KEMI Report

No 1/03

HUMAN HEALTH RISK ASSESSMENT

Proposals for the use of assessment (uncertainty) factors

Application to risk assessment for plant protection products, industrial chemicals and biocidal products within the European Union

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Preface

This project was conducted within the framework of KOMET, the Swedish National Chemicals Inspectorate (KemI) body for Competence and Methodology Development. The intention of the project was to develop proposals for the use of assessment factors in the human health risk assessment of plant protection products, new and existing industrial chemicals and biocidal products. Another important aim was to provide support and guidance for staff of KemI in their risk assessment work within the EU as well as in all other national and international work.

The work was carried out by KemI in collaboration with the Institute of Environmental Medicine on a consultant basis (IMM, Karolinska Institutet, Solna, Sweden). The project group included Maria Wallén (project leader), Helena Casabona, Claes Debourg, Gregory Moore, and Lena Rosén from KemI, and Agneta Falk Filipsson, Annika Hanberg, and Katarina Victorin from IMM. The project was administrated by Sten-Åke Svensson (KemI) and in the early phase was directed by Malik Altahir (KemI). In addition, Sven Eric Dahlén, Magnus Ingelman Sundberg, Marie Vahter, and Margareta Warholm, all from IMM, and Carola Lidén from the Department of Medicine, Karolinska Institutet and Stockholm County Council, Stockholm, Sweden, contributed to specific sections in Section 5.2.7 “Inter-individual variations in sensitivity”. The Annexes were prepared by Agneta Falk Filipsson, IMM (“The benchmark dose method”) and by Rodolfo Avila, Facilia AB, Bromma (“Probabilistic methods for assessment of health risk of chemicals”).

N.B. The present document does not include any policy laid down in KemI but is intended to serve as a basis for future policy-making.

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1.1 Overall conclusions and recommendations

There is an obvious need to harmonise the current use of assessment factors within the different sets of legislation for plant protection products, industrial substances and biocidal products, and this is also what we recommend. If no harmonisation takes place, the result may be levels of protection for a specific population depending on which directive or regulation is applied.

One of the main conclusions drawn from the evaluation of the available data on default assessment factors is that the conventionally used factor of 100 (10 for animal-to-human and 10 for human-to-human variations) is probably an underestimate. Based on current scientific data, it is likely that the animal-to-human extrapolation is greatly underestimated. In the case of human-to-human variability, an assessment factor of 10 – 16 is suggested as a minimum.

Furthermore, there is some other factors not included in the traditional assessment factor of 100, which need to be allowed for. Such factors are adequacy of the database, nature of the effect, duration of exposure, route-to-route extrapolation and considerations of extra-sensitive sub-populations such as children, the elderly and patients under medical treatment.

In this respect, children have been identified as a subgroup to be considered with particular care in risk assessment. Emphasis should be laid on vulnerability to chemical toxicity during development and maturation and also on possible deficiencies in the databases for young individuals. As only scanty data are often available in young experimental animals, the lack of data needs to be compensated for by an appropriate assessment factor. Our recommendation is a factor of 1 - 10 to compensate for a poor database.

Conditions not directly associated with assessment factors, which might also affect the outcomes of the risk assessments of plant protection products, industrial substances and biocidal products are, for example, the general approach and information requirements. The information requirements therefore need to be harmonised and strengthened, especially in relation to long-term studies, reproductive toxicity studies and toxicokinetics.

Although the scientific background for default assessment (uncertainty) factors in general remains unsatisfactory, we recommend the inclusion of assessment factors in risk assessment, when justifiable.

Finally, it should be emphasised that the proposed assessment factors in the document are not precise figures and should only be used as a guide. In addition, policy aspects such as level of protection are not included here. Expert judgement should always be applied in the risk assessment procedure, whether assessment factors are being used or not. It is also of the utmost importance to ensure transparency in the performance of the risk assessment irrespective of the approach being applied.

1.1.1 Summary of methods for human health risk assessment

There are similarities but also basic differences between the risk assessment procedures for plant protection products, for new and existing industrial chemicals and for biocidal products. The differences mainly relate to goals and objectives laid down in differing sets of legislation. An overview of risk assessment procedures used in the European Union collaboration on plant protection products, on new and existing industrial chemicals and on biocidal products is given in Table 1.1.

At present, a new system of chemicals control - the REACH system (Registration, Evaluation and Authorisation of CHemicals) - is under development by the European Commission in collaboration with the Member States. The REACH system, to be applied to new and existing industrial chemicals, is intended to be able to cope with the large number of substances, which has become far too demanding on resources for the authorities involved.

Use of assessment factors

Assessment factors (10×10 for animal-to-human and human-to-human variation) are currently used in the risk assessment of plant protection products and are also recommended for biocides. However, in the current version of the Technical Guidance Document for the risk assessment of new and existing industrial substances[1], there is no recommendation that pre-set (default) assessment factors should be used. Instead, expert judgement is recommended and substances are considered to be "of concern for risk reduction measures" when the quotient between the NOAEL value obtained from animal experiments and the human exposure is less than the value of 1 (the margin of safety < 1). If the margin of safety is greater than 1, the degree by which the N(L)OAEL exceeds the estimated exposure needs to be considered.

A comparison between the outcomes from the risk assessment of plant protection products and existing industrial substances shows that the assessment factor used for setting Acceptable Operator Exposure Levels (AOEL) for workers exposed to plant protection products is rarely below 100. In contrast, for workers exposed to industrial chemicals the Margin of Safety (MOS) considered large enough for the population potentially exposed not to be harmed has always been lower than 100. This may assume a higher protection level for workers exposed to plant protection products compared to workers exposed to industrial chemicals.

The use of pre-set assessment factors could, on the other hand, in some cases lead to the acceptance of too small a quotient between the NOAEL value and the human exposure. This can be exemplified by the use of the same level of assessment factor (normally 100) as for deriving AOELs when setting the Acceptable Daily Intake

[1] the current version of the Technical Guidance Document (EC1996) is at present undergoing revision

(ADI) for food and drinking water in the plant protection products programme. This means that the general public – including all types of subgroups exposed via food and water - will not be protected more than workers unless an extra safety factor is added. In the existing substances programme, where expert judgement is used on a case-by-case basis for each population, practice in risk assessment is that the general public exposed via food, water and air ought always to be protected to a greater degree than workers and also to a greater degree than the general public exposed to commercially available products.

In cases where the active substance is associated with severe toxicological properties such as toxicity to reproduction, the use of an additional assessment factor of 10 is proposed for the risk characterisation of biocidal products. The proposal for an extra assessment factor is included as a recommendation in the guidance document to the Biocidal Products Directive 98/8/EC (EC 1998). No such recommendations on a quantified additional assessment factor are laid down in the Technical Guidance Document (EC 1996) for new and existing industrial chemicals. However, in the case of severe toxicological properties the Technical Guidance Document recommends extra prudence in the risk assessment. For plant protection products, common practice has developed towards the use of an additional assessment factor ranging from approximately 2 to 10.

No more details on the possible inclusion of risk assessment factors are given in the various sets of legislation or in the applicable guidance documents.

Conditions not directly associated with assessment factors, which might also affect the outcomes of the risk assessments are, for example, the general approach and the information requirements.

The general approach

Plant protection products and biocides are subject to Annex 1 listing/authorisation procedures resulting in approval/non-approval. The process is initiated by an application from a company. The procedure for new industrial chemicals is based on an application for notification made by industry. However, risk assessment on existing industrial chemicals is based on current use and results in possible risk reduction measures.

Priority setting for substances to be risk-assessed also differs between the procedures. For new plant protection products, new biocidal products and new industrial substances, the manufacturer or the importer makes the application and thus sets the priorities. However, in the case of existing industrial chemicals, the Commission and the Member States draw up priority lists of substances to be risk-assessed.

The information requirements

The information requirements differ with respect to the availability of long-term studies and also with respect to reproductive toxicity studies. Long-term studies (i.e. exposure throughout lifetime, beginning with exposure of experimental animals as young adults) and studies on reproductive toxicity are requested for plant protection products, for high-volume new industrial chemicals and for biocides, but not as a base-set for existing industrial chemicals regardless of marketed quantities. Furthermore, there is a lack of proper toxicokinetic data in the basic information requirements for existing industrial chemicals.

Exemption from basic information requirements

Exemption from basic information requirements is possible if fully justified by industry in all the procedures described. For new industrial chemicals, the exemption is referred to as *defer*. Defer of testing may be possible if there are negligible levels of human exposure. For existing industrial chemicals, *derogation* from testing can be granted on the grounds that a certain item of information is either unnecessary for the risk assessment or impossible to obtain. The Biocidal Products Directive also states that the data/test requirements should suit the individual circumstances, and that information which is not necessary owing to the nature or the proposed use of the biocidal product need not be submitted and may therefore be *waived*. According to the Directive for the risk assessment of plant protection products, refusal to submit data must be justified.

For all processes, effects assessment is based on common methodology on hazard assessment. With regard to exposure assessment, there are some differences in how exposure data are to be obtained and also how personal protective equipment is considered in the risk assessment/management. In addition, there are minor differences in how relevant populations are specified.

Human populations to be assessed

The populations to be considered in the risk assessment are the same for industrial chemicals and biocidal products. Exposure has to be assessed for workers, consumers and humans exposed indirectly via the environment, including exposure via food, water and air. In this case, consumer exposure means exposure due to products which can be purchased from retail outlets by members of the general public. With regard to plant protection products, risk assessment has to be carried out for operators (users of the product), workers (other than the operator), bystanders (persons in the vicinity of a product application) and also for man exposed indirectly via the environment.

No other specific subgroups to be assessed for risk are identified in the legislation or in the guidance documents. There are only general recommendations to consider human populations, to which the information on exposure are available. In addition, only general guidance is available for considerations of inter-individual variations.

Exposure data

Exposure data are obtained both from measured data and from modelled data. With regard to industrial chemicals and biocides, measured data are preferred to modelled data. For the risk assessment of plant protection products, a step-wise or tiered approach is used to refine the exposure assessment. For dermal exposure, modelled data are consequently used in the initial steps and measured data in the later steps.

Personal protective equipment

In the risk assessment of plant protection products, various types of personal protective equipment, including respiratory protective equipment, are taken into account and may be considered in the exposure models as well as in the field studies. For industrial chemicals and biocidal products, personal protective equipment should not be part of the risk assessment. Personal protective equipment should instead be considered at subsequent stages of risk reduction or risk management. The Biocidal Products Directive furthermore states that the product shall not normally be

authorised if, for non-professional users, the wearing of personal protective equipment would be the only possible method for reducing exposure.

Conclusions and recommendations

In conclusion, the approaches to pre-set default factors vary in the different legislation for the risk assessment of plant protection products, new and existing industrial chemicals and biocidal products. As a serious consequence, this may lead to differences in the level of protection depending on the directive or regulation applied. Furthermore, the differences between the procedures related to the general approaches and the information requirements might also affect the outcome of the risk assessments. The information requirements therefore need to be harmonised and strengthened. Although this is beyond the scope of the present project, the need for proper and improved toxicokinetic data can be highlighted as being of very high concern.

There is also a lack of guidance in the risk assessment procedures on how to make risk assessments for extra-vulnerable sub-populations, such as children, the elderly and patients under medical treatment.

There is therefore an obvious need to harmonise the current use of assessment factors under the different sets of legislation, and this is also our recommendation. It is also necessary to evaluate in which parts of the total risk assessment the application of pre-set default factors would be preferable to a non-quantified assessment factor approach.

Table 1.1 An overview of risk assessment procedures used in the European Union collaboration applicable to plant protection products, new and existing industrial chemicals and biocidal products.

Directive/Regulation	**Plant Protection Products (PPP;** see Section 4.3.1**) Directive 91/414/EC (EC 1991)**	**New Industrial Subst. (NS;** see Section 4.3.2**) Directive 67/548/EC (EC 1967)**	**Existing Industrial Subst. (ExS;** see Section 4.3.2**) Regulation EEC 793/93 (EC 1993a)**	**Biocidal Products (BP;** see Section 4.3.3**) Directive 98/8/EC (EC 1998)**
General				
Approach	Application for approval for specified uses	Notification	Risk assessment on current uses	Application for approval for specified uses
Priority setting	Existing PPP: priority list New PPP: application	Notification	Priority list	Existing BP: priority list New BP: application
Consequences	Approval Non-approval	Risk reduction No concern Further testing Defer	Risk reduction No concern Further testing	Approval Non-approval
Information				
Information requirements	Extensive basic data set	Base set (based on production volume)	Base set (not based on production volume)	Extensive basic data set
Exemption from basic information requirement	Possible if fully justified	Possible if fully justified Defer [1)]	Possible if fully justified Derogation [1)]	Possible if fully justified Waiving [1)]
Exposure assessment				
Populations	Professionals (operator, worker) Non-professionals (operator) Man exposed via the environment (food, water) Bystanders	Workers Consumers Man exposed via the environment (food, water, air)	Workers Consumers Man exposed via the environment (food, water, air) Combined exposure (occup + consumers + man exp v env)	Workers Consumers Man exposed via the environment (food, water, air) Combined exposure (occup + consumers + man exp v env)
Exposure levels	Modelled data Measured data	Prim: measured data Sec: modelled data	Prim: measured data Sec: modelled data	Prim: measured data Sec: modelled data

cont.

Directive/Regulation	**Plant Protection Products (PPP;** see Section 4.3.1**) Directive 91/414/EC (EC 1991)**	**New Industrial Subst. (NS;** see Section 4.3.2**) Directive 67/548/EC (EC 1967)**	**Existing Industrial Subst. (ExS;** see Section 4.3.2**) Regulation EEC 793/93 (EC 1993a)**	**Biocidal Products (BP;** see Section 4.3.3**) Directive 98/8/EC (EC 1998)**
Effects assessment	Common effects assessment methodology (see Section 4.2)			
Risk characterisation				
Method	Acceptable Operator Exposure Level AOEL = NOAEL/AF Acceptable Daily Intake ADI = NOAEL /AF Acute Reference Dose ARfD = NOAEL/AF	Margin of Safety MOS = NOAEL/Exposure;	Margin of Safety MOS = NOAEL/Exposure	Margin of Exposure MOE = NOAEL/Exposure; Acceptable Operator Exposure Level AOEL = NOAEL/AF Acceptable Daily Intake ADI = NOAEL /AF
Use of default assessment factors in general	Yes (normally 100)	No (case-by-case)	No (case-by-case)	Yes (normally 100)
Use of additional assessment factors for severe effects	Might be included	No[2)]	No[2)]	An extra factor of 10 might be included in the case of thereshold effects
Effects exemption from the risk characterisation	Acute toxicity[3)] Irritation[3)] Sensitisation[3)]	No	No	No
Exposure refinement	Refinement from generic to scientific data	Reasonable worst case (may be refined)	Reasonable worst case (may be refined)	Reasonable worst case (may be refined)
Further information	To cover unforeseeable gaps in information	By request as outcome of the conclusion	By request as outcome of the conclusion	To cover unforeseeable gaps in information

Abbreviations: AF = assessment factor; ARfD = Acute Reference Dose

1) "defer", "derogation", and "waiving" are used as synonymous terms for exemption from basic information requirement

2) Although no assessment factors are used, extra caution is recommended in the risk assessment for example of reproductive toxicants and carcinogens

3) included for classification purposes only

1.1.2 Summary of risk assessment factors

In the health risk assessment of chemicals, the basis for a NOAEL (No Observed Adverse Effect Level) is often only data from animal experiments. The identified NOAEL is then divided by an assessment factor to obtain an exposure dose that is considered to be without appreciable health risks (of no concern) for a given human population. Historically, an assessment factor of 100 intended to cover the inter-species (animal-to-human) and inter-individual (human-to-human) variations has often been used as a default. This simple approach has many aspects that are open to discussion, especially concerning the scientific foundation for some of the assumptions made. It should be noted, moreover, that other factors are not directly included in the assessment factor 100, such as the adequacy of the database, nature of the effect, duration of the exposure, route-to-route extrapolation, and consideration of extra-sensitive sub-populations such as children, the elderly and patients under medical treatment.

Adequacy of the toxicological database

The adequacy of the database first has to be evaluated. It is natural that a poor database will need an extra assessment (uncertainty) factor to compensate for possible toxic effects not studied or observed in the animal experiments. Transparent expert judgement of the adequacy of the database in a case-by-case manner is at present the most useful tool in considering the database. Based on other organisations' default values, a factor of 1 – 5 could be chosen for practical reasons when judging the adequacy of the database. If effects on the endocrine, reproductive, immune and the nervous systems are suspected or indicated, particularly in young individuals, specially designed animal studies including the relevant age period are necessary to make an adequate risk assessment. If adequate knowledge is lacking, we propose an extra assessment factor of 1-10 as a default value to protect children.

Adverse effect – the NOAEL

The NOAEL is the observed threshold for adverse effects in animal experiments. Not all detected effects are adverse, but we recommend a conservative approach to the question of when an effect should be regarded as adverse or not.

