2.3.5 in the initial screening the consumer product categories in which substances of interest occur have to be identified. **Table 1** provides a list of categories that can be used for that purpose. This list is not an official list but a first attempt to build one. Further discussion at international level is needed to agree on a harmonised categorisation.

Table 1 Product category list

Category of Product/ general characterisation	Subcategories
CLEANER / POLISH The category covers all products that are used in the household for cleaning, polishing and care. Some subcategories can be defined by different use characteristics. A comprehensive overview on household cleaners and its subcategories has been published by the IKW (IKW,2001)	• Cleaning of machines and vehicles (e.g. cars, bikes, motorbikes) • General household (All Purpose Cleaners) • Dish washing, manual • Dish washing, machine • Sanitary cleaners • Textile cleaners e.g. Powder Laundry Detergents, Laundry Liquid Detergents • Oven cleaners • Shoe and leather cleaner • Furniture cleaners • Drain cleaners • Metal cleaners
ADHESIVE / SEALANT The category covers all products that are used in the household as adhesives or sealants. Some subcategories can be defined by different use characteristics (The list of subcategories of adhesives has been prepared by the WHO/IPCS).	• General purpose adhesive • Floor covering adhesives • Dental plate cement • Fabric adhesive • Film cement, photographic • Leather adhesive • Metal adhesive • Paper adhesive • Plastic adhesive • Rubber adhesive • Wallboard joint cement • Wallpaper adhesive • Wood adhesive
PRINTING / WRITING MATERIAL The category covers all products that are used in the household for writing and printing. Some subcategories can be defined by different use characteristics: (The list of subcategories of adhesives has been prepared by the WHO/IPCS).	• Dye • Ink • Etching fluid • Correction fluid • Crayon • Pen marker • Toner

Category of Product / general characterisation	Subcategories
PAINTING MATERIAL AND ADDITIVES The category covers all products that are painted to an area for renewing, or to protect the areas against wetness or corrosion etc. Some subcategories can be defined by different use characteristics. The classification of subcategories has been prepared according to Baumann & Muth (1997) and Bremmer and van Veen (2000a).	• Solvent based paint • Water based paint • Resin based paints • Aerosol paints • Paints for special purposes • Industrial paints • Varnish • Bleaching paints • Paints for conservation • Thinner • Paint remover
FUELS This category covers products that are used for feed machines (cars, motorbikes) or lamps or to lighten fires.	• Gasoline • Fuel oil • Liquid lamp oils and grill lighters • Solid grill lighters • Solid lighteners, other
BLEACH / DISINFECTANT / STERILIZER The category covers all products that are used in the household as a bleach or for sterilisation. Some subcategories can be defined by different use characteristics.	• Bleaches • Sterilisers
REMOVERS The category covers all products that are used in the household to remove substances, from surfaces and thus cleaning them. Some subcategories can be defined by different use characteristics:	• Adhesive/glue remover • Dye/ink remover • Seal remover • Polish remover • Limescale remover/descaler • Oil/grease remover • Rust remover • Stain remover • Wall paper remover
PHOTOGRAPHIC CHEMICAL The category covers all products in the household that are referred to photography. Some subcategories can be defined by different use characteristics:	• Photographic chemicals • Photographic paper
TEXTILE CHEMICAL The category covers all products that are exposure related to the use of textiles. Some subcategories can be defined by different use characteristics.	• Textile colours/dyes • Emission from textiles • Residues from cleaning textiles • Fabric softeners • Fire protecting agents in textiles
VEHICLE MAINTENANCE The category covers all products that are used in the household to make vehicles (cars, bikes, motorbikes, caravans, boats etc.) ready for use. Cleaning is covered by the category "cleaner/polish". Some subcategories can be defined by different use characteristics:	• Lubricants • Repairing material • Antifreeze (vehicle) • Screen wash • Brake fluid • Fuel additive • Radiator fluid • Transmission fluid
COSMETIC / PERSONAL HYGIENE PRODUCT The category covers all products that are used in the household to clean and care the body in particular e.g. hair and skin. Some subcategories can be defined by different use characteristics. Categories of cosmetics are extensively described by the compilation of cosmetic frame formulations (COLIPA, 2000). For composition of cosmetic products and for further use levels see also section 4.	• Rinse off products (e.g. Hand Dishwashing Liquids) • Non-rinse products • Spraying • Products that can contact mucous membranes
CONTAMINATION OF FOOD The category covers exposures that can be referred to the consumption of food. In particular, it is referred to contaminations of food and is subcategorised to the different kinds of food. Most of the data referring to this type of exposure are available from food surveillance studies (e.g. BGVV, 1995).	Categories of food consumption should be taken according to the EFG food grouping system (EFCOSUM, 2001). • Contamination of food by processing and packaging material
AIR CONTAMINANT / POLLUTANT The category covers all exposures that are referred to the emission of chemicals from materials in the household except textiles	• Furniture chemicals • Building chemicals • Emissions from vehicles (e.g. cars)
TOY / JOKE / CHILDREN'S PLAYTHING (Bremmer and van Veen, 2001)	

Category of Product/ general characterisation	Subcategories
OTHER CATEGORIES NOT MENTIONED OTHERWISE	• Refrigerant, coolant • Solvent • Water softener • Aerosol propellant • Aquarium product • Art/craft material • Deodorizer/air freshener • Sports product • Swimming pool product • Waterproofing compound • Agricultural products other than pesticides • Medical devices • Piercings

3 MODELS FOR CONSUMER EXPOSURE ASSESSMENTS

3.1 SIMPLE MODEL APPROACHES

This section provides the exposure models that can be applied to perform the quantitative exposure assessment as described in Section 2.3.6 of the human health chapter. Simple algorithms are given that are useful for calculating the exposure from consumer products via inhalation or the dermal or oral route. Basically five different sets of equations are given representing five different exposure scenarios:

- **inhalation:** a substance that is released as a gas, vapour or airborne particulate into a room (e.g. a component of an aerosol insecticide, a carrier/solvent in a cosmetic formulation, a powder detergent). Release may be the result of direct release as a gas, vapour or particulate, or by evaporation from liquid or solid matrices. In the latter case, the equation represents a worst-case situation by assuming that the substance is directly available as a gas or vapour;
- **dermal (1):** a substance contained in a medium. This dermal scenario also applies to i) a non-volatile substance in a medium used without further dilution and ii) a non-volatile substance in a volatile medium;
- **dermal (2):** a non-volatile substance migrating from an article (e.g. dyed clothing, residual fabric conditioner, dyestuff/newsprint from paper);
- **oral (1):** a substance in a product unintentionally swallowed during normal use (e.g. toothpaste);
- **oral (2):** a substance migrating from an article into food or drink (e.g. plastic film, plastic-coated cups/plates).

In addition Sections 3.1.4 and 3.1.5 provide information on how to deal with acute vs. chronic exposures and how to calculate the total uptake from a product via different routes.

Note that the algorithms that are presented in the following sections have been implemented in the EUSES computer program (EUSES, 1997) and in CONSEXPO (van Veen, 2001).

3.1.1 Inhalation exposure

A substance that is released as a gas, vapour or airborne particulate into a room (e.g. a component of an aerosol insecticide, a carrier/solvent in a cosmetic formulation, a powder detergent)

Release may be the result of direct release as gas, vapour or particulate, or by evaporation from liquid or solid matrices. In the last case, the equation represents a worst-case situation by assuming that the substance is directly available as a gas or vapour. The equation applies to both volatile substances and airborne particulates. When the inhalable and/or respirable fraction is known, it should be taken into account. It should be remembered that the non-respirable fraction can be swallowed and oral exposure may also need to be considered (see equations 10 and 11, below). The concentration in air after using an amount Q_{prod} of the product becomes:

$$C_{inh} = \frac{Q_{prod} \cdot Fc_{prod}}{V_{room}} \quad (1)$$

The air concentration C_{inh} results in an inhalatory intake of:

$$I_{inh} = \frac{F_{resp} \cdot C_{inh} \cdot IH_{air} \cdot T_{contact}}{BW} \cdot n \quad (2)$$

Explanation of symbols

Q_{prod}	amount of product used	[kg]	
Fc_{prod}	weight fraction of substance in product	[kg · kg_{prod}^{-1}]	
V_{room}	room size	[m^3]	
F_{resp}	respirable fraction of inhaled substance	[-]	1
IH_{air}	ventilation rate of person	[$m^3 \cdot d^{-1}$]	
$T_{contact}$	duration of contact per event	[d]	
BW	body weight	[kg]	cf Section 5.2.1
n	mean number of events per day	[d^{-1}]	
I_{inh}	inhalatory intake of substance	[kg · $kg_{bw}^{-1} \cdot d^{-1}$]	
C_{inh}	concentration in air of room	[kg · m^{-3}]	

It should be noted that for short-term local exposure the value for V_{room} could be reduced (e.g. to 2 m^3) to represent the volume of air immediately surrounding the user.

Inhalation exposure can also occur to a substance that is released relatively slowly from a solid or liquid matrix (e.g. solvent in paint, plasticiser or monomer in a polymer, fragrance in furniture polish). This is further described in Section 3.2.2.

3.1.2 Dermal exposure

Dermal (1): A substance contained in a medium

The concentration in the product as used can be calculated using the following equation. Depending on how the parameters are provided, three analogous calculations are used:

$$C_{der} = \frac{C_{prod}}{D} = \frac{\varphi_{prod} \cdot Fc_{prod}}{D} = \frac{Q_{prod} \cdot Fc_{prod}}{V_{prod} \cdot D} \quad (3)$$

The total amount to which the skin is exposed is then given by (two options, depending on format of available data):

$$A_{der} = C_{der} \cdot V_{appl} = C_{der} \cdot TH_{der} \cdot AREA_{der} \quad (4)$$

The potential uptake per kilogram body weight per day is derived as:

$$U_{der,pot} = \frac{A_{der} \cdot n}{BW} \quad (5)$$

Explanation of symbols

C_{prod}	concentration of substance in product before dilution	[kg · m^{-3}]	
D	dilution factor	[-]	
RHO_{prod}	density of product before dilution	[kg · m^{-3}]	
Q_{prod}	amount of product used	[kg]	
Fc_{prod}	weight fraction of substance in product before dilution	[-]	
V_{prod}	volume of product used before dilution	[m^3]	
V_{appl}	volume of diluted product actually contacting the skin	[m^3]	
TH_{der}	thickness of product layer on skin	[m]	10^{-4}
$AREA_{der}$	area of contact between product and skin	[m^2]	
BW	body weight	[kg]	cf Section 5.2.1
n	mean number of events per day	[d^{-1}]	
C_{der}	dermal concentration of substance on skin	[kg · m^{-3}]	
A_{der}	amount of substance on skin per event	[kg]	
$U_{der,pot}$	amount of substance that can potentially be taken up	[kg · kg$_{bw}^{-1}$ · d^{-1}]	

In case of contact with a large volume of (diluted) product, the amount of substance which is actually in contact with the skin will be less than A_{der}. Risk characterisation will then be based on C_{der}, mode, duration and frequency of exposure, the toxicity of the substance and what is known (or can be deduced) about the toxicokinetics of the substance.

The above dermal equations apply also to:

- a non-volatile substance in a medium used without further dilution. In this case the dilution factor (D) is set to 1;
- a non-volatile substance contained in an undiluted medium removed from the skin by, for example, wiping or rinsing and drying (e.g. cosmetic cleansing cream, shampoo, liquid soap). Recalculate the volume of application as $V^*_{appl}=V_{appl} \cdot Fc_{der}$; where Fc_{der} is the fraction of the product remaining on the skin;
- a non-volatile substance in a volatile medium. The concentration C_{der} (equation (3)) is only valid at the very beginning of exposure. However, this concentration can still be used to calculate A_{der} (equation (4)), because the substance is non-volatile.

The above equations for dermal exposure can also be used for volatile substances, but in that case they represent a worst-case situation as the evaporation rate is neglected. At the risk

characterisation stage, the area of skin involved and the known/derived dermal absorption of the product/substance will be taken into account. The balance between evaporation and skin permeation will determine the dermal exposure.

Dermal (2): A non-volatile substance migrating from an article (e.g. dyed clothing, residual fabric conditioner, dyestuff/newsprint from paper)

The exposure calculation will involve estimating the amount of substance which will migrate from the area of the article in contact with skin during the time of contact. The concentration in the product as used can be calculated according to Equation (3) in case the density of the product and the fraction of substance in the product are known. Dyestuff levels in fabrics and paper are usually given as weight of product per unit area (e.g. mg/m^2). The total amount is then calculated by multiplying by $AREA_{der}$. The amount to which the skin is exposed is given by:

$$A_{der} = W_{der} \cdot AREA_{der} = C_{der} \cdot TH_{der} \cdot AREA_{der} \quad (6)$$

where $C_{der} \cdot TH_{der}$ is equal to weight per unit of area:

$$W_{der} = C_{der} \cdot TH_{der} \quad (7)$$

Extractability in simulated body fluids for several classes of dyestuffs and different fabric types has been evaluated by ETAD (1983). For migrating substances, only part of the total amount A_{der} is able to reach the skin. The amount to be used is:

$$A_{migr,der} = A_{der} \cdot Fc_{migr} \cdot T_{contact} \quad (8)$$

where $Fc_{migr} \cdot T_{contact}$ must be much smaller than 1. The potential uptake per kilogram body weight per day is then derived as:

$$U_{der,pot} = \frac{A_{migr,der} \cdot n}{BW} \quad (9)$$

Explanation of symbols

Fc_{migr}	fraction of substance migrating per unit time	[kg · kg^{-1} · d^{-1}]	
$T_{contact}$	duration of contact per event	[d]	
TH_{der}	thickness of product	[m]	
W_{der}	weight of substance on skin per event	[kg · m^{-2}]	
$AREA_{der}$	area of contact between product and skin	[m^2]	
C_{der}	concentration of substance	[kg · m^{-3}]	
BW	body weight	[kg]	cf Section 5.2.1
n	mean number of events per day	[d^{-1}]	
A_{der}	total amount of comp. to which skin is pot. exposed	[kg]	
$A_{migr,der}$	amount of substance to which skin is expected to be exposed due to migration	[kg]	
$U_{der,pot}$	potential uptake	[kg · kg$_{bw}^{-1}$ · d^{-1}]	

3.1.3 Oral exposure

Oral (1): A substance in a product unintentionally swallowed during normal use (e.g. toothpaste)

The concentration in the product as swallowed is calculated from:

$$C_{oral} = \frac{C_{prod}}{D} = \frac{\varphi_{prod} \cdot Fc_{prod}}{D} = \frac{Q_{prod} \cdot Fc_{prod}}{V_{prod} \cdot D} \quad (10)$$

and the intake is then given by:

$$I_{oral} = \frac{F_{oral} \cdot V_{appl} \cdot C_{oral} \cdot n}{BW} \quad (11)$$

If an undiluted product is swallowed, $D = 1$.

Explanation of symbols

C_{prod}	concentration of substance in product before dilution	[kg · m^{-3}]	
D	dilution factor	[-]	
RHO_{prod}	density of product before dilution	[kg · m^{-3}]	
Q_{prod}	amount of product before dilution	[kg]	
Fc_{prod}	weight fraction of substance in product before dilution	[-]	
V_{prod}	volume of product before dilution	[m^3]	
V_{appl}	volume of diluted product per event in contact with mouth	[m^3]	
F_{oral}	fraction of V_{appl} that is ingested	[-]	
BW	body weight	[kg]	cf Section 5.2.1
n	mean number of events per day	[d^{-1}]	
C_{oral}	concentration in ingested product	[kg · m^{-3}]	
I_{oral}	intake	[kg · kg$_{bw}^{-1}$ · d^{-1}]	

These equations may also be used to estimate exposures arising from ingestion of the non-respirable fraction of inhaled airborne particulates.

Oral (2): A substance migrating from an article into food or drink (e.g. plastic film, plastic-coated cups/plates)

The following equation can be used to obtain a conservative estimate of substance uptake from a defined volume of food. The value of Fc_{migr} will be influenced by the type of food (e.g. fatty/dry/moist), the period of exposure and the temperature at which migration occurs. The consumer exposure level will be influenced by the proportion of the contaminated food eaten. The concentration in the food as a result of migration from an article is given by:

$$C_{oral} = \frac{AREA_{art} \cdot TH_{art} \cdot C_{art} \cdot Fc_{migr} \cdot T_{contact}}{V_{prod}} \quad (12)$$

and the oral intake is given by:

$$I_{oral} = \frac{V_{appl} \cdot C_{oral} \cdot n}{BW} \quad (13)$$

Explanation of symbols

$AREA_{art}$	surface area of article in contact with food	[m^2]	
TH_{art}	thickness of article in contact with food	[m]	
C_{art}	concentration of substance in article	[$kg \cdot m^{-3}$]	
Fc_{migr}	fraction migrating per time	[$kg \cdot d^{-1}$]	
V_{prod}	volume of food	[m^3]	
V_{appl}	volume of diluted product actually ingested	[m^3]	
$T_{contact}$	contact duration between article and food	[d]	
BW	body weight	[kg]	cf Section 5.2.1
n	mean number of events per day	[d^{-1}]	
C_{oral}	concentration in ingested product	[$kg \cdot m^{-3}$]	
I_{oral}	intake	[$kg \cdot kg_{bw}^{-1} \cdot d^{-1}$]	

3.1.4 Acute versus chronic exposure

Consumer exposure may be acute or chronic. Many consumer products are used lifelong. If they are used at regular intervals e.g. daily or weekly, and/or if steady concentrations are built up in the body, the lifetime average exposure is well approximated by using the annual average exposure, averaging out seasonal usage differences. Exposure estimations from seldom events (e.g. twice a year) should not be extrapolated to a daily year average exposure! With regard to acute exposures, the equations used for consumer exposure, model exposures as resulting from a constant concentration, thereby setting mean and maximum event concentrations equal. Therefore, acute exposure is characterised by the inhalatory, dermal, and oral concentrations, C_{inh}, C_{der}, and C_{oral} respectively, which are given in the model descriptions. For chronic exposures, the intake and potential uptake rates I_{inh}, $U_{der,pot}$ and I_{oral} usually represent annual average measures of exposure. Where chronic exposure is measured with reference to concentration, the annual average exposure concentrations can be used:

$$C_{route,ann} = \frac{\int_0^{365} C_{route}(t)\,dt}{365} \qquad route \in \{inh, der, oral\} \tag{14}$$

where C_{route} represents the exposure concentration via the inhalatory, dermal or oral route. Both the acute and the chronic characterisation of exposure are given. Because the equations model exposure with reference to constant concentration, the equation can be written as:

$$C_{route,ann} = C_{route} \cdot n \cdot T_{contact} \qquad route \in \{inh, der, oral\} \tag{15}$$

Explanation of symbols

C_{route}	exposure concentration through route *route*	[$kg \cdot m^{-3}$]
$T_{contact}$	event duration	[d]
n	mean number of events per day	[d^{-1}]
$C_{inh,ann}$	annual average inhalation exposure concentration	[$kg \cdot m^{-3}$]
$C_{der,ann}$	annual average dermal exposure concentration	[$kg \cdot m^{-3}$]
$C_{oral,ann}$	annual average oral exposure concentration	[$kg \cdot m^{-3}$]

Both the acute and the chronic characterisations are given per route. The acute concentrations are compared to the appropriate acute toxicity value, the chronic intakes or concentrations to the appropriate N(L)OAEL.

3.1.5 Total exposure

If a consumer is exposed to a substance in a particular consumer product via different routes, the contribution of each route to the total uptake can be summed. Normally the summation is done for each time scale separately (acute and -sub-chronic).

Differences in bioavailability for the various routes can be accounted for by multiplying the intakes (or potential uptakes) with absolute absorption factors. A first approximation of the total uptake from one product via different routes can be obtained by the following equation:

$$U_{tot} = I_{inh} \cdot BIO_{inh} + U_{der,pot} \cdot BIO_{der} + I_{oral} \cdot BIO_{oral} \quad (16)$$

Explanation of symbols

I_{inh}	inhalatory intake of substance	$[kg \cdot kg_{bw}^{-1} \cdot d^{-1}]$
$U_{der,pot}$	potential uptake	$[kg \cdot kg_{bw}^{-1} \cdot d^{-1}]$
I_{oral}	intake	$[kg \cdot kg_{bw}^{-1} \cdot d^{-1}]$
BIO_{oral}	bioavailability for oral intake	[-]
BIO_{inh}	bioavailability for inhalation	[-]
BIO_{der}	bioavailability for dermal uptake	[-]
U_{tot}	total uptake for one product via different routes	$[kg \cdot kg_{bw}^{-1} \cdot d^{-1}]$

3.2 REFINEMENT OF EXPOSURE ANALYSES

3.2.1 Introduction

As described in Section 2.3.9 of human health (Chapter 2), there may be situations were it is decided to improve the exposure assessment using more sophisticated exposure models. This section provides useful background information on possible models that can be used for this purpose. No recommendations for the use of special algorithms or models will be given. There are many models available, either in computer programs or published in the scientific literature. The assessor has to decide which model is taken, and should give conclusive and transparent reasons for the decision. A list of computer programs that partly cover the described models is given in Section 4.

3.2.2 Description of the release of the substance

Some examples of releases of substances which can be attributed to uses of consumer products with respect to the paths of exposure and a short description of the characteristics is given in **Table 2** below.

Table 2 Detailed characterisation of possible exposure scenarios

Kind of release	Characterisation	Relevant exposure paths
Evaporation from a liquid surface:	Occurs if liquid consumer products (e.g. liquid cleaners, adhesives, bleaches, removers) containing volatile ingredients are applied which contain a high liquid fraction e.g. water, water soluble liquids or organic solvents. Normally, the release will lead to air concentrations that can be inhaled. Use can be short and long term. The release of volatile substances are evaluated in a number of publications (Chinn (1981), Dunn (1987), Dunn and Tichenor (1998), Gmehling et al. (1989), Sparks et al. (1996). Computer programs that cover this scenario are CONSEXPO, THERdBASE, CEM.	Evaporation from a liquid surface leads to inhalation exposure as well as to dermal exposure via air.
Evaporation from a layer/coating	Very similar to evaporation from a liquid surface. The difference of this release scenario is that the matrix is based on a composition of substances to form a solid layer while the liquid part (solvents) evaporates. Occurs by the transport of a substance from a layer e.g. paint, adhesive to air or contacting skin. The layer may change its solidity with time. A migration of the substance through the layer takes place Evaporation from a layer may occur after the following categories of chemical products (e.g. adhesives, paints, removers) have been used. This release has also been evaluated in a number of publications. One is based on the model presented by Jayjock (1994), and is included as the “evaporation from pure substance” and the “evaporation from mixture” models in CONSEXPO. Numbers of further evaluations covering thin film source emission, application of paint, emission from solid and liquid sources, VOC's have been published: Bjerre (1989), Bremmer and van Veen (2000b), Clausen et al. (1990), Dunn and Chen (1992), Evans (1996), Guo et al. (1996), Guo et al. (1998), Tichenor et al. (1993), Sullivan (1975), Van Veen et al. (1999), Zimmerli (1982).	Evaporation from layer/coating leads to inhalation exposure as well as to dermal exposure via air.
Contact of layer (liquid/semi-liquid/semi solid) with body surface	This scenario can be applied for all uses where the skin comes into contact with liquids or semiliquid products. There may be short-time-uses (cleaners, liquid soaps), and rarely long time contacts (e.g. lotions) with high frequence. There are some publications that evaluated dermal exposure: Howes (1975), Kasting and Robinson (1993), Thongsinthusak et al. (1999), as well as dermal absorption: Weegels and van Veen (2000), Wilschut et al. (1995). Dermal exposure may also be estimated by use of computer programs e.g. CONSEXPO, CEM, MCCEM, THERdbASE. Models of dermal exposure by contact with fluids have been evaluated by McKone and Howd (1992).	Contact of layer (liquid/semi-liquid/semi solid) with body surface leads to dermal exposure and, sometimes to oral exposure by hand-to-mouth contact
Contact of skin with solid articles	Contact of skin by touching solid materials, in particular textiles, paper, toys. A publication of ETAD deals with the extractability of dyestuffes from textiles (ETAD (1983)); computer models: CONSEXPO. Contact of skin with solids may also be applicable for dermal exposure to soil which has been evaluated for modelling by McKone et al. (1990; 1992).	Contact of skin with solid articles leads to dermal exposure and, sometimes to oral exposure by direct oral contact.
Migration from articles	Migration of a substance from solid material with permanent emission. Exposure occurs indirectly via air, particles or food. This scenario estimates the amount of a substance which is migrating. It should be combined with the scenarios mentioned above. In many cases, measurements of room concentrations are available. This scenario may be attributed to emissions of chemicals from furniture, wood, and other solid materials in the home such as textiles (e.g. carpets). Some models have been published dealing with emissions from furniture (HCHO, (Panzhauser et al. (1992)), emission of VOCs from PVC flooring (Christianson et al. (1993)), release from carpets (Little et al. (1994), and studies on contaminant diffusion in the gase phase (Zimmerli (1982)).	Migration from articles may lead to inhalation exposure as well as to dermal and oral exposure.

Kind of release	Characterisation	Relevant exposure paths
Spraying	Exposure to clouds of substances due to the use of spray, the cloud distributes into the total room volume after finishing spraying. Exposure may occur via the dermal route and via inhalation. It is valid for numbers of applications of consumer products e.g. adhesives, paints, cleaners, deodorizers, air fresheners, cosmetics. The exposure to aerosols has been evaluated in a small number of publications (Hartop et al. (1991); Jennings et al. (1987)), and is also considered in the CONSEXPO model.	Spraying leads to inhalation exposure and to dermal exposure
Contaminations	Many exposures to substances occur indirectly via contaminations of food or drinking water. The pathways that lead to exposure should be described and exposure estimates may be performed taking data from measurements of substances in the above mentioned media. Food consumption data can be gathered from literature (e.g. AUH (1995); Andelmann (1985); Jennings et al. (1987), Legrand et al. (1991)), as well as data from national food consuming monitoring studies.	Contamination is the most important source for oral exposure
Solid particles in air	(1) Transport of solid ultrafine particles from a container to surrounding air (2) Adsorption of substances (in particular non-volatiles) to dust particles Exposure towards particles may occur via unhaltion of dust, as well by the dermal (by touching) dust/soil or orally (eating dust or soil). The latter exposure is of special importance in children. Data that can be useful for estimating exposure to solid particles e.g. has been published by the German Ausschuss für Umwelthygiene (AUH, 1995), giving a critical overview on existing evaluations on dust intake.	Solid particles in air lead to inhalation exposure from particles

3.2.3 Models for refined exposure assessment

Inhalation exposure

Additionally to the above mentioned estimations, the refined assessment should consider time. Modelling exposure therefore requires data that describe the duration of use and the duration of primary and secondary exposure. In general, these times depend on the category of the chemical products and should be given in detail. For instance, 1 kg of paint may be used over a period of 2 hours, followed by secondary exposure of 10 hours, which must be considered by the model chosen for estimating this exposure. As a further additional variable, room ventilation has to be taken into account for inhalation exposure.

For instance for highly volatile substances the velocity of evaporation may be limited by the velocity of application.

In general, the concentration of a substance in a room can be simply described by the formula according to Guo et al. (1996; 1998) and Tichenor et al. (1991):

$$R_t = R_0 * \exp^{-k_e * t} \qquad (17)$$

In this equation, Rt reflects the contaminant emission at any time, Ro the contaminant emission rate at t = 0, t is the time and k_e is the 1st order decay constant from the room, which can be expressed e.g. by the room ventilation rate (Q_{vent}).

Other models for inhalation exposure published in the scientific literature are mentioned above under description of the scenarios “Evaporation from a liquid surface”, “Evaporation from a layer/coating”, “Spraying”.

A number of multi-route programs exists for assessing exposures to consumer products: CONSEXPO (van Veen, 2001) and THERdbASE (Pandian et al., 1990). CONSEXPO version 3.0 has been set up to reflect the algorithms in this appendix, but also allows more refined exposure assessment. In addition, a number of programs directed to a specific route of exposure exist, such as the US EPA household models SCIES, DERMAL, CEM, and MCCEM (see also Section 4).

As a worst-case approach, the release is assumed to be immediate and equations 1 and 2 are to be used. Another possibility is the use of the 2-zone indoor air model as developed by the US EPA. There are several versions of this model, the SCIES and the CEM program from Versar (1994), and the 2-zone model implemented in THERdbASE. The CONSEXPO and the US EPA models are based on different scenarios, they differ significantly in the contact duration.

3.2.4 Dermal exposure

Contact of skin with surfaces

A critical review on methods for assessing dermal exposure has been described by van Hemmen (1993). Transfer of contaminants from surfaces to skin has been studied by Brouwer et al. (1999). A single hand contact to a powdered glass surface resulted in 4 to 16%, 12 contacts in 40% of contamination of the palm surface.

Dermal uptake/percutaneous absorption

Wilschut et al. (1995) have compared five different models estimating skin absorption rates simulating the penetration of substances from aqueous solutions. They developed a database containing absorption constants for 123 substances, which can be applied for dermal uptake studies. The maximum flux through the skin (mg/cm²/hour) is estimated as the product of Kp (permeation coefficient (cm/hour) and water solubility (mg/cm³). The algorithms are also implemented in the CONSEXPO software, within the boundaries of the model.

Derivations of dermal absorption rates have been evaluated by Stubenrauch et al. (1995) giving data for dermal exposure rates for children and adults from playgrounds and in the garden referencing primary data sources.

Principles of assessing dermal exposure have also be published by the US EPA (1992).

3.2.5 Oral exposure

Oral exposure may result from swallowing of substances in cosmetics (lipsticks) and toothpaste, ingestion of phthalates or other substances from teethers/toys/clothing or via hand-mouth contact for exposure to substances on carpets. In addition, intake of air and water as indirect sources of exposure via the environment as well as from contamination of food may occur. Substances having low vapour pressure that do not evaporate leading to air concentrations frequently are carried by solid particles, in particular dust. For this reason, dust may represent a major source of exposure having importance in special situations. For instance, the oral route via ingestion of dust may represent a significant path of exposure to children playing on the ground and sucking their fingers which can be contaminated via dust and soil. It is very uncertain how much dust or soil children will ingest. An extensive and proper description of this type of exposure is given in the report Standards zur Expositionsabschätzung (AUH, 1995).

4 COMPUTER TOOLS FOR ESTIMATION OF CONSUMER EXPOSURE

4.1 INTRODUCTORY REMARKS

All the computer tools mentioned in this section can be very helpful for performing exposure assessments. They are designed from different views of exposure monitoring and thus reflect different scientific approaches which has to be kept in mind when using them. First of all, the assessor must be aware that the scenarios governing the model characterisation are different. For example, the EUSES programme is based on the scenario that a volatile substance (without characteristion of volatility) is spread out into a room which can be taken for worst-case estimations. Other computer models are more complex but the results are hard to compare because the model algorithms are based on different scenarios. For instance, the CONSEXPO inhalation exposure scenarios are based on a one room spread-out with a user directed virtual volume, while e.g. the SCIES as well as the CEM programme consider exposure in a whole house with different rooms and differentiated scheme of times staying in the rooms throughout a day of users and non-users. It is clear that these differences of the scenario must lead to different results and the assessor has to point out the reasons for favouring a specific model.

4.2 CONSEXPO 3.0

Features

The CONSEXPO (CONSumer EXPOsure models) program is being developed at the RIVM (The Dutch National Institute of Public Health and the Environment) to provide estimation routines for exposure to consumer products including pesticides (Van Veen, 2001). CONSEXPO contains also the screening models that are given in Section 3.1. These models are also part of EUSES.

- total exposure is defined from the combination of contact, exposure and uptake scenarios for each route of entry and dose measures are calculated. These dose measures contain concentration estimates, and short and long-term average doses in terms of milligram chemical per day per kilogram bodyweight;
- the program allows for stochastic parameters and each parameter can attain a normal, lognormal or uniform distribution, or an empirical distribution defined by data. Exposure and dose distributions reflect stochastic parameters and these distributions can be depicted and percentiles can be quantified;
- the program provides sensitivity analyses for each stochastic parameter, where mean exposures or doses as function of the value of a selected stochastic parameter are depicted and analysed. Sensitive parameters will cause big differences in model outcome, while others will cause hardly any differences.

Theoretical

It is based on a modelling framework that contains the components of (1) contact, (2) exposure and (3) uptake. For each component, the user selects a model and provides its parameters. The contact component does not contain a mathematical model but specifies duration of actual use, duration of contact with the product, and frequency of use. The duration of actual use and the duration of contact might differ if actual usage is short, like using a spray, but compounds from the product fill the air around a person, causing a prolonged exposure.

The exposure component contains multiple models to estimate the concentration of compound in the medium that directly contacts the human body. These estimation models range from simple screening models to advanced models describing specific exposures. Exposure includes the inhalatory, dermal, and oral routes and the software provides the possibility to model exposure through multiple routes of exposure. For the inhalatory route, the advanced models include painting, evaporation, exhaust gas production, and a continuous source. For the dermal route, the models include transfer factors, contact rates and fixed volume of product. For the oral route, models include ingestion, leaching from materials into food or into the mouth and hand-mouth contact.

The uptake component estimates the amount taken up through the skin, the lungs or the gastrointestinal wall. This denotes the amount that reaches systemic circulation. If information on the fraction taken up is available, this can be specified. Otherwise, simple diffusion models can be used to estimate the fraction taken up. As an alternative, uptake can be set to 100%, in which case potential doses are calculated by the program.

Validation

Van Veen et al. (1999, in press) report two experiments to test the CONSEXPO painting model that contains evaporation as source term. The model predicts upper room concentrations well. Model predictions for paint were within 80% of the measured concentrations.

Remarks

One requirement for using CONSEXPO in an appropriate way is that the user has to select appropriate models and find parameter values. The translation from product type, e.g. spray can, to exposure, e.g. source and ventilation model is therefore the responsibility of the user. At the moment, research is done to set up a defaults-database for CONSEXPO. The database will refer to product types and assign default models and default parameter values to product type. These product types will include ready-to-use spray cans, spraying after dilution of a concentrate, dusting, evaporation from a matrix, etc. Inclusion of the database will enable a product based use of the program, although the user will never be relieved of checking the match between (default) model and the product to be assessed.

CONSEXPO 3 can be obtained as Report 612810.011 from the RIVM, Bilthoven, The Netherlands, which describes all models incorporated into the program.

4.3 US EPA WALL PAINT EXPOSURE ASSESSMENT MODEL (WPEM)

The Wall Paints Exposure Assessment Model (WPEM) estimates the potential exposure of consumers and workers to the chemicals emitted from wall paint which is applied using a roller or a brush. WPEM is a user-friendly, flexible software product that uses mathematical models developed from small chamber data to estimate the emissions of chemicals from oil-based (alkyd) and latex wall paint. This is then combined with detailed use, workload and occupancy data (e.g., amount of time spent in the painted room, etc,) to estimate exposure. The output of WPEM was evaluated in a home used by EPA for testing purposes and, in general, the results were within a factor of 2. The WPEM provides exposure estimates such as Lifetime and Average Daily Doses, Lifetime and Average Daily Concentrations, and peak concentrations.

Specific input parameters include: the type of paint (latex or alkyd) being assessed, density of the paint (default values available), and the chemical weight fraction, molecular weight, and vapour

pressure. Occupancy and exposure data are provided by the model as default values but the model is designed to be flexible and the user may select other values for these inputs: activity patterns on weekdays/weekends for workers or occupants, and during the painting event; number of exposure events and years in lifetime; room size (volume); building type (e.g., office, single family home); number of rooms being painted; air exchange rates; etc. For those chemicals in which the mathematical emissions model does not apply, you can enter emissions data.

Status and availability

WPEM Version 3.2, a Windows-based tool is available. The model has been peer reviewed by experts outside EPA. This model was developed under contract for the EPA's Office of Pollution Prevention and Toxics, Economics, Exposure, and Technology Division, Exposure Assessment Branch. WPEM was developed under the Design for the Environment Program, Designing Wall Paints for the Indoor Environment. This project was accomplished in coordination and cooperation with the National Paint and Coatings Association (NPCA), in addition to paint manufacturers and chemical suppliers.

The model, user's guide and background document is available as a pdf file via http://www.epa.gov/oppt/exposure/

4.4 CONSUMER EXPOSURE MODEL (CEM)

The Economics, Exposure and Technology Division (EETD) of the Office of Pollution Prevention and Toxics (OPPT) is responsible for conducting specific activities in support of the Agency's risk assessment process. One of these responsibilities is to assess new and existing chemical substances under the Toxic Substances Control Act (TSCA). CEM, developed by Drewes and Peck (1999) is designed to provide EETD's Exposure Assessment Branch and Chemical Engineering Branch with an easy way to perform consumer inhalation and dermal exposure assessments for OPPT's new and existing chemical programs. The methods used to perform these assessments often involve generic screening-level techniques to allow exposures to be estimated rapidly. CEM has been programmed in C++/Windows and is designed to be run on a personal computer.

CEM is an interactive model which calculates conservative estimates of potential inhalation exposure and potential and absorbed dermal exposure to consumer products. Consumer inhalation exposures modeled in CEM use the same approach and calculations as the Multi-Chamber Concentration and Exposure Model (MCCEM), as well as scenarios depicted in the Screening -Level Consumer Inhalation Exposure Software (SCIES). Dermal exposures are modeled using the same approach and equations as the DERMAL Exposure Model. CEM allows for screening-level estimates of acute potential dose rates, and average and lifetime average daily dose rates. Because the model incorporates upper percentile and mean input values for various exposure factors in the calculation of potential exposures / doses, the exposure / dose estimates are considered “high end” to “bounding” estimates.

The dermal portion of CEM uses a film-thickness approach which assumes that exposure occurs from a thin layer of the consumer product on a defined skin surface area to determine potential exposure. Few data exist on the actual thickness of films of various products on human skin. Therefore, due to the uncertainty associated with the amount of product forming a film on the skin the dermal exposure estimates are considered less certain than those calculated in the inhalation portion of CEM. Absorbed dermal dose rates can be calculated using a permeability coefficient or a log octanol water coefficient, but these values and their use in calculating

exposure also involves uncertainty. Absorbed exposure can only be calculated for the User-Defined Scenario in CEM.

The consumer exposure scenarios were selected for inclusion in the model by EETD because they are products or processes for which exposure assessments are most frequently performed during the new chemical review process. In addition to these scenarios, users are able to create their own scenario. CEM is user friendly and provides on-line help to assist the user in optimizing model use.

The CEM programme covers most of the scenarios needed for consumer exposure modelling. It should be noted that input data are needed for 50^{th} and 95^{th} percentiles.

4.5 US EPA MULTI-CHAMBER CONCENTRATION AND EXPOSURE MODEL (MCCEM),

Features

The Multi-Chamber Concentration and Exposure Model (MCCEM) Version 1.2 (GEOMET, 1995) was developed for the US EPA Office of Pollution Prevention and Toxics to estimate indoor concentrations for chemicals released in residences). The feature of MCCEM is as follows:

- MCCEM needs time-varying emission rates for a chemical in each zone of the residence and outdoor concentrations. The emission rates of pollutants can be entered into the model either as numbers or as formulas;
- inhalation exposure levels are calculated from the estimated concentration if the user specifies the zone where an individual is located in a spreadsheet environment;
- MCCEM has data sets containing infiltration and interzonal airflow rates for different types of residences in various geographic areas. The user can select from the data sets, or can input zone descriptions, volumes and airflow rates;
- concentrations can be modeled in as many as four zones (chambers) of a residence;
- the programme is capable of performing Monte Carlo simulation on several input parameters (i.e., infiltration rate, emission rate, decay rate, and outdoor concentration) for developing a range of estimates for zone-specific concentrations or inhalation exposures;
- the programme has an option to conduct sensitivity analyses of the model results to a change in one or more of the input parameters;
- the percentage of cases for which modeled contaminant concentrations are at or above a user-specified level of possible concern or interest is determined.

Theoretical

This multi-chamber mass-balance model has been developed by using air infiltration rates and corresponding interzonal air flows for a user-selected residence or a user-defined residence. This model provides a spreadsheet environment to the user for entering time-service data for emission rates in one or more zones, the zone of exposure, and concentration values of the contaminant outdoors.

Information assembled by Brookhaven National Laboratory concerning measured infiltration/exfiltration airflow, interzonal airflow, and the volume and description of each zone for different types of structures in various geographic areas has been incorporated in the software for access by users. Two generic houses represent average volume (408 m^3) and flow

information in summer or fall/spring that has been compiled from a large number of residences. One generic house has a bedroom as the first zone and the remainder of the house as the second zone. The other, with the same total volume as the first, has a kitchen as the first zone and the remainder of the house as the second zone. The features of the generic houses are noted in the Exposure Factors Handbook (US EPA, 1997).

Remarks

The user's guideline listing good examples enable risk assessors to handle easily the full items within MCCEM. In addition, MCCEM contains a database of various default house data that are needed to complete each calculation such as air-exchange rates, geographically based inter-room air flows, and house/room volumes. However, the so many data might cause a confusion to risk assessors who aim to evaluate the risk tendency of pesticides for a typical population at the first tier approach. Therefore, it seems reasonable that the user's guide suggests that a two-storey residence will be chosen by default, and that US EPA recommends a fixed storey using the above generic house in Summer to estimate a high-end assessment.

The MCCEM model is available via http://www.epa.gov/oppt/exposure.

4.6 FURTHER DEVELOPMENTS FROM THE US

Newly emerging exposure models are set up to accommodate aggregated residential exposure scenarios, containing multiple sources of a chemical. These models are mostly initiated in response to the demands of the Food Quality Protection Act in the United States. They aggregate exposure from multiple sources, at the cost of needing good input data for each source. LIFELINE is an effort by the Hampshire Research Institute, funded by US EPA, to develop a dietary and nondietary residential exposure model that will estimate aggregate exposure over a lifetime. CALENDEX is a model under development by Novigen that can estimate daily to annual nondietary residential exposure and works in a probabilistic environment. CALENDEX does link with Novigen's DEEM dietary model to produce an aggregate model. CARES is an industry effort to create an aggregate and cumulative risk assessment model. CARES stands for Cumulative and Aggregate Risk Evaluation System. It intended to contain the dietary components from DEEM (it will not contain DEEM itself) and the REx model. The REx model itself is another model for aggregated exposure assessment and it is structured according to the US EPA SOPs for pesticidal residential exposure assessment (for the latter, see REx Model, 2000). All these models are under development and first results are expected in the near future.

5 DATA REFERENCES

5.1 DESCRIPTION OF PEOPLES BEHAVIOUR (TIME BUDGETS)

This TGD does not give parameters on time budgets. There are substantial differences between the European countries and regions that are not documented sufficiently. Some information on time budgets can be found in American Industrial Health Council (AIHC, 1994), Standards zur Expositionsabschätzung (AUH, 1995), Dörre and Knauer (1994), Dörre et al. (1999) or Groot et al. (1998).

5.2 ANTHROPOMETRIC DATA

5.2.1 Body weight

For performing the calculations with the equations given in Section 3 default bodyweights of 70 kg for adult males and 60 kg for adult females may in principle be used. For further analyses, particularly for estimations of children's exposure, more detailed compilations of body weights (including distributions) are available for Germany (AUH, 1995), The Netherlands (Bremmer and van Veen, 2000b), as well as for the US (AIHC, 1994; US EPA, 1997).

5.2.2 Surface area

An overview of distributions of body surfaces is given in the AIHC "Exposure Factors Sourcebook" (AIHC, 1994), in the EPA Exposure factors handbook (US EPA, 1997), in Standards zur Expositionsabschätzung (AUH, 1995), as well as in the RIVM publication "Factsheet algemeen" (Bremmer and van Veen, 2000b).

The total body surface ($S_{der,tot}$) can be calculated from the bodyweight (BW) and the body height (BH) by the formula:

$$S_{der,tot} = 0.0239 \cdot BH^{0.417} \cdot BW^{0.517} \quad (18)$$

The mean of body surfaces, given for adult men and women, and referred to the different body parts, is given in **Table 3.** For females, it was anticipated that the ratio of body part surfaces to total body surface is similar as for men. According to a report from the German Ausschuss für Umwelthygiene the 50th percentile of the body surface is 6,030 cm^2 for children between 2 and 3 years, 10,700 cm^2 for children between 9 and 10 years, and 14,700 cm^2 for adolescents (AUH, 1995).

Table 3 Body surface areas for adult humans (US EPA, 1997)

Body Part	Mean surface area, men (cm^2)	Mean surface area, women (cm^2)
head (face)	1,180	1,028
trunk	5,690	4,957
upper extremities	3,190	2,779
arms	2,280	1,984
upper arms	1,430	1,244
forearms	1,140	992
hands (fronts and backs)	840	731
lower extremities	6,360	5,533
legs	5,060	4,402
thighs	1,980	1,723
lower legs	2,070	1,801
feet	1,120	1,001
total	**19,400**	**16,900**

5.2.3 Respiration volume

For performing the calculations with the equations given in Section 3 a default respiration volume (IH_{air}) of 20 m^3 should normally be used. It should be noted however, that persons are not necessarily staying at the same level of activity during the use of consumer products, neither for the whole day. Hence it may be necessary to adapt the default respiration rates for short-term or long-term exposures, the latter considering the daily changes of activity levels. The tables below provide some useful information on respiration rates for different subpopulations during different activity patterns.

Table 4 Respiration volume (all data as m^3/d), related to activity levels (AUH, 1995)

subject age		Resting	Light activity	Medium activity	Heavy activity
adults females)	20 - 30	6.5 – 8.6	23 - 27	36	130
Pregnant women)		14			
adults males	20 - 33	6.5 – 10.8	29 - 42	62	160
adults		6.47	17.6	35.9	

Table 5 Inhalation rates which are applicable for estimating short-term exposures (AUH, 1995).

	Age	Resting	Light activity	Medium activity	Heavy activity
children	<1	1.4	2.9	5.8	10
children	1-3	2.9	5.8	12	20
children	4-6	5.8	12	23	40
children	7-9	8,6	12	35	61
children	10-14	12	23	46	81
childen	15-19	13	26	51	91
adults	20-75	13	26	51	91

Table 6 Respiration rates concerning a whole day exposure (AUH, 1995)

Age	<1 y	2-3 y	4-6 y	7-9 y	10-14 y	15-19 y	20-75 y
Breathing volume	3	7	11	14	18	20	18

5.2.4 Room volume

The room volume that needs to be used for calculating the exposure of consumer is of course related to where the activity takes place. No default values can be given. Some information on room volumes for the Netherlands and for Germany is given in **Table 7** below. This table shows that only minor differences exist between these countries. Further data considering room volumes are available from the US (Jennings et al., 1987) but not from other EU member states.