Dose-response curve and extrapolation from LOAEL to NOAEL

Due to the experimental design, the NOAEL cannot always be identified. In such cases the Lowest Observed Adverse Effect Level, the LOAEL, can be used instead, but in that case compensated for by an extra assessment factor (3 - 10 has been recommended in different guidance documents). As the correct size of such an assessment factor is unknown and depends on the design of the experiment, it can only be assigned using expert judgement, where the shape of the dose-response curve and the magnitude of the LOAEL are taken into account. In the case of a consensus on which response or effect size best represents the NOAEL, the use of the benchmark dose approach would be the most appropriate. Otherwise, a default factor of 3 - 10 for extrapolation from LOAEL to NOAEL is recommended.

Nature of the effect

The severity of the effect should also be taken into account, and a number of bodies, including WHO, JECFA and JMPR, have incorporated en extra assessment factor of 1-10 in cases where the NOAEL is derived for an effect which is of a high degree of severity, such as reproductive toxicity or carcinogenicity, especially if associated with a shallow dose-response relationship. Others may argue that the severity of the effect is irrelevant from the statistical point of view

when the purpose of the risk assessment is to extrapolate a NOAEL in animal experiments to a human no-effect level. In comparison with other organisations' default values for severity a factor of 1 – 10 could be chosen for practical reasons when judging the nature of the effect.

Route-to-route extrapolation

Route-to-route extrapolations sometimes have to be made, for example if the animal experiments are performed using oral exposure but the significant human exposure is absorption through the skin or via the lungs. As a default, we suggest 100% degree of absorption when extrapolating from oral exposure to dermal exposure or inhalation. It should be noted that an assumed degree of absorption of 100% (i.e. 100% of oral absorption) is probably conservative for dermal absorption. However, in the case of inhalation such an assumption may underestimate the actual absorption if the degree of absorption via inhalation is higher than via the oral route. If kinetic data are available, these should be used for comparisons**.**

Duration of exposure

Another type of extrapolation concerns the duration of exposure. A lifetime is often considered proportionally for laboratory animals and man, so chronic exposures in animals can be directly transferred to chronic exposures in man. However, chronic studies are often not available. A strict comparison of time gives a factor of 3 in comparing subacute (1 month) to subchronic (3 months) exposure and a factor of 8 subchronic to chronic (24 months) exposure. As the NOAEL is expected to be lower in chronic studies than in shorter-term studies, default assessment factors ranging from 2-10 have been proposed by different organisations. From studies examining the ratios between NOAELs from subchronic and chronic studies, a distribution was calculated (log-normal distribution; GM=2; GSD=3.5). The NOAEL in subchronic studies was on average two times higher than the NOAEL in chronic studies. A particular percentile can be chosen from the distribution for the derivation of an assessment factor. The choice of percentile is a matter of policy and beyond the scope of this document. If the level of 95% is chosen from the distribution (covering 95% of the substances compared), the corresponding assessment factor for extrapolation from subchronic to chronic exposure would be 16. Extrapolation from subacute to chronic exposure should preferably not be performed, but if it is necessary a similar approach is suggested.

Inter-species (animal-to-human) extrapolation

An important extrapolation is from the NOAEL in animals to an equivalent no-effect level in humans. The inter-species assessment factor is generally recognised as providing an extrapolation from experimental animals to humans, assuming that humans may be ten times more sensitive than animals. Based on limited data, this inter-species factor of 10 has been divided into 4 for differences in toxicokinetics and 2.5 for toxicodynamic differences. These factors are related to the NOAEL being expressed in mg per kg bodyweight per day. An alternative to extrapolation on a mg/kg bw basis using default assessment factors is to extrapolate on the basis of surface area or caloric demand by means of allometric scaling, which would account for differences in metabolic size. Because of their larger body size, humans will always be regarded as more sensitive than laboratory animals in such allometric scaling. If expressed in mg/kg body weight, the scaling factor from mouse to man would be 7 on the basis of caloric demand. From rat to man, the corresponding scaling factor would be 4. If inhalation is the relevant route of exposure in both animals and man, the common basis for comparison is the concentration in inhaled air, and no assessment factor is required. Scaling by caloric demand

does not include factors influencing the toxicity at the site of action (the toxicodynamics), so the total inter-species extrapolation factor should also include a factor for toxicodynamic differences. A distribution was calculated from studies examining the actual relationship between NOAELs in rats, mice and dogs after adjustment for metabolic size, (log-normal distribution; GM 1; GSD 4.5). If the level of 95% is chosen from the distribution (covering 95% of the substances compared), the corresponding assessment factor would be 12.

We suggest that a species-specific default factor should be used for inter-species extrapolation regarding toxicokinetics (basic metabolic rate). This factor should be based on differences in caloric demand and is thus 4 for extrapolation from rats, 7 from mice, 3 from guinea pigs, 2.4 from rabbits and 1.4 from dogs. Regarding toxicodynamics and remaining toxicokinetic differences, and if the level of 95% is chosen (covering 95% of the substances compared), an assessment factor of 12 is recommended. As an example, this results in a total inter-species assessment factor of 48 from rats to humans and 84 from mice to humans. The ten-fold inter-species assessment factor, which is generally used for differences in both toxicokinetics and toxicodynamics, has been estimated to account for 73 % of the variability among different substances.

Inter-individual (human-to-human) variations in sensitivity

The inter-individual variation in sensitivity towards toxic agents can be expected to be much higher within the human population than in inbred strains of laboratory animals. At present, there is not enough data on such differences in sensitivity for the derivation of probabilistic distributions. Potentially sensitive subgroups include the foetus, children, particularly infants, teenagers, the elderly, people with certain illnesses and people with certain genetic polymorphisms. A default assessment factor of 10 has traditionally been used, and it has been suggested that this factor should be divided equally into a factor of 3.16 for variations in toxicokinetics and a factor of 3.16 for toxicodynamics. We suggest that an inter-individual factor of 3-5 might be sufficient to reflect the toxicokinetic variability between healthy adults. Differences in sensitivity due to enzymatic polymorphisms are not included in this estimate. The data available on toxicodynamic differences between individuals are limited, and we do not consider that it is possible, based on present-day knowledge on inter-individual variability, to suggest an alternative to the default value of 3.2 for toxicodynamics for the general population. In view of the state of current knowledge, a total inter-individual assessment factor of 10-16 (3-5 times 3.2) is therefore suggested as a minimum. No attempt is made to provide a factor covering the inter-individual differences in sensitivity with the inclusion of all the various risk groups. Instead it is important to make a qualitative case-by-case expert judgement, depending on the effect studied, the mechanism of action and the exposure of concern.

Chemical-specific and metabolic pathway-related assessment factors

The WHO/IPCS default inter-species subfactors of 2.5 for toxicodynamics and 4.0 for toxicokinetics and the inter-individual factors of 3.16 each could be replaced by chemical-specific adjustment factors, as proposed in a recent IPCS document. The chemical-specific approach is attractive because it attempts to use scientific data. Although sound in principle, the proposed approach has limitations. It would, if applied, rely very heavily on scarce data that are not regularly investigated for. In most cases the experimental data will be weak or non-existent (particularly toxicodynamic data). The approach might nevertheless be applied in those instances where the required quantitative data may be derived. In such cases, the quality of the underlying studies must be carefully scrutinised and criteria established. The chemical-specific

approach requires that human studies and studies using human tissue samples must be used. For ethical reasons, such studies may be controversial.

If the metabolic fate of a chemical is known but the toxicokinetics of the chemical has not been quantified a pathway-related factor could be used to refine the risk assessment process. Such pathway-related factors would, however, need to be developed for each test-species in relation to humans, as well as for human variability in the pathway. For ethical reasons, such studies might be controversial.

Derivation of an overall assessment factor

If all the assessment factors discussed here are multiplied as point estimates to obtain an overall assessment factor, one might end up with a very large factor, which would probably lead to a very high level of protection. It is recommended that distributions of the assessment factors should be used, if available, in the calculation of an overall assessment factor. In addition, distributions and point estimates can be used in parallel and be combined when necessary. Distributions are only available at present for the inter-species extrapolation factor and the factor for duration of exposure. Which percentile of the distribution should be chosen is a matter of policy.

Two of the most important assessment factors are those for inter-species and inter-individual variation. If, for example, the 95th percentile of the inter-species distribution is selected, and combined with the point estimate of the inter-individual assessment factor, the overall factor would become 500-800 for rat experiments. This factor is higher than the traditional assessment factor of 100. For mouse experiments, the combined inter-species and inter-individual assessment factor would become 800-1300.

Table 1.2 includes our own conclusions regarding assessment factors. In the case of assessment factors derived from probabilistic distributions of existing data (duration of exposure and inter-species extrapolations), estimates are given for the 90th, 95th and 99th percentiles to illustrate the outcome depending on the desired level of protection.

It should be noted that the scientific foundation for the assessment factors is still unsatisfactory, although the influence on the NOAEL of species and duration of exposure based on actual animal experiments is a step forward. Recent and ongoing studies on inter-species and inter-individual variability in pharmacokinetics provide information that may form the basis for new species- and metabolic pathway-related default assessment factors. More information, primarily concerning human inter-individual variation in sensitivity, is still needed.

We are aware that the risk assessment process as outlined in this chapter leaves scope for different interpretations of the underlying scientific data, and that different risk assessors may come to different conclusions. As risk assessment is one important part of different actions with the common goal of the protection of human beings, we feel that it is prudent to adopt a conservative approach. It is also of the utmost importance to ensure transparency in the performance of the risk assessment.

Table 1.2 Summary of deterministic and probabilistic assessment factors suggested in this report for use in human health risk assessment. Our recommendation is to use assessment factors derived from probabilistic distributions in favour of deterministic assessment factors.

Ref. to Section	Area to be extrapolated	Assessment factor	
		Deterministic approach	Probabilistic approach[1, 2)]
5.2.1	**Adequacy of the toxicological database**		
	– relevance, validity and reliability	1 - 5	-
	– children	1 - 10	-
5.2.2	**Nature of the effect**		
	– adversity, severity, and potency	1 - 10	-
5.2.3	**Duration of exposure**		
	– subacute (1 month) → subchronic (3 months)	3	-
	– subchronic (3 months) → chronic (24 months)	8	10 (90^{th}); 16 (95^{th}); 37 (99^{th})
	– subacute (1 month) → chronic (24 months)	24	25 (90^{th}); 39 (95^{th}); 92 (99^{th}) [5)]
5.2.4	**Route-to-route extrapolation**		
	– dermal NOAEL from oral NOAEL	100% or case-by-case	-
	– inhalation NOAEL from oral NOAEL	100% or case-by-case	-
5.2.5	**Dose-response curve**		
	– shape of the curve	case-by-case	-
	– LOAEL → NOAEL	BMDL5 or 3 – 10	-
5.2.6	**Inter-species extrapolation**		
	– rat → human	4 (TK) × 2.5 (TD) = 10	28 (90^{th}); 48 (95^{th}); 132 (99^{th}) [3)]
	– mouse → human	-	49 (90^{th}); 84 (95^{th}); 231 (99^{th}) [3)]
5.2.7	**Inter-individual extrapolation**	3 - 5 (TK) × 3.16 (TD) = 10 - 16[4)]	-

Abbreviations: TK = toxicokinetics; TD = toxicodynamics; BMDL5 = 5% lower confidence limit of the benchmark dose

1) Based on log-normal distributions; percentile given in brackets

2) Note that the percentile values are not directly multiplicative

3) Including an inter-species scaling factor based on caloric demand (rat/human = 4, mouse/human = 7; rabbit/human = 2.4; dog/human = 1.4)

4) A minimum value not covering all the various risk groups.

5) Extrapolation from subacute exposure to chronic exposure should preferably not be performed

2 Sammanfattning och rekommendationer

Hälsoriskbedömning
Förslag till användning av bedömnings- (osäkerhets-) faktorer.
Tillämpning av bedömningsfaktorer vid riskbedömning av växtskyddsmedel, industrikemikalier och biocidprodukter i det europeiska samarbetet.

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OBS: Föreliggande dokument innehåller ingen policy fastställd av KemI utan är avsett att tjäna som bas för framtida policydiskussioner.

2.1 Allmänna slutsatser och rekommendationer

Det finns ett klart behov av att harmonisera den nuvarande användningen av bedömningsfaktorer inom de olika lagstiftningarna för växtskyddsmedel, industrikemikalier och biocidprodukter. Detta är även vår rekommendation. Om ingen harmonisering görs kan en riskbedömning leda till olika skyddsnivå för en särskild population, beroende på vilket direktiv eller vilken förordning som tillämpas.

En av huvudslutsatserna som har dragits av utvärderingen av tillgängliga uppgifter om bedömningsfaktorer är att den vanligen använda faktorn 100 (10 för variationer mellan djur och människa och 10 för individuella variationer mellan människor) sannolikt är för liten. Baserat på aktuella vetenskapliga uppgifter är det troligt att bedömningsfaktorn djur-till-människa är kraftigt underskattad. När det gäller variabiliteten människa-till-människa föreslås en bedömningsfaktor på 10–16 som ett minimum.

Vidare finns faktorer som inte inkluderas i den traditionella bedömningsfaktorn 100 och som behöver beaktas. Sådana faktorer är databasens tillräcklighet, effektens beskaffenhet, exponeringens varaktighet, extrapolering mellan olika tillförselvägar och beaktande av extrakänsliga populationer såsom barn, äldre och patienter under medicinsk behandling.

I detta hänseende har barn identifierats som en grupp som bör beaktas särskilt noggrant i en riskbedömning. Tyngdpunkten bör läggas på sårbarheten för kemisk toxicitet under utvecklingen och mognaden av vissa organsystem liksom på eventuella bristfälligheter i databasen för unga individer. Oftast finns endast begränsade uppgifter tillgängliga från försök på unga försöksdjur. Därför anser vi att bristen på uppgifter bör kompenseras med hjälp av en lämplig bedömningsfaktor. Vår rekommendation är en faktor på 1–10 för att kompensera för en bristfällig databas.

Förhållanden som inte är direkt förknippade med bedömningsfaktorer, som också kan påverka resultatet av riskbedömningen av växtskyddsmedel, industriella ämnen och biocidprodukter, är till exempel det allmänna tillvägagångssättet och dokumentationskraven. Dokumentationskraven bör därvid harmoniseras och stärkas, särskilt när det gäller långtidsstudier, studier av reproduktionstoxicitet och toxikokinetik.

Även om den vetenskapliga bakgrunden för bedömningsfaktorer i allmänhet fortfarande har stora luckor rekommenderar vi att bedömningsfaktorer inkluderas i riskbedömningen där detta är möjligt.

Slutligen bör betonas att de föreslagna bedömningsfaktorerna i dokumentet endast bör ses som vägledning. Vidare ingår inga politiska aspekter såsom t ex val av skyddsnivå. En expertbedömning måste alltid tillämpas i riskbedömningsförfarandet, oberoende av om bedömningsfaktorer används eller ej. Det är också av yttersta vikt att riskbedömningsproceduren är tydlig och öppen, oberoende vilken metod som tillämpas.

2.1.1 Sammanfattning av metoder för hälsoriskbedömning

Det finns likheter men också grundläggande skillnader mellan riskbedömningsförfarandena för växtskyddsmedel, för nya och existerande industrikemikalier och för biocidprodukter. Skillnaderna hänför sig i huvudsak till mål och målsättningar som fastställts i de olika lagstiftningarna. En översikt över de riskbedömningsmetoder som används i samarbetet inom Europeiska unionen om växtskyddsmedel (EC 1991), om nya och existerande industrikemikalier (EC 1967, 1993a) och om biocidprodukter (EC 1998) finns i tabell 2.1.

För närvarande är ett nytt system för kemikaliekontroll – REACH-systemet (Registration, Evaluation and Authorisation of CHemicals) – under utveckling av Europeiska kommissionen i samarbete med medlemsstaterna. REACH-systemet, som kommer att tillämpas på nya och existerande industrikemikalier, är bl a avsett att kunna hantera ett stort antal substanser, vilket har blivit alltför resurskrävande för berörda parter.

Användning av bedömningsfaktorer

Bedömningsfaktorer (10×10 för variationer djur-till-människa och människa-till-människa) används för närvarande inom riskbedömningen av växtskyddsmedel och rekommenderas också för biocider. I den nuvarande versionen av det tekniska vägledningsdokumentet för riskbedömning av nya och existerande industriella ämnen (EC 1996)[2], finns det ingen rekommendation om att förbestämda bedömningsfaktorer bör användas. I stället rekommenderas expertbedömning och substanser betraktas vara ”föremål för riskbegränsande åtgärder” när kvoten mellan NOAEL-värdet som erhållits från djurförsök och människors exponering är mindre än 1 (säkerhetsmarginalen < 1). Om säkerhetsmarginalen är större än 1 måste hänsyn tas till i vilken grad som effektvärdet överskrider den uppskattade exponeringen.

En jämförelse mellan resultaten från riskbedömningen av växtskyddsmedel och existerande industriella ämnen visar att bedömningsfaktorn som använts för att bestämma AOEL-värdena (Acceptable Operator Exposure Levels = acceptabel exponeringsgrad för användare) för arbetstagare som exponeras för växtskyddsmedel sällan är under 100. När det gäller arbetstagare som exponerats för industrikemikalier har däremot säkerhetsmarginalen (MOS), som anses tillräckligt stor för en population som eventuellt exponerats men ej skadats, alltid varit lägre än 100. Detta kan leda till en högre skyddsnivå för arbetstagare som exponeras för växtskyddsmedel jämfört med arbetare som exponeras för industrikemikalier.