Table 7 Room volumes (m^3) in the Netherlands (Bremmer and van Veen, 2000), and Germany (medians)

Room	Netherlands	Germany [1)]
Living room	58	64
Room 1	40	43 (children's room)
Room 2	30	
Sleeping room 1	16	
Kitchen	15	
Toilet	2.5	
Bathroom	4	

1) The Statistisches Bundesamt (Wiesbaden) has published a list of means of room areas. From these data an estimate of room volume has been performed by multiplying the areas with a height of 2.8 – 3.5 m. The median of this estimate is 64 m^3. These data cannot be taken for worst-case scenarios, because they do not cover extreme values.

5.2.5 Ventilation

An overview on ventilation rates is given in Bremmer and van Veen (2000b) and Klobut (1993). For The Netherlands, room ventilation varies between 0.5 and 2.5 (h^{-1}), depending on the room Bremmer and van Veen (2000b). According to evaluations made in a test house by Guo et al. (1995) the room ventilation rate accounts for 0.382 ± 0.084 h^{-1} under "normal" conditions and 2.06, respectively 4.20 h^{-1} when all doors and windows are kept open. In another experimental study van Veen (1995) estimated a room ventilation rate of 6.2 h^{-1} (all doors and windows open).

5.3 USE AND COMPOSITION DATA FOR SOME SPECIFIC CONSUMER PRODUCTS

The following tables give information about use instructions and compositions of some laundry and house cleaning products and amounts and migration rates of textile dyes.

Table 8 Habits and practices for consumer products in Western Europe (AISE, 2002) (International Association for soap, detergents and maintenance products)

CATEGORY	Grams/Task			Use Frequency: # Tasks per week			Duration of Task			Other intended uses of category
	Min.	Max.	Typ.	Min.	Max.	Typ.				
LAUNDRY REGULAR										
Powder	55	290	150	1	18	5	Machine wash: < 1 min.			Laundry pretreatment: 10 min. / task,
Liquid	78	230	150	1.8	10	4	Hand wash (b): 10 min.			50-60% paste (powder); neat liquid
LAUNDRY COMPACT										
Powder	20	200	75	1	21	5	Machine wash: < 1 min.			Laundry pretreatment: 10 min. / task,
Liquid/gel	40	140	90	2.8	10	4	Hand wash (b): 10 min.			50-60% paste (powder); neat liquid
Tablet	45	135	90	3	10	4				
FABRIC CONDITIONERS										
Liquid Regular	50	140	135	3.3	10	4	Machine: < 1 min.			Not applicable
Liquid Concentrate	11	90	44				Hand wash (b): 10 min.			
LAUNDRY ADDITIVES										
Powder Bleach	50	70	60				Machine: < 1 min.			
Liquid Bleach (ml)	40	100	70	1.5	4	3	Hand wash (b): 5 - 10 min.			Laundry pretreatment liquid (neat)
Tablet	20	30	25							
HAND DISHWASHING							Min.	Max.	Typ.	
Liquid Regular (a)	3	10	--	3	21	14	10	45	30	Not applicable
Liquid Concentrate (a)	2	5	--				10	45	30	
MACHINE DISHWASHING										
Powder	20	46	--							
Liquid	20	40	--	3	7	5	< 1 min.			Not applicable
Tablet	20	50	--							
SURFACE CLEANERS							Min.	Max.	Typ.	
Liquid (a)	30	110	60							
Powder (a)	20	40	--	1	7	2	10	20	--	Not applicable
Gel (neat)	20	40	--							
Spray (neat)	5	30	--				2	10	--	
TOILET CLEANERS										
Powder	15	30	20							
Liquid (ml)			30	1	2	1	< 1 min.			Not applicable
Gel	20	35	25							
Tablet	25	50	35							

(a) per 5 l of wash water volume Min. = minimum value Max. = maximum value Typ. = typical value

(b) 0.1 - 1% wash solution

5.3.1 Composition tables for laundry and cleaning products

The following tables provide information on the composition of different kinds of laundry and cleaning products. All data were provided by AISE (1996) (International Association for soap, detergents and maintenance products).

Table 9 Powder laundry detergents composition (% by weight)

Ingredients	Regular	Compact	Colour
Anionic surfactants Nonionic surfactants	10 – 20	10 - 20	12 - 25
Builders	20 – 40	20 - 40	20 - 40
Co-builders	3 – 5	3 - 8	2 – 7
Bleaching agent	10 – 25 [1)]	10 – 20 [2)]	-
Bleach activator	1 – 3	3 - 8	-
Anti-deposition agents	0 – 1	0 - 1	0 – 1
Corrosion inhibitor	2 – 6	2 - 7	1 - 5
Stabilizers	0 – 1	0 - 1	0 - 1
Foam inhibitors	1 – 4	0.1 - 2	1 - 3
Enzymes	0.3 - 0.8	0.5 - 2	<2
Optical brighteners	0.1- 0.3	0.1- 0.3	-
Dye transfer inhibitor	-	-	0 - 3
Processing aids ("inert" substances e.g. Na2SO4)	0 – 20	-	-
Minors (e.g. perfume, dye)	0 – 1	0 - 1	0 - 1
Water	Balance	Balance	Balance
Bulk density (g/1)	500 – 600	600 - 900	550 - 800

[1)] Predominantly Tetrahydrate
[2)] Predominantly Monohydrate

Table 10 Fabric softeners composition (% by weight)

Ingredients	Regular softener	Concentrated softener
Cationics or fabric softener actives	3 – 7	15 - 25
Nonionic surfactants	0 – 2	0 - 2
Anionic surfactants	0 – 2	0 - 2
Perfume *	1.1- 0.6	0.5 - 1
Preservative	0 - 0.1	0 - 0.1
Dye	~0.001	~0.001
Alcohols	0 – 10	0 - 10

* Usually comprises a mixture of substances

Table 11 All purpose cleaners

Ingredients	Composition (% by weight)
Anionic surfactants and soaps	2 - 10
Nonionic surfactants	0 - 3
Amphoteric surfactants	0 - 1
Builders/chelators	1 - 15
Solvents and hydrotropes	0 - 15
Perfume *	0 - 1
Dyes	>1
Preservatives	>1
Water	Balance

* Usually comprises a mixture of substances

Table 12 Laundry liquid detergents

Ingredients	Composition (% by weight)
Anionic surfactants	7 - 18
Soap	3 - 22
Nonionic surfactants	0 - 25
Amphoteric surfactants	0 - 2
Softening agents	0 - 12
Builder	0 - 34
Alcohols	8 - 15
Enzymes	1 - 2
Optical brighteners	0.005 - 0.3
Stabilizers	<1
Minors (e.g. perfume, dye)	<1
Water	Balance

Table 13 Hand washing Liquids

Ingredients	Composition (% by weight)
Anionic surfactants	10 - 40
Nonionic and amphoteric surfactants	0 - 15
Additional ingredients	<2
Hydrotrope	<10
Perfume *	<1
Dye	0.1
Preservatives	<0.1
Water	Balance

* Usually comprises a mixture of substances

5.3.2 Use levels of cosmetics

The typical amount is the average quantity used per application. For some product types the quantity left on the skin/scalp (e.g. rinse-off products, hair styling products) is much less (default = 10%). The frequency of use depends on the product type. Data are from 1981 or 1993 (1993 data are indicated by asterisks).

Table 14 Typical use levels of cosmetics

Product type	Typical amount per application	Frequency of use
	(grams)	(per day, week or year)
I. Mucous membrane contact		
- Toothpaste	1.4 *	1-2/day
- Mouthwash (ready to use)	10.0 *	1-5/day *
- Eye make-up: powder	0.01	1-3/day
mascara	0.025	1/day
liner	0.005	1/day
- Eye make-up remover (wiped off)	0.5	1-2/day
- Lipstick	0.01	2-6/day
II. Non-rinse products		
- Face cream	0.8	1/day
- After-shave	1.2	1-2/day
- General purpose cream	1.0 mg cm^{-2}	1-2/day
- Body lotion	7.5	1-2/day
- Setting product	12.0	1-2/week
- Hairspray (as sprayed)	10.0	1-2/day
- Hair styling products*	5.0*	1-2/day*
- Temporary hairdye	12.0	1-2/week
- Toilet water	0.75	1-5/day
- Talcum powder	2.5	1-2/day
- Anti-perspirant/deodorant spray (as sprayed)	3.0	1-3/day
- Make-up remover	2.5	1-2/day
- Anti-perspirant/deodorant (roll-on) *	0.5	1/day *
- Nail product	0.25	2-3/week
- Sun cream	8.0	2-3/day for 2 weeks/year +1
- Sun lotion	10.0	week in Winter on face only
III. Rinse-off products		
- Shaving cream	2.0	1/day
- Soap bar	0.8	3-6/day
- Foam bath (undiluted)	17.0	1-2/week
- Shower gel	5.0*	1-2/day *
- Shampoo	12.0	2-7/week *
- Hair conditioner	14.0	1-2/week *
- Semi-permanent hair-dye	30.0	8-18/year
- Permanent hair-dye (ready to use)	50.0	8-12/year

5.3.3 Consumer exposure to textile dyes

Table 15 Consumer exposure to textile dyes

Dye Class	Reactive Dyes	Disperse Dyes	Direct Dyes	Acid Dyes	Basic Dyes
Application and W (assuming 4% deep shade) – in g/m²	0.5	0.5	0.5	0.2	0.5
Weight fraction (Wf)	0.7	0.4	0.8	0.8	0.8
Migration (Mf) for both wash and perspiration [1), 2)]	0.001% (>4)	0.001% (>4)	0.01% (3)	0.005% (4)	0.005% (4)

1) Based as published grey scale scores (in brackets) and data contained in ETAD Report A4007 (ETAD, 1983)
2) Figures refer to first washing only

6 LIST OF VALUABLE SOURCES ON EXPOSURE DATA

Abbreviation	Full title	Country	Remarks	Contact
AIHC	American industrial health council (1994). Exposure factors handbook	US	Anthropometric data on adults and children, behaviour data, given as distributions	Update coordinator, Suite 760, 2001 Pennsylvania Ave. NW, Washington DC 20006-1807
AUH	Standards zur Expositionsabschätzung	D	Compilation of anthropometric data, partly referred to other sources (US), focus on children	Dr. R Fehr, LAUG Bielefeld, Germany 49 521 8007 253
BgVV-ZEBS	Zentralstelle zur Erfassung und Bewertung von Stoffen in Lebensmitteln	D	Food monitoring, focus to Germany	Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany 49 1888 412 0
CEPA	Air toxic Hot Spots Program Risk Assessment Guidelines Californian Environmental Protection Agency.	US	Part IVTechnical Support for Exposure Assessment and StochasticAnalysis	http://www.oehha.ca.gov/air/hot_spots/finalStoc.html
CH-PR	Swiss product register	CH	Product information, given on request	Dr. J. De Peyer, Swiss Federal Health Office, Geneva
ECETOC	Exposure Factors Sourcebook for European Populations (with focus on UK data)	EU/ ECETOC	Probability analysis Anthropometrics Time activity patterns	www.ecetoc.org
IFL	Industrieverband Farben und Lacke	D	National industrial association, focus on paints, lacquors	Karlstrasse 21 D-60329 Frankfurt 49 69 2556 0
IKW	Industrieverband Körperpflege und Waschmittel	D	National industrial association, focus on household preparations	Karlstrasse 21 D-60329 Frankfurt 49 69 2556 0
IVA	Industrieverband Agrar	D	National industrial association, focus on agricultural preparations	Karlstrasse 21 D-60329 Frankfurt 49 69 2556 0
PR-D	Product data base according to regulations of chemical law	D	Product information	Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany 49 1888 412 0
PR-FIN (KETU)	Finnish product register	FIN	Product information	www.sttv.fi

Abbreviation	Full title	Country	Remarks	Contact
PR-S	Swedish product register	S	Product information	www.kemi.se
RIVM	Bremmer HJ, van Veen MP (2000b). Factsheet allgemeen. Randvoorwaarden en betrouwbaarheid, ventilatie kamergroote, lichaamsoppervlak. RIVM report 612810009, factsheet allgemeen	NL	General informations, room volumes, room ventilation data	RIVM (2000)
RIVM-paint	Bremmer HJ, van Veen MP (2000a). Factsheet verf.Ten behoeve van de schatting van de risico's voor de consument.	NL	Use data on paints, paint classification, characterisation of paint use, focus to NL	RIVM (2000) Bilthoven, The Netherlands
RIVM-toys	Bremmer HJ, van Veen MP (2000a). Factsheet verf.	NL	Toys, characterisation of children, childrens behaviour	RIVM (2000)
US EPA	Environmental Protection Agency (1997). Exposure Factors Handbook.	US	Substantial compilation of exposure factors	EPA (1997) www.epa.gov
VCI	Verband der chemischen Industrie	D	National industrial association (all chemical industries)	Karlstrasse 21 D-60329 Frankfurt 49 69 2556 0

Appendix III Model calculations for indirect exposure via the environment

In this appendix, the equations are given to calculate indirect exposure through the environment. The tables below list the required information to perform the assessment. The properties of the substance are partly derived from the data set provided by the notifier. The secondary data are derived in Chapter 3, Environmental Risk Assessment. The environmental concentrations necessary to perform the assessment are also derived in Chapter 3. Both local and regional concentrations are used. Bioconcentration and biotransfer factors are derived from physico-chemical properties using (Q)SAR approaches. It should be noted that reliable and relevant measured data are always preferable considering the large uncertainties in the (Q)SARs.

Input chemical properties

Kow	octanol-water partitioning coefficient	[-]
HENRY	Henry's law constant	[Pa · m³ · mol-1]
$K_{air\text{-}water}$	air-water partitioning coefficient	[m³ · m-³]
$F_{ass\text{-}aer}$	fraction of the substance associated with aerosol particles	[-]
$DT50bio_{water}$	half-life for biodegradation in surface water	[d]

Input local concentrations

PEClocalwater,ann	annual average local PEC in surface water (dissolved)	[mg · l-1]
PEClocalair,ann	annual average local PEC in air (total)	[mg · m-3]
PEClocalgrassland	local PEC in grassland (total), averaged over 180 days	[mg · kg-1]
PEClocalagr.soil,porew	local PEC in porewater of agricultural soil	[mg · l-1]
PEClocalgrassland,porew	local PEC in porewater of grassland	[mg · l-1]
PEClocalgrw	local PEC in groundwater under agricultural soil	[mg · l-1]

Input regional concentrations

$PECregional_{water}$	regional PEC in surface water (dissolved)	[mg · l-1]
$PECregional_{air}$	regional PEC in air (total)	[mg · m-3]
$PECregional_{agr.soil}$	regional PEC in agricultural soil (total)	[mg · kg-1]
$PECregional_{agr.soil,porew}$	regional PEC in porewater of agricultural soils	[mg · l-1]

The regional model does not distinguish between grassland and other agricultural soils. Only $PECregional_{agr.soil}$ is used for the regional grassland concentration. $PECregional_{agr.soil,porew}$ is used for the concentration in groundwater. The indirect exposure calculations are identical for the local and regional scales. Therefore, the indirect exposure equations in the following sections use generalised symbols:

Symbols used in indirect exposure equations

C_{water}	concentration in surface water	[mg · l-1]
C_{air}	concentration in air	[mg · m-3]
$C_{grassland}$	concentration in grassland soil	[mg · kg-1]
$C_{agr.porew}$	concentration in porewater agric. soil	[mg · l-1]
$C_{grass.,porew}$	concentration in porewater grassland soil	[mg · l-1]
C_{grw}	concentration in groundwater	[mg · l-1]

Bioconcentration water-fish

Numerous studies on the estimation of BCFs have been published. The methods that estimate a BCF from a log Kow are widely used and, in general, most reliable. However, because these methods are based on several assumptions, such as a constant water concentration and no metabolism of the substance by the organism, their resulting values should be considered as a

relative measure for the bioaccumulation potential of a substance. Furthermore, these methods may not have the same accuracy for different classes of substances. The following QSAR is advised in Chapter 4 (in this chapter BCF for fish is discussed in more detail):

$$\begin{aligned} \log Kow \leq 6: \quad & \log BCF_{fish} = 0.85 \cdot \log Kow - 0.70 \\ \log Kow > 6: \quad & \log BCF_{fish} = -0.20 \cdot (\log Kow)^2 + 2.74 \cdot \log Kow - 4.72 \end{aligned} \tag{1}$$

Explanation of symbols

Kow	octanol-water partition coefficient	[$m^3 \cdot m^{-3}$]	data set
BCF_{fish}	bioconcentration factor for fish on wet weight basis	[$l \cdot kg^{-1}$]	

The steady-state concentration in fish is now given by:

$$C_{fish} = BCF_{fish} \cdot water \tag{2}$$

Explanation of symbols

BCF_{fish}	bioconcentration factor for fish on wet weight basis	[$l \cdot kg^{-1}$]
C_{water}	dissolved concentration in surface water	[$mg \cdot l^{-1}$]
C_{fish}	concentration in wet fish	[$mg \cdot kg^{-1}$]

Biotransfer to plants

The field of chemical uptake by plants is complicated and processes are not very well understood. Furthermore, we are dealing with a heterogeneous variety of plant species, acting as food crops for humans and cattle. The modelling approach proposed by Trapp and Matthies (1995) is used to estimate levels in plants due to uptake from porewater and air (gas-phase).

This approach integrates uptake from porewater and air into a consistent, one-compartment model. The sink term in the model is formed by diffusive transfer from leaf to air, elimination in the plant tissue, and dilution by growth. The source term is formed by the uptake and translocation from soil and gaseous uptake from air.

The model is a simplification of the PLANTX model, as described in Trapp & McFarlane (1995). The original PLANTX model is validated by Trapp and co-workers in short-term experiments. It should be noted that this model is a simplified, generic representation of plant uptake. This model cannot give more than an indication for levels that may occur in plants in the field. Only the concentrations in leaf and root tissue are estimated, fruit is not accounted for. Several plant- specific parameters are required for the model. As there are many different plant species acting as food crops for humans and cattle, an average or typical plant definition is not scientifically feasible. Therefore, the suggested defaults in the Table below are “typical values” but arbitrary and should be evaluated in the future. Aerosol deposition is not considered in the model. Although this route may be important for some substances, it is not yet clear how this route can be satisfactorily quantified and incorporated into the model.

Table 1 Default settings for plant-specific parameters

Plant properties, taken from Riederer (1990), values from Brassica oleracea (rounded)

F_{water}	volume fraction water in plant tissue	[$m^3 \cdot m^{-3}$]	0.65
F_{lipid}	volume fraction lipids in plant tissue	[$m^3 \cdot m^{-3}$]	0.01
$Fair_{plant}$	volume fraction air in plant tissue	[$m^3 \cdot m^{-3}$]	0.30
RHO_{plant}	bulk density of plant tissue	[$kg \cdot m^{-3}$]	700

Plant properties, taken from Trapp and Matthies (1995), values referenced to 1 m²

$AREA_{plant}$	leaf surface area	[m^2]	5
g_{plant}	conductance (0.001 $m \cdot s^{-1}$)	[$m \cdot d^{-1}$]	86.4
V_{leaf}	shoot volume	[m^3]	0.002
Q_{transp}	transpiration stream (1 $l \cdot d^{-1}$)	[$m^3 \cdot d^{-1}$]	1.10^{-3}
b	correction exponent for differences between plant lipids and octanol	[-]	0.95
$kgrowth_{plant}$	growth rate constant for dilution by growth	[d^{-1}]	0.035

The partitioning between water and plant tissue is a key property for the fate of compounds in the soil-plant-air system. This partitioning is assumed to be based on hydrophobic sorption to plant lipids. The Kow is corrected slightly for the differences between plant lipids and octanol. The $K_{plant\text{-}water}$ can than be calculated as:

$$K_{plant\text{-}water} = Fwater_{plant} + Flipid_{plant} \cdot Kow^{\,b} \qquad (3)$$

Explanation of symbols

F_{water}	volume fraction water in plant tissue	[$m^3 \cdot m^{-3}$]	0.65
F_{lipid}	volume fraction lipids in plant tissue	[$m^3 \cdot m^{-3}$]	0.01
Kow	octanol-water partitioning coefficient	[$m^3 \cdot m^{-3}$]	
b	correction for differences between plant lipids and octanol	[-]	0.95
$K_{plant\text{-}water}$	partition coeff. between plant tissue and water	[(mg · m plant^{-3})/(mg · m water^{-3})]	

Concentration in root tissue is mainly governed by physical sorption, and is given by:

$$Croot_{plant} = \frac{K_{plant-water} \cdot C_{porewater}}{ROH_{plant}} \qquad (4)$$

Explanation of symbols

$K_{plant\text{-}water}$	partition coeff. between plant tissue and water	[$m^3 \cdot m^{-3}$]	
C_{porew}	concentration in porewater of soil	[$mg \cdot m^{-3}$]	
RHO_{plant}	bulk density of plant tissue	[$kg \cdot m^{-3}$]	700
$Croot_{plant}$	concentration in root tissue of plant	[$mg \cdot kg^{-1}$]	

The transpiration stream concentration factor (TSCF) is the ratio between the concentration in the transpiration stream and the concentration in porewater. TSCF is given by (Briggs et al., 1982):

$$TSCF = 0.784 \cdot \exp\left[\frac{-(\log Kow - 1.78)^2}{2.44}\right] \qquad (5)$$

Explanation of symbols

Kow	octanol-water partitioning coefficient	[-]
TSCF	transpiration stream concentration factor	[-]

This estimation for TSCF was derived for small group of pesticides in one plant species (Barley). The range of log Kow where this equation can be used is -0.5 - 4.5. Outside this range, the maximum or minimum Kow value should be used. Gaseous exchange leaf-air can be described by a leaf-air partitioning coefficient. $K_{leaf\text{-}air}$ is given by:

$$K_{leaf-air} = Fair_{plant} + \frac{K_{plant-water}}{K_{air-water}} \quad (6)$$

Explanation of symbols

$K_{plant\text{-}water}$	partition coefficient between plant tissue and water	[$m^3 \cdot m^{-3}$]	
$K_{air\text{-}water}$	air-water partitioning coefficient	[$m^3 \cdot m^{-3}$]	
$Fair_{plant}$	volume fraction air in plant tissue	[$m^3 \cdot m^{-3}$]	0.30
$K_{leaf\text{-}air}$	partition coeff. between leaves and air	[$m^3 \cdot m^{-3}$]	

Elimination of the substance may take place in the leaf tissue by metabolism or photolysis. If rate constants are known for these processes, they may be added:

$$K_{elim\,lant} = kmetab_{plant} + kphoto_{plant} \quad (7)$$

Explanation of symbols

$kmetab_{plant}$	pseudo-first order rate constant for metabolism in plants	[d^{-1}]	data set
$kphoto_{plant}$	pseudo-first order rate constant for photolysis in plants	[d^{-1}]	data set
$Kelim_{plant}$	pseudo-first order rate constant for total elimination in plants	[d^{-1}]	

During the process of chemical uptake in the plant, the concentration will also be influenced by growth of the plant. Growth acts as a dilution process by increasing the volume of plant tissue. For the (volumetric) growth rate constant, a default is set (according to Trapp and Matthies, 1995):

$kgrowth_{plant}$	growth rate constant for dilution by growth	[d^{-1}]	0.035

The actual one-compartment model, calculating the concentration in the leaf, can be described with a simple, differential equation:

$$\frac{dC_{leaf}}{dt} = -\alpha \cdot C_{leaf} + \beta \quad (8)$$

Explanation of symbols

C_{leaf}	concentration in leaf tissue of plant	[$mg \cdot m^{-3}$]
α	sink term	[d^{-1}]
β	source term	[$mg \cdot m^{-3} \cdot d^{-1}$]

The sink term is formed by diffusive transfer from leaf to air, elimination in the plant tissue, and dilution by growth:

$$\alpha = \frac{AREA_{plant} \cdot g_{plant}}{K_{leaf-air} \cdot V_{leaf}} + Ke\lim_{plant} + kgrowth_{plant} \quad (9)$$

Explanation of symbols

$AREA_{plant}$	leaf surface area	[m^2]	5
g_{plant}	conductance (0.001 m.s^{-1})	[$m \cdot d^{-1}$]	86.4
$K_{leaf-air}$	partition coeff. between leaves and air	[-]	
V_{leaf}	shoot volume	[m^3]	0.002
$kelim_{plant}$	pseudo-first order rate constant for elimination in plants	[d^{-1}]	
$kgrowth_{plant}$	pseudo-first order rate constant for dilution by growth	[d^{-1}]	

Naturally, the leaf surface area increases during growth, but the model is valid as long as the ratio area to volume stays the same.