[2] Den nuvarande versionen av det tekniska vägledningsdokumentet (EC 1996) är för närvarande under omarbetning.

Användningen av förbestämda bedömningsfaktorer skulle å andra sidan i vissa fall kunna leda till acceptans av en alltför liten kvot mellan effektvärdet från djurexperiment och människors exponering. Detta kan exemplifieras genom att samma bedömningsfaktor (normalt 100) som vid används för härledning av AOEL-värden används när man bestämmer det acceptabla dagliga intaget (ADI) för livsmedel och dricksvatten inom växtskyddsmedelsprogrammet. Detta innebär att allmänheten – omfattande alla typer av delgrupper som exponeras via livsmedel och vatten – inte kommer att vara mer skyddade än arbetstagare om inte en extra säkerhetsfaktor används. I programmet för existerande ämnen, där expertbedömning används för varje enskilt fall för varje population, är praxis för riskbedömningen att allmänheten som exponeras via livsmedel, vatten och luft alltid skyddas i högre grad än yrkesexponerade och även i högre grad än allmänheten som exponeras för kommersiellt tillgängliga produkter.

I fall då den aktiva substansen förknippas med svåra toxikologiska egenskaper (såsom reproduktionstoxicitet) föreslås att en extra bedömningsfaktor på 10 används vid riskbedömningen av biocidprodukter. Förslaget om en extra bedömningsfaktor ingår som en rekommendation i vägledningsdokumentet till direktiv 98/8/EG (EC 1998) om biocidprodukter. Inga sådana rekommendationer om en kvantifierad extra bedömningsfaktor är fastställda i det tekniska vägledningsdokumentet (EC 1996) för nya och existerande industrikemikalier. I fråga om allvarliga toxikologiska egenskaper rekommenderas i det tekniska vägledningsdokumentet extra försiktighet vid riskbedömningen. För växtskyddsmedel har den allmänna praxisen utvecklats mot användningen av en extra bedömningsfaktor som sträcker sig från cirka 2 till 10.

Ingen ytterligare instruktion eller vägledning om den eventuella användningen av bedömningsfaktorer ges i de olika lagstiftningarna eller i de tillämpliga vägledningsdokumenten.

Omständigheter som inte direkt förknippas med bedömningsfaktorer som också kan påverka resultaten från riskbedömningar är till exempel *det allmänna tillvägagångssättet* och *uppgiftskraven*.

Det allmänna tillvägagångssättet

Växtskyddsmedel och biocider är föremål för ansökningsförfaranden som resulterar i godkännande/avslag. Processen påbörjas genom en ansökan från ett företag. Förfarandet för nya industrikemikalier baseras på en ansökan om anmälan av industrin. Riskbedömningen av existerande industrikemikalier baseras på den nuvarande användningen och leder till eventuella åtgärder för att minska riskerna.

Prioriteringen av substanser som skall riskbedömas skiljer sig också mellan förfarandena. För nya växtskyddsmedel, nya biocidprodukter och nya industrikemikalier lämnar tillverkaren eller importören in ansökan och sätter därmed prioriteten. När det gäller existerande industrikemikalier gör kommissionen och medlemsstaterna prioriteringslistor för substanser som skall riskbedömas.

Uppgiftskraven

Uppgiftskraven skiljer sig åt beträffande tillgängligheten av långtidsstudier och även när det gäller studier om reproduktionstoxicitet. Långtidsstudier (dvs. exponering under hela livet, med början med exponering av försöksdjur som unga vuxna) och studier av reproduktionstoxiciteten krävs för växtskyddsmedel, nya industrikemikalier (om de framställs i stora volymer) och för biocider, men utgör

inget baskrav för existerande industrikemikalier oberoende av saluförd kvantitet. Vidare finns det en brist på lämpliga toxikokinetiska uppgifter i de grundläggande uppgiftskraven för existerande industrikemikalier.

Befrielse från grundläggande uppgiftskrav

Befrielse från grundläggande uppgiftskrav är möjligt om det helt kan motiveras av industrin enligt alla de metoder som beskrivs. För nya industrikemikalier kallas befrielsen för *uppskov*. Uppskov med testning kan vara möjligt om exponeringsnivåerna för människan är försumbara. För existerande industrikemikalier kan *undantag* från testning beviljas mot bakgrund av att en viss informationspost antingen inte är nödvändig för riskbedömningen eller omöjlig att få fram. Även i direktivet om biocidprodukter fastslås att uppgifts- och testkraven bör anpassas till förhållandena i det enskilda fallet och uppgifter som inte är nödvändiga på grund av biocidproduktens beskaffenhet eller den avsedda användningen inte behöver lämnas in och därför kan *åsidosättas*. Enligt direktivet om riskbedömning av växtskyddsmedel måste vägran att lämna in uppgifter motiveras.

Bedömning av effekter orsakade av växtskyddsmedel, industrikemikalier och biocider baseras på gängse metodik för farobedömning. Beträffande exponeringsbedömningen finns vissa skillnader i hur *uppgifterna om exponering* inhämtas och hur *personlig skyddsutrustning* betraktas i riskbedömningen. Vidare finns det smärre skillnader i hur de relevanta *populationerna*, som riskbedöms, specificeras.

Humana populationer som skall bedömas

De populationer som ingår i riskbedömningen är samma för industrikemikalier som för biocidprodukter. Exponeringen skall bedömas för yrkesexponerade, konsumenter och människor som exponeras indirekt via miljön genom livsmedel, vatten och luft. I detta fall innebär konsumentexponering exponering som kommer från produkter som kan köpas i detaljhandeln av allmänheten. När det gäller växtskyddsmedel måste riskbedömningar utföras för användare (yrkesmässig eller inte yrkesmässig användning), arbetstagare (andra än användaren), övriga vid användningen närvarande personer (personer i närheten där produkten används) och även för människor som exponeras indirekt via miljön.

Inga andra särskilda delgrupper som skall riskbedömas har identifierats i lagstiftningen eller i vägledningsdokumenten. Det finns endast allmänna rekommendationer om att hänsyn skall tas till humana populationer där det finns information om exponering tillgänglig. Vidare finns det endast allmän vägledning tillgänglig om beaktande av variationen mellan individer.

Uppgifter om exponering

Uppgifter om exponering inhämtas både från uppmätta data och från modellerade data (simuleringsmodeller). När det gäller industrikemikalier och biocider föredras uppmätta data framför modellerade data. När det gäller riskbedömning av växtskyddsmedel används en stegvis metod för att förfina exponeringsbedömningen. Vid hudexponering används modellerade data i de inledande stegen och uppmätta data i de senare stegen.

Personlig skyddsutrustning

Vid riskbedömning av växtskyddsmedel tas hänsyn till olika typer av personlig skyddsutrustning och kan tas under övervägande i exponeringsmodellerna liksom i fältstudierna. Beträffande industrikemikalier och biocidprodukter ska personlig skyddsutrustning inte vara en del av riskbedömningen. Personlig skyddsutrustning ska i stället övervägas i de efterföljande stegen för riskreduktion eller riskhantering. I direktivet om biocidprodukter konstateras att produkten normalt inte skall godkännas om användning av personlig skyddsutrustning är den enda möjliga metoden att minska exponeringen för icke yrkesmässiga användare.

Slutsatser och rekommendationer

Användningen av förbestämda bedömningsfaktorer varierar i de olika lagstiftningarna för riskbedömning av växtskyddsmedel, nya och existerande industrikemikalier och biocidprodukter. Som en allvarlig följd kan detta leda till skillnader i skyddsnivå beroende på vilket direktiv eller vilken förordning som tillämpas. Vidare kan skillnaderna mellan förfarandena som hör samman med de allmänna tillvägagångssätten och uppgiftskraven också påverka resultatet av riskbedömningen. Uppgiftskraven bör därför harmoniseras och förstärkas. Även om detta är utom räckvidden för det aktuella projektet är det viktigt att framhålla att behovet av relevanta toxikokinetiska uppgifter är mycket stort.

Det finns också brist på vägledning om hur man gör riskbedömningar för särskilt sårbara populationer, såsom barn, äldre och patienter under medicinsk behandling.

Det föreligger ett klart behov av att harmonisera den nuvarande användningen av bedömningsfaktorer i olika lagstiftningar, vilket vi också rekommenderar. Det är också nödvändigt att utvärdera i vilka delar av den totala riskbedömningen som tillämpningen av förbestämda bedömningsfaktorer är att föredra framför ett förfarande med icke-kvantifierade bedömningsfaktorer.

Tabell 2.1 Översikt över riskbedömningsmetoder som används i samarbetet inom den europeiska unionen tillämpade på växtskyddsmedel, nya och existerande industrikemikalier och biocidprodukter.

Direktiv/förordning	**Växtskyddsmedel** (se avsnitt 4.3.1) **Direktiv 91/414/EG (EC 1991)**	**Nya industriella ämnen** (se avsnitt 4.3.2) **Direktiv 67/548/EG (EC 1967)**	**Existerande industriella ämnen** (se avsnitt 4.3.2) **Förordning (EEG) 793/93 (EC 1993a)**	**Biocidprodukter** (se avsnitt 4.3.3) **Direktiv 98/8/EG (EC 1998)**
Allmänt				
Tillvägagångssätt	Ansökan om godkännande för specificerad användning	Anmälan	Riskbedömning av nuvarande användning	Ansökan om godkännande för specificerad användning
Prioritering	Existerande växtskyddsmedel: prioriteringslista Nya växtskyddsmedel: ansökan	Anmälan	Prioriteringslista	Existerande biocidprodukter: prioriteringslista Nya biocidprodukter: ansökan
Följder	Godkännande Avslag	Riskreduktion Inga åtgärder Ytterligare testning Uppskov	Riskreduktion Inga åtgärder Ytterligare testning	Godkännande Avslag
Information				
Uppgiftskrav	Omfattande grundläggande uppgifter	Baskrav (baserat på produktionsvolym)	Baskrav (ej baserat på produktionsvolym)	Omfattande grundläggande uppgifter
Befrielse från grundläggande uppgiftskrav	Möjligt om helt motiverat	Möjligt om helt motiverat Uppskov [1)]	Möjligt om helt motiverat Undantag [1)]	Möjligt om helt motiverat Åsidosättande [1)]
Exponeringsbedömning				
Populationer	Användare (yrkesmässig eller inte yrkesmässig användning) Arbetstagare (andra än användaren) Övriga vid användningen närvarande Människor exponerade indirekt via miljön	Arbetstagare Konsumenter Människor exponerade via miljön (livsmedel, vatten, luft)	Arbetstagare Konsumenter Människor exponerade via miljön (livsmedel, vatten, luft) Kombinerad exponering (arbetstagare. + konsumenter + människor exp. via miljön)	Arbetstagare Konsumenter Människor exponerade via miljön (livsmedel, vatten, luft) Kombinerad exponering (arbetstagare. + konsumenter + människor exp. via miljön)
Exponeringsnivåer	I första hand: Modellerade data I andra hand: Uppmätta data	I första hand: Uppmätta data I andra hand: Modellerade data	I första hand: Uppmätta data I andra hand: Modellerade data	I första hand: Uppmätta data I andra hand.: Modellerade data

forts.

Direktiv/förordning	**Växtskyddsmedel** (se avsnitt 4.3.1) **Direktiv 91/414/EG (EC 1991)**	**Nya industriella ämnen** (se avsnitt 4.3.2) **Direktiv 67/548/EG (EC 1967)**	**Existerande industriella ämnen** (se avsnitt 4.3.2) **Förordning (EEG) 793/93 (EC 1993a)**	**Biocidprodukter** (se avsnitt 4.3.3) **Direktiv 98/8/EG (EC 1998)**
Bedömning av effekter	Gängse metodik för effektbedömning (se avsnitt 4.2)			
Riskkaraktärisering				
Metod	Acceptabel exponeringsgrad för användare AOEL = NOAEL/ bedömn.faktor Acceptabelt dagligt intag ADI = NOAEL/bedömn.faktor Akut referensdos ARfD = NOAEL/ bedömn.faktor	Säkerhetsmarginal MOS = NOAEL/exponering;	Säkerhetsmarginal MOS = NOAEL/exponering;	Exponeringsmarginal MOE = NOAEL/exponering; Acceptabel exponeringsgrad för användare AOEL = NOAEL/ bedömn.faktor Acceptabelt dagligt intag ADI = NOAEL/bedömn.faktor
Användning av bedömningsfaktorer	Ja (normalt 100)	Nej (för varje enskilt fall)	Nej (för varje enskilt fall)	Ja (normalt 100)
Ytterligare bedömningsfaktorer för allvarliga effekter	Kan inkluderas	Nej[2)]	Nej[2)]	En extra faktor på 10 kan inkluderas i de fall då det finns tröskeleffekter
Effekter som ej beaktas vid riskkarakteriseringen	Akut toxicitet[3)] Irritation[3)] Sensibilisering[3)]	Nej	Nej	Nej
Förfining av exponeringsdata	Förfining från allmänna till vetenskapliga uppgifter	Rimligt värsta fall-scenario (kan förfinas)	Rimligt värsta fall-scenario (kan förfinas)	Rimligt värsta fall-scenario (kan förfinas)
Ytterligare information	För att täcka oförutsebara informationsbrister	På begäran som ett resultat av slutsatsen	På begäran som ett resultat av slutsatsen	För att täcka oförutsebara informationsbrister

1) ”uppskov”, ”undantag” och ”åsidosättande” är termer som används för befrielse från grundläggande uppgiftskrav

2) Även om inga bedömningsfaktorer används, rekommenderas extra försiktighet i riskbedömningen av exempelvis reproduktionstoxiska ämnen och carcinogener

3) Endast inkluderad av klassificeringsskäl

2.1.2 Sammanfattning av bedömningsfaktorer

Vid hälsoriskbedömning av kemikalier består underlaget för NOAEL-värdet (No Observed Adverse Effect Level) vanligen endast av uppgifter från djurförsök. Det identifierade NOAEL-värdet divideras med en bedömningsfaktor för att få en exponeringsdos som inte bedöms medföra hälsorisker hos människor (baserat på dagens kunskap).

Historiskt har en bedömningsfaktor på 100, som ska täcka variationer i känslighet mellan arter (djur-till-människa) och mellan individer (människa-till-människa), använts i de flesta fall. Denna enkla metod kan diskuteras, särskilt när det gäller den vetenskapliga grunden för några av antagandena. Det bör dessutom noteras att andra faktorer inte är medräknade i bedömningsfaktorn 100, såsom databasens tillräcklighet, effektens beskaffenhet, exponeringens varaktighet, extrapolering mellan olika tillförselvägar och beaktande av extra känsliga grupper såsom barn, äldre och patienter under medicinsk behandling.

Den toxikologiska databasens tillräcklighet

Först måste databasens tillräcklighet utvärderas. Det är naturligt att en bristfällig databas kräver en extra bedömningsfaktor (osäkerhetsfaktor) för att kompensera för eventuella toxiska effekter som inte har studerats eller observerats i djurförsök. Expertbedömning är för närvarande det mest användbara redskapet när det gäller att bedöma databasens tillräcklighet i varje enskilt fall. Baserat på andra organisationers rekommendationer kan en faktor på 1–5 kunna väljas av praktiska skäl då databasens tillräcklighet skall bedömas. Om man misstänker effekter på endokrin-, reproduktions-, immun- eller nervsystemen, särskilt ifråga om unga individer, måste särskilt utformade djurstudier som omfattar den relevanta åldersperioden utföras för att kunna göra en riktig riskbedömning. Om tillräcklig kunskap saknas föreslår vi en extra bedömningsfaktor på 1–10 för att skydda barn.

NOAEL-värdet

NOAEL-värdet är det observerade tröskelvärdet för toxikologiska effekter i djurförsök. Inte alla effekter som kan påvisas är emellertid skadliga (adverse). Vi rekommenderar ett konservativt synsätt vid bedömningen om när en effekt skall anses vara negativ eller ej.

Extrapolering från LOAEL till NOAEL

Beroende på försöksutformningen är det inte alltid möjligt att identifiera NOAEL-värdet. I sådana fall har det föreslagits att LOAEL-värdet (Lowest Observed Adverse Effect Level) skulle kunna användas i stället. Då används en extra bedömningsfaktor (3–10 har rekommenderats i olika vägledningsdokument) för att kompensera för detta. Eftersom den korrekta storleken för en sådan bedömningsfaktor är okänd och beror på försöksutformningen kan den endast sättas med hjälp av expertbedömning, där hänsyn tas till bl a dos-responskurvans form. Om ett samförstånd kan nås om vilken respons- eller effektstorlek som bäst representerar NOAEL-värdet är benchmarkmetoden lämplig att använda för dos-responsmodellering. I annat fall rekommenderas en faktor på 3–10 för extrapolering från LOAEL till NOAEL.

Effektens beskaffenhet

Hänsyn skall också tas till effektens beskaffenhet. Ett antal organisationer, däribland WHO, JECFA och JMPR, har lagt till en extra bedömningsfaktor på 1–10 i de fall då NOAEL-värdet härleds från en allvarlig effekt, t.ex. reproduktionstoxicitet eller cancer, särskilt om den förknippas med ett dos-responsförhållande som har en svag lutning.

Andra kan hävda att effektens allvarlighetsgrad är irrelevant sett ur ett statistiskt perspektiv då syftet med riskbedömningen är att extrapolera ett NOAEL-värde från djurförsök till en nivå utan effekt för människor. I jämförelse med andra organisationers rekommendationer skulle en faktor på 1-10 kunna väljas då effektens beskaffenhet skall bedömas.

Extrapolering mellan olika tillförselvägar

Ibland måste man göra extrapoleringar mellan olika tillförselvägar, t.ex. om djurförsök utförs med oral exponering men den relevanta exponeringen för människan är via huden eller lungorna. Som en standardmetod föreslår vi 100 % absorptionsgrad vid extrapolering från oral exponering till hudexponering eller inandning. Det bör noteras att en antagen absorptionsgrad på 100 % (dvs. 100 % av oral absorption) troligen är konservativ för hudabsorption. När det gäller inandning kan däremot ett sådant antagande underskatta den verkliga absorptionen, om absorptionsgraden via inandning är högre än via den orala vägen. Om det finns kinetiska uppgifter tillgängliga bör dessa användas som jämförelse.

Exponeringens varaktighet

En annan typ av extrapolering hänför sig till exponeringens varaktighet. En livstid anses ofta proportionell för laboratoriedjur och människor, så att kronisk exponering hos djur direkt kan överföras till kronisk exponering hos människan. Ofta finns inga kroniska studier tillgängliga. En strikt jämförelse av tid ger en faktor på 3 vid jämförelse av subakut (1 månad) och subkronisk (3 månader) exponering och en faktor 8 för subkronisk jämfört med kronisk (24 månader) exponering. Eftersom NOAEL-värdet förväntas vara lägre i studier av kronisk exponering än i korttidsstudier har bedömningsfaktorer som sträcker sig från 2 till 10 föreslagits av olika organisationer vid extrapolering från korttidsstudier till studier under längre tid. Från studier där man undersökte förhållandet mellan NOAEL-värden från subkroniska och kroniska studier, beräknades en fördelning (log-normalfördelning; geometriskt medelvärde=2; geometrisk standardavvikelse=3,5). NOAEL-värdet i subkroniska studier var i genomsnitt två gånger högre än NOAEL-värdet i kroniska studier. En viss percentil kan väljas från fördelningen för härledning av en bedömningsfaktor. Valet av percentil är en policyfråga och ligger utanför detta dokument. Om t.ex.95-percentilen väljs från fördelningen (täcker 95 % av de jämförda substanserna) skulle motsvarande bedömningsfaktor för extrapoleringen från subkronisk till kronisk exponering vara 16. Extrapolering från subakut till kronisk exponering bör helst inte göras men om det är nödvändigt föreslås ett liknande tillvägagångssätt.

Extrapolering mellan arter (försöksdjur till människa)

En viktig extrapolering är från NOAEL-värdet från djurförsök till en motsvarande icke-effektnivå för människor. Vanligtvis bygger en sådan mellan-artsfaktor på antagandet att människor är tio gånger mer känsliga än djur. Baserat på begränsade uppgifter har denna mellan-artsfaktor på 10 delats upp i 4 för skillnader i toxikokinetik och 2,5 för toxikodynamiska skillnader. Dessa faktorer hänför sig till NOAEL-värdet uttryckt som mg per kg kroppsvikt och dag. Ett alternativ till faktorn 10 med mg/kg som bas är att extrapolera baserat på ytstorleken eller kaloribehovet genom s.k. allometrisk skalning, vilket tar hänsyn till skillnader i basal metabolism. På grund av den större kroppsstorleken kommer människor alltid att anses vara känsligare än försöksdjur i en sådan allometrisk skalning. Uttryckt i mg/kg kroppsvikt blir skalningsfaktorn från mus till människa 7 baserat på kaloribehovet. Motsvarande skalningsfaktor från råtta till människa blir 4. Om inandning är den relevanta exponeringsvägen för både djur och människor är koncentrationen i den inhalerade luften den gemensamma basen för jämförelse och ingen skalningsfaktor krävs. Skalning baserat på kaloribehov omfattar inte andra

toxikokinetiska skillnader eller faktorer som påverkar toxiciteten i målorganet (toxikodynamiska skillnader). Detta innebär att den totala extrapoleringsfaktorn mellan arter också bör omfatta en kompensation för dessa skillnader. En fördelning beräknades från studier där förhållandet mellan NOAEL-värden hos råttor, möss och hundar efter justering för kaloribehovet studerades (log-normalfördelning; geometriskt medelvärde 1; geometrisk standardavvikelse 4,5). Om t.ex. 95-percentilen väljs från fördelningen (täcker 95 % av de jämförda substanserna) skulle motsvarande bedömningsfaktor, som inte inkluderar skillnader i basal metabolism, vara 12.

Vi föreslår att en artspecifik bedömningsfaktor används för extrapolering mellan arter när det gäller toxikokinetiken (basal metabolism). Denna faktor bör baseras på skillnader i kaloribehov och är 4 för extrapolering från råttor, 7 från möss, 3 från marsvin, 2,4 från kaniner och 1,4 från hundar. När det gäller toxikodynamiken och övriga toxikokinetiska skillnader, och om en nivå på 95 % väljs (täcker 95 % av de jämförda substanserna), rekommenderas en bedömningsfaktor på 12. Detta leder till en total mellan-artsfaktor på 48 från råtta till människa och 84 från mus till människa. Faktorn 10, som normalt används för skillnader i både toxikokinetik och toxikodynamik, har uppskattats stå för 73 % av variabiliteten mellan olika substanser.

Variationer i känslighet mellan individer (människa-till-människa)

Variationen i känslighet mellan individer för giftiga ämnen kan antas vara högre inom den humana populationen än i inavlade stammar av försöksdjur. För närvarande finns det inte tillräckligt med uppgifter om sådana skillnader i känslighet för att kunna härleda fördelningar. Potentiellt känsliga grupper omfattar foster, barn (särskilt spädbarn), tonåringar, äldre människor, personer med vissa sjukdomar och personer med vissa genetiska polymorfier. En bedömningsfaktor på 10 har traditionellt använts och det har föreslagits att denna faktor bör delas upp lika, i en faktor 3,16 för variationer i toxikokinetik och i en faktor 3,16 för toxikodynamik. Vi föreslår att en faktor för variationer mellan individer på 3–5 skulle kunna vara tillräcklig för att återspegla den toxikokinetiska variabiliteten mellan friska vuxna personer. Skillnader i känslighet som beror på enzymatisk polymorfism ingår inte i denna uppskattning. Tillgängliga uppgifter om toxikodynamiska skillnader mellan individer är begränsade och vi anser det inte möjligt, baserat på dagens kunskap om variabilitet mellan individer, att föreslå ett alternativ till den traditionellt använda faktorn 3,2 för toxikodynamik för den allmänna populationen. Mot bakgrund av den nuvarande kunskapen föreslås därför en total bedömningsfaktor mellan individer på 10–16 (3–5 gånger 3,2) som ett minimum. Inget försök görs att ge en faktor som täcker skillnader i känslighet mellan individer där alla olika riskgrupper räknas med. I stället är det viktigt att göra en kvalitativ expertbedömning för varje enskilt fall, beroende på den effekt som studeras, mekanismen för effekten och exponeringen i fråga.

Kemikaliespecifika bedömningsfaktorer och bedömningsfaktorer som hänför sig till metabolismvägen

Bedömningsfaktorerna för skillnader mellan arter enligt WHO/IPCS (2,5 för toxikodynamik och 4,0 för toxikokinetik) och faktorerna för skillnader mellan individer (3,16 vardera) kan ersättas av kemikaliespecifika faktorer, enligt vad som föreslås i ett nyligen framtaget IPCS-dokument. Det kemikaliespecifika angreppsssättet är intressant eftersom det bygger på att använda vetenskapliga data. Även om den föreslagna metoden är principiellt riktig har den sina begränsningar. Om den skulle tillämpas blir den i hög grad beroende av fåtaliga uppgifter som ofta saknas i den tillgängliga databasen. I de flesta fall kommer försöksdata att vara bristfälliga eller saknas (särskilt toxikodynamiska uppgifter). Metoden kan likväl tillämpas i de fall där det går att inhämta de kvantitativa

uppgifter som behövs. I sådana fall måste kvaliteten på de bakomliggande studierna granskas noggrant och kriterier fastställas. För att ta fram kemikaliespecifika faktorer krävs studier på människa och studier där man använder vävnadsprover från människa. Sådana studier kan vara kontroversiella av etiska skäl.

Om den huvudsakliga metabolismvägen för en kemikalie är känd men toxikokinetiken inte har kvantifierats, så skulle en faktor som generaliserats utifrån ämnen med gemensam metabolismväg kunna användas för att förfina riskbedömningsprocessen. Sådana metabolism-relaterade faktorer behöver tas fram för varje djurart i förhållande till människan liksom för metabolismvägens variabilitet hos människor. Sådana studier kan vara kontroversiella av etiska skäl.

Härledning av en övergripande bedömningsfaktor

Om alla de bedömningsfaktorer som diskuteras här multipliceras som punktskattningar för att få en övergripande bedömningsfaktor blir resultatet en mycket stor faktor, som förmodligen skulle leda till en mycket hög skyddsnivå. Vi rekommenderar att fördelningar för bedömningsfaktorer används, om sådana finns tillgängliga, vid beräkningen av en övergripande bedömningsfaktor. Vidare kan fördelningar och punktskattningar användas parallellt och kombineras om det behövs. Fördelningar, baserade på experimentella data, finns för närvarande bara tillgängliga för bedömningsfaktorn för skillnader mellan arter och för faktorn för exponeringens varaktighet. Vilken percentil för fördelningen som bör väljas är en policyfråga.

Två av de viktigaste bedömningsfaktorerna är dels faktorn för variation mellan arter, dels faktorn för variation mellan individer. Om exempelvis 95-percentilen för fördelningen för mellan-artsfaktorn väljs och kombineras med punktskattningen av bedömningsfaktorn för skillnader mellan individer skulle den övergripande faktorn bli 500–800 för försök med råttor. Denna faktor är högre än den traditionella bedömningsfaktorn på 100. För försök med möss skulle den kombinerade bedömningsfaktorn mellan arter och mellan individer bli 800–1300.

I tabell 2.2 ingår våra egna slutsatser om bedömningsfaktorn. När det gäller bedömningsfaktorer som härletts från probabilistiska fördelningar av existerande data (exponeringens varaktighet och extrapoleringar mellan arter) ges uppskattningar för den nittionde, den nittiofemte och den nittionionde percentilen för att illustrera resultatet beroende på den önskade skyddsnivån.

Det bör noteras att den vetenskapliga grunden för bedömningsfaktorerna fortfarande är otillfredsställande, även om jämförelserna av NOAEL-värden från försök med olika djurarter och med olika varaktighet är ett steg framåt. Nyligen utförda och pågående studier om variabiliteten mellan arter och mellan individer när det gäller toxikokinetiken ger information som skulle kunna utgöra grunden för nya art- och metabolismvägsrelaterade bedömningsfaktorer. Mer information behövs, framförallt om variationen i känslighet mellan individer.

Vi är medvetna om att riskbedömningsprocessen lämnar utrymme för olika tolkningar av de bakomliggande vetenskapliga uppgifterna, och olika riskbedömare kan komma till olika slutsatser. Eftersom riskbedömning är en viktig del av olika åtgärder med det gemensamma målet att skydda människors hälsa, anser vi det vara klokt att anta en konservativ hållning. Det är också av yttersta vikt att se till att det finns öppenhet och insyn i utförandet av riskbedömningen.

Tabell 2.2 Sammanfattning av deterministiska och probabilistiska bedömningsfaktorer som föreslås i denna rapport för användning vid bedömning av hälsorisker för människor. Vi rekommenderar användning av bedömningsfaktorer som härletts från probabilistiska fördelningar i första hand och för deterministiska bedömningsfaktorer i andra hand.

Hänvisning till kapitel	Område som skall extrapoleras	Bedömningsfaktor	
		Deterministiskt förfarande	**Probabilistiskt förfarande[1, 2)]**
4.2.1	**Den toxikologiska databasens tillräcklighet**		
	– relevans, giltighet och tillförlitlighet	1–5	-
	– barn	1–10	-
4.2.2	**Effektens beskaffenhet**		
	– skadlig effekt, svårighetsgrad och styrka	1–10	-
4.2.3	**Exponeringens varaktighet**		
	– subakut (1 månad) → subkronisk (3 månader)	3	-
	– subkronisk (3 månader) → kronisk (24 månader)	8	10 (*90:e*); 16 (*95:e*); 37 (*99:e*)
	– subakut (1 månad) → kronisk (24 månader)	24	25 (*90:e*); 39 (*95:e*); 92 (*99:e*)[5)]
4.2.4	**Extrapolering mellan olika tillförselvägar**		
	– dermalt NOAEL-värde från oralt NOAEL-värde	100 % eller för varje enskilt fall	-
	– NOAEL-värde för inandning från oralt NOAEL-värde	100 % eller för varje enskilt fall	-
4.2.5	**Dos-responskurva**		
	– kurvans form	för varje enskilt fall	-
	– LOAEL → NOAEL	BMDL5 eller 3–10	-
4.2.6	**Extrapolering mellan arter**		
	– råtta → människa	4 (TK) × 2,5 (TD) = 10	28 (*90:e*); 48 (*95:e*); 132 (*99:e*)[3)]
	– mus → människa	-	49 (*90:e*); 84 (*95:e*); 231 (*99:e*)[3)]
4.2.7	**Extrapolering mellan individer (människa)**	3–5 (TK) × 3,16 (TD) = 10–16[4)]	-

Förkortningar: TK = toxikokinetik; TD = toxikodynamik; BMDL5 = det nedre 5-procentiga konfidensintervallet av benchmarkdosen

1) Baserat på log-normalfördelning; percentil inom parentes.

2) Lägg märke till att percentilvärdena inte kan multipliceras direkt.

3) Omfattar en skalfaktor mellan arter baserad på kaloribehov (råtta/människa = 4, mus/människa = 7; kanin/människa = 2,4; hund/människa = 1,4).

4) Ett minimivärde som inte täcker alla olika riskgrupper.

5) Extrapolering från subakut exponering till kronisk exponering bör helst inte göras.

3 Background

Within the European Union legislation on chemicals, harmonised human health risk assessment schemes have been adopted for plant protection products, following Directive 91/414/EC (EC 1991), new and existing industrial chemicals following Directive 67/548/ EC (EC 1967) and Regulation EEC 793/93 (EC 1993a), and for biocides following Directive 98/8/EC (EC 1998). Plant protection products include pesticides used in agriculture, while biocidal products include pesticides used for other purposes. Plant protection products and biocidal products, regardless of marketed volumes, are subject to approval procedures implying that the risk of the active substance has to be adequately assessed with respect to the intended use. Both directives lay down approval procedures for biologically active (and consequently potentially toxic) substances and formulated products. All new industrial chemicals, including substances placed on the European Union market after 1981, should be notified and risk assessment should be carried out on them. Existing chemicals subject to risk assessment are what are referred to as high-production-volume chemicals produced or imported in quantities exceeding 1000 tonnes per year, and placed on specific priority lists prepared by the Commission in consultation with the Member States. The directives and regulation for plant protection products, new and existing industrial substances have been in force since 1991, 1981 and 1993 respectively, while the directive for biocides came into effect in 1998.

At present, a new system of chemicals control - the REACH system (Registration, Evaluation and Authorisation of CHemicals) - is under development by the European Commission in collaboration with Member States (EC 2001). The REACH system, to be applied to new and existing industrial chemicals, is intended to cope with the large number of substances, which has become far too demanding on resources for the authorities involved.

The approaches in risk assessment differ in some respects between plant protection products, new and existing industrial chemicals and biocides. For plant protection products and biocides, quantified assessment factors (10×10 for inter-species and inter-individual variations) are used to derive the health-based exposure limits Acceptable Operator Exposure Level (AOEL) and Acceptable Daily Intake (ADI). Risk assessment of biocides may also imply considerations of the size of toxicity/exposure ratios, and these are initially based on an assessment factor of 100 (10 x10). In the risk assessment of new and industrial chemicals, margins of safety (MOS), which means the degree by which the no observed adverse effect value (NOAEL) obtained from animal experiments exceeds the estimated chemical exposure in humans, is considered on a case-by-case basis by expert judgement in a non-quantified assessment factor approach.

When comparing and estimating different approaches in risk assessment, it is important to consider variability, consistency and transparency. For example, the approach of using well-based default assessment factors can be expected to give better consistency and transparency to the conclusions. However, a lower degree of freedom is left for expert judgement, which means that the variability is often small.