The source term is formed by the uptake and translocation from soil and gaseous uptake from air:

$$\beta = C_{porew} \cdot TSCF \cdot \frac{Qtransp}{V_{leaf}} + (1 - Fass_{aer}) \cdot C_{air} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}} \quad (10)$$

Explanation of symbols

Qtransp	transpiration stream (1 1 $\cdot d^{-1}$)	[$m^3 \cdot d^{-1}$]	0.10^{-3}
C_{porew}	concentration in porewater of soil	[$mg \cdot m^{-3}$]	
C_{air}	concentration in air	[$mg \cdot m^{-3}$]	
TSCF	transpiration stream concentration factor	[-]	
V_{leaf}	shoot volume	[m^3]	0.002
$Fass_{aer}$	fraction of substance adsorbed to aerosol	[-]	

The concentration in the leaf can be calculated at any given moment in time by the analytical solution of the differential equation:

$$C_{leaf}(t) = V_{leaf}(0) \cdot e^{-\alpha \cdot t} + \frac{\beta}{\alpha}\left[1 - e^{-\alpha \cdot t}\right] \quad (11)$$

The steady-state concentration is given by:

$$C_{leaf}^{(\infty)} = \frac{\beta}{\alpha} \quad (12)$$

The time required to reach steady-state (95%) is given by:

$$T_{95\%} = -\frac{\ln 0.05}{\alpha} \quad (13)$$

Explanation of symbols

α	sink term in one-compartment model	[d^{-1}]
$T_{95\%}$	time required to reach 95% of steady-state situation	[d]

RHOplant is required to translate from $mg \cdot m^{-3}$ to $mg \cdot kg^{-1}$. According to Riederer (1990), RHOplant = 700 $kg \cdot m^{-3}$ (this is consistent with the definition of 0.65 volume fraction water and 0.30 volume fraction air in plants).

NOTE: the time required to reach 95 % of steady-state is only governed by α. As shown in eq. (7), α cannot be lower than $kgrowth_{plant}$. This implies that 95% of steady-state will always be achieved within 86 days.

Biotransfer to meat and milk

According to Travis and Arms (1988), the uptake of substances by cattle, resulting in concentrations in meat and milk, can be modelled by means of biotransfer factors, defined as the steady state concentration in a receiving medium (meat, milk) divided by the animals' daily intake of the substance in source media (air/grass/soil/drinking water). It should be noted that at this moment, for all dairy products, the concentration in milk is used. Travis and Arms performed a log-linear regression analysis between experimentally derived biotransfer factors and the octanol-water partitioning coefficient:

The BTF for meat is derived on data of 36 organic compounds. It should be noted that the uncertainty in this estimation is considerable. The estimation can be used for substances with a log Kow range of 1.5 - 6.5. Outside this range, the maximum or minimum Kow value should be used.

$$BTF_{meat} = 10^{-7.6 + \log kow} \tag{14}$$

Explanation of symbols

Kow	octanol-water partitioning coefficient	[-]	data set
BTF_{meat}	biotransfer factor for meat	$[(mg \cdot kg^{-1})/(mg \cdot d^{-1})]$	

The BTF for milk was derived on data of 28 organic compounds. It should be noted that the uncertainty in this estimation is considerable. The estimation can be used for substances with a log Kow range of 3 - 6.5. Outside this range, the maximum or minimum Kow value should be used.

$$BTF_{milk} = 10^{-8.1 + \log Kow} \tag{15}$$

Explanation of symbols

Kow	octanol-water partitioning coefficient	[-]	data set
BTF_{milk}	biotransfer factor for milk	$[(mg \cdot kg^{-1})/(mg \cdot d^{-1})]$	

The concentrations in meat and milk can be calculated by applying the biotransfer factors and summing the contributions from air, soil, grass, and drinking water:

$$C_{meat} = BTF_{meat} \cdot \sum C_i \cdot IC_i \qquad i \in \{grass, soil, air, drw\} \tag{16}$$

$$C_{milk} = BTF_{milk} \cdot \sum C_i \cdot IC_i \qquad i \in \{grass, soil, air, drw\} \tag{17}$$

Explanation of symbols

BTF_{meat}	biotransfer factor for meat	[d · kg^{-1}]
BTF_{milk}	biotransfer factor for milk	[d · kg^{-1}]
C_i	concentration in exposure medium i (wet weight)	[mg · kg^{-1} or mg · m^{-3} or mg · l^{-1}]
ICi	daily intake of exposure medium i (wet weight)	[kg · d^{-1} or m^3 · d^{-1} or l · d^{-1}]
Cmeat	concentration in meat (wet weight)	[mg · kg^{-1}]
Cmilk	concentration in milk (wet weight)	[mg · kg^{-1}]

Table 2 Intake rates for cattle, conversions from dry weight to wet weight

Parameter	Dry weight	Conversion dry to wet	Wet weight	Unit
IC_{grass}	16.9 a)	4 a)	67.6	[kg · d^{-1}]
IC_{soil}	0.41 a)	1.13 b)	0.46	[kg · d^{-1}]
IC_{air}			122 a)	[m^3 · d^{-1}]
IC_{drw}			55 c)	[l · d^{-1}]

a) Source: McKone and Ryan (1989).
b) According to: RHOsoil /(Fsolidsoil RHOsolid) with standard values from Chapter 3.
c) Source: ECETOC (1990).

Purification of drinking water

Drinking water is produced from surface water or groundwater, and will be modelled as described by Hrubec and Toet (1992). The drinking water module in the present version of the program, assumes a complete removal of suspended particles from surface water and groundwater. The effects of the treatment processes used for purification of groundwater, which are generally not intended for the removal of organic pollutants, can be neglected.

Dependent on the type of storage, two different water treatment systems for surface water can be distinguished: system 1 includes storage in open reservoirs, system 2 includes dune recharge. Removal of the dissolved fraction of a xenobiotic from the surface water is modelled by means of purification factors. For the choice between the two systems and the choice between surface water or groundwater, a worst-case approach will be followed.

$$F_{pur} = \max\left(Fsys1_{pur}, Fsys2_{pur}\right) \tag{18}$$

Explanation of symbols

$Fsys1_{pur}$	purification factor system 1	[-]
$Fsys2_{pur}$	purification factor system 2	[-]
F_{pur}	worst-case purification factor for surface water	[-]

Purification factors for both systems can be extracted from the table below. The factors from each relevant column should be multiplied to give the resulting purification factor for both systems ($Fsys1_{pur}$ and $Fsys2_{pur}$).

Table 3 Purification factors, based on Henry's law constant and biodegradation rate

Treatment process	log Kow			Henry's law constant ($Pa \cdot m^3 \cdot mol^{-1}$)		Aerobic biodegradation rate (days)	
	<4	4-5	>5	<100	>100	>10	<10
System 1	1	1/4	1/16	1	½	1	1
System 2	1	1/2	1/4	1	½	1	1/4

Source: Hrubec and Toet (1992)

$$C_{drw} = \max\left(C_{water} \cdot F_{pur}, C_{grw}\right) \tag{19}$$

Explanation of symbols

Cwater	dissolved concentration in surface water	[mg · l^{-1}]
Cgrw	groundwater concentration	[mg · l^{-1}]
Cdrw	concentration in drinking water	[mg · l^{-1}]

Total daily intake for humans

The exposure of humans to xenobiotics originates from several sources. The exposure assessment includes 6 pathways: drinking water, fish, crops, meat, milk and air. The daily intake by humans is calculated by means of the daily intake values. This approach implies that each of these intake media is retrieved exclusively from within the contaminated system.

$$DOSE_i = \frac{C_i \cdot IH_i}{BW} \qquad DOSE_{air} = \frac{C_{air} \cdot IH_{air}}{BW} \cdot \frac{BIO_{inh}}{BIO_{oral}} \tag{20}$$

$$i \in \{drw, fish, stem, root, meat, milk\}$$

Explanation of symbols

C_i	concentration in medium i	[mg · kg^{-1} or mg · m^{-3} or mg · l^{-1}]	
IH_i	daily intake of medium i	[kg · d^{-1} or m^3 · d^{-1} or l · d^{-1}]	
BIO_{inh}	bioavailability for chemical through inhalation	[-]	0.75
BIO_{oral}	bioavailability for chemical through oral route	[-]	1
BW	bodyweight of the human considered	[kg]	70
$DOSE_i$	daily dose through intake of i	[mg · kg · bw^{-1} · d^{-1}]	
$DOSE_{air}$	daily dose through inhalation	[mg · kg^{-1} · d^{-1}]	

$$DOSE_{tot} = \left(\sum DOSE_i\right) + DOSE_{air} \tag{21}$$
$$i \in \{drw, fish, stem, root, meat, milk\}$$

Explanation of symbols

$DOSE_i$	daily dose through intake of i	[mg · kg · bw^{-1} · d^{-1}]
$DOSE_{tot}$	total daily intake for humans	[mg · kg · bw^{-1} · d^{-1}]
$DOSE_{air}$	daily dose through inhalation	[mg · kg^{-1} · d^{-1}]

Table 4 Standard defaults-for indirect exposure of humans

Parameter	Symbol	Value	Unit	Source
Drinking water	IH_{drw}	2	$[l \cdot d^{-1}]$	US EPA (1989)
Fish	IH_{fish}	0.115	[kg wwt $\cdot d^{-1}$]	ECETOC (1994)
Leaf crops(incl. fruit and cereals)	IH_{stem}	1.20	[kg wwt $\cdot d^{-1}$]	ECETOC (1994)
Root crops	IHroot	0.384	[kg wwt $\cdot d^{-1}$]	ECETOC (1994)
Meat	IHmeat	0.301	[kg wwt $\cdot d^{-1}$]	ECETOC (1994)
Dairy product	IHmilk	0.561	[kg wwt $\cdot d^{-1}$]	ECETOC (1994)
Inhalation rate	IHair	20	$[m^3 \cdot d^{-1}]$	US EPA (1989)
Bioavail. inhalation	BIOinh	0.75	[-]	Vermeire et al. (1993)
Biaovail. oral route	BIOoral	1.0	[-]	Vermeire et al. (1993)
Body weight adult	BW	70	[kg]	

Table 5 Total average food-consumption in kg per capita per year (Euromonitor, 1992; according to ECETOC, 1994)

Intake of	EU	B	DK	F	D	GR	IRL	I	NL	P	SP	UK	min. EU	max.EU
Meat *	94.0	105.0	108.9	109.9	104.9	76.4	87.3	87.5	88.8	68.4	95.0	77.3	68.4	109.9
Beef and veal	22.0	22.3	18.1	30.7	23.7	19.0	19.0	26.8	19.3	13.6	11.5	17.0		
Pork	40.7	49.1	69.1	37.6	63.2	22.2	34.7	30.8	47.6	25.9	47.4	24.8		
Other	21.7	19.3	13.3	25.2	12.6	29.5	26.7	20.9	18.5	21.9	27.4	25.0		
Fish	10.1	10.2	41.8	7.5	7.4	7.2	12.6	10.1	9.8	24.7	20.3	3.5	3.5	41.8
Milk + Yoghurt	82.4	67.8	74.2	65.8	65.8	66.9	173.6	69.7	79.3	52.1	102.6	122.3	52.1	173.6
Butter	4.9	8.6	6.9	8.6	8.5	1.0	5.5	2.4	3.9	0.9	0.6	4.2	0.6	8.6
Cheese	14.5	12.6	12.8	22.4	17.4	22.1	5.4	17.6	15.0	5.2	5.5	8.0	5.2	22.4
Fruit + Vegetables	205.6	173.8	149.8	194.0	196.4	248.3	148.3	277.5	242.5	165.4	252.6	131.6	131.6	277.5

* Total does not reflect the sum of the individual subgroups

Appendix IV Toxicokinetics

A Predicting toxicokinetics in the absence of experimental toxicokinetic data

B Dermal absorption

C Physiological factors

Appendix IVA Predicting toxicokinetics in the absence of experimental toxicokinetic data

The following document is a guide to the use of physico-chemical data and, if available, toxicological data to produce a qualitative assessment of the toxicokinetic behaviour of a substance. For most substances the available data will be sufficient to enable qualitative judgements to be made about their potential for absorption via the inhalation, oral and dermal routes. However, the physico-chemical characteristics of the substance will change if the substance undergoes metabolic transformation and the physico-chemical characteristics of the parent substance may not provide any clues as to the identity, distribution, retention and elimination of its metabolites.

Absorption

Qualitative information on the potential for a substance to be absorbed by the inhalation, oral and dermal routes can be found from a variety of sources. The observation of systemic toxicity following exposure by any route is one indication that a substance has been absorbed. However, this will not provide any quantitative information about the amount of substance that has been absorbed since it could be that a very small amount of a very toxic substance has been absorbed or a large amount of a much less toxic substance has been absorbed. Also some clinical signs, e.g. hunched posture, could be due to discomfort caused by irritation or simply the presence of a large volume of test substance in the stomach. Reduced feed intake could be due to an unpalatable test substance. It must therefore be clear that the effects that are being cited as evidence of systemic absorption are genuinely due to absorbed test substance and not localised site of contact effects. It is also important to note that an absence of systemic toxicity after exposure does not indicate a lack of absorption, but may indicate that the substance is of low toxicity.

In order for a substance to be absorbed, it must cross biological membranes. Most substances cross by passive diffusion. This process requires a substance to be soluble both in lipid and water. The most useful parameters providing information on the potential for a substance to diffuse across biological membranes are the octanol/water partition coefficient (Log P) value and the water solubility. The Log P value provides information on the relative solubility of the substance in water and the hydrophobic solvent octanol (used as a surrogate for lipid) and is a measure of lipophilicity. Log P values above 0 indicate that the substance is more soluble in octanol than water i.e. lipophilic and negative values indicate that the substance is more soluble in water than octanol i.e. hydrophilic. In general, moderate Log P values (between 0 – 4) are favourable for absorption. However, a substance with a Log P value around 0 and low water solubility (around 1 mg/l) will also be poorly soluble in lipids and hence not readily absorbed. It is therefore important to consider both the water solubility of a substance and its Log P value when assessing the potential of that substance to be absorbed.

Inhalation

Substances that can be inhaled include gases, vapours, liquid aerosols (both liquid substances and solid substances in solution) and finely divided powders/dusts. Such substances may be absorbed from the respiratory tract or, through the action of clearance mechanisms, may be transported out of the respiratory tract and swallowed. This means that absorption from the gastrointestinal tract will contribute to the total body burden of substances that are inhaled. Some factors that determine the extent to which a substance may be absorbed by the inhalation route are discussed below and summarised in **Table 1.**

Gases and vapours

Most gases and vapours are readily absorbed across the lungs. Absorption of gases and vapours occurs predominantly in the alveoli by passive diffusion along a concentration gradient. The key determinant of absorption of gases and vapours in the respiratory tract is solubility in blood. For gases and vapours that readily dissolve into blood, a large proportion of what is inhaled per breath will be absorbed. As solubility in blood decreases the amount that dissolves into the blood per breath will also decrease (Rozman and Klaassen, 1996). To be readily soluble in blood, a gas or vapour must be soluble in water and increasing water solubility would increase the amount absorbed per breath. However, the gas or vapour must also be sufficiently lipophilic to cross the alveolar and capillary membranes therefore a moderate Log P value (between 0 - 4) would be favourable for absorption. The rate of systemic uptake of very hydrophilic gases or vapours may be limited by the rate at which they partition out of the aqueous fluids (mucus) lining the respiratory tract and into the blood. Such substances may be transported out of the lungs with the mucus and swallowed or may pass across the respiratory epithelium via aqueous membrane pores. Highly reactive gases or vapours can react at the site of contact thereby reducing the amount available for absorption. Beside the physico-chemical properties of the compound physical activity has a great impact on absorption rate and must also be addressed (Csanady and Filser, 2001).

Liquid aerosols and finely divided powders/dusts

The potential for liquid aerosols or finely divided powders to be inhaled will be determined by their particle size. The ability of a liquid to form an aerosol and the characteristics of that aerosol will depend on the way the liquid is being used and the process that generated the aerosol. This is not necessarily the case for dusts. Precise deposition patterns for dusts will depend not only on the particle size of the dust but also the hygroscopicity, electrostatic properties and shape of the particles and the respiratory dynamics of the individual. Thus it is only possible to make very general statements about sites of deposition for inhaled dusts. Note that these generalisations apply only to particles which are compact and symmetrical in shape. As a rough guide, particles with aerodynamic diameters below 100 μm have the potential to be inhaled. Particles with aerodynamic diameters of above 1- 5 μm have the greatest probability of settling in the nasopharyngeal region whereas particles with aerodynamic diameters below 1- 5 μm are most likely to settle in the tracheobronchial or pulmonary regions (Velasquez, 1990). Therefore any powder that contains particles with aerodynamic diameters below 100 μm is potentially of concern. It should be noted that particle size distribution data generally relates to the bulk material and not the airborne material. The particle size distribution of the airborne material will be different to that of the bulk material, a greater percentage of smaller particles will be present. On this basis, the particle size distribution data will only indicate the presence of particles of concern but not the percentage of substance that can be inhaled.

Once a liquid droplet or dust particle has deposited in the airways, it can be absorbed across the respiratory tract epithelium, cleared from the lungs via the mucociliary mechanism or lymphatic system or retained within the lungs (Inchiosa, 1987). Highly reactive substances may react at the site of contact thereby reducing the amount available for absorption. Generally, liquids, solids in solution and water-soluble dusts would readily diffuse/dissolve into the mucus lining the respiratory tract. Lipophilic substances (Log P >0) would then have the potential to be absorbed directly across the respiratory tract epithelium. There is some evidence to suggest that substances with higher Log P values may have a longer half-life within the lungs but this has not been extensively studied (Cuddihy and Yeh, 1988). Very hydrophilic substances might be absorbed through aqueous pores (for substances with molecular weights below around 200) or be retained

in the mucus and transported out of the respiratory tract. For poorly water-soluble dusts, the rate at which the particles dissolve into the mucus will limit the amount that could be absorbed directly. Poorly water-soluble dusts depositing in the nasopharyngeal region could be coughed or sneezed out of the body or swallowed (Schlesinger, 1995). Such dusts depositing in the tracheobronchial region would mainly be cleared from the lungs by the mucociliary mechanism and swallowed. However a small amount may be phagocytosed by macrophages and transported to the blood via the lymphatic system. Poorly water-soluble dusts depositing in the alveolar region would mainly be engulfed by alveolar macrophages. The macrophages will then either translocate particles to the ciliated airways or carry particles into the pulmonary interstitium and lymphoid tissues. Particles may also migrate directly to the pulmonary interstitium where clearance depends on the rate at which the particle dissolves. Those particles most likely to be retained are those that are poorly soluble in both water and lipids.

Table 1. Information on the potential for a substance to be absorbed from the respiratory tract

Data source	What it tells us
Vapour pressure	Indicates whether a substance may be available for inhalation as a vapour. As a general guide, highly volatile substances are those with a vapour pressure greater than 25 KPa (or a boiling point below 50°C). Substances with low volatility have a vapour pressure of less than 0.5 KPa (or a boiling point above 150°C)
Particle size	Indicates the presence of inhalable/respirable particles. As a rough guide, particles with aerodynamic diameters below 100 µm have the potential to be inhaled. Particles with aerodynamic diameters of above 1- 5 µm have the greatest probability of settling in the nasopharyngeal region whereas particles with aerodynamic diameters below 1- 5 µm are most likely to settle in the tracheobronchial or pulmonary regions.
Log P	Log P values above 0 indicate the potential for absorption directly across the respiratory tract epithelium.
Water solubility	Very hydrophilic substances may be retained within the mucus or for low molecular weight substances (MW <200) could be absorbed through aqueous pores. Very low water solubility (1 mg/l or less) and small particle size (below 1 µm) indicates the potential for accumulation.
Inhalation toxicity data	If signs of systemic toxicity are present then absorption has occurred. This is not a quantitative measure of absorption.
Oral toxicity data	If signs of systemic toxicity are present in an oral toxicity study or there are other data to indicate the potential for absorption following ingestion it is likely the substance will also be absorbed if it is inhaled.
Hydrolysis test	Hydrolysis data are not always available. The hydrolysis test conducted for a "VIIA" substance notified under Directive 67/548/EEC (7th Amendment) provides information on the half-life of the substance in water at 50°C and pH values of 4.0, 7.0 and 9.0. The test is conducted using a low concentration, 0.01M or half the concentration of a saturated aqueous solution (whichever is the lower). Since the temperature at which this test is conducted is much higher than that in the respiratory tract, this test will not provide an estimate of the actual hydrolysis half-life of the substance in the respiratory tract. However, it may give an indication that the parent compound may only be present in the respiratory tract for a limited period of time. Hence, toxicokinetic predictions based on the characteristics of the parent compound may be of limited relevance.

Oral

For many substances, a single oral dose study and a repeated dose toxicity study usually by the oral route will be available. From the effects and the dose-response relationship observed, it

might be possible to determine that absorption has occurred rather than relying upon predictions. Absorption can occur along the entire length of the gastrointestinal tract. Since most substances are absorbed by passive diffusion, some general physico-chemical characteristics can be identified which favour absorption (D'Souza, 1990). These are listed in **Table 2**. However, substances with physico-chemical characteristics that are not favourable for absorption could still reach the systemic circulation because specific mechanisms exist to enable for example dietary fats and electrolytes to be absorbed. When assessing the potential of a substance to be absorbed in the gastrointestinal tract it should be noted that substances could undergo chemical changes in the gastrointestinal fluids (chymus) as a result of metabolism by gastrointestinal flora, by enzymes released into the gastrointestinal tract or by simple hydrolysis. These changes will alter the physico-chemical characteristics of the substance and hence predictions based upon the physico-chemical characteristics of the parent substance may no longer apply.