3.1 The purpose of the project

The main objectives of this project were to

- point out similarities and discrepancies between the different risk assessment procedures which may be due to current instructions in directives and regulations and also in practice within the working groups for plant protection products, for new and existing industrial chemicals and for biocides,
- review the scientific background for the use of risk assessment factors and evaluate the options for introducing the use of more elaborated assessment factors in the risk assessment,
- produce recommendations for the use of risk assessment factors,
- provide brief reviews on specific methods (the benchmark dose method and probabilistic assessment of risks) used for health risk assessment (presented in an Annex to this document).

The present publication covers

- risk assessment procedures for those chemical covered by the directives for plant protection products, new and existing industrial substances and biocidal products (substances not included are, for example, pharmaceuticals, food additives and cosmetic and hygiene products),
- human health risk assessment only,
- threshold-based effects only; mutagens and genotoxic carcinogens are not included,
- only single substance exposure (exposure to mixtures of chemicals is not considered).

4 Methods for human health risk assessment

The summary of "Methods for human risk assessment" is found in Section 1.1.1 (in English) and in Section 2.1.1 (in Swedish).

4.1 Introduction

The general steps in risk assessment for plant protection products, new and existing industrial substances and biocidal products are outlined in Figure 4.1. Data requirements on exposure, toxic effects, toxicokinetics and physico-chemical properties are needed to enable the establishment, for example, of exposure levels, no-adverse observed effect levels and dose-response relationships. Data required for the separate assessment processes are laid down in the respective directives (see sections on data requirements in Section 4.3). Risk assessment also includes considerations of margins of safety and different types of health-based exposure limits. In addition, with classification and labelling following Directive 67/548/ EC (EC 1967) and protective technical devices, the risk assessment serves as the basis for conclusions on further measures (for example more testing or risk reduction) and decision-making in the area of chemicals control in the EU.

4.1.1 The precautionary principle

While the risk assessment is mainly scientific in nature, the subsequent risk management (conclusions and decisions) is influenced by political considerations. In this context, it should be noted that the element of prudence or conservatism that scientists apply in their risk assessment of scientific data must not be confused with the precautionary principle. The concept of the precautionary principle was laid down as long ago as 1957 in the Treaty establishing the European Community, Article 174 (EC 1999), and is an example of a political stance in risk management. An elaborated version of the precautionary principle is contained in the "Communication from the Commission on the Precautionary Principle" (EC 2000a), where the main triggering factors to invoke the principle include

- insufficient, inconclusive or uncertain scientific evidence and
- indications through scientific evaluation that there are reasonable grounds for concern that the potentially dangerous effects on man or the environment may be inconsistent with the chosen level of protection.

There are many diverging interpretations at present on how and when the principle should be applied.

4.1.2 Ethical issues

Both science and ethics have roles in environmental decision-making. This is reflected for example in the White Paper "Strategy for a future Chemicals Policy", presented by the EU Commission in 2001 (EC 2001), where it is stated that ethical

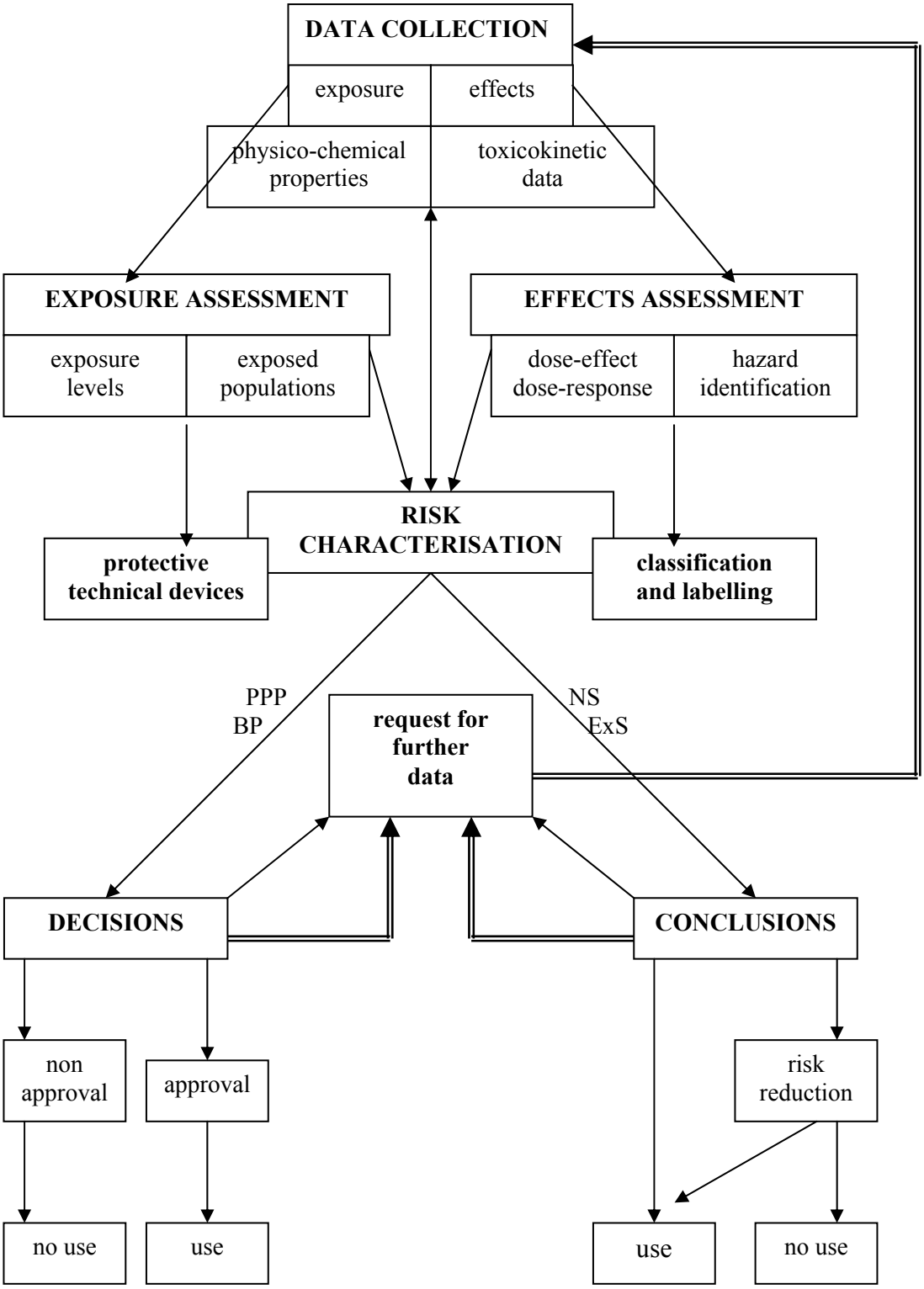

Figure 4.1 General steps in risk assessment and subsequent conclusions and decision-making for plant protection products (PPP), new industrial substances (NS), existing industrial substances (ExS) and biocidal products (BP).

considerations on animal welfare as well as the costs of testing strongly advocate a balanced approach to the testing of chemicals so that the acquired knowledge offers proportionate benefits in terms of managing risks.

The EU White Paper identifies the tests needed for hazard identification and potency of chemicals (substances and preparations as defined in Directive 67/548/EEC (EC 1967)). As well as physico-chemical tests and tests using *in vitro* systems, tests in experimental animals are used. Information from epidemiological studies should be used if available, but is not a test requirement.

Testing of chemicals in humans has long been discussed as a possible addition to tests in experimental animals. The ethical problems related to testing of chemicals in humans are obvious and would need to be discussed in appropriate fora before such tests could be performed. However, while the EU White Paper (EC 2001) addresses ethical considerations related to testing of chemicals in experimental animals, no considerations on ethical issues linked to testing of chemicals in humans are included. There is therefore a need to develop guidance on possible testing of chemicals in humans and also on how to utilise already existing human data.

Before such guidance can be developed, an issue of high ethical importance that needs to be considered is the justification of the use of human data for research purposes versus the justification of the use of human data for regulatory purposes (in legislation, in generally adopted guidelines, or by expert judgement on a case-by-case basis).

4.2 General methods for health risk assessment

The health risk assessment of chemicals has two main objectives, either to assess the health risks in connection with certain exposures (an assessment of the Margin of Safety, MOS[3] or the Margin of Exposure, MOE)[3] or to establish recommended health-based guidance values (for example Acceptable Daily Intakes, ADIs). Although the outcomes of these two procedures are different, the principles are essentially the same. After all relevant scientific data have been collected, the toxicokinetics and the toxicological properties of the chemical are described and evaluated (hazard identification). Dose-response relations are then scrutinised with the primary aim of estimating No Observed Adverse Effect Levels (NOAEL) for threshold effects. In the case of the derivation of recommended health-based guidance values based on effect data, the actual exposure is not part of the assessment procedure. However, exposure estimates will be very important when the MOS/MOE values are calculated for different exposure scenarios by comparing the anticipated exposure with the NOAEL (risk characterisation). There must be a discussion on how to extrapolate toxic effects seen in laboratory animals to the human population using assessment factors to compensate for differences in sensitivity between animals and humans (inter-species variation) and also in differences in sensitivity between individuals (inter-individual variation). This is applicable both in setting health-based guidance values and in judging whether a margin between the NOAEL seen in animals and the human exposure is wide enough to be protective.

[3] The terms margin of safety (MOS) and Margin of Exposure (MOE) both reflect the quotient between an effect value (NOAEL) in experimental animals and the chemical exposure of the human population. The choice in using the expression MOS or MOE is a matter of risk communication.

The terminology used for the different parts of risk assessment as outlined above has varied between times, countries and organisations. The different parts are briefly described below, and in greater detail in the following chapter.

4.2.1 Effects assessment

There are different requirements for the testing of chemicals. The completeness (and quality) of the available studies will depend on the type of substance being assessed. The present document focuses on regulated chemicals, for which the industry is responsible to deliver data according to specified requirements within the EU (see Section 4.3). This means that testing has to be performed according to guidelines and formal testing requirements. However, even if the requirement of testing is met, there might be important toxicological endpoints that have not been covered. For example, there are, as yet, no adopted tests for endocrine disruptor effects or immunotoxicity (other than skin sensitisation). The risk assessor therefore has to evaluate for each chemical the adequacy of the overall database. This will be discussed in greater depth in Section 5.2.

High-volume existing substances not included on priority lists are usually poorly investigated. Environmental pollutants such as those formed in combustion processes and as by-products in industrial processes may also be very poorly investigated. This means that the toxicological database sometimes may be very poor and not suitable for health risk assessment purposes.

4.2.1.1 Hazard identification

The purpose of the hazard identification is to identify the adverse effects, which a substance has an inherent capacity to cause. Basic data requirements are specified for the groups of chemicals covered in this document, and will be described in the following chapter.

Toxicological studies with experimental animals are the main basis for risk assessment of chemicals. Human epidemiological studies or case reports may also be available, especially for environmental pollutants and chemicals that have been used for a long time in the working environment. Such data have the advantage over toxicological studies in animals that no extrapolation from animals to humans has to be made in the risk assessment. In some cases there may also be data from controlled studies with human volunteers. For ethical considerations on testing in humans, see Section 4.1.2.

Animal experiments can provide basic data on uptake, distribution, metabolism and excretion of the chemical (toxicokinetics). Both local and systemic toxic effects should be investigated as well as both acute toxicity and repeated dose toxicity. There are specific tests for different endpoints such as reproductive toxicity. There are both test guidelines and guidelines for Good Laboratory Practice (GLP). These should normally be followed, but toxicological results are obtained in many different research projects apart from testing according to GLP and test guidelines protocols, so the risk assessor always has to try to evaluate the adequacy of the study.

In vitro studies are useful for mechanistic information and for certain toxicological effects, particularly genotoxicity/mutagenicity. Tests with cell cultures from different animal species can provide information on inter-species differences in metabolism, and recently it has been suggested that *in vitro* experiments can also generate data on

inter-species differences in toxicodynamics (interaction with target cells). It has been proposed, that such information could be used for extrapolation between species (and between individuals), see Section 5.1.2).

4.2.1.2 Dose-response assessment

Chemicals showing a threshold for effects, including non-genotoxic carcinogens.

For most toxicants except genotoxic carcinogens, it is generally assumed that there is a threshold dose below which no toxic effects will occur. In animal experiments, the threshold may be approximated by the NOAEL. In the dose-response assessment, an attempt is made to identify the NOAEL and then transfer this NOAEL to an equivalent dose that is considered to be of no concern for human health. The assessment factors that can be used in this process to account for uncertainties concerning differences in sensitivity between animals and humans etc., are discussed in Section 5.2. An alternative to the NOAEL approach, the benchmark dose method, is also discussed (see Section 5.2.5 and Annex 1).

In order to identify a NOAEL for different toxicological endpoints, the design of the study must be carefully evaluated. For statistical reasons, the number of animals in each dose group and the spacing between doses are particularly important. In some cases only a Lowest Observed Adverse Effect Level (LOAEL) can be identified. The term critical effect may be used for the effect that develops at the lowest dose. This concept implies that protection from the critical effect will protect against all other effects. However, in practice it is more useful to examine all the toxicological studies and give NOAELs for as many endpoints as possible. Some of the studies will usually be deemed more important than others from the human health point of view, and this or these studies will provide information on the critical effect(s).

In establishing a NOAEL or LOAEL, there is a problem as to whether an effect should be judged as being “adverse” or not. This will be discussed in more detail in Section 5.2.2.

Genotoxic carcinogens

For mutagenic substances and for those carcinogenic substances that have been judged to have a genotoxic mode of action, it is generally assumed that there will be a risk of tumour initiation even at very low doses, and that the risk is proportional to the dose. This means that no threshold can be identified below which there is zero risk. Mathematical methods have been developed for the purpose of quantitative risk assessment of such carcinogens. This will not be discussed in greater depth, as the focus in the present document is on non-genotoxic chemicals.

Allergens

Following initial sensitisation, subsequent exposure to extremely low levels of the chemical can trigger severe, life-threatening reactions. This is the main reason why allergenic substances are difficult to evaluate in the risk assessment process. Concerning specific considerations of allergens, see below (Section 5.2.7.4).

4.2.2 Exposure assessment

The aim of exposure assessment is to determine the nature and extent of human exposure to chemical substances under different conditions. However, the actual exposure of individuals is rarely measured, other than for occupational exposure and for the registration of plant protection products. More often, population exposures are

estimated indirectly, using data on measured or predicted concentrations of the chemical in air, food or water. In addition, dermal absorption may be an important exposure pathway, especially in the working environment.

Exposure models are in use for both plant protection products and existing and new chemicals. Exposure models are also under development for biocides. Such models predict reasonable worst-case exposures for workers, consumers and humans indirectly exposed via the environment to new and existing substances. Concerning plant protection products, exposure models are used to provide an estimated exposure but direct exposure measurements may also be formally required in certain instances. Modelling is based to some extent on the physico-chemical properties of the chemical product, but mainly on the use and release patterns, the estimated frequency and duration of contact and the distribution in the environment. It should be noted that there may be very wide variations in the actual exposure to chemicals so that a small proportion of the population is highly exposed.

4.2.3 Risk characterisation

One of the purposes of the risk characterisation is to compare the NOAEL to the estimated exposure for different population groups (assessment of the margin of safety or the margin of exposure) in order to obtain information on the potential for adverse health effects. Another is to identify doses that can be considered to be without appreciable health risks for humans (for example ADI). Risk characterisation is an evaluation and integration of the available scientific evidence obtained at the previous stages of the risk assessment process, including various uncertainties.

Where the aim of the risk assessment is to recommend health-based guidance values, ADI values and the like, safety/uncertainty/assessment/extrapolation factors are used to extrapolate from animal experimental data to humans and/or to compensate for inter-individual differences in human sensitivity (discussed in Section 5.2). An important question is whether all members or a certain percentile of the general population should be included. In the case where the aim is to derive and to value the margin of safety between the NOAEL and the anticipated exposures, the same type of assessment factors can be used, at least implicitly. Information on the use and/or non-use of pre-set default assessment factors for risk assessment of plant protection products, new and existing industrial chemicals and biocidal products is presented in Section 4.3.

Very seldom will it be possible to estimate the incidence and severity of the adverse effects likely to occur in a human population due to actual or predicted exposure to a chemical substance. The lower the MOS, the higher the likelihood for a potential adverse health effect to occur, but because of the uncertainties in animal versus human sensitivities, along with uncertainties in the exposure assessment, it is difficult to make a quantitative estimate of the number of people who might be affected.

4.3 Current application of specific methods for health risk assessment in EU legislation

4.3.1 The Council Directive for Plant Protection Products

4.3.1.1 Basic Provisions

Directive

Commission Directive 91/414/EEC (EC 1991) requires that a human health risk assessment be carried out on plant protection products consisting of an active substance, responsible for the desirable biological activity, and substances with other functions such as emulsifiers, wetting agents, stabilisers and adhesive additives.

According to Directive 91/414/EEC (EC 1991), active substances can be approved at EU level, provided that the risk assessment of at least one product including the active substance has been shown it to be acceptable. After such an approval is made, the use of other products containing the active substances can be authorised in the Member States on a national basis. Instructions on how the risk characterisation should be carried out, in a stepwise or tiered approach, are laid down in an amendment, 94/79/EC (EC 1994a), to the Directive.