One consideration that could influence the absorption of ionic substances (i.e. acids and bases) is the varying pH of the gastrointestinal tract. Not only does the pH of the gastrointestinal fluid change in different sections of the gastrointestinal tract, but the pH of the bulk material within the gastrointestinal tract may be different to that at the mucosal surface. It is generally thought that ionised substances do not readily diffuse across biological membranes. When assessing the potential for an acid or base to be absorbed, knowledge of its pKa is advantageous but this information is not a data requirement for substances notified under Directive 67/548/EEC (7^{th} Amendment). The pKa of a substance is the pH at which 50% of the substance is in ionised and 50% in non-ionised form. Absorption of acids is favoured at pHs below their pKa whereas absorption of bases is favoured at pHs above their pKa. In theory this would suggest that weak acids would be preferentially absorbed in the stomach. However, the large surface area of the small intestine makes this the predominant site of absorption for both weak acids and bases. A substance that is ionised at a pH of around 5 - 6 (i.e. that of the small intestine) would be anticipated to be poorly absorbed. Strong organic acids and bases generally show incomplete absorption because they are extensively ionised at all pH values in the gastrointestinal tract (Renwick, 1994).

Other mechanisms by which substances can be absorbed in the gastrointestinal tract include the passage of small water-soluble molecules (MW up to around 200) through aqueous pores or carriage of such molecules across membranes with the bulk passage of water (Renwick, 1994). The absorption of highly lipophilic substances (Log P of 4 or above) may be limited by the inability of such substances to dissolve into gastrointestinal fluids and hence make contact with the mucosal surface. However, the absorption of such substances will be enhanced if they undergo micellular solubilisation by bile salts (Aungst and Shen, 1986). Substances absorbed as micelles enter the circulation via the lymphatic system, bypassing the liver. Although particles and large molecules (with molecular weights in the 1000's) would normally be considered too large to cross biological membranes, small amounts of such substances may be taken into epithelial cells by pinocytosis or persorption (passage through gaps in membranes left when the tips of villi are sloughed off) (Aungst and Shen, 1986). Absorption of surfactants or irritants may be enhanced because of damage to cell membranes. Occasionally a substance may be sufficiently similar to a nutrient substance to compete with that nutrient for a carrier mediated or active transport mechanism. It is rare that an exogenous compound will be taken up in this manner and it is not generally possible to predict which substances could be absorbed by such a mechanism. Active transport mechanisms also exist to remove exogenous substances from gastrointestinal epithelial cells (efflux mechanisms) thereby limiting entry into the systemic circulation. It is not possible to identify which substances could be removed by efflux mechanisms from physico-chemical data.

The sites and mechanisms of uptake along the gastrointestinal tract are:

Mouth - Uptake is minimal, occurs by passive diffusion, substances pass directly to the systemic circulation, some enzymatic degradation may occur.

Stomach - Uptake is minimal, occurs by passive diffusion, the acidic environment favours uptake of weak acids and there is the potential for hydrolysis and, very rarely, metabolism (by endogenous enzymes) prior to uptake. Substances absorbed at this point will go to the liver before entering the systemic circulation. Hence, first pass metabolism may limit the systemic bioavailability of the parent compound.

Small intestine - The small intestine has a very large surface area and the transit time through this section is the longest, making this the predominant site of absorption within the gastrointestinal tract. Most substances will be absorbed by passive diffusion. However, lipophilic compounds may form micelles and be taken into the lymphatic system and larger molecules/particles may be taken up by pinocytosis. Metabolism prior to absorption may occur by gut microflora or enzymes in the gastrointestinal mucosa. Since substances that enter the blood at this point pass though the liver before entering the systemic circulation, hepatic first pass metabolism may limit the amount of parent compound that enters the systemic circulation.

Large intestine - Uptake is mainly by passive diffusion but active transport mechanisms for electrolytes are present. Compared to the small intestine, the rate and extent of absorption within the large intestine is low. Most blood flow from the large intestine passes through the liver first.

Table 2 Information on the potential for a substance to be absorbed from the gastrointestinal tract

Data source	What it tells us
Structure	It may be possible to identify ionisable groups within the structure of the molecule. Groups containing oxygen, sulphur or nitrogen atoms e.g. thiol (SH), sulphonate (SO_3H), hydroxyl (OH), carboxyl (COOH) or amine (NH_2) groups are all potentially ionisable.
Molecular weight	Generally the smaller the molecule the more easily it will be taken up. Molecular weights below 500 are favourable for absorption; molecular weights in the 1000's do not favour absorption.
Particle size	Generally solids have to dissolve before they can be absorbed. It is possible for small amounts of particles in the in the nanometer size range to be taken up by pinocytosis. The absorption of very large particles, several hundreds of micrometers in diameter, that were administered dry (e.g. in the diet) or in a suspension may be reduced because of the time taken for the particle to dissolve. This would be particularly relevant for poorly water-soluble substances.
Water solubility	Water-soluble substances will readily dissolve into the gastrointestinal fluids. Absorption of very hydrophilic substances by passive diffusion may be limited by the rate at which the substance partitions out of the gastrointestinal fluid. However, if the molecular weight is low (less than 200) the substance may pass through aqueous pores or be carried through the epithelial barrier by the bulk passage of water.
Log P	Moderate Log P values (between 0 – 4) are favourable for absorption by passive diffusion. Any lipophilic compound may be taken up by micellular solubilisation but this mechanism may be of particular importance for highly lipophilic compounds (Log P >4), particularly those that are poorly soluble in water (1 mg/l or less) that would otherwise be poorly absorbed.
Dosing vehicle	If the substance has been dosed using a vehicle, the water solubility of the vehicle and the vehicle/water partition coefficient of the substance may affect the rate of uptake. Compounds delivered in aqueous media are likely to be absorbed more rapidly than those delivered in oils, and compounds delivered in oils that can be emulsified and digested e.g. corn oil or arachis oil are likely to be absorbed to a greater degree than those delivered in non-digestible mineral oil (liquid petrolatum) (D'Souza, 1990).
Oral toxicity data	If signs of systemic toxicity are present, then absorption has occurred. Also coloured urine and/or internal organs can provide evidence that a coloured substance has been absorbed. This information will give no indication of the amount of substance that has been absorbed. Also some clinical signs such as hunched posture could be due to discomfort caused by irritation or simply the presence of a large volume of test substance in the stomach and reduced feed intake could be due to an unpalatable test substance. It must therefore be clear that the effects that are being cited as evidence of systemic absorption are genuinely due to absorbed test substance and not localised site of contact effects.
Hydrolysis test	If a substance undergoes hydrolysis in the GI tract, the bioavailability of the parent compound will be reduced and toxicokinetic predictions based on the characteristics of the parent compound may not be relevant.

Dermal

(Risk assessment of dermally absorbed substances is also addressed in Annex B). There are three possible routes for substances to penetrate the skin. Firstly there is the intercellular route in which the chemical penetrates via the lipid filled medium between the compact corneocytes of the stratum corneum. Secondly there is the trans-cellular route in which the chemical crosses through the corneocytes and finally the trans-appendageal route in which chemicals penetrate through the lipids surrounding hairs in the follicles or sebum in sweat ducts. Penetration via the first two routes requires the chemical to enter the stratum corneum and then partition into the more aqueous epidermis and into the dermis and the systemic circulation. For substances that penetrate the skin by these routes, the rate of transfer between the stratum corneum and the epidermis is thought to be the rate-limiting step for dermal absorption. The trans-appendageal route allows the substance to penetrate directly to the dermis. The contribution that absorption via the trans-appendageal route makes to overall dermal absorption has not been systematically studied for a wide range of chemicals and it is not possible to identify specific physico-chemical

properties that would favour absorption by this route. Consequently absorption via the trans-appendageal route will not be addressed further in this document.

Substances that can potentially be taken up across the skin include gases and vapours, liquids and particulates. Evidence that dermal uptake has or might occur is listed in **Table 3.** Most of the information in this section has been taken from Pryde and Payne (1996).

Table 3 Evidence that uptake following dermal exposure has or might occur

Data source	What it tells us
Physical state	Liquids and substances in solution are taken up more readily than dry particulates. Dry particulates will have to dissolve into the surface moisture of the skin before uptake can begin. Absorption of volatile liquids across the skin may be limited by the rate at which the liquid evaporates off the skin surface (Pryde and Payne, 1999).
Molecular weight	Less than 100 favours dermal uptake. Above 500 the molecule may be too large.
Structure	As a result of binding to skin components the uptake of chemicals with the following groups can be slowed: certain metal ions, particularly Ag+, Cd2+, Be2+ and Hg2+ acrylates quaternary ammonium ions heterocyclic ammonium ions sulphonium salts. A slight reduction in the dermal uptake of chemicals with the following groups could also be anticipated for the same reason: quinones dialkyl sulphides acid chlorides halotriazines dinitro or trinitro benzenes.
Water solubility	The substance must be sufficiently soluble in water to partition from the stratum corneum into the epidermis. Therefore if the water solubility is below 1 mg/l, dermal uptake is likely to be low. Between 1-100 mg/l absorption is anticipated to be low to moderate and between 100-10,000 mg/l moderate to high. However, if water solubility is above 10,000 mg/l and the Log P value below 0 the substance may be too hydrophilic to cross the lipid rich environment of the stratum corneum. Dermal uptake for these substances will be low.
Log P (octanol/water)	For substances with Log P values below 0, poor lipophilicity will limit penetration into the stratum corneum and hence dermal absorption. Values below –1 suggest that a substance is not likely to be sufficiently lipophilic to cross the stratum corneum, therefore dermal absorption is likely to be low. Log P values between 1 and 4 *favour* dermal absorption (values between 2 and 3 are optimal) particularly if water solubility is high. Above 4, the rate of penetration may be limited by the rate of transfer between the stratum corneum and the epidermis, but uptake into the stratum corneum will be high. Above 6, the rate of transfer between the stratum corneum and the epidermis will be slow and will limit absorption across the skin. Uptake into the stratum corneum itself may be slow.
Vapour pressure	The rate at which gases and vapours partition from the air into the stratum corneum will be offset by the rate at which evaporation occurs therefore although a substance may readily partition into the stratum corneum, it may be too volatile to penetrate further. This can be the case for substances with vapour pressures above 100-10,000 Pa (ca. 0.76-76 mm Hg) at 25°C, though the extent of uptake would also depend on the degree of occlusion, ambient air currents and the rate at which it is able to transfer across the skin. Vapours of substances with vapour pressures below 100 Pa are likely to be well absorbed and the amount absorbed dermally may be more than 10% of the amount that would be absorbed by inhalation.
Surface tension	If the surface tension of an aqueous solution is less than 10 mN/m, the substance is a surfactant and this will enhance the potential dermal uptake. Surfactants can also substantially enhance the absorption of other compounds, even in the absence of skin irritant effects.
Skin irritation / Corrosivity	If the substance is a skin irritant or corrosive, damage to the skin surface may enhance penetration.
Dermal toxicity data	Signs of systemic toxicity indicate that absorption has occurred. However, if steps have not been taken to prevent grooming, the substance may have been ingested and therefore signs of systemic toxicity could be due to oral rather than dermal absorption.
Skin sensitisation data	If the substance has been identified as a skin sensitiser then, providing the challenge application was to intact skin, some uptake must have occurred although it may only have been a small fraction of the applied dose.

Distribution

It is sometimes possible to get an indication of how widely the parent compound may distribute in the body from the available physico-chemical and toxicological data. The sites to which the parent compound distributes (pattern of distribution) once it has entered the systemic circulation are likely to be similar for all routes of administration. However, because of first pass effects, differing amounts of parent substance may be available for distribution depending on route of administration. It is important to note that the pattern of distribution for any metabolites is likely to be different to the pattern of distribution predicted for the parent substance. Therefore, if a substance undergoes extensive first-pass metabolism, predictions made on the basis of the physico-chemical characteristics of the parent substance may not be applicable. Sometimes, the available information may not allow a judgement to be made about the potential distribution of the parent compound.

In general, substances and their metabolites that readily diffuse across membranes will distribute throughout the body and may be able cross the blood-brain and blood-testes barriers, although the concentrations within the brain or testes may be lower than that in the plasma (Rozman and Klaassen, 1996). The rate at which highly water-soluble molecules distribute may be limited by the rate at which they cross cell membranes and access of such substances to the central nervous system (CNS) or testes is likely to be restricted (though not entirely prevented) by the blood-brain and blood-testes barriers. It is not clear what barrier properties the placenta may have. However, species differences in transplacental transfer may occur due to differing placental structure and also differing metabolic capacity of the placenta in different species. Although protein binding can limit the amount of a substance available for distribution, it will generally not be possible to determine from the available data which substances will bind to proteins and how avidly they will bind. Sources of information about the potential distribution of a substance are listed in **Table 4**.

Table 4 Sources of information on the extent to which a substance might distribute

Data source	What it tells us
Molecular weight	In general, the smaller the molecule, the wider the distribution.
Water solubility	Small water-soluble molecules and ions will diffuse through aqueous channels and pores. The rate at which very hydrophilic molecules diffuse across membranes could limit their distribution.
Log P	If the molecule is lipophilic (Log P >0), it is likely to distribute into cells and the intracellular concentration may be higher than extracellular concentration particularly in fatty tissues.
Target organs	If the parent compound is the toxicologically active species, it may be possible to draw some conclusions about the distribution of that substance from its target tissues. If the substance is a dye, colouration of internal organs can give evidence of distribution. This will not provide any information on the amount of substance that has distributed to any particular site. Note that anything present in the blood will be accessible to the bone marrow.
Signs of toxicity	Clear signs of CNS effects indicate that the substance (and/or its metabolites) has distributed to the CNS. However, not all behavioural changes indicate that the substance has reached the CNS. The behavioural change may be due to discomfort caused by some other effect of the substance.

Bioaccumulative potential

It is also important to consider the potential for a substance to accumulate or to be retained within the body. Lipophilic substances have the potential to accumulate within the body if the dosing interval is shorter than 4 times the whole body half-life. Although there is no direct correlation between the lipophilicity of a substance and its biological half-life, substances with high log P values tend to have longer half-lives. On this basis, there is the potential for highly lipophilic substances (Log P >4) to accumulate in individuals that are frequently exposed (e.g. daily at work) to that substance. Once exposure stops the concentration within the body will decline at a rate determined by the half-life of the substance. Other substances that can accumulate within the body include poorly soluble particulates that deposited in the alveolar region of the lungs, substances that bind irreversibly to endogenous proteins and certain metals and ions that interact with the crystal matrix of bone (Rozman and Klaassen, 1996). The properties of these substances are such that the body cannot readily remove them, hence they gradually build up with successive exposures and the body burden can be maintained for long periods of time. Factors that indicate the potential for accumulation and retention are outlined in **Table 5**.

Table 5 Sites and physico-chemical indicators of bioaccumulation

Site	Characteristics of substances of concern
Lung	Poorly water and lipid soluble particles (i.e. Log P values around 0 and water solubility around 1 mg/l or less) with aerodynamic diameters 1 µm or below have the potential to deposit in the alveolar region of the lung. Here particles are likely to undergo phagocytosis by alveolar macrophages. The macrophages will then either translocate particles to the ciliated airways or carry particles into the pulmonary interstitium and lymphoid tissues. Particles can also migrate directly to the pulmonary interstitium and this is likely to occur to the greatest extent where the particle is toxic to alveolar macrophages or inhaled in sufficient quantities to overwhelm the phagocytic capabilities of alveolar macrophages. Within the pulmonary interstitium clearance depends on solubilisation alone, leading to the possibility of long-term retention (Snipes, 1995).
Adipose tissue	Lipophilic substances will tend to concentrate in adipose tissue and depending on the conditions of exposure may accumulate. If the interval between exposures is less than 4 times the whole body half-life of the substance then there is the potential for the substance to accumulate. It is generally the case that substances with high log P values have long biological half-lives. On this basis, daily exposure to a substance with a log P value of around 4 or higher could result in a build up of that substance within the body. Substances with Log P values of 3 or less would be unlikely to accumulate with the repeated intermittent exposure patterns normally encountered in the workplace but may accumulate if exposures are continuous. Once exposure to the substance stops, the substance will be gradually eliminated at a rate dependent on the half-life of the substance. If fat reserves are mobilised more rapidly than normal, e.g. if an individual or animal is under stress or during lactation there is the potential for large quantities of the parent compound to be released into the blood.
Bone	Certain metals e.g. lead and small ions such as F^- can interact with ions in the crystal matrix of bone. In so doing they can displace the normal constituents of bone, leading to retention of the metal or ion.
Stratum corneum	Highly lipophilic substances (Log P between 4 and 6) that come into contact with the skin can readily penetrate the lipid rich stratum corneum but are not well absorbed systemically. Although they may persist in the stratum corneum, they will eventually be cleared as the stratum corneum is sloughed off. A turnover time of 12 days has been quoted for skin epithelial cells (Valburg, 1990).

Metabolism

Differences in the way substances are metabolised by different species and within different tissues is the main reason for species and route specific toxicity. The liver has the greatest capacity for metabolism and a common cause of route specific effects is presystemic (first pass) metabolism by the liver of substances absorbed following oral intake. However, route specific

toxicity may result from several phenomena, such as hydrolysis within the gastrointestinal or respiratory tracts, also metabolism by gastrointestinal flora or within the gastrointestinal tract epithelia (mainly in the small intestine), respiratory tract epithelia (sites include the nasal cavity, tracheobronchial mucosa [Clara cells] and alveoli [type 2 cells]) and skin. It is very difficult to predict what metabolic changes a substance may undergo on the basis of physico-chemical data alone. Although it is possible to look at the structure of a molecule and identify potential metabolites, it is by no means certain that these reactions will occur *in vivo*. The molecule may have the wrong three-dimensional shape or may not reach the necessary site for a particular reaction to take place. It is even more difficult to predict the extent to which it will be metabolised along different pathways and what species differences may exist. Therefore, although predictive models have been developed, at present such models are not able to mimic the complexities of the *in vivo* situation and give at best a qualitative overview of possible metabolites that might be formed. On this basis, experimental data are much preferred for any assessment of potential metabolic pathways. Provision of such data is not a requirement for new substances notified under directive 67/548/EEC (7th Amendment) until they have reached the "Level 2" tonnage trigger.

Evidence that metabolic changes may occur can be found from the *in vitro* genotoxicity tests where differences in mutagenic activity or cytotoxicity between cultures prepared with or without S9 may be seen. The hydrolysis test which is sometimes conducted as part of the ecotoxicity testing programme may also provide information on the potential for the substance to hydrolyse *in vivo*. Mucosal irritation may be a consequence of exposure to a substance which rapidly hydrolyses, evidence of this might be found in the eye irritation test or toxicity tests by the oral or inhalation routes. Hydrolysis occurring at different rates at different pHs may be one possible explanation for route specific toxicity. If a large proportion of the parent molecule is likely to undergo hydrolysis in the body, this will affect the reliability of toxicokinetic predictions based on the physico-chemical characteristics of the parent compound, since these characteristics may be very different to the physico-chemical characteristics of any hydrolysis products.

Excretion

There are a limited number of conclusions that can be drawn from physico-chemical data about the excretion of a substance from the body. Depending on the metabolic changes that may have occurred, the compound that is finally excreted may have few or none of the physico-chemical characteristics of the parent compound. Also, depending on whether the substance is conjugated, the molecular weight of the final product may be smaller or greater than that of the parent compound.

The major routes of excretion for substances from the systemic circulation are in the urine and/or the faeces (via bile and directly from the gastrointestinal mucosa (Rozman, 1986). For volatile substances and metabolites exhaled air is an important route of excretion. Substances that are excreted in the urine tend to be water-soluble and of low molecular weight (below 300 in the rat). Most will have been filtered out of the blood by the kidneys though a small amount may enter the urine directly by passive diffusion and there is the potential for reabsorption into the systemic circulation across the tubular epithelium. Substances that are excreted in the bile tend to have higher molecular weights. In the rat it has been found that substances with molecular weights below around 300 do not tend to be excreted into the bile (Renwick, 1994). However, it is not clear if a similar cut-off exist for humans and if so where this cut-off lies. Substances in the bile pass through the intestines before they are excreted in the faeces and as a result may undergo enterohepatic recycling which will prolong their biological half-life. This is particularly

a problem for conjugated molecules that are hydrolysed by gastrointestinal bacteria to form smaller more lipid soluble molecules that can then be reabsorbed from the gastrointestinal tract. Those substances less likely to recirculate are substances having strong polarity and high molecular weight of their own accord. Other substances excreted in the faeces are those that have diffused out of the systemic circulation into the gastrointestinal tract directly, substances which have been removed from the gastrointestinal mucosa by efflux mechanisms and non-absorbed substances that have been ingested or inhaled and subsequently swallowed.

Small amounts of substances and their metabolites may also be excreted in other biological fluids for example breast milk, saliva and sweat. However, these may have significant toxicological consequences. For example, excretion in breast milk is of particular toxicological importance because of the resulting exposure of the neonate. In the skin, substances that have accumulated within the stratum corneum are gradually removed by exfoliation. Some substances are incorporated into hair and nails. **Table 6** outlines a few general rules about factors governing routes of excretion and sources of information that might indicate that excretion by a particular route is occurring.

Table 6 Physico-chemical determinants of potential routes of excretion

Route	Favourable physico-chemical characteristics
Urine	Characteristics favourable for urinary excretion are low molecular weight (below 300 in the rat), good water solubility, and ionisation of the molecule at the pH of urine.
Exhaled air	Vapours and gases are likely to be excreted in exhaled air. Also volatile liquids and volatile metabolites may be excreted as vapours in exhaled air.
Bile	In the rat, molecules that are excreted in the bile are amphipathic (containing both polar and non-polar regions), hydrophobic/strongly polar and have a high molecular weight. In general, in rats for organic cations with a molecular weight below 300 it is unlikely that more than 5-10% will be excreted in the bile, for organic anions e.g. quaternary ammonium ions this cut off may be lower (Smith, 1973). Substances excreted in bile may potentially undergo enterohepatic circulation. This is particularly a problem for conjugated molecules that are hydrolysed by gastrointestinal bacteria to form smaller more lipid soluble molecules that can then be reabsorbed from the gastrointestinal tract. Those substances less likely to recirculate are substances having strong polarity and high molecular weight of their own accord. Little is known about the determinants of biliary excretion in humans.
Faeces	The faeces will contain those substances that are excreted in the bile plus substances that have diffused out of the systemic circulation into the gastrointestinal tract, substances that have been removed from gastrointestinal mucosal cells by efflux and non-absorbed substances that have been ingested or inhaled and subsequently swallowed.
Breast milk	All substances present in plasma are found in the breast milk. Lipid soluble molecules may be at a higher concentration than in blood/plasma. Although excretion in breast milk is likely to be a minor route, given the limited amount of milk produced, it may have great toxicological significance for the neonate.
Saliva/sweat	Non-ionised and lipid soluble molecules may be excreted in the saliva, where they may be swallowed again, or in the sweat.
Hair/nails	Metal ions may be incorporated into the hair and nails.
Exfoliation	Highly lipophilic substances that have penetrated the stratum corneum but not penetrated the viable epidermis may be sloughed off with skin cells.

Appendix IVB Dermal absorption

Dermal absorption

The dermal route is a major route of exposure in many occupational and consumer exposure scenarios. In order to determine the contribution dermal exposure may make to systemic body burden, it is necessary to estimate the potential for a substance to be absorbed across the skin. For this purpose, dermal absorption studies are conducted. It should be noted however, that experimental dermal absorption data are not necessarily suitable for dermal risk assessment if an inappropriate experimental protocol has been used.

Dermal absorption, the process by which a substance is transported across the skin and taken up into the living tissue of the body (US EPA, 1992), is a complex process. The extent to which a substance is absorbed across the skin is influenced by many factors. The skin is a dynamic, living multilayered biomembrane and as such its permeability may vary as a result of changes in hydration, temperature, and occlusion. In order to cross the skin, a compound must first penetrate into the dead *stratum corneum* and may subsequently reach the viable epidermis, the dermis and the vascular network. During the absorption process, the compound may be subject to biotransformation (for review see Noonan and Wester, 1989). The *stratum corneum* provides its greatest barrier function against hydrophilic compounds, whereas the viable epidermis is most resistant to penetration by highly lipophilic compounds (Flynn, 1985).

Dermal absorption is influenced by many factors e.g. physico-chemical properties of the substance, vehicle, occlusion, concentration, exposure pattern, skin site of the body, etc. (for review see Howes et al., 1996; Schaefer and Redelmeier, 1996; ECETOC, 1993). It is generally tested in studies according to methodologies described by international platforms (OECD, 2000b,c,d; US EPA, 1996, 1999; ECETOC, 1993; Howes et al., 1996; Diembeck et al., 1999). These documents provide a certain amount of standardisation and thereby improve the ability to compare data between studies. These guidelines, however, give only a general description of the experimental design, whereas a proper study protocol (be it *in vivo* or *in vitro*) should take the anticipated exposure conditions into account (Benford et al., 1999). With respect to the anticipated exposure scenarios it is important to realise that the duration and frequency of exposure as well as the level of exposure may vary tremendously. Exposure may be incidental or may be almost continuous. In view of the tremendous differences in exposure conditions, studies addressing more than one relevant concentration per unit area are highly recommended, as well as use of various exposure times, and vehicles. In general, the percentage absorption is the most useful parameter to use for risk assessment. Since the percentage absorption is amongst others dependent on the exposure duration and concentration, the latter being inversely related to the percentage absorption, it is recommended that *1)* the exposure duration of the study should be as long as or longer than the anticipated exposure duration and, *2)* the concentrations tested should include the lowest concentration anticipated, on a precautionary basis. In general, this will result in rather conservative estimates of dermal absorption.

An estimate of dermal absorption cannot be deduced from the results of acute toxicity studies because of the fact that differences in e.g. oral and dermal LD_{50} values are not necessarily a result of differences in absorption. First, the result in a dermal LD_{50} study is dependent on the size of the exposed area and can be changed by altering the exposed area. Second, differences in toxicity after oral and dermal exposure could be the result of first-pass effects (i.e. substance is (in)activated in the liver). Furthermore, the toxicity of a substance is also influenced by the rate of absorption. Generally, and especially in acute (gavage) studies, oral absorption may be relatively fast, resulting in a peak concentration in the body, whereas the absorption after dermal exposure may generally be more gradual. Finally, in establishing LD_{50} values usually high levels

of test compound are given. Since absorption percentages are highly dependent on the applied dose, this may very well lead to underestimation of absorption percentages at (low) occupational exposure levels. Based on these considerations, it can be concluded that the results of acute toxicity studies can only be used to indicate high, but not low, dermal absorption.

An estimate of dermal absorption can be made by considering data on physico-chemical properties of the substance (molecular weight (MW), log P_{ow}) possibly in combination with oral absorption data (see below) or, preferably, by considering experimental dermal absorption data. If an initial assessment ends up with a prediction of a risk, more refinement can be obtained if more information is provided on the actual exposure and tailor made dermal absorption studies are conducted.

Default-values for dermal absorption

Based on theoretical considerations on skin permeation, it might be expected that there should be an optimum in log P_{ow} and a maximum in MW for facilitating percutaneous absorption. Unfortunately, clear cut-off values for negligible, low and/or high dermal absorption of chemicals cannot be derived from data presented in the literature. The following criteria were proposed by De Heer et al. (1999) to discriminate between chemicals with high and low dermal absorption:

- 10% dermal absorption is used in case MW >500 and log P_{ow} is smaller than -1 or higher than 4, otherwise
- 100% dermal absorption is used.

The lower limit of 10% was chosen, because there is evidence in the literature that substances with MW and/or log P values at these extremes can to a limited extent cross the skin. If data are available (e.g. data on water solubility, ionogenic state, 'molecular volume', oral absorption and dermal area dose in exposure situations in practice) which indicate the use of an alternative dermal absorption percentage value is appropriate, then this alternative value can be used. Scientific justification for the use of alternative values should be provided.

If a default value for dermal absorption of 100% is applicable based on the physico-chemical properties of a substance and an appropriate oral absorption/ADME study (with an acceptable total recovery (OECD, 1984)) is available, the results of the oral absorption study may be used to refine the default value for dermal absorption. It is required that the oral absorption is determined at appropriate dose levels in bile duct cannulated experimental animals, to get an accurate estimate of the oral absorption. Based on theoretical grounds and supported by a comparison of oral and dermal absorption data available for 12 pesticides, it is assumed that dermal absorption will not exceed oral absorption established by means of bile duct cannulation (unpublished data). In that case the oral absorption value may be adopted for dermal absorption as well.

The use of mathematical skin permeation models for quantitative risk assessment purposes is limited because these models have generally been validated by *in vitro* data ignoring the fate of the skin residue levels (see below). However, these models may prove useful as a screening tool or for qualitative comparison of skin permeation potential. On a case-by-case basis, and if scientifically justified, the use of (quantitative) structure activity relationships may prove useful, especially within a group of closely related substances.

In vitro dermal absorption studies

In vitro dermal absorption studies should preferably be performed according to OECD guideline 428 (OECD 2000c,d). Although *in vitro* studies using various methods with respect to e.g. the

type of diffusion cell and the skin membrane used are increasingly being submitted, there is still debate over the way in which *in vitro* data could or should be used in risk assessment. Recently, an evaluation of available data on *in vitro* dermal absorption was performed under auspices of the OECD (OECD, 2000a). Because the available studies, comparing *in vitro* and *in vivo* test results, contained too many variables (different species, thickness and types of the skin, exposure duration, vehicles, etc.), evaluation of *in vitro* test methods by means of data available from public literature appeared to be difficult (OECD, 2000a). A major issue of concern relating to *in vitro* methods was the presence of test substance in the various skin layers, i.e., absorbed into the skin but not passed into the receptor fluid. It was noted that it is especially difficult to examine very lipophilic substances *in vitro*, because of their low solubility in most receptor fluids. By including the amount retained in the skin *in vitro*, a more acceptable (but probably conservative) estimation of skin absorption can be obtained. Alternatively, this issue can be addressed by conducting additional *in vitro* or *in vivo* studies. Water-soluble substances can be tested more accurately *in vitro* because they more readily diffuse into the receptor fluid (OECD, 2000a). At present, provided that skin levels are included in the overall percentage absorption figure, results from *in vitro* methods seem to adequately reflect those from *in vivo* experiments supporting their use as a replacement test to measure percutaneous absorption. Preferably, *in vitro* studies should include reference compounds to increase the confidence of the results (see OECD, 2000d).

Other measures of dermal absorption that can be derived from *in vitro* dermal absorption studies are the (maximum) flux and the permeability coefficient (Kp). The maximum flux (defined as the (maximum) mass of test chemical passing through a unit area of skin per unit of time (in $\mu g/cm^2/h$) and calculated from the linear part of the absorption vs. time curve) at relevant exposure levels can be used for semi-quantitative comparison of absorption of chemicals between species, between compounds within one species, and between different vehicles within one species. In this regard, it is important to realise that *in vitro* studies give relative results, i.e. that they should in first instance be compared with results generated within the same test system at comparable and relevant test conditions. If appropriate dermal penetration data are available for rats *in vivo* and for rat and human skin *in vitro*, the *in vivo* dermal absorption in rats may be adjusted in light of the relative absorption through rat and human skin *in vitro*. The latter adjustment may be done because the permeability of human skin is often lower than that of animal skin (e.g., Howes et al., 1996). A generally applicable correction factor for extrapolation to humans can however not be derived, because the extent of overestimation appears to be dose, substance, and animal specific (Bronaugh and Maibach, 1987; ECETOC, 1993).

The permeability coefficient (Kp) is a value (in cm/h) that represents the rate at which a chemical penetrates the skin. This is calculated from the flux divided by the applied concentration (OECD, 2000d). Because, by definition, the permeation constant (Kp in cm/hr) is established at infinite dose levels, the usefulness of the Kp for dermal risk assessment is limited.

In vivo dermal absorption studies

In vivo dermal absorption studies should preferably be performed according to OECD guideline 427 (OECD 2000b,d). *In vivo* dermal absorption studies performed in laboratory animals can be used to estimate percutaneous absorption in humans. *In vivo* skin absorption studies for risk assessment purposes are most often performed in laboratory rats. While US EPA (1996) states that the rat is the only acceptable species, OECD (2000b) mentions that also other animal species can be used when they have been proven to have more similar skin absorption rates to humans. Although it is known that data from rat studies generally overestimate human skin absorption (e.g. ECETOC, 1993; Van de Sandt et al., 2000), no validation of the rat *in vivo* study has been performed in which reproducibility and the relationship to human skin absorption have been

established. Unlike for *in vitro* studies, no inter-laboratory validation studies have been performed. Since the results of *in vivo* dermal absorption studies are heavily influenced by experimental conditions, such as dermal area dose (in $\mu g/cm^2$), exposure duration, occlusion, vehicle, species, application site, etc. (ECETOC, 1993), test results can only be used with confidence if the experimental conditions match the exposure scenario of interest with regard to these aspects.

A general problem is the fate of the test substance remaining in the application site at the termination of the experiment, i.e., should it be included in the output value of the study or no? Unless data are available from which the total absorbed dose can be calculated e.g. plasma kinetics or an absence of parent compound and/or metabolites in excreta, it seems scientifically justified to use the "potentially absorbed dose" i.e. substance remaining in the skin at the site of application plus already absorbed substance as a basis for risk assessment calculations (see e.g. Chu et al, 1996). The decision about the quantity that remains bound in the skin can be based e.g. on the excretion curve - a decline of radioactivity in the excreta at the end of experiment indicates that the dose at the dosed skin site may not become (completely) systemically available (Thongsinthusak et al., 1999; De Heer et al*.,* 1999). In this case, the skin bound dose at the end of the exposure period may not or only partly be included as being absorbed.

Finally, nearly all of the available dermal absorption studies have used single exposure regimes. Information on the effects of repeated exposure on dermal absorption is scarce and no general conclusion can be drawn from this. Repeated exposures may increase dermal absorption (e.g. Wester et al., 1994) or may not affect the absorption characteristics (e.g. Tauber and Matthes, 1992). The experiments addressing repeated application further indicate that the results may be influenced by experimental conditions such as skin washing and direct effects of the test compound on the skin.

Appendix IVC Physiological factors

Inventory of physiological factors that could be useful for the toxicokinetic assessment

This inventory has been compiled to provide a source of information on physiological parameters for various species that may be useful for interpreting toxicokinetic data. The list is not exhaustive and data from other peer-reviewed sources may be used. If study-specific data are available then this should be used in preference to default data.

In a recent publication Zwart et al. (1999) have reviewed anatomical and physiological differences between various species used in studies on pharmacokinetics and toxicology of xenobiotics. A selection of the data presented by these authors that may be relevant in the context of the EU Risk assessment is quoted below. The tables are adapted from Zwart et al. (1999).

The authors however, focus on the oral route of administration and data relevant for other routes may have to be added. Some of those are already quoted in the section on repeated dose toxicity and are therefore not repeated here.

Data on stomach pH-values

Qualitative Aspects to be considered in the stomach

Rodents have a non-glandular forestomach that has no equivalent in humans. It is thin-walled and transparent. In the non-glandular stomach the pH is typically higher than in the glandular part and it contains more microorganisms. The glandular stomach has gastric glands similar to the human stomach but is a relatively small part of the total rodent stomach. Data on stomach pH for different species are rare and most stem from relatively old sources.

Table 1 Data on stomach pH for different species

	Human	Rhesus monkey	Rat	Mouse	Rabbit	Dog	Pig
Median							2.7 (3.75-4)
Median anterior portion	2.7 (1.8-4.5)	4.8	5.0	4.5	1.9	5.5	4.3
Median posterior portion	1.9 (1.6-2.6)	2.8	3.0	3.1	1.9	3.4	2.2
Fasted	1.7 (1.4-2.1)					1.5	1.6-1.8 (0.8-3.0)
Fed	5.0 (4.3-5.4)					2.1± 0.1 [1)]	<2 [2)]

1) Standard deviation
2) Data from one animal only

Data on intestine pH and transit times

Table 2 Data on Intestine pH

pH (fasted)	Human	Rat (Wistar)	Rabbit	Dog	Pig	Monkey
Intestine		6.5-7.1	6.5-7.1	6.2-7.5	6.0-7.5	5.6-9
Duodenum	5-7	6.9[1]		4.5-7.5	7.2	
Jejunum	6-7					
Ileum	7-8					
Jejunum/ileum		7.8[1]				
Caecum	5.9	6.8	6.6	6.4	6.3	5.0
Colon	5.5-7	6.6, 7.1 [1)]	7.2	6.5	6.8	5.1
Rectum	7					

1) Fed state

Table 3 Calculated transit times in the intestine

Transit time (hours)	Human	Rat	Rabbit	Dog
small intestine	2.7 to 5 [1)] Children (8 to 14 years): 5.1-9.2	1.5		0.5-2
Colon	Children (8 to 14 years): 6.2-54.7	6.0-7.2	3.8	

1) From various authors, after fasting or a light meal

Physiological parameters for inhalation

Table 4 A comparison of physiological parameters relating to the upper airways of rat, humans and monkeys (DeSesso, 1993)

Species	body weight (kg)	Body surface area (m^2)	Nasal cavity volume (cm^3)	Nasal cavity surface area (cm^2)	Relative nasal surface area	Pharynx surface area (cm^2)	Larynx surface area (cm^2)	Trachea surface area (cm^2)	Tidal volume (cm^3)	Breaths per min	Minute volume (l/min)
Human	70	1.85	25	160	6.4	46.6	29.5	82.5	750-800	12-15	9-12
Rhesus monkey	7	0.35	8	62	7.75	-	-	-	70	34	2.4
Rat	0.25	0.045	0.26	13.44	51.7	1.2	0.17	3	2	120	0.24

The US EPA in the Exposure factors handbook, 1997 has reviewed a number of studies on inhalation rates for different age groups and activities. The activity levels were categorised as resting, sedentary, light, moderate and heavy. Based on the studies that are critically reviewed in detail in the US EPA document, a number of recommended inhalation rates can be derived. One bias in the data is mentioned explicitly, namely that most of the studies reviewed were limited to the Los Angeles area and may thus not represent the general US population. This should also be

born in mind when using those data in the European context. The recommended values were calculated by averaging the inhalation rates (arithmetic mean) for each population and activity level from the various studies. Due to limitations in the data sets an upper percentile is not recommended. The recommended values are given below:

Table 5 Summary of recommended values from US EPA (1997)

Population	Mean ventilation rates [m^3/24 h]
Long-term exposures	
Infants <1 year [1)]	4.5
Children 1-2 years [1)]	6.8
3-5 years [1)]	8.3
6-8 years [1)]	10
9-11 years males females	 14 13
12-14 years males females	 15 12
15-18 years males females	 17 12
Adults 19 – 65+ years males females	 15.2 11.3
Short-term exposures	m^3/h
Children	
Rest	0.3
Sedentary activities	0.4
Light activities	1.0
Moderate activities	1.2
Heavy activities	1.9
Adults	
Rest	0.4
Sedentary activities	0.5
Light activities	1.0
Moderate activities	1.6
Heavy activities	3.2
Outdoor workers	
Hourly average	1.3 (3.3 m^3/h) [2)]
Slow activities	1.1
Moderate activities	1.5
Heavy activities	2.5

1) No sex difference found
2) Upper percentile

The document also mentions that for a calculation of an internal dose using the alveolar ventilation rate it has to be considered that only the amount of air available for exchange via the alveoli per unit time has to be taken into account, accounting for approximately 70% of the total ventilation. This should also be considered in the risk assessment.

Using a respiratory tract dosimetry model (ICRP66 model, 1994), Snipes et al. (1997) calculated respiration rates for male adults, see **Table 6**.

Table 6 Calculated respiration rates for male adults (Snipes et al., 1997)

Activity	Breathing rate m^3/h
Sleeping	0.45
Sitting	0.54
Light activity	1.5
Heavy activity	3.0

Based on these breathing rates estimated daily volumes of respiration were derived for different populations:

General population: 8 h sleep, 8 h sitting, 8 h light activity: 19.9 m^3
Light work: 8 h sleep, 6.5 h sitting, 8.5 h light activity, 1 h heavy activity: 22.85
Heavy work: 8 h sleep, 4 h sitting, 10 h light activity, 2 h heavy activity: 26.76

The same authors also mention that in humans breathing pattern changes from nose breathing to nose/mouth breathing at a ventilation rate of about 2.1 m^3/h (60% through nose, 40% through the mouse). At a ventilation rate of 5 m^3/h about 60% of air is inhaled through the mouth and 40% through the nose. However these model calculations seem to overestimate the ventilation rates compared to the experimental data reviewed by US EPA (1992).

Physiological parameters used in PBPK modelling

Literature on PBTK- or PBPK modelling also contains a number of physiological parameters that are used to calculate tissue doses and distributions. Brown et al. (1997) have published a review of relevant physiological parameters used in PBPK models. This paper provides representative and biologically plausible values for a number of physiological parameters for common laboratory species and humans. It constitutes an update of a document prepared by Arms and Travis (1988) for US EPA and also critically analyses a compilation of representative physiological parameter values by Davies and Morris (1993). Those references are therefore not reviewed here, but given in the reference list for consultation. In contrast to the other authors Brown et al. (1997) also try to evaluate the variability of the parameters wherever possible, by giving mean values plus standard deviation and/or the range of values identified for the different parameters in different studies. The standard deviations provided are standard deviations of the reported means in different studies, in other words they are a measure of the variation among different studies, not the interindividual variation of the parameters themselves. This variation may therefore include sampling error, interlaboratory variation, differences in techniques to obtain the data. The authors also provide some data on tissues within certain organs which will not be quoted here.

Table 7 Organ weights as percent of body weight (adapted from Brown et al., 1997) (Typically the values reflect weights of organs drained of blood)

Organ	Mouse mean ± standard deviation	Mouse range	Rat mean ± standard deviation	Rat range	Dog mean ± standard deviation	Dog range	Human reference value mean ± standard deviation	Human range
Adipose tissue[1]		5-14 [1a)]		5.5-7 [1b)]			13.6 ±5.3 [1c)] 21.3 [1d)], 32.7 [1e)]	5.2-21.6 [1c)]
Adrenals	0.048 [2)]		0.019 ± 0.007	0.01-0.031	0.009 ± 0.004	0.004-0.014	0.02 [3)]	
Bone	10.73 ± 0.53	10.16-11.2		5-7 [4)]	8.10 [2,5)]		14.3 [3)]	
Brain	1.65 ± 0.26	1.35-2.03	0.57±0.14	0.38-0.83	0.78±0.16	0.43-0.86	2.00 [3)]	
Stomach	0.60 [2)]		0.46±0.06	0.40-0.60	0.79±0.15	0.65-0.94	0.21 [3)]	
Small intestine	2.53 [2)]		1.40±0.39	0.99-1.93	2.22±0.68	1.61-2.84	0.91 [3)]	
Large intestine	1.09 [2)]		0.84±0.04	0.80-0.89	0.67±0.03	0.65-0.69	0.53 [3)]	
Heart	0.50±0.07	0.40-0.60	0.33±0.04	0.27-0.40	0.78±0.06	0.68-0.85	0.47 [3)]	
Kidneys	1.67±0.17	1.35-1.88	0.73±0.11	0.49-0.91	0.55±0.07	0.47-0.70	0.44 [3)]	
Liver	5.49±1.32	4.19-7.98	3.66±0.65	2.14-5.16	3.29±0.24	2.94-3.66	2.57 [3)]	
Lungs	0.73±0.08	0.66-0.86	0.50±0.09	0.37-0.61	0.82±0.13	0.62-1.07	0.76 [3)]	
Muscle	38.4±1.81	35.77-39.90	40.43± 7.17	35.36-45.50	45.65±5.54	35.20-53.50	40.00 [3)]	
Pancreas	no reliable data		0.32±0.07	0.24-0.39	0.23±0.06	0.19-0.30	0.14 [3)]	
Skin	16.53± 3.39	12.86-20.80	19.03± 2.62	15.80-23.60	no representative value		3.71 [3)] (3.1 female, 3.7 male) [3)]	
Spleen	0.35±0.16	0.16-0.70	0.20±0.05	0.13-0.34	0.27±0.06	0.21-0.39	0.26 [3)]	
Thyroid	no data		0.005± 0.002	0.002-0.009	0.008± 0.0005	0.0074-0.0081	0.03 [3)]	

1) Defined mostly as dissectible fat tissue,
1a) Strongly dependent on strain and age in mice,
1b) Male Sprague Dawley rats equation: Fat content = 0.0199 · body weight + 1.664, for male F344 rats: Fat content = 0.035 · body weight + 0.205
1c) Males, 30-60 years of age
1d) ICRP, 1975 reference value for 70 kg man,
1e) ICRP, 1975 reference value for 58 kg women
2) One study only
3) ICRP, 1975 reference value
4) In most of the studies reviewed by the authors
5) Mongrel dogs

To derive the organ volume from the mass for most organs a density of 1 can reasonably be assumed. The density of marrow free bone is 1.92 g/cm^3 (Brown et al., 1997).

Brown et al. (1997) also give values for cardiac output and regional blood flow as a percentage of cardiac output or blood flow rate/100 g tissue weight for the most common laboratory species and humans. The data used are data from non-anaesthetised animals using radiolabelled

microsphere technique. For humans data using various techniques to measure perfusion were compiled.

Table 8 Cardiac output (ml/min) for different species (adopted from Brown et al., 1997).

Mouse mean ± standard deviation	Mouse range	Rat mean ± standard deviation	Rat range	Dog mean ± standard deviation	Dog range	Human reference value
13.98± 2.85	12-16	110.4± 15.60	84-134	2,936 [1)]	1,300-3,000 [1)]	5,200 [1)]

1) One study only

Regional blood flow distribution in different species

According to the authors giving blood flow in units normalised for tissue weight can result in significant errors if default reference weights are used instead of measured tissue weights in the same study.