Data requirements

Data are required for both the active substance and at least one product, representative of use in Europe. Data regarding the active substance are mainly used for the hazard assessment, and hence for establishing health-derived limits, while the product data are mainly used for the exposure assessment in the risk assessment. Requirements are laid down in Annex II (for the active substance) and Annex III (for the product) of Directive 91/414/EEC (EC 1991). The requirements are quite stringent, which means that the same type of data are required for all types of plant protection products. Data on efficacy are also required but restricted to the description of the intended purpose of the active ingredient.

The data required are presented in Table 4.1. Studies should be performed according to guidelines and Good Laboratory Practice, GLP (EC 1987). However, the Member States and experts at technical meetings have an opportunity to require further data, for example mechanistic studies, if necessary.

Exemption from formal data requirement

According to Directive 91/414/EEC (EC 1991) the data package should at least contain the information and results of the studies referred to in Annexes II and III to the Directive. Refusal to submit data must be justified.

Priority setting of active substances to be risk-assessed

Active substances already existing on the European market in 1993 are subsequently characterised in terms of risk in special EU programmes, while active substances introduced onto the market at a later date are risk-characterised when an application for approval is received in a Member State.

Responsibilities

The notifier, usually the company behind the product, is responsible for data generation, and for completion of a documentation file (a dossier). The notifier selects a product representative for European use, and this selection has to be

confirmed by the Rapporteur Member State, appointed by the EU Commission to assess the active substance and the product. The result of the assessment, known as the draft assessment report, is presented at technical meetings. Other Member States have the opportunity to comment upon the conclusions drawn at or before the technical meetings. Finally, when all the technical issues are considered to be resolved, the Commission will provide a proposal for decision. This proposal will be discussed and voted on (qualified majority) by all 15 Member States in the Standing Committee on the Food Chain and Animal Health.

Table 4.1 Data requirements for hazard assessment of the active substance and for a representative product (Annexes II and III to Directive 91/414/EEC (EC 1991) respectively)

Data/information	Data needed for the active substance	Data needed for a representative product
Toxicokinetics	x	-
Acute toxicity[1)]: oral, and dermal inhalation data might be required.	x	x
Irritation[1)]: skin and eye	x	x
Skin sensitisation[1)]	x	-
Repeated dose toxicity - 90 days in rat and dog	x	-
Repeated dose toxicity –1 year in dog[2)]	x	-
Genotoxicity	x	-
Long-term toxicity and carcinogenicity (rats and mice)	x	
Reproduction toxicity[3)]	x	-
Neurotoxicity (might be required)	x	-
Medical data (if available)	x	x
Dermal absorption (might be required)	-	x
Exposure data of operators, workers and bystanders	-	x

1) not included in the risk characterisation; only for classification purposes
2) performed when dog is considered to be more sensitive than rat in the 90-day repeated dose toxicity study
3) covering fertility and developmental toxicity studies

In cases when there is need for (extra) scientific advice, the Scientific Committee on Plants, which consists of independent experts from Member States, may comment on problems put forward by the Commission. The Scientific Committee on Plants is also consulted by the Commission as a final check on scientific conclusions drawn during the process.

Risk assessment

After the completeness of the submitted dossier has been checked/confirmed, the active substance is assessed with respect to its intended use. As regards existing active substances, these are assessed by the Rapporteur Member State, designated by the Commission (i.e. DG Health and Consumer Protection in the context of plant protection products). The Competent Authority of the Member State, which first receives the application, would normally carry out the risk assessment of a new active substance.

The assessment has to address the risk of the active substance when used as intended, which means with respect to representative uses of the product under investigation. The risk assessment involves the following and subsequent general activities: hazard identification, dose-response assessment, exposure assessment, and risk characterisation.

Consequences/Decision-making

The objective of the risk assessment is to investigate whether the use of a representative product is acceptable. If so, the active substance can be included in Annex I of Directive 91/414/EEC (EC 1991). Other products containing the active substance can then be approved and marketed on a national basis in the Member States, provided that an application for use in that Member State has been made and that the conditions of use are shown to be acceptable. In general, subject to a provision of mutual recognition of authorisations, a plant protection product authorised in one Member State should, as a rule, be authorised in a second Member State, which has received an application for the same product, provided that the active substance (with mandatory conditions for use) is already included in the relevant positive list. A Member State may request, by invoking special national conditions, that certain conditions for authorisation be adjusted to the different circumstances of that Member State. A Member State may also refuse to mutually authorise a plant protection product, if it believes that the authorisation of the product granted by another Member States does not meet the requirements of the Directive. In these cases the Member States must communicate the reasons for restriction/refusal to the Commission and other Member States.

4.3.1.2 Effect assessment

Hazard assessment and dose-effect relationship

The toxicological endpoints that should normally be evaluated for the assessment of human health effects are listed in Table 4.1. Depending on the outcome of the studies, additional relevant studies may be required.

The hazard assessment follows the lines laid down in Section 4.2 of this document.

4.3.1.3 Exposure assessment

Exposure scenarios

Residue data are needed for the assessment of the risk due to consumption of residues in food, while exposure data are required for the assessment of the risk due to handling and use of products containing the active substance. Regarding the use of plant protection products, exposure data are required for three categories of groups of persons (Directive 91/414/EEC, Appendix III. 7.1nn, EC 1991):

- users of the plant protection products, that is mixer/loader or applicator (*operators*),
- persons re-entering crops after application and thereby indirectly exposed *(workers*),
- persons in the vicinity of a pesticide application (*bystanders*).

Operators include professionals and non-professionals, and the duration and frequency may vary between subgroups of operators. Workers are professionals, while bystanders may be professional or non-professional, who happen to be accidentally exposed.

Operator exposure data can be obtained either by direct field measurements of the exposure of the product under investigation, or by calculations based on collected data from a database.

Field studies can be conducted either by measuring the amount of product in the clothing (patches) and in the breathing zone, or by biomonitoring; measured concentrations of parent compound and possible metabolites in urine, faeces and blood.

Regarding the exposure of workers and bystanders, a case-by-case assessment has to be made for each crop and task to be carried out.

Exposure data

In the database, the collected data are composed of exposure measurements for products with a variety of use conditions ("scenarios"). Based on the different databases, models have been developed which are used for the generation of exposure estimates for specific products. At present, there are a number of operator exposure models in use, which differ in the way they handle and rank technical parameters. In the EU programme on plant protection products, two models have mainly been used, the United Kingdom Predictive Operator Exposure Model (UKPOEM 1992) and the German model (Lundehn *et al* 1992). These models have mainly been developed on the basis of the conditions in the United Kingdom and Germany respectively. A European model, the European Predictive Operator Exposure Model (EUROPOEM 1996; draft version), is under development for use in the future.

Protective technical devices

Various types of personal protective equipment (PPE), including respiratory protective equipment (RPE), are taken into account and may be considered in the exposure models as well as in the field studies. PPE and RPE are assumed to significantly reduce the external exposure. For example, the most common PPE, protective gloves, can decrease the exposure by 90 – 99 %, depending on which model is considered. These figures are partly based on laboratory tests with new material.

4.3.1.4 Risk characterisation

General aspects

Principles of risk characterisation of plant protection products are laid down in Appendix VI of Directive 91/414/EEC (EC 1991), known as the Uniform Principles, and in a further amendment 94/79/EC (EC 1994a) to the Directive. At the time when the EU programme on plant protection products started, no valid technical guidance documents were available. However, various proposals for such documents have been put forward since. Guidelines are under development with regard to the setting of the health-derived limits (see also below; "Quantitative and qualitative risk characterisation" in this chapter) of Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD). Guidances are also under development for the use of European Predictive Operator Exposure Model (EUROPOEM 1996; draft version), production and use of dermal absorption data, and definition of relevant metabolites. Regarding the Acceptable Daily Intake (ADI), technical guidance has long been available.

As no final valid versions of these guidance documents are available at present (besides for ADIs), up to now the outcome from expert meetings has mainly guided the EU programme.

Risk characterisation should be performed for each sub-population exposed to the active substance, either from direct use of the product, as a worker or bystander, or by residues in food. With regard to ADI and ARfD these values will be compared with pesticide residues in foodstuff. The national Food Administration takes on the primary responsibility for this assessment, although in close contact with National Chemicals Inspectorate. Up to now, the risk assessment has covered the exposure from each source separately, which means that the combined exposure from several different sources for example via foodstuff *and* contaminated application equipment has not been taken into consideration.

The method by which risk characterisation is carried out depends on the nature of the relevant toxicological endpoints. If there is a threshold dose below which the effect is not observed and a NOAEL or alternatively a LOAEL can be identified, then a calculated or quantitative risk characterisation can be carried out. If the mechanism of the effect is not threshold-based or insufficient evidence of a threshold has been identified (i.e. it is not possible to establish ADI or AOEL values), the use of the active substance cannot be considered acceptable. A non-quantitative risk characterisation is therefore not applicable for plant protection products.

Choosing a NOAEL and derivation of health based limit values

The choice of NOAEL depends on the toxicological findings in studies with the active substance. The exercise to select the highest level at which no adverse effect is observed, in the most sensitive relevant species, needs to be assessed on a case-by-case basis, and requires expert judgement. The chosen NOAEL can then be used for the derivation of AOEL, ADI and ARfD by dividing the NOAEL or NOEL[4] by the appropriate assessment factor, normally 100 (Equation 4.1).

$$\text{AOEL} = \frac{\text{NOAEL}}{100}$$

Equation 4.1. Estimation of Acceptable Operator Exposure Level (AOEL) by dividing the no observed effect level (NOAEL) by an assessment factor normally of 100 (10×10 for inter-species and inter-individual variations).

The AOEL is the Acceptable Operator Exposure Level at which no harmful effects to health are expected to occur. The ARfD is an estimate of the amount of a substance in food or drinking water that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risks to the consumer on the basis of all known facts, at the time of the evaluation. The ADI is the comparative level for long-term consumption. The AOEL is compared with the estimated exposure from handling and use of the product itself, or from handling contaminated crops, while ADI and the ARfD are compared with the estimated daily intake of residues. The comparison is in most cases done in terms of internal or systemic dose.

Only studies from which a NOAEL can be derived are considered relevant as a basis for AOEL. NOAELs from studies with the relevant route of administration should be used whenever possible. When the most appropriate study does not provide a NOAEL, the LOAEL may be used, although this ought to be reflected in the choice and justification of the assessment factor.

[4] NOEL is usually used for the calculation of the ADI

It is under discussion whether the AOEL should be derived solely from the study with the most critical effect, or whether the duration of exposure to the product in use should decide the choice of study. The present draft on Technical Guidance on AOEL Setting (DG SANCO 2001) indicates both possibilities. However, the AOEL is usually derived from a short-term study. Although it can also be established on the basis of other toxicity studies. Determination of the ADI is generally based on a long-term study, while the ARfD is usually derived from a sub-acute or short-term study. In a few cases, mostly for organophosphorus compounds, the AOEL, ARfD and ADI have been derived from studies on humans.

Oral data have generally been used for setting AOELs, since the major part of the data file for toxicology is of oral origin. However, it is possible to use dermal and inhalatory data in deriving the AOEL, especially when the German model (Lundehn *et al* 1992) is used for the exposure calculation.

The risk characterisation for each sub-population is carried out separately, which means that the effect level for the critical toxicological endpoint of the active substance is identified and compared with the estimated human exposure of the product selected.

Qualitative risk characterisation is mainly applicable to active substances for which the critical effect(s) is/are not threshold-based. Since assessment of non-threshold effects is beyond the scope of the project, it will not be further addressed in this document.

Approaches in the risk characterisation of plant protection products

It has generally been agreed that when deciding upon an appropriate/adequate margin of exposure (assessment factor), this should normally be 100 (that is a factor of 10 to account for inter-species (animal-to-human) extrapolations multiplied by a factor of 10 for inter-individual (human-to-human) differences. This default value of 100 has usually been applied for the calculation of the AOEL, ARfD and ADI.

A value higher than 100 may be appropriate, and has been proposed in cases where great deficiency in the toxicological database has been identified, or due to the nature of the critical effect. The justification for the choice of a certain assessment factor, for a given risk characterisation, should be based on expert judgement and must always be clearly stated.

The AOEL, ARfD and ADI are derived by selection of the most relevant NOAEL (or LOAEL if necessary) and application of an appropriate assessment factor. These levels are expressed as mg active substance per kg body weight per day and compared with an internal exposure. Regarding characterisation of exposure by residues in food, ARfD and ADI are based on oral studies, and as the exposure is mediated via the oral route, the comparison can consequently be done directly. With regard to the AOEL, a correction factor for systemic availability, based on gastro-intestinal absorption data, needs to be applied on a case-by-case basis. This is due to the fact that the AOEL is compared with an external exposure, mainly dermal, which consequently is converted to an internal exposure (or systemic availability) by the use of a factor for dermal absorption, i.e. 100% (DG SANCO 2001). Regarding exposure via the inhalatory route, 100% is considered to be systemically available.

Regarding characterisation of the risk for the user, a step-wise or *tiered* approach (Figure 4.2) is used (Directive 91/414/EEC (EC 1991)). At each tier in the system, the AOEL is compared with the exposure. The tier system is intended to refine the risk and is theoretically applicable to all kinds of users. Risk characterisation is generally carried out for operators only.

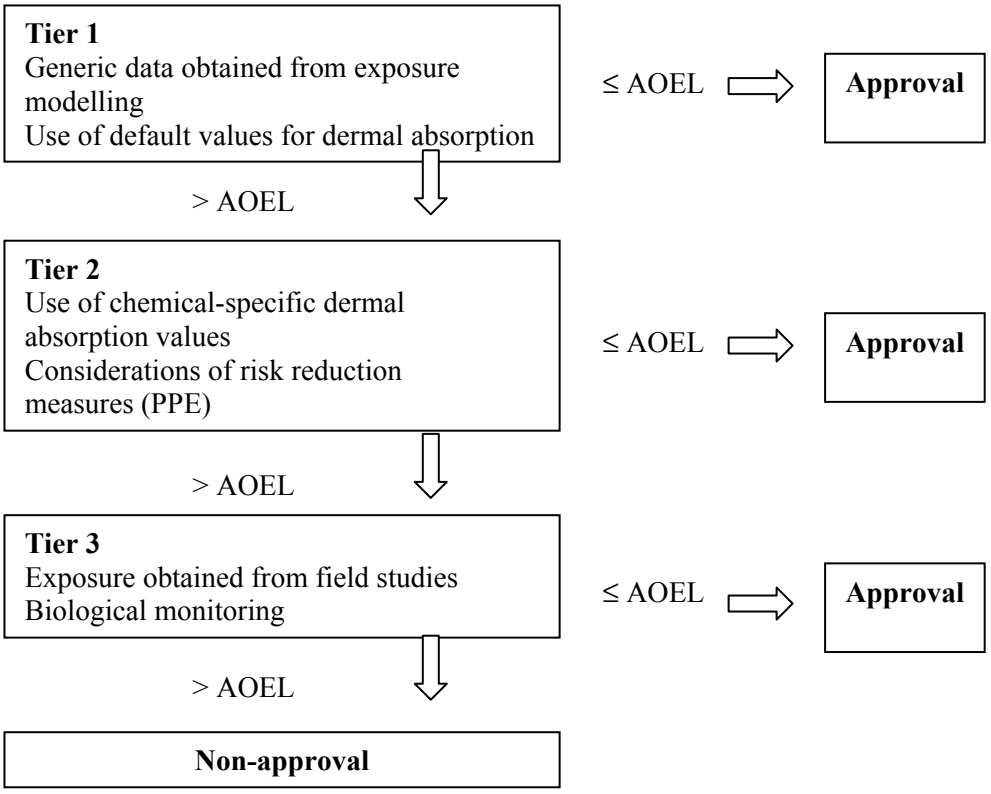

Figure 4.2 Tiered regulation of operator exposure and risk assessment

A general feature of the tier system is that the exposure is modified at each tier. The internal exposure is mainly due to the exposure source, presence or absence of personal protective equipment, and the extent of dermal absorption. By assuming various conditions for these factors, different internal exposure values can be calculated. With regard to dermal absorption, default data are initially used, while the more refined exposure assessment includes measured data.

The basic principle in the tier system is that if the established (internal) AOEL is not exceeded by the estimated exposure, the risk is considered acceptable. Otherwise, one continues to the next tier and to a more refined assessment. If the AOEL is still exceeded at the last tier, the risk is considered unacceptable.

At Tier I, the estimated exposure, based on surrogate values derived from all available exposure data, is generated by a model, and it is assumed that conventional field clothing and no personal protective equipment is worn. A default value of 100% for dermal absorption is used, unless the active substance is known to have low dermal penetration.

At Tier II, the use of personal protective equipment is taken into consideration, which will significantly reduce the estimated exposure generated by the model. With

regard to dermal absorption, either a default value (100%) or a value achieved from a dermal absorption study with the actual product or active substance is used.

At Tier III, two major options are possible:
(a) A field study with the actual product, with or without personal protective equipment, is carried out and dermal absorption is either of default or, more often, of measured origin.
(b) A field study with the actual product, using the biomonitoring technique, is conducted, and for this reason there is no need for absorption data.