Table 9 Regional blood flow distribution in different species (ml/min/100g tissue) (adopted from Brown et al., 1997)

Organ	Mouse mean ± standard deviation	Mouse range	Rat mean ± standard deviation	Rat range	Dog mean ± standard deviation	Dog range
Adipose tissue[1]			33±5	18-48	14±1	13-14
Adrenals			429±90	246-772	311±143	171-543
Bone			24±3	20-28	13±1	12-13
Brain	85±1	84-85	110±13	45-134	65±4	59-76
Heart	781±18	768-793	530±46	405-717	79±6	57-105
Kidneys	439±23	422-495	632±44	422-826	406±37	307-509
Liver	131					
Hepatic artery	20		23±44	9-48	21±3	12-30
Portal vein	111±9	104-117	108±17	67-162	52±4	42-58
Lungs	35[1]		127±46 [1)]	38-147 [1)]	79±43 [1)]	36-122
Muscle	24±6	20-28	29±4	15-47	11±2	6-18
Skin	18±12	9-26	13±4	6-22	9±1	8-13

1) Bronchial flow
2) Based on animal studies

Table 10 Regional blood flow distribution in different species (% cardiac output) (adopted from Brown et al., 1997)

Organ	Mouse mean ± standard deviation	Mouse range	Rat mean ± standard deviation	Rat range	Dog mean ± standard deviation	Human reference value mean, male	Human reference value mean, female	Human range
Adipose tissue [1)]			7.0 [2)]			5.0	8.5	3.7-11.8
Adrenals			0.3±0.1	0.2-0.3	0.2[2]	0.3	0.3[2]	
Bone			12.2 [2)]			5.0	5.0	2.5-4.7
Brain	3.3±0.3	3.1-3.5	2.0±0.3	1.5-2.6	2.0 [2)]	12.0	12.0	8.6-20.4
Heart	6.6±.0.9	5.9-7.2	4.9±0.1	4.5-5.1	4.6 [2)]	4.0	5.0	3.0-8.0
Kidneys	9.1±2.9	7.0-11.1	14.1±1.9	9.5-19.0	17.3 [2)]	19.0	17.0	12.2-22.9
Liver	16.2		17.4	13.1-22.1	29.7 [2)]	25.0	27.0	11-34.2
Hepatic artery	2.0		2.4	0.8-5.8	4.6 [2)]			
Portal vein	14.1	13.9-14.2	15.1	11.1-17.8	25.1 [2)]	19.0	21.0	12.4-28.0
Lungs	0.5[1]		2.1±0.4 [1)]	1.1-3.0 [1)]	8.8 [1,2)]	2.5[1]		
Muscle	15.9±5.2	12.2-19.6	27.8 [2)]		21.7 [2)]	17.0	12.0	5.7-42.2
Skin	5.8±3.5	3.3-8-3	5.8 [2)]		6.0 [2)]	5.0	5.0	3.3-8.6

[1)] Bronchial flow

[2] One study only

The blood flow rates to some organs such as the liver are highly variable and can be influenced by factors including anaesthesia, posture, food intake, exercise.

Gerlowski and Jain (1983) have published a compilation of different organ volumes and plasma flows for a number of species at a certain body weight from other literature sources.

Table 11 Organ volumes, plasma flow rates used in PBTK-models

Parameter	Mouse	Hamster	Rat	Rabbit	Monkey	Dog	Human
Body weight (g)	22	150	500	2,330	5,000	12,000	70,000
Volume (ml)							
Plasma	1	6.48	19.6	70	220	500	3,000
Muscle	10	-	245	1,350	2,500	5,530	35,000
Kidney	0.34	1.36	3.65	15	30	60	280
Liver	1.3	6.89	19.55	100	135	480	1,350
Gut	1.5	12.23	11.25	120	230	480	2,100
Gut lumen	1.5	-	8.8	-	230	-	2,100
Heart	0.095	0.63	1.15	6	17	120	300
Lungs	0.12	0.74	2.1	17	-	120	-
Spleen	0.1	0.54	1.3	1	-	36	160
Fat	-	-	34.9	-	-	-	10,000
Marrow	0.6	-	-	47	135	120	1,400
Bladder	-	-	1.05	-	-	-	-
Brain	-	-	-	-	-	-	1,500
Pancreas	-	-	2.15	-	-	24	-
Prostate	-	-	6.4	-	-	-	-
Thyroid	-	-	0.85	-	-	-	20
Plasma flow rate (ml/min)							
Plasma	4.38	40.34	84.6	520	379	512	3,670
muscle	0.5	-	22.4	155	50	138	420
Kidney	0.8	5.27	12.8	80	74	90	700
Liver	1.1	6.5	4.7	177	92	60	800
Gut	0.9	5.3	14.6	111	75	81.5	700
Heart	0.28	0.14	1.6	16	65	60	150
Lungs	4.38	28.4	2.25	520	-	512	-
Spleen	0.05	0.25	0.95	9	-	13.5	240
Fat	-	-	3.6	-	-	-	200
Marrow	0.17	-	-	11	23	20	120
Plasma flow rate (ml/min)							
Bladder	-	-	1.0	-	-	-	-
Brain	-	-	0.95	-	-	-	380
Pancreas	-	-	1.1	-	-	21.3	-
Prostate	-	-	0.5	-	-	-	-
Thyroid	-	-	0.8	-	-	-	20

Table 12 A number of physiological parameters for different species compiled by Nau and Scott (1987)

Parameter	Mouse	Rat	Guinea pig	Rabbit	Dog	Monkey	Human
Bile flow (ml/kg per day)	100	90	230	120	12	25	5
Urine flow (ml/kg per day)	50	200		60	30	75	20
Cardiac output (ml/min per kg)	300	200		150	100	80-300	60-100
Hepatic blood flow (l/min)	0.003	0.017	0.021	0.12	0.68	0.25	1.8
Hepatic blood flow (ml/min per kg)	120	100		50	25	25	25-30
Liver weight (% of body weight)	5.1	4.0	4.6	4.8	2.9	3.3	2.4
Renal blood flow (ml/min per kg)	30				22	25	17
Kidney clearance (ml/min per kg)	5				3.2	3	1.3

Gad and Chengelis (1992) have summarised a number of physiological parameters for different species. The most important data of the most common laboratory test species are summarised below.

Table 13 A number of physiological parameters for different species (Gad and Chengelis, 1992)

	Rat	Mouse	Guinea Pig	Rabbit	Dog (Beagle)
Blood volume whole blood (ml/kg)	57.5-69.9	78	75	45-70	-
Blood volume Plasma (ml/kg)	36.3-45.3	45	30.6-38.2	-	-
Respiratory frequency min^{-1}	66-114	84-230	69-160	35-65	10-30[1]
tidal volume (ml)	0.6-1.25	0.09-0.38	1.8	4-6	18-35[1]
Urine volume (ml/kg/24 h)	55			20-350	-
Urine pH	7.3-8.5	-	-	8.2	-

1) In Beagles of 6.8 to 11.5 kg bw

Appendix V Strategy for selecting the appropriate route of exposure for toxicity testing

Objective of the strategy

The objective of this strategy is to give guidance on the selection of the appropriate route(s) of exposure in tests for hazard identification conducted on substances. The strategy is applicable to new substances which are tested according to the requirements of Annex V to Directive 67/548/EEC, but may also be of use for existing substances when the data which is already available has been reviewed and it has been decided that further testing would be beneficial. The strategy focuses particularly on the circumstances in which testing via the inhalation or dermal route will be required. It relates primarily to acute (single dose) and repeated dose (28-day and 90-day) toxicity studies conducted to investigate the overall local and systemic toxicity of a substance. However, the principles are also applicable to the selection of exposure route for other types of study, such as those for reproductive toxicity, chronic toxicity and carcinogenicity.

Due to differences between new substances and biocides with regard to data requirements as laid down in Directive 67/548/EEC and Directive 98/8/EC, the strategy for new substances discussed below is not fully compatible with biocide requirements. Detailed guidance on the selection of appropriate routes in toxicity testing of biocidal active substances and products is given in the TNsG on Data Requirements (2000).

Why guidance is needed on selection of exposure route

In order to facilitate the systematic testing of substances, taking into account any existing data which may be available, it is necessary to adopt a strategic approach towards testing. This appendix sets out step-by-step guidance with regard to selecting the most appropriate exposure routes for the studies to be carried out.

Humans may be exposed to substances by three routes: inhalation, dermal or oral. Taking into account animal welfare, costs and scientific considerations, it is not justifiable to require each substance to be tested for acute and repeated dose toxicity by all three routes. Use of the inhalation route of administration in animal tests should be considered when inhalation exposure of humans is of relevance. However, the final decision on which route of exposure to use in a particular test will be taken in the light of several other parameters (e.g. the physico-chemical properties of the substance; the results of already-conducted toxicity tests).

General principles for testing substances

In relation to testing at the base-set level for new substances (1 tpa or 5 t cumulative), Annex VIIA to Directive 67/548/EEC states that, for acute toxicity tests, substances other than gases shall be administered via at least two routes, one of which should be the oral route. The choice of the second route will depend on:

- the nature of the substance; and
- the likely route of human exposure.

Gases and volatile liquids should be administered by the inhalation route."

Directive 67/548/EEC does not deal with existing substances but these should be tested for acute toxicity using the same guidelines to obtain data not already available.

Similarly, for repeated dose toxicity testing, where a 28-day repeated dose toxicity study involving administration by one route is required, Annex VIIA to Directive 67/548/EEC states that the route of administration should be the most appropriate having regard to

- the likely route of human exposure;
- the acute toxicity;
- the nature of the substance.

In the absence of contra-indications the oral route is usually the preferred one.

The same considerations apply to repeated dose toxicity testing of existing substances.

In testing for local and systemic toxicity at higher tonnage levels for both new and existing substances, it will also be necessary to select the most appropriate exposure route from the three potential alternatives. Annex VIII to Directive 67/548/EEC gives little guidance on selection of exposure route in this context.

Accordingly, this guidance document sets out detailed criteria, expanding those given in Annexes VIIA and VIII to Directive 67/548/EEC for the selection of exposure route for acute and repeated dose toxicity testing of substances, so that the most appropriate studies are performed at each stage.

In particular, the document addresses selection of the inhalation route of exposure. Inhalation exposure of humans occurs frequently and yet, to date, corresponding inhalation toxicity tests have seldom been performed. Examples of substances that should have been tested by inhalation, but were not, are given in Examples A - C.

Comments on the selection strategy

In general, route-to-route extrapolation is considered to be a poor substitute for toxicity data obtained using the appropriate route of exposure. Uncertainties in extrapolation increase when it becomes necessary to perform a risk assessment with toxicity data obtained by an administration route which does not correspond to the human route of exposure. Insight into the reliability of the current methodologies for route-to-route extrapolation has not been obtained yet (Wilschut et al., 1998).

When route-to-route extrapolation is to be used, the following aspects should be carefully considered:

- *nature of effect:* route-to-route extrapolation is only applicable for the evaluation of systemic effects. For the evaluation of local effects after repeated exposure, only results from toxicity studies performed with the route under consideration can be used;
- *toxicokinetic data (absorption, distribution, metabolism and elimination):* The major factors responsible for differences in toxicity due to route of exposure include (see also Section 3.5 of human health (Chapter 2)):
  - differences in bioavailability (absorption),
  - differences in metabolism (a.o. first pass effects),
  - differences in internal exposure pattern.

In practice, relevant data on toxicokinetics and metabolism, especially after dermal and inhalation exposure, are frequently missing. As a consequence, corrections can only be made for differences in bioavailability.

Hence, it is strongly advised that, if no additional data exist which allow a reliable route-to-route extrapolation, a substance should be tested by administration routes which correspond to the likely routes of human exposure.

For the selection strategy, the following general principles should be applied:

- an inhalation toxicity testing is mandatory for gases;
- an acute oral toxicity test has to be performed for all substances except gases. For acute toxicity testing, discussion therefore concentrates on the selection of a second administration route;
- for repeated dose toxicity studies, the oral route is the preferred one except where contraindications exist. Contraindications must be given if an inhalation or dermal repeated dose toxicity study is preferentially indicated.

To structure the decision-making process, two categories of criteria have been established:

- criteria related to the anticipated toxicity of the substance (corresponding to "nature of the substance");
- criteria related to the anticipated exposure situation in production and use (corresponding to "likely route of human exposure").

For each category, the assessor should decide whether the given criteria support or contraindicate a certain administration route. Thereafter, the criteria in the two categories are combined to decide on the need for a particular route of administration.

As the individual criteria may have differing weights, it is necessary to use expert judgement. A written explanation of the rationale behind the selection of the administration route(s) for acute and repeated dose toxicity studies should be given in the notification dossiers.

In the criteria related to the anticipated toxicity of the substance, reference is made to the classification of the substance. The criteria for classification are presented in Annex VI to Directive 67/548/EEC. It should be noted that the classification for acute oral, dermal and inhalation toxicity are primarily based on lethality. It is expected that other toxic effects might occur at lower concentrations or dose levels. These should be taken into account if reported.

Although this document emphasises the importance of conducting studies employing the inhalation route of exposure wherever appropriate, it is acknowledged that inhalation testing of a substance is more demanding than testing using dermal or oral administration. The design of the acute inhalation toxicity test, as given in Annex V to Directive 67/548/EEC, does not fulfil in all aspects the requirements which would be helpful for decisions on further testing. The obtainable information could be optimised by increased emphasis on physiological, biochemical and histopathological examinations and the investigation of concentration-time-effect relationships. A meaningful interpretation of the parameters may require the use of a concurrent control group. It may be useful, therefore, to review the acute inhalation toxicity test guideline in Annex V to Directive 67/548/EEC.

In addition, an LC_{50} test should not be performed with substances classified as corrosive, as the results are predictable. Instead a repeated dose toxicity study, using non-corrosive concentrations, should be considered. For such substances, appropriate classification and labelling with respect to the potential inhalation hazard of acute exposure is necessary.

Insoluble and sparingly soluble dusts may cause adverse effects in the lung which can be explained by overload phenomena. Overload phenomena in low and intermediate exposure

groups should be avoided by selecting appropriate concentrations, especially in repeated dose studies. It may be useful to investigate overload phenomena in the high exposure group. However the toxicological significance of such overload phenomena should be carefully evaluated when considering classification and labelling.

It should be mentioned that currently there are no *in vitro* methods available which would be adequate alternatives to the *in vivo* acute or repeated dose inhalation toxicity studies.

Selection strategy

Base-set (≥1 tpa or 5 t cumulative) testing: acute toxicity

All substances, other than gases, must be examined in an acute oral toxicity study. The decision to be made is which exposure route to employ in the second acute toxicity test. For some substances (volatile liquids or respirable dusts), it may be required to cover all three exposure routes. Before this decision is made, the results of the skin and eye irritation tests and some physico-chemical properties of the substance should be known. This also applies to existing substances when further acute toxicity data are required.

Criteria for an acute dermal toxicity study

Criteria related to the anticipated toxicity

Physico-chemical properties:

- appreciable lipid solubility and a non-extreme octanol/water partition coefficient, either or not in combination with a relatively low molecular weight, suggesting a significant rate of absorption through the skin.

Toxicological data:

- toxicity observed in the acute oral toxicity study at low doses (e.g. classified as toxic, very toxic);
- systemic effects or other evidence of absorption observed in skin and/or eye irritation studies;
- *in vitro* tests indicate significant dermal absorption.

Structure-activity relationships:

- significant acute dermal toxicity recognised for structurally-related compounds;
- significant dermal penetration recognised for structurally-related compounds.

Criteria related to the anticipated exposure

Exposure characteristics:

- likely skin contact in production and/or use (e.g. manual handling, splashing);
- possible dermal exposure in an accident.

Criteria for an acute inhalation toxicity study

Criteria related to the anticipated toxicity

Toxicological data:

- results of irritation tests suggest local effects in the respiratory tract;
- toxicity observed in the acute oral toxicity study at low doses (i.e classified as toxic, very toxic).

Structure-activity relationships:

- significant acute inhalation toxicity recognised for structurally-related compounds.

Criteria related to the anticipated exposure

Physico-chemical parameters:

- significant vapour pressure, e.g. $>1 \cdot 10^{-2}$ Pa at 20° C, which is also the value for which biocides inhalation toxicity must be reported for active substances or preparations);
- substance as used contains particles in the inhalable size range (e.g. MMAD ≤100µm) i.e. they may be deposited anywhere in the respiratory tract; the inhalable size range of particles is important in determining not only if the situation poses an inhalation problem, but also where in the respiratory tract the particles may deposit (compare **Figure 1** and **Table 1**).

Exposure characteristics:

- dust or aerosol formation in production and use (e.g. filling, mixing, spraying); handling of a volatile liquid in an open system;
- handling of the substance at elevated temperatures;
- possible inhalation exposure in an accident.

If the overall evaluation of criteria related to toxicity and exposure clearly indicates either the dermal or the inhalation administration route, that route should be used. If there is evidence favouring both routes but no clear-cut decision is possible the inhalation route should be used, because, in general, inhalation exposure is more of a problem for human health than dermal exposure.

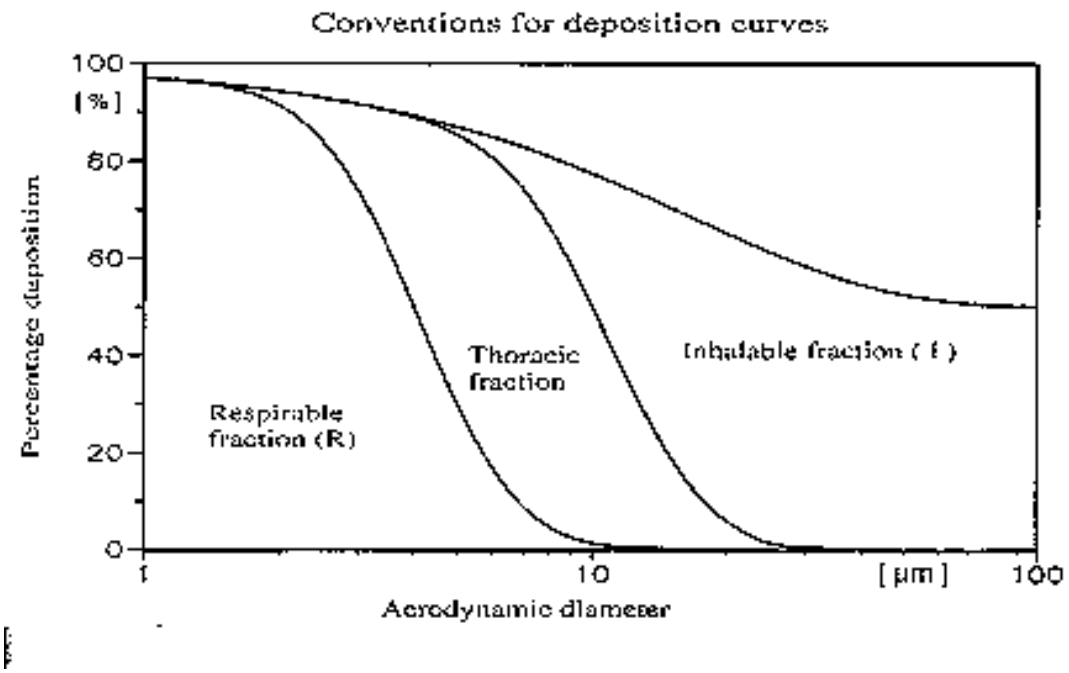

Figure 1: Curves for the division of the airborne dust in the breathing zone into inhalable, thoracic and respirable fractions. Because the X-axis is on a logarithmic scale, the aerodynamic diameter with the value „zero“ cannot be included. The deposition fraction corresponding to this value would be 100%.

from DFG (2001): List of MAK- and BAT-Values 2001, Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Report No. 37 , page 164

Table 1 Data for the curves defined in DIN EN 481* for the division of the total airborne aerosol into fractions of occupational medical relevance

Aerodynamic diameter [μm]	Inhalable fraction [%]	Thoracic fraction [%]	Respirable fraction [%]
0	100,0	100,0	100,0
1	97,1	97,1	97,1
2	94,3	94,3	91,4
3	91,7	91,7	73,9
4	89,3	89,0	50,0
5	87,0	85,4	30,0
6	84,9	80,5	16,8
7	82,9	74,2	9,0
8	80,9	66,6	4,8
9	79,1	58,3	2,5
10	77,4	50,0	1,3
11	75,8	42,1	0,7
12	74,3	34,9	0,4
13	72,9	28,6	0,2
14	71,6	23,2	0,2
15	70,3	18,7	0,1
16	69,1	15,0	0,0
18	67,0	9,5	
20	65,1	5,9	
25	61,2	1,8	
30	58,3	0,6	
35	56,1	0,2	
40	54,5	0,1	
50	52,5	0,0	
60	51,4		
80	50,4		
100	50,1		

* European Committee for Standardization (CEN): EN 481 Workplace atmospheres - Size fraction definitions for measurements of airborne particles. Brussels 1993, Beuth Verlag, Berlin 1993 quoted from DFG (2001): List of MAK- and BAT-Values 2001, Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Report No. 37, page 165.

Base-set (≥1 tpa or 5 t cumulative) testing: repeated dose toxicity

For the base-set repeated dose toxicity study, the oral route is the preferred one, except where contra-indications exist. For some substances, the criteria related to physico-chemical characteristics, toxicity and exposure patterns clearly indicate that either the inhalation or the dermal route is the most appropriate for the base-set repeated dose toxicity study, as indicated below.

Generally, the categories of criteria are the same as those for acute toxicity. Those criteria are not repeated here; instead additional criteria are described.

There are certain substances whose physico-chemical characteristics are such that a 28-day oral repeated dose toxicity study may be inappropriate. These include the following:

- insoluble substances which are extremely unlikely to be absorbed from the gastro-intestinal tract (e.g. certain polymeric materials, organoclays). The presence of impurities of toxicological relevance must, however, be taken into consideration. Criteria for the testing of polymeric materials are being established;
- highly reactive substances (e.g. alkyls of aluminium, lithium and magnesium). For such substances, appropriate classification and labelling are necessary with respect to the possible health hazards.

Criteria for a 28-day dermal repeated dose toxicity study (in addition to those given for the acute dermal toxicity study)

Criteria related to the anticipated toxicity

Toxicological data:

- systemic toxicity observed in the acute dermal toxicity study (e.g. classified as at least harmful);
- acute dermal toxicity greater than acute oral or inhalation toxicity (e.g. more severe classification after dermal administration).

Structure-activity relationships:

- clear indications from chemical analogues that severe local effects on the skin (except sensitisation) may arise from repeated exposure which were not produced by single exposure.

Criteria related to the anticipated exposure

Exposure characteristics:

- prolonged/repeated skin contact likely during production and/or use.

Criteria for a 28-day –inhalation repeated dose toxicity study (in addition to those given for the acute inhalation toxicity study)

Criteria related to the anticipated toxicity

Toxicological data:

- systemic or local effects were observed in the acute inhalation toxicity study (e.g. classified as at least harmful);
- local effects in the respiratory tract are anticipated because the substance is classified as skin irritant, eye irritant or corrosive;
- acute inhalation toxicity greater than acute oral or acute dermal toxicity (e.g. more severe classification after administration by inhalation);

- indications that special activity of the respiratory tract may increase bioavailability (e.g. metabolic activation by macrophages); indications that accumulation of the substance may occur in the lung (e.g. sparingly soluble dusts).

Structure activity relationships:

- clear indications from chemical analogues that severe effects (except respiratory sensitisation) may arise from repeated exposure which were not produced by single exposure.

Criteria related to the anticipated exposure

Exposure characteristics:

- significant vapour pressure (e.g. ≥5 Pa at 20°C corresponding to a saturated air concentration of 1 mg/l for a substance with a molecular weight of 500);
- prolonged/repeated inhalation exposure during production and/or use.

According to Directive 67/548/EEC, in base-set testing, the oral route is preferred for the repeated dose toxicity study if no contraindications exist. When the overall evaluation of criteria related to toxicity and exposure clearly indicate that either the dermal or the inhalation administration route should be used, that route should be used in preference to the oral route. If there is evidence favouring both the inhalation and dermal routes but no clear-cut decision between the two is possible, the inhalation route should be used because, in general, inhalation exposure is more of a problem for human health than dermal exposure.

Post-base-set testing (Levels 1 and 2, i.e. 10/100, and 1,000 tpa, or 50/500 and 5,000 t cumulative)

The administration route chosen for post-base-set repeated dose studies for both new and existing substances may be oral, dermal or inhalation. As in the decision-making process for the base-set 28-day repeated dose toxicity study, all available information on toxicological effects and exposure characteristics has to be considered, so the criteria already taken into account at the base-set level for acute and repeated dose studies are again of relevance.

Criteria which indicate the need to continue using the administration route chosen for the base-set repeated dose toxicity study

Criteria related to toxicity

Toxicological data:

- base-set repeated dose toxicity study shows relevant systemic or local effects which need to be further evaluated (e.g. concentration-time-effect relationships, no observed adverse effect level (NOAEL), reversibility etc.).

Criteria related to exposure

Exposure characteristics:

- modifications in production and use of the substance shift the original exposure situations towards a scenario characterised by repeated/prolonged exposure by the administration route chosen for the base-set repeated dose toxicity study.

Criteria which indicate the need for a different administration route from that used in the base-set repeated dose toxicity study

Criteria related to toxicity

Toxicological data:

- base-set repeated dose toxicity study shows no relevant systemic or local effects which need to be evaluated further;
- another administration route was previously also indicated but not chosen;
- base-set repeated dose toxicity study confirms toxicological effects which are relevant to the choice of another administration route (e.g. local effects observed in an oral repeated dose toxicity study could indicate potential inhalation toxicity).

Criteria related to exposure

Exposure characteristics:

- modification in production and use of the substance lead to changes in the predominant route of exposure and shift an original short-term exposure situation towards a scenario characterised by repeated/prolonged exposure.

If the overall evaluation of the criteria related to toxicity and exposure clearly indicates a certain administration route, that route should be used. If no such clear-cut decision is possible, the inhalation route is preferable to the dermal one as, in general, inhalation exposure is more of a problem for human health than other routes of exposure.

Example A

Decision about the administration route for a second acute toxicity study: inhalation versus dermal

Substance: water-soluble granular solid

	Criteria	for inhalation	for dermal
Anticipated toxicity	Skin and eye irritant	pro	-
	LD_{50} (oral) >5,000 mg/kg	contra	contra
	SAR to a substance which is classified as toxic by inhalation	pro	-
Anticipated exposure	Inhalable dust formation in production and use	pro	-

Conclusion: an acute inhalation toxicity study is indicated.

Example B

Decision about the administration route for a 28-day repeated dose toxicity study (oral or inhalation)

Substance: volatile amine

	Criteria	for inhalation	for oral
Anticipated toxicity	Amine	pro	-
	LD_{50} (oral) = 300 mg/kg	pro	pro
	LC_{50} (inh.) >7.6 mg/l/4h	contra	-
	Signs of toxicity at 7.6 mg/l/4h: nose bleeding	pro	-
	Corrosive	pro	contra
	Employees complain of respiratory symptoms on handling comparable amines	pro	-
Anticipated exposure	Volatile	pro	contra
	Long-term inhalation exposure of employees to volatile amines in polyurethane production is documented in the literature	pro	contra

Conclusion: A subacute inhalation toxicity study is indicated.

Example C

Decision about changing the administration route from oral in the 28-day repeated dose toxicity study to another administration route in a further repeated dose toxicity study (dermal or inhalation)

Substance: liquid of low volatility

	Criteria	for inhalation	for dermal
Anticipated toxicity	LD_{50} (oral) >2,000 mg/kg. LD_{50} (dermal) >2,000 mg/kg.	-	contra
	SAR to ethylacrylate which shows predominantly toxicity to airways	pro	contra
	28-day oral repeated dose toxicity study reveals irritant effects in the forestomach like ethylacrylate; no other relevant effects observed	pro	-
	Local irritant effects in the airways occurred in a preliminary (14-day) inhalation toxicity study	pro	-
Anticipated exposure	Liquid, low volatility	contra	pro
	Higher levels of production and use shift the inhalation exposure from short-term to prolonged exposure at the workplace	pro	-

Conclusion: A repeated-dose inhalation toxicity study is indicated even though the original 28-day repeated dose toxicity study was performed using oral administration.

Appendix VI Default reference values for biological parameters

It has been agreed that where actual measured data are not provided in the study report, for consistency and transparency a standard single set of default biological parameter values should be applied for the determination of NOAELs/LOAELs.

Further information on the basis for the biological parameter default values listed below can be found in Paulussen et al. (1998).

Table 1 Default values for body weights in oral / inhalation toxicity studies

Species	Sex	Body weights				
		28-days	90-days	chronic	pregnant	lactating
Rat	M	250	325	475		
	F	175	200	275	300	300
	M+F	200	275	375		
Mouse	M	30	35	45		
	F	25	30	35	35	35
	M+F	30	35	40		
Dog	M	11	12	N/a		
	F	9	10	N/a		
	M+F	10	11	N/a		

Body weights are given in grams (g) for rats and mice, and kilograms (kg) for dogs

NB: Proposed body weight defaults for the 28 and 90-day studies in the mouse, and the 28, 90-day and chronic studies in the rat are based on data from more than one strain.

Table 2 Default values for body weights in dermal toxicity studies

Species	Sex	Body weights	
		28-day	90-day
Rabbi [a)]	M	3	
	F	3	
	M+F	3	
Rat [b)]	M	350	400
	F	250	275
	M+F	300	350
Guinea Pig [c)]	M+F	475	

Body weights are given in g for rats and guinea pigs, and kg for rabbits

[a)] Defaults based on New Zealand White rabbit strain

[b)] Defaults based on Sprague-Dawley rat strain

[c)] Defaults based on Strain 13 guinea pigs where only joint male and female data were available

Water and food consumption

Because experimental conditions (e.g. type of diet) may affect the water and food consumption, default values are not explicitly stated for these parameters. Instead allometric equations are provided in order to make it possible to derive default values on a case-by-case basis.

Allometric equations provided below have been divided into general equations (see **Table 4**), as well as species-specific equations (see **Table 3**, from US EPA, 1988).

It is recommended that where possible the equations that are species-specific should be applied as they represent the most realistic approach, although they do not always correlate well.

Table 3 Species-specific equations for food consumption according to the US EPA (1988)

Animal group	**Allometric equation**
Rat	$F=0.040\ W^{0.479}$
Mouse	$F=0.064\ W^{0.7242}$
Dog	$F=5.13\ W^{-0.918}$
Guinea pig	$F=0.041\ W^{0.3308}$
Rabbit	$F=0.041\ W^{0.7898}$
Hamster	$F=0.082\ W^{0.9285}$
Laboratory mammals *	$F=0.056\ W^{0.6611}$
All species combined **	$F=0.065\ W^{0.7919}$

Where F = Food consumption in kg/day, and W = Body weight in kg
* Laboratory mammals include gerbils, guinea pigs, hamsters, mice, rats, cats, dogs and rabbits
** All species combined also includes chickens in addition to laboratory mammals

Table 4 General equations according to the US EPA (1988)

Animal group	**Allometric equation**	**Equation number**
FOOD AND WATER CONSUMPTION		
Dry diet: all species	$F=0.31\ L^{0.7923}$ (kg)	1a
	$L= 3.59\ F^{1.2041}$ (l)	1b
Wet diet: all species	$F= 2.09\ L^{0.7389}$ (kg)	2a
	$L= 0.39\ F^{1.2447}$ (l)	2b
Laboratory mammals: (dry diet)	$F= 0.28\ L^{0.7613}$ (kg)	3a
	$L= 0.31\ F^{1.2226}$ (l)	3b
Laboratory rodents : (dry diet)	$F= 0.16\ L^{0.6426}$ (kg)	4a
	$L= 0.25\ F^{1.2943}$ (l)	4b
Animal group	**Allometric equation**	**Equation number**
BODY WEIGHT TO FOOD OR WATER CONSUMPTION		
Dry diet: all species	$F= 0.049\ W^{0.6087}$ (kg)	5
	$L= 0.093\ W^{0.7584}$ (l)	6
Unknown diet: all species	$F= 0.065\ W^{0.7919}$ (kg)	7
	$L= 0.11\ W^{0.7872}$ (l)	8

Where F = Food consumption in kg/day, L = Liquid (water) consumption in l/day and W = Body weight in kg

The equations that describe the food and water consumption data cannot be applied to pregnant females used in reproductive multi-generation studies.

When either food or water consumption is known for animals on a wet or dry diet, equations 1- 4 should be used to estimate the missing value. If diets are specified or can be reasonably assumed to have been dry or moist, equations 1 and 2 are recommended. If body weight is known or can be estimated, equations 5-8 are recommended for estimating food and water consumption.

Body surface areas

With certain assumptions, it can be shown that the body surface area is proportional to two-thirds of the body mass. The following body surface areas are applicable for dermal repeated-dose toxicity data (see **Table 5**).

Table 5 Body surface areas applicable for dermal repeated dose toxicity data

Species	Sex	Surface areas	
		28-day	90-day
Rat	M	450	500
	F	350	350
	M+F	400	425
Rabbit	M+F	2600	
G. Pig	M+F	500	600

Body surface areas are given in cm^2

Inhalation volumes in ml/min

Table 6 Inhalation volumes ml/min

Species	Sex	Inhalation volumes		
		28-day	90-day	chronic
Rat	M	175	200	300
	F	125	150	200
	M+F	150	175	250
Mouse	M	30	35	50
	F	25	30	40
	M+F	25	30	45
Rabbit	M	750		
	F	750		
	M+F	750		
G. Pig	M+F	200	225	
Dog	M	2,550	2,700	
	F	2,250	2,350	
	M+F	2,400	2,500	

Inhalation volumes are given in ml/min
These values are based on species-specific allometric relationships

Appendix VII Human health risk assessment of petroleum substances

Introduction

Petroleum substances are complex and variable mixtures of hydrocarbons. In this respect, they differ from pure substances. Therefore, although the TGD on the health risk assessment of substances is relevant for single substances, they may not be appropriate for the assessment of complex mixtures such as the petroleum substances. The purpose of this appendix is to identify, for the petroleum substances, those areas which are adequately addressed by the TGD and those which may give rise to difficulties and, where appropriate, suggest suitable alternative pragmatic approaches. It should be noted that this appendix only addresses exposure to substances and not to their degradation or combustion products which are formed during use.

Petroleum substances

The petroleum substances are derived (separated) from crude oil (petroleum) by distillation and may be further separated and/or refined depending on the desired technical properties of the final product. Petroleum substances are complex and variable mixtures comprised primarily of hydrocarbons of two general types:

- saturated species - linear aliphatics (normal paraffins), branched aliphatics (iso-paraffins), cycloaliphatics (cycloparaffins or naphthenes);
- unsaturated species - olefins and aromatics.

Depending on its physical/chemical characteristics, any specific petroleum substance can contain any or all of these chemical species in varying ratios. In addition, some of these substances may also include sulphur- and nitrogen-containing compounds. Residues also contain vanadium, nickel and other metallic compounds.

Each of the 510 petroleum substances included in Annex 1 of Regulation 793/93 has been placed into one of 32 product groups. The groups are numbered from 1 to 14; some are divided into subgroups e.g. 4A and 4B; definitions for the groups are given in the HEDSET literature. Each group contains substances which have been derived from similar feedstocks by similar refining processes. As a consequence, all of the substances in each group are of similar physical and chemical properties and would normally be expected to have similar toxicological characteristics. Exceptions to this rule are groups 3G, 3J, 5B, 7C, 10 and 12 which contain petroleum substances of a more heterogeneous character and may, therefore, be more difficult to deal with collectively.

Toxicity of petroleum substances

The toxicity of petroleum hydrocarbons including commercial products has been reviewed by Cavender (Cavender, 1994) and the carcinogenicity of mineral oils (IARC, 1984), bitumen (IARC, 1985), hydrocarbon solvents (IARC, 1989) and crude oil and fuels (IARC, 1989) has been evaluated by IARC.

Some constituents contained in petroleum substances are the known carcinogens 1,3-butadiene, benzene and certain 3 to 7 fused-ring polycyclic aromatic compounds. Some petroleum substances contain n-hexane which is neurotoxic. In terms of the product groups these may be found as follows:

- 1,3-butadiene may be present in group 2 substances;
- benzene and n-hexane may be found in substances of groups 3A to 3G;
- 3 to 7 fused-ring polyaromatic compounds may be found in substances of groups 4A to 14.

Assessment of mammalian toxicity

The products produced by the petroleum industry are, in general, intended for use either as fuels, lubricants, intermediates or feedstocks for the chemicals industry. When exposure occurs, it is usually either by inhalation or by dermal contact, depending on the intended use and physical/chemical properties of the specific substance. Exposure by the oral route generally only occurs under exceptional circumstances and normally involves accidental exposure by domestic consumers. There are a few, more highly refined products, such as white oils which are exceptions since they may be used in food or cosmetic applications.

If there is contact by either the oral or the dermal routes, humans are normally exposed to all of the constituents of the substance. Toxicity studies assessing effects by these routes, normally utilise whole material as the test substance, and the tests themselves are normally carried out in precisely the same way as would be studies on single substances. Thus, the results of such studies are directly applicable for use in hazard assessment and risk characterisation processes in the same way as for single substances.

A common route of exposure to petroleum substances is by inhalation. For gaseous substances, exposure will be to the whole substance. For substances of low vapour pressure, exposure by inhalation is not common but when it occurs, is to either aerosols or mists. Regardless, for both gases and low vapour pressure substances, the exposure is to all components of the complex mixture. Thus toxicity studies of the entire substance are, therefore, directly relevant to the human exposure situation, potentially simplifying the subsequent risk assessment.

A potential problem arises, however, for those complex substances such as the gasoline naphthas, kerosines, and some gas oil streams which contain both volatile and non-volatile components spanning a relatively wide distillation range. For these substances, humans will normally be exposed, by inhalation, predominantly to the lower molecular weight, more volatile constituents. Exposure will also be influenced by environmental conditions influencing vaporisation. Much of the available toxicity data is from studies which have utilised wholly vaporised material as the test substances and there is a significant problem in deciding how to relate the experimental data to the "real life" conditions of exposure in order to carry out risk assessments. An additional, related problem, is to decide how inhalation toxicity studies of these volatile substances should be carried out in the future to be more representative of actual exposures.

Considering the inhalation toxicity testing question first, there are three general approaches:

- conduct studies on each of the identified individual constituents of the substance;
- conduct studies on wholly vaporised material; or
- define the vaporised fraction representing, actual exposure and conduct appropriate studies on this fraction.

In principle, regardless of the option, the test procedures are well established, and do not normally differ from those used for single substances. The associated analytical procedures may be complex, but, nevertheless, these also are reasonably well established.

The first option, to conduct studies on each of the individual constituents of the petroleum substance, is the most complex, the most expensive in terms of time and animal usage, and is not the preferred approach. It should be noted that in practice

- it would be difficult, if not impossible, to actually identify all constituents of a mixture;
- not all identified constituents could be synthesised in necessary quantities; human exposure to most of these individual constituents is at such low levels that the data would have little practical significance.

In addition, conclusions about the toxicity of a complex substance comprising these constituents requires assumptions about potential interactive effects which might or might not be correct. On the other hand, it is also true that certain constituents are substances in their own right, and some of these have been extensively studied (and will often be separately considered as part of the risk assessment process). Thus, existing data on individual constituents may be useful in some circumstances. Similarly, if a significant toxicological finding is made in a study of a complex mixture, it may be necessary to assess individual constituents to identify the specific chemical species responsible.

The second option, to test wholly vaporised material, is the easiest of the three options to carry out from an experimental view point. Indeed, the majority of existing data have been generated in this way. Total volatilisation of test material can usually be accomplished and it is relatively easy to define the test material to which the test species is exposed. One significant drawback to this approach however, is that the atmosphere generated is not always the same as that to which humans are exposed, and this might complicate subsequent risk characterisation.

The third option, to define and then generate an experimental atmosphere which closely resembles that to which humans are exposed, is the most straightforward for risk assessment as it entails the fewest assumptions. However, in order to generate an appropriate atmosphere for a toxicity study, there is a prior need to carry out thorough analytical monitoring to establish the nature of the substance to which humans are exposed. In principle this is possible for existing substances but impossible for new substances. Further, this approach may result in a test material which is not formally a “substance”, or at least would not be precisely the same substance as that defined for the risk assessment process. There may also be additional problems if the data are to be used for other purposes, e.g., classification, which by definition should reflect hazard and not take account of potential for exposure.

Health risk assessment

The initial approach to the risk assessment of petroleum substances should consider the toxicity of the whole substances and the available data on human exposure. In many cases this process will be assisted by knowledge of the generic hydrocarbon compositions of the substances and the presence of particular hydrocarbons having specific modes of action. In considering particular target populations, it will be important to identify the specific elements of the available hazard and exposure data that will enable risk characterisation to be done. Ultimately this can only be achieved on a case-by-case basis.

Guidance in relation to each route of exposure follows.

Oral exposure

Assessment of health risks resulting from exposures to petroleum substances by the oral route is straightforward and does not differ from the approach described in existing technical guidance

documents for single substances. The NOAEL and exposure data will relate to the whole substance and the established rules for single substances will apply.

Dermal exposure

The dermal exposure route may be used to determine acute or chronic toxicity as well as to investigate potential irritant, sensitising or corrosive effects. Whilst established protocols exist for investigating these end-points from a classification standpoint, the exposure regimes are often considered artificial in the context of real-world exposures thus making extrapolations for risk assessment purposes problematic.

This is particularly so when investigating the case of substances that are themselves mixtures of a range of substances of widely-varying volatilities. Each particular scenario should be considered on a case-by-case basis.

Inhalation exposure

The assessment of risks arising by inhalation exposure to gases or aerosols is straightforward. In either case the NOAEL and exposure data will relate the whole substance and established rule for single substances will again apply.

Potentially more difficult, however, is the situation in which individuals are exposed by inhalation to relatively volatile, complex materials of wide boiling range. In this situation, exposure is often to the more volatile constituents. Therefore, risk characterisation can be somewhat problematic since existing inhalation toxicology studies have most commonly been conducted with wholly vaporised material.

One should bear in mind that the more volatile constituents represent a subset of the wholly vaporised substance. Thus it is reasonable to assume that the toxicological properties of the more volatile constituents are similar if not identical to those of the wholly vaporised material and that these toxic effects, if present, would be identified in studies of wholly vaporised material.

To carry out risk characterisation for inhalation to complex, volatile petroleum substances, the following situations can be envisaged:

- if studies have been conducted with material which closely approximates that to which humans are exposed, these will be the most relevant for hazard assessment and risk characterisation. In this case, the NOAEL and the exposure concentration data will relate to the same substance. However, these data will only relate to a volatile fraction of the petroleum substance for which the risk characterisation is being done and not the total substance;
- if the only studies which exist are from those utilising wholly vaporised material, one should ascertain whether there is any reason to believe that the toxicology of the whole substance is not similar to that of the more volatile constituents (data on fractionated material or individual molecular species, if available, may be useful at this stage). If no differences are expected, then equivalence between wholly vaporised material and the more volatile fractions should be assumed for hazard assessment and for risk characterisation purposes. In this case, the NOAEL will only apply to the whole substance whilst the exposure concentration data only to its volatile components;
- if the volatile fraction is expected to be more toxic than the wholly vaporised substance, it should be determined if adequate hazard characterisation can be inferred from the existing

data. If so, this should be used in the risk characterisation process. If not, the possibility of additional testing to fill critical data caps must be considered;
- in either case, the NOAEL and the exposure concentration data will relate to the same substance. However, these data will only relate to a volatile fraction of the whole substance for which the risk characterisation is being done;
- if wholly vaporised material is expected to be more toxic than its volatile fraction, data from studies of wholly vaporised substance could be used in an initial risk characterisation process on a conservative "worst-case" basis. In this case, the NOAEL will apply to the whole substance and the exposure concentration data to its volatile fraction. Unless this leads to the conclusion that the potential risk is unacceptable, these data should be adequate for the purposes required;
- if the risk assessment process requires that additional data be generated, a decision on the substance to be tested should be based on the purpose for which data are being collected. In particular, to avoid unnecessary duplication of effort and animal usage, there needs to be clear agreement that both classification and risk assessment decisions for the substance in question, can be based on the data which will be produced.

Hydrocarbon solvents

Many hydrocarbon solvents consist of complex mixtures of hydrocarbons and are described by EINECS numbers that are also used for petroleum refinery streams. EINECS numbers used for hydrocarbon solvents are found in some of the groups from 3A to 5B of the Existing Substances Regulation.

Hydrocarbon solvents usually differ significantly from petroleum refinery streams:

- they are more highly refined;
- they cover a narrower range of carbon number;
- they contain virtually no 1,3-butadiene, benzene and 3 to 7 fused-ring polycyclic aromatic compounds;
- they contain virtually no olefins.

Hydrocarbon solvents have a wider spectrum of use and hence, a different exposure pattern compared to petroleum refinery streams. For example, hydrocarbon solvents are used in industrial coating formulations where human exposure in the application of these materials may be significant. In contrast, petroleum refinery streams are blended into fuels at the refinery and the finished products are marketed to both industry and the consumer; human exposure to these products is likely to be more limited.

The general guidance given in the previous section for the human health risk assessment of petroleum refinery substances requires some modification for hydrocarbon solvents and the following differences are emphasised:

- there is less of a problem of preferential volatilisation of lower molecular weight components from hydrocarbon solvents than from petroleum refinery streams, because the former contain a narrower range of carbon numbers;
- accordingly, for hydrocarbon solvents, the results of animal inhalation studies and measured human exposure values will both relate to the same substance;
- as they are generally more highly refined, hydrocarbon solvents may be less toxic than the corresponding petroleum refinery streams. As such, hazard assessments for hydrocarbon solvents may need separate consideration;

- hydrocarbon solvents are not classified as carcinogenic in terms of the Dangerous Substances Directive (67/548/EEC). This is because hydrocarbon solvents in groups 3A to 3G contain less than 0.1% benzene. Those in group 5B are also not classified when derived from non-carcinogenic feedstocks;
- in both industrial and consumer use, human exposure to hydrocarbon solvents may be significantly greater than for petroleum refinery substances.

In summary, the petroleum substance groups from 3A to 5B primarily consist of refinery streams which are used to blend fuels, but some contain hydrocarbon solvents. In conducting risk assessments for a particular group, hydrocarbon solvents are likely to merit separate consideration, since their hydrocarbon compositions, toxicities and exposure patterns will be different from petroleum refinery streams.

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Directive 98/8/EC of the European Parliament and of the Council
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