4.3.2 The Council Directive for New Industrial Chemicals and The Council Regulation of Existing Industrial Chemicals

4.3.2.1 Basic Provisions

Directive

Council Directive 67/548/EEC (EC 1967) and Council Regulation EEC 793/93 (EC 1993a) require that a human health risk assessment be carried out on notified new industrial substances or on priority existing industrial substances respectively.

New industrial chemicals
A ”new industrial chemical substance” is defined as any chemical substance, which has been placed on the market after 18 September 1981. These chemicals are cumulatively listed in ELINCS (European List of Notified Chemical Substances), which is periodically updated as an Official Journal publication. Council Directive 67/548/EEC (EC 1967) (as amended for the seventh time by Directive 92/32/EEC (EC 1992)) 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.

A technical dossier for new substance notification should provide results from the analysis of physico-chemical properties, and test reports from toxicological and ecotoxicological assays. Proposals for classification and labelling should be submitted, including recommended precautions relating to safety. A risk assessment may be drafted by the notifier, facilitated by decision support software EUSES (European Union System for the Evaluation of Substances). Requisite dossier detail increases according to substance quantity, see Table 4.2.

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 (EC 1993b). Results from the risk assessment of new substances are assigned four available conclusions (Table 4.3)

Summary dossiers are prepared by the Competent Authorities in a standard format using Data Entry Screen (DES) and Summary Notification Interchange Format (SNIF). Dossier information is stored and handled through the New Chemicals Database (NCD) at the European Chemicals Bureau (ecb.jrc.it/)). New and updated summary dossiers are regularly distributed to all Member States. The risk assessments are also included in the summary dossiers.

Approximately 5000 notifications in total, representing about 3000 substances, have been submitted since 1981. More than 800 risk assessments of new substances have been prepared since 1993.

Existing industrial chemicals

An "existing chemical substance" is defined as any chemical substance listed in the EINECS, European INventory of Existing Commercial Substances. EINECS is an inventory containing 100195 substances (OJ 1990) between 1971 and 1981. In 1993, Council Regulation EEC 793/93 or the Existing Substances Regulation (ESR) (EC 1993a) on the evaluation and control of existing substances under Article 10 requires the real or potential risk to man and the environment of priority substances (see below; *Priority substances to be risk-assessed*) to be assessed. Principles used in the assessment have been laid down in Commission Regulation EC 1488/94 (EC 1994b) on risk assessment for existing substances.

Regulation 793/93 foresees that the evaluation and control of the risks posed by existing chemicals will be carried out in the four steps, namely priority setting, data collection, risk assessment and risk reduction.

All the data on chemicals produced or imported by companies have to be submitted in a specific electronic format HEDSET (Harmonised Electronic Data Set), which is managed by IUCLID (International Uniform ChemicaL Information Database). Data have been collected in three phases concerning: High Production Volume Chemicals (HPVCs) (quantities exceeding 1000 tonnes/year) in phase 1 (1990) and II (1994), and Low Production Volume Chemicals (LPVCs) (quantities between 10 and 1000 tonnes/year) in Phase III (1998). All companies are required to update the information at least once every three years.

Following the framework set out in Commission Regulation 1488/94 (EC 1994b) implemented in the current Technical Guidance Document (TGD)[5] on Risk Assessment for New and Existing Substances (EC 1996), risk assessment is conducted on a case-by-case basis by an iterative process using expert judgement. The risk assessment is discussed by Member States and industry at Technical Meetings mediated by the Commission. Further testing to add to the database set and/or collect other information (for example exposure) may be necessary. When a consensus on conclusions has been reached in the Technical Meetings, a final report is submitted to CSTEE (Scientific Committee on Toxicity, Ecotoxicity and the Environment) for peer review. The completed risk assessment report may arrive at one or more conclusions (see Table 4.3).

[5] The Technical Guidance Document (EC 1996) is currently under revision in the EU Commission.

After adoption by the Commission of the risk assessment, three publications are produced:

- the comprehensive risk assessment report (as a book, on the European Chemicals Bureau homepages and in the International Uniform ChemicaL Information Database),
- a summary thereof (as an European Commission report and on the ECB homepages)
- a listing of the conclusions in the Official Journal of the European Communities.

Data requirements

The hazard assessment of new and existing industrial chemicals have to address the potential toxic effects as outlined in Table 4.2 for the human populations of workers, consumers and humans exposed indirectly via the environment.

According to Article 9(2) of Regulation 793/93 (EC 1993a), 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 (EC 1967). For a new substance, further data are foreseen at increasing production volumes (Annex VIII of Directive 67/548/EEC (EC 1967)) (Table 4.2). These data for health assessment only concern non-human studies.

For new industrial chemicals, there is no formal requirement to provide measured human exposure data, as generic scenarios are applied in the risk assessment and refined where specific information is available. Where measured data are available, the adequacy has to be determined. Under the Regulation for existing industrial substances, manufacturers and importers are obliged to provide information on exposure for the exposure assessment.

Exemption from formal data requirement; defer or derogation

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 (EC 1967). 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.

By way of *derogation* referring to Directive 793/93 (EC 1993a), manufacturers and importers may request of the rapporteur that they be exempted from some or all of the testing on the grounds that a given piece of information is either unnecessary for risk assessment or is impossible to obtain. They may also request an extended time period to conduct the studies where circumstances so require. Full justification must be provided to support such derogation, and the rapporteur has to decide whether the request should be accepted. Where derogations are allowed in conformity with this Article, the rapporteur has to immediately inform the Commission of his decision. The Commission has to inform the other Member States. If the decision of the rapporteur is contested by one of the other Member States, a final decision has to be taken in conformity with the committee procedure laid down in Article 15.

Table 4.2 Data/information needed for hazard assessment of new and existing industrial substances (Annex VII A and Annex VIII level 1 and Annex VIII level 2 to Council Directive 67/548/EEC (EC 1967).

Data/information	**New industrial substances (tonnes/year per manufacturer)**						**Existing industrial substances**
	0.01-0.1	0.1-1	1-10	10–100 [1)] *level 1*	100–1000 [2)] *level 1*	>1000 [3)] *level 2*	substances included in *priority lists* "base set"
acute toxicity	a	a	a	a	a	a	a
irritation	---	a	a	a	a	a	a
corrosivity	---	---	a	a	a	a	a
skin sensitisation	---	a	a	a	a	a	a
repeated dose toxicity - 28 days	---	---	a	a	a	a	a
subchronic/chronic toxicity	---	---	---	b[4)]	c[4)]	d[5)]	---
carcinogenesis	---	---	---	---	---	d	---
mutagenicity	---	a	a	b	c	d	a
fertility study	---	---	---	b	c	d	screening
teratology/ developmental toxicity	---	---	---	b	c	d	---
toxicokinetics	---	---	---	b	c	d	to be derived from base set and other data

1) or when the total quantity placed on the market reaches 50 tonnes per year per manufacturer

2) or when the total quantity placed on the market reaches 500 tonnes per year per manufacturer

3) or when the total quantity placed on the market reaches 5000 tonnes per year per manufacturer

4) subchronic and/or chronic toxicity

5) chronic toxicity

a) data required from the Competent Authority unless the notifier can give good reason why a given test is not appropriate or could be replaced by an alternative

b) data which may be required from the Competent Authority unless the notifier can give good reason why a given test is not appropriate or could be replaced by an alternative

c) data which are to be required from the Competent Authority

d) data required unless there are strong reasons to the contrary, supported by evidence, that they should not be provided

Priority setting of substances to be risk-assessed

New Industrial Chemicals

No formal priority setting exists, as new industrial chemicals are risk-assessed following the notification procedure.

Existing Industrial Chemicals

Lists of priority substances in IUCLID are drawn up by the Commission and the Member States. Factors other than imported or manufactured quantities, which should be taken into account in preparing the priority lists, are

- the effects of the substance on man or the environment
- the exposure of man or the environment to the substance
- the lack of data on the effects of the substance on man and the environment
- work already carried out in other international fora
- other Community legislation and/or programmes relating to dangerous substances

By mid-2002, Member States Rapporteurs had completed the first draft Risk Assessment Reports on 91 out of a total of 141 priority substances included in the first four priority lists since the ESR programme started in 1993. In Technical Meetings, 57 substances have been finalised and overall conclusions (Table 4.3) for further actions have been agreed. A need for further limiting of the risks was identified for 46/57 substances, and no further actions were recommended for 10/57 substances. New information was required for one substance out of 57 (EC 2002).

Responsibilities

Manufacturers and importers of new and existing industrial chemicals are obliged to submit data to be used for the risk assessment. Such data are quantities of the substance imported or produced, classification and labelling of the substance, information on reasonable foreseeable use, physico-chemical data, ecotoxicity-related data and human health toxicity-related data

Manufacturers and importers may also be required to submit further data if necessary for the purpose of the risk assessment.

Risk Assessment

The risk assessment involves the following and subsequent general activities: hazard identification, dose (concentration)-response assessment and dose (concentration)-effects assessment, exposure assessment and risk characterisation.

A risk assessment containing all steps must be carried out for all priority existing substances. In contrast, for notified new substances the risk assessment process in relation to a particular effect or property can be stopped. Reasons for stopping are that the hazard identification related to that effect/property does not lead to classification in accordance with Directive 67/548/EEC (EC 1967) and if there is no other reasonable ground for concern. Where investigations of an effect have not yet been conducted, the risk assessment does not need to consider this effect for new substances unless there is cause for concern

Risk assessment is an iterative process for both 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/EEC (EC 1967).

A risk assessment also needs to be reviewed and, where necessary, revised for existing substances 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 (EC 1993a).

Consequences/Decision-making

Having followed the required steps, assessors will come to integrated conclusions/results separately for human health and the environment, which in a subsequent step will 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 conclusions/results outlined in Table 4.3.

Although conclusion (i) (there is a need for further information and/or testing) may be reached, the progress of the risk assessment process is not delayed because new information/test results are assessed in a separate annex in the future or the recommendations leading to conclusion (i) may not be presently feasible. Conclusion (iii) (there is a need for limiting the risks) triggers the development of a risk reduction strategy. Risk reduction is not discussed in further detail here.

Table 4.3 Summary of overall conclusions, which can be reached in the risk assessment of new and existing industrial chemicals.

New substances	Existing substances
(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/EEC (EC 1967).	(i) There is need for further information and/or testing.
(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/EEC (EC 1967).	(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) The substance is of concern and further information should be requested immediately.	iii) There is a need for limiting the risks; risk reduction measures which are already being applied, shall be taken into account.
(iv) The substance is of concern and the Competent Authority should immediately make recommendations for risk reduction.	

4.3.2.2 Effects assessment

Hazard Assessment

The hazard assessment procedure is mainly carried out in accordance with the description in Section 4.2. The toxicological endpoints that should normally be evaluated for the assessment of human health effects are listed in Table 4.2.

4.3.2.3 Exposure Assessment

Exposure scenarios

Populations

The Technical Guidance Document (EC 1996) for new and existing industrial substances identifies three human populations that should be assessed for exposure. These are:

- occupational exposure
- consumer exposure (which means exposure from products which can be purchased from retail outlets by members of the general public)
- humans exposed indirectly via the environment, including exposure via food, water and air)

In addition, the exposure levels for each of these populations may be added together to determine the combined exposure (cumulative exposure). However, the combined exposure often tends to be unrealistic high depending on the summation of three "realistic worst cases" and is thus seldom usable for a plausible risk assessment.

Occupational exposure

Occupational exposure is considered to occur in the workplace and include all working activities. In practice, the population is considered to include both males and females but does not include, for example, particularly susceptible groups, the young or the elderly. Based on the type of working area and type of data available, a division can be made into two areas, relating to upstream activities, such as operations of process plants, and downstream uses such as professional operations.

Consumer exposure

The consumers (the general public) may be exposed to substances by using consumer products. A consumer product is one, which can be purchased from retail outlets by members of the public and may be either the substance itself, a preparation or an article containing the substance. In practice, exposure to a substance present in medical products and equipment has also been evaluated among this population.

Humans exposed via the environment

Indirect exposure of humans via the environment may occur by the consumption of food and drinking water, inhalation of air and ingestion of soil, and concerns all humans. Indirect exposure is principally assessed on two spatial scales: locally near a point source of the substance, and regionally using averaged concentrations over a large area.

Routes of exposure

Three main routes of exposure are routinely considered, namely oral, dermal and inhalation exposure. However, other routes may also be important; for example the transplacental and intravenous routes should be assessed where appropriate. Oral exposure occurs either directly, due to ingestion of the substance present for example in food, breast milk, infant formulas and water, or by the mucocilliary route.

Mucocilliary exposure may be an important exposure route for inhaled dusts in the occupational environment

Exposure description

To estimate the human exposure for a substance, information from several different sources including physico-chemical data, general substance information, toxicokinetics and measured data are required. As a first step, possible human exposure scenarios are identified and secondly the exposure is quantified. Information on duration, frequency, route of exposure, human habits and practices, technological processes and ways in which a substance is used need to be considered. The spatial scale of the exposure (for example personal, local, regional level) also has to be taken into account.

Exposure data

Measured and modelled exposure data

Exposure levels may be derived on the basis of measured data or in its absence by model or algorithm calculations. Relevant monitoring data from substances with analogue use (surrogate) and exposure patterns or analogue properties, if available, should also be considered when applying model calculations. When using monitored data, these have to be evaluated for reliability, and their relevance validity should also be considered.

Different exposure models are available to estimate the potential human exposure. Where possible, measured or estimated values should be used for each of the numerical parameters, but when this is not possible default values may be derived from available data sources or expert judgement. It is important to consider the limitations of each model used and where possible compare the results with measured data. The results have to be critically evaluated to avoid overestimating or underestimating exposure.

The prediction of the exposure levels should describe a reasonable worst-case situation in the normal use patterns. Upper estimates of extreme use and reasonably foreseeable misuse should also be considered. However, exposure as a result of accidents or due to abuse is not to be addressed. Since there are multiple pathways of exposure, including different exposure scenarios and populations, a cumulative worst-case estimate is also possible.

External and internal exposure

The present TGD (EC 1996) specifies that the exposure should normally be understood as external exposure, which means the amount of substance ingested, in contact with the skin or present in the atmosphere, and that the internal exposure (systemic exposure) may be considered at the risk characterisation stage. However, for many substances this is not a practical approach, as aggregate and cumulative exposures have to be estimated. This is important for the determination of the total body burden and corrects for route-to-route differences.

Biomonitored data may also be available, providing information directly on internal exposure. Two main factors, which should be considered when estimating internal exposure, are bioavailability and biotransformation, particularly first-pass metabolism in the liver. Information derived from toxicokinetic studies can be of particular importance.

Protective technical devices

Personal Protective Equipment (PPE)

Personal Protective Equipment (PPE) should not be considered as part of the risk characterisation of existing and new industrial substances. PPE should instead be considered subsequently at the stage of risk reduction or risk management, as stated in Directive 89/656/EEC "Use by Workers of Personal Protective Equipment at the Work Place, Article 3" (EC 1989).

4.3.2.4 Risk Characterisation

General aspects

General principles for the risk characterisation of new and existing industrial substances are given in the Technical Guidance Document (EC 1996) referring to Regulation 1488/94 (EC 1994b) and Directive 93/67/EEC (EC 1993b).

In essence, once a NOAEL or a LOAEL has been identified in animal experiments for any of the effects set out in Regulation 1488/94 (EC 1994b) or Directive 93/67/EEC (EC 1993b), it will be used in comparison with the exposure estimate for the exposed human population.

For both the exposure assessment and the effects assessment, data on physico-chemical properties may be needed. The physico-chemical properties are required, for example, to estimate emissions and the human exposure scenarios and to assess the design of toxicity tests, and may also provide pointers to the absorption of the substance for various routes of exposure. In particular, the chemical reactivity is most often of special importance, as it has an impact on the toxicokinetics – an area that is not included in the formal data requirement.

Choosing a N(L)OAEL and derivation of Margin of Safety (MOS)

NOAELs or LOAELs are to be established for all effect areas included in the hazard assessment (Table 4.2). No specific critical effect is primarily identified.

Referring to the current TGD (EC 1996), the assessor carries out the risk characterisation by comparing the N(L)OAEL to the quantitative information on exposure for a human population, thus establishing a Margin of Safety (MOS) value (Equation 4.2). Where it is not possible to determine an N(L)OAEL, a qualitative evaluation of the likelihood that an effect will occur at the given exposure is carried out on the basis of relevant quantitative or qualitative exposure information.

$$\text{MOS} = \frac{\text{N(L)OAEL}}{\text{human exposure}}$$

Equation 4.2 Calculation of the Margin of Safety (MOS) by dividing the No Observed Adverse Effect Level (NOAEL) or the Lowest Observed Adverse Effect Level (LOAEL) by the exposure level of the human population.

The quantitative or qualitative risk characterisation is done separately for each human population exposed, or likely to be exposed, to the substance, and for each effect and route of administration. It should be noted that, in any particular human population, sub-populations might be identified (for example with different exposure scenarios and/or different susceptibility: very young, elderly, infirm, pregnant women) which may need to be considered individually during risk characterisation. Exposure levels are therefore derived separately for each

relevant population/sub-population, different N(L)OAELs, where appropriate, are identified for the different endpoints, and respective N(L)OAEL/ exposure level values are established.

Where it is not possible to determine an N(L)OAEL (for example acute toxicity, sensitisation, genotoxicity), the likelihood that the effect will occur is evaluated on the basis of the information on exposure relevant to the human populations under consideration. Where, despite an N(L)OAEL not having been determined, the test results nevertheless demonstrate a relationship between dose or concentration and 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 degree of the adversity of the effect, such information also has to be taken into account in evaluating the likelihood of the effect occurring.

Approaches in the risk characterisation for new and existing industrial substances

Depending on the N(L)OAEL/exposure level ratio (that is the Margin of Safety; MOS) or the qualitative evaluation of the likelihood that an effect will occur at the given exposure, the risk assessor will decide which of the possible conclusions (Table 4.3) for each population potentially exposed are applicable.

Where the exposure estimate is less than the N(L)OAEL, the risk assessor will need to decide which of the possible results applies (Table 4.3). For this step, the magnitude by which the N(L)OAEL exceeds the estimated exposure needs to be considered taking account of the following parameters:

- the uncertainty arising, among other factors, from the variability in the experimental data and intra- and inter-species variation;
- the nature and severity of the effect;
- the human population to which the quantitative and/or qualitative information on exposure applies.
- the differences in exposure (route, duration, frequency and pattern);
- the dose-response relationship observed;
- the overall confidence in the database.

When all the effects and all the expected human exposure patterns are considered, on a case-by-case basis, further testing requirements to refine the risk assessment at each stage of risk assessment should be considered. For instance, more than one route of exposure may be relevant. This should be done in the light of all the available information (including toxicokinetic studies), 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.

Expert judgement is required to weigh these individual parameters on a case-by-case basis. The approach adopted should be transparent and a justification should be provided by the assessor for each conclusion reached.

4.3.3 The Council Directive for Biocidal Products

4.3.3.1 Basic provisions

The Directive

In Directive 98/8/EC (EC 1998) for Biocidal Products, harmonised positive approval/authorisation schemes for active substances and biocidal products are laid down for 23 biocidal product types. The Biocidal Products Directive entered into force on 14 May 2000 in the EU Member States. The biocidal products that are covered by the Biocidal Products Directive comprise pesticidal products that are used for other purposes than plant protection. By definition, biocidal products are active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of or otherwise exert a controlling effect on any harmful organism by chemical or biological means.

The Biocidal Products Directive has many general features in common with Directive 91/414/ EEC (EC 1991), which regulates the marketing and use of agricultural pesticides, also known as plant protection products, but there are also differences. Both directives lay down positive approval procedures for biologically active (and thereby potentially toxic) substances and formulated products. It is stated in both directives that an active substance/biocidal product under assessment has to be authorised for use only if it has no unacceptable effect or influence on health or the environment and that assessments and decisions as far as possible have to be mutually recognised between the Member States. Under both schemes extensive basic data sets are required; the active substances are approved at EU level, whilst the formulated products are authorised at Member State level. Both directives state, as a prerequisite for acceptance of an active substance, that health-based exposure limits have to be established, where appropriate.

In comparison with the Plant Protection Products Directive, the Biocidal Products Directive lays down provisions that reflect a higher standard of protection in some respects. For example, *the principle of comparative assessment/substitution* is introduced in the legal text of the Biocidal Products Directive as a risk reduction tool in the decision-making on *active substances*, while the Plant Protection Products Directive in its present form does not accommodate the principle of substitution at any level of decision. The Biocidal Products Directive explicitly states that products intended for general public use should not normally be authorised if the use of personal protective equipment is the only possible method to reduce the risk to an acceptable level.

The Biocidal Products Directive also states that, even though authorised, the use of a biocidal product should be limited to the minimum necessary by rational combination with other measures of control.

Risk-based decisions

The Biocidal Products Directive stipulates that decisions on authorisation/non-authorisation of a biocidal product be risk-based. There are a few exceptions to this approach. Fulfilment of the criteria/classification according to Directive 67/548/EEC (EC 1967) *as very toxic, toxic or carcinogenic, mutagenic or toxic to reproduction (CMR substances) category 1 and 2* of the active substance and the corresponding

product is a sufficient basis for non-approval, if the product is intended to be used solely by the general public.

For professional uses, active substances classified as carcinogenic or mutagenic category 1 and 2 may be approved only if exposure is unlikely. An extra assessment factor is applied with regard to substances toxic to reproduction.

Data requirements/information collection

Data requirements for assessment of active substances and biocidal products are laid down in annexes to the Biocidal Products Directive. The data should cover all relevant toxicological (and ecotoxicological) endpoints, detailed description of the use and emission scenarios of the biocidal product and treated material, as well as data needed to estimate exposure at the intended use(s) of the product and treated material (Table 4.4).

Data on efficacy of the active substance/biocidal product for the intended use should also be submitted.

Under the Biocidal Products Directive, information on the benefits of using an active substance as intended has to be accounted for in the decision process.

Table 4.4 Data needed for hazard assessment of biocidal active substances and product data.

Data/information	Data needed for the active substance	Data needed for the product
Acute toxicity: oral, dermal. Inhalation data might be required	x	x
Irritation (skin and eye) and corrosivity	x	x
Skin sensitisation	x	x
Subchronic toxicity	x	
Genotoxicity	x	
Carcinogenicity	x	
Reproductive and developmental toxicity	x	
Dermal absorption	-	x
Exposure data for the operator	-	x

The data should be compiled and assessed by the applying company and presented with the aid of an “all-in-one” electronic format. The all-in-one approach implies that the format is also to be used for the verifications/conclusions of the responsible authority.

Exemption from formal data requirement; waiving

The Biocidal Products Directive also states that the data/test requirements should suit the individual circumstances and that information which is not necessary owing to the nature or the proposed use of the biocidal product need not be submitted and may therefore be *waived*. However, to have a requested data waiver accepted the applicant must present a well-based justification as to why the omitted data are not considered necessary for the risk assessment. Due to the diversity of the 23 different biocidal product types and the way they are handled and used, the exposure patterns and other product characteristics may vary considerably both between and within the different product types. The product types involve products used not only in industrial or other professional settings, but also a wide range of products mainly intended for use by the general public. As a consequence, the ultimate data requirements/possibility of data waivers for an active substance may vary. In

Technical Notes for Guidance (TNsG) (EC 2000b) in support of the Biocidal Products Directive, the data requirements are amplified/specified with regard to the different product types and some principles for non-submission (waiving) of data are given for guidance.

Priority-setting for existing biocides to be risk-assessed.

As for plant protection products, as well as for existing and new industrial chemicals, the (EU) regulatory work on biocides will cover active substances that already existed on the market before a certain date (in the case of biocides the date when the Biocidal Products Directive entered into force), as well as active substances/biocidal products introduced on the EU market after that date. Priority-setting will, in practice, only be used in the process of assessing existing active substances. As regards new active substances these should be assessed (within time periods stated in the Biocidal Products Directive) by the Member States that receive an application for authorisation of the first biocidal product containing the active substance of concern.

The general procedures for assessment of existing active substances, (planned to be carried out within a ten-year review programme as outlined in Article 16 of the Biocidal Products Directive), are further specified in two Commission Regulations.

Existing biocidal active substances that have been adequately notified (according to requirements specified in the 1st Regulation) for full assessment under the Biocidal Products Directive will be prioritised according to various factors including a simplistic risk assessment by means of a proposed Biocidal Active Substances Ranking Method (BASRAM 2002). The substances will be listed for full evaluation in the order of priority. To enable comparative assessment to be carried out in connection with the evaluation of existing active substances, the substances to be fully evaluated will be grouped by biocidal product type/field of use.

The listed active substances will be allocated to Rapporteur Member States and time-tables set for the submission of complete dossiers. The lists will be published in the 2nd Commission Regulation draft version. However, the review programme is, at the time of writing, still in the notification phase. This means that, to date, experience of regulatory work on biocides has only been gathered under a few national schemes.

Responsibilities

In a positive approval procedure, as provided in the Biocidal Products Directive, the principle of the reversed burden of proof is applied. It is the responsibility of the applying company to submit all information needed for a reliable and adequate risk assessment of the active substance and the formulated product and to show that the risk of the product when used as intended is low enough to be acceptable.

The role/responsibility of the Competent Authority (Authorities) is to verify the information submitted, including the risk assessment carried out by the company and to make its own assessment and draw its own final conclusion.

A separate authorisation of the product will be granted by the Competent Authority of the Member States where the product will be placed on the market if it is judged that the product meets the requirements of the Biocidal Products Directive and can be used safely.

An authorisation should be seen as an endorsement, that the risk connected to the authorised use, as judged from the information available at the time of decision, is low enough to be acceptable.

Risk assessment

After completeness of the submitted dossier has been checked/confirmed, the active substance is assessed with respect to the intended use. As regards existing active substances, these are assessed by the Rapporteur Member State designated by the Commission (i.e. DG Environment in the context of biocides). Regarding new active substances, the Competent Authority of the Member State that first receives the application for a biocidal product containing that substance will normally carry out the risk assessment.

The assessment has to address the risk of the active substance when used as intended, that is with respect to representative uses of the product under investigation.

A formal assessment of the corresponding biocidal product is carried out by the Member State that first received the application. The product risk assessment has to take account of the risk of the active substance(s) and of any other substance of concern in the product. If the provisions of the Biocidal Products Directive are met, the biocidal product is authorised.

Consequences/Decision-making

The outcome of the assessment of the active substance for its intended use(s), and a recommendation for a decision, is presented by the Rapporteur Member State in a report, for further deliberations between all Member States and the Commission (COM/DG Environment for biocides).

A decision is taken on the basis of a qualified majority vote by the Standing Committee on Biocides, for or against the inclusion of the active substance in Annex 1, Annex 1A, or Annex 1B (EC 1998) (the positive lists) of the Biocidal Products Directive.

The decision to authorise a biocidal product is made by the individual Member States. Subject to the provision of mutual recognition of authorisations, a biocidal product that has been authorised in one Member State should, as a rule, be authorised in another Member State that has received an application for the same product, provided that the active substance (with mandatory conditions for use) is already included in the relevant positive list. A Member State may request, by invoking special national conditions, that certain conditions for authorisation be adjusted to the different circumstances of that Member State. A Member State may also refuse to mutually authorise a biocidal product if it believes that the authorisation of the product granted by another Member States does not meet the requirements of the Biocidal Products Directive. In these cases, the Member States must communicate the reasons for restriction/refusal to the Commission and other Member States.

4.3.3.2 Effects assessment

Hazard identification and dose-effects assessment

The elements of risk assessment of a chemical substance are described in general terms in Section 4.2 of this document.

As regards the hazard identification and the dose-effects assessment, it has been decided that the revised version of the current Technical Guidance Document for risk assessment of existing and new industrial chemicals (EC 1996) should also be applicable for hazard assessment of biocidal active substances (see also Section 4.3.2.1).

The toxicological endpoints that should normally be evaluated for the assessment of human health effects from biocides are listed in Table 4.4. Depending on the outcome of the studies, additional relevant endpoints such as neurotoxicity or endocrine disturbances may have to be assessed.

4.3.3.3 Exposure assessment

To enable a risk assessment of the active substance be carried out, information about the nature of the exposure and exposure levels is needed for representative uses of the active substance within the product type(s) applied for.

The objective should be to make a quantitative or a qualitative estimate of the dose/concentration of each active substance to which a population is/may be exposed.

Technical Notes for Guidance on how to estimate human exposure to biocidal products are being prepared under a Commission contract.

Exposure scenarios

A detailed description of use/exposure is required, including, for example, relevant physico-chemical characteristics, life cycle/service life of the substance in treated material, product description, frequency and duration of use, possibility of combined/cumulative exposure etc.

An exposure assessment has to be carried out for each of the following human populations

- professional users,
- non-professionals including the general public, of which the latter should be addressed as a separate group, and
- those exposed via the environment for which exposure to the biocidal product occurs or can be reasonably foreseen.

Where relevant, the assessment has to cover aggregate exposure from different routes of exposure, specifically oral and dermal exposure and exposure via inhalation. When assessing the exposure, realistic (worst-case) variations in exposure have to be accounted for.

The information also has to address the total exposure from both primary and secondary exposure (that is exposures that the exposed person/population is unaware of), and where relevant take account of combined and/or cumulative exposure of the active substance under assessment.

Exposure data

Quantitative estimation of exposure

The Biocidal Products Directive states that measured or modelled data are to be used for the exposure assessment of the active substance. When adequate and

representative measured exposure data are available, these should be given special consideration in the exposure assessment.

When calculation methods are used, adequate models are to be applied. These models are to

- make a best possible estimation of all relevant processes, taking into account parameters and assumptions,
- be subjected to an analysis taking into account possible elements of uncertainty,
- be reliably validated with measurements carried out under conditions relevant to the use of the model,
- be relevant to the conditions in the area of use.

External versus internal exposure
See Section 4.3.3.4 – Choosing a NOAEL.

Qualitative estimation of exposure
A qualitative estimation of exposure could be that the use/application of the substance is carried out in such a confined/closed way that negligible exposure can be foreseen. A judgement that exposure "is likely" may also be sufficient for decision-making.

Protective technical devices

Personal technical equipment
The Biocidal Products Directive states, that "if for non-professional users the wearing of personal protective equipment would be the only possible method for reducing exposure, the product shall not normally be authorised". For professionals, personal protective equipment would be expected to be used as a safety routine in the work process. The use of personal protective equipment may therefore, as a means of risk management, be considered in decision-making of active substances/biocidal products that are solely intended for professional use. However, it should be emphasised that any measures of risk mitigation/management that have an influence on decision-making must be made distinct from other factors that affect the outcome of the risk assessment.

4.3.3.4 Risk Characterisation

General aspects

It has been agreed, in the context of biocides assessment, that the term "Margin of Safety, MOS" is to be substituted by more accurate terminology such as "Margin of Exposure, MOE". This term is preferable since it does not include any indication of ultimate safety, even though it may be less effective for risk communication purposes.

The procedure for risk characterisation of biocidal active substances is indicated in general terms by the contents of Annex VI to the Biocidal Products Directive (EC 1998). The process is amplified in the draft version of the Technical Notes for Guidance, TNsG (EC 2000b), which describes principles and practical procedures, including criteria for the inclusion of active substances in the positive lists of the Biocidal Products Directive. This TNsG was adopted in the spring of 2002.

Risk characterisation of biocides will be necessary for each population exposed to the active substance either from direct use of the product, as a bystander or someone entering a treated area, from exposure to treated material or from indirect exposure via the environment. The latter can also include exposure to residues in food or drinking water. The risk needs to be considered from *aggregate* exposure (i.e. when exposure can occur via more than one route) and from *combined* exposure, for example a person may have used a product and remained exposed following use and/or consumed food and/or water containing residues. The method by which risk characterisation is carried out depends in part on the nature of the relevant toxicological endpoints. If there is a threshold dose below which the effect is not observed and a NOAEL (or LOAEL) can be identified, then a calculated or quantitative risk characterisation can be conducted. If the mechanism of the effect is not threshold-based or insufficient evidence of a threshold has been identified, then the risk characterisation should instead be based on whether the exposure is considered unlikely or not.

Health risk characterisation of the active substance of a biocidal product should be based on the toxic effect(s) that is considered most critical in relation to the use pattern of the product.

Choosing a NOAEL and derivation of health based limit values and Margin of Exposure (MOE)

The choice of NOAEL depends above all on the toxicological profile of the active substance. Selection of the most sensitive (normally the lowest) NOAEL on which to base an AOEL, for example, needs to be assessed on a case-by-case basis, and requires expert judgement. The choice of NOAEL should derive from the identification of the no-effect levels in the most relevant and sensitive animal species. The duration of the study from which the NOAEL is chosen should be appropriate to the pattern of use of the product but only if serious effects such as carcinogenicity, toxicity to reproduction or other specific effects have not been identified. In such cases, a NOAEL should be based on these critical effects.

If the chosen NOAEL is an external dose (applied dose) it has to be converted to an internal value by using a correction factor for systemic availability, and adequate absorption data should be provided for this purpose.

For single exposures or exposures that happen very rarely, LD_0-LD_{50} values or subacute NOAELs should be chosen, while for (working) life-long, intermittent exposures at least subchronic studies should be used. For long-term exposure, for example from indoor use of preservatives/preservative-treated material, NOAELs should be derived from chronictoxicity/carcinogenicity studies. NOAELs from studies with the relevant route of administration should be used where possible. When the most appropriate study does not provide a NOAEL, the lowest dose may be used as an LOAEL but this situation must be reflected in choosing and justifying the assessment factor.

Quantitative and qualitative risk characterisation.

In a quantitative risk characterisation, the estimated human exposure and the toxicological effects of the biocide have to be compared for each population exposed, product type and method of application relevant for the accompanying product(s) as indicated by the exposure assessment. Qualitative risk assessment is mainly

applicable to active substances where the critical effect(s) is not threshold-based. Since assessment of non-threshold effects is beyond the scope of this report, it will not be addressed in more detail. The process of risk characterisation is worked through as described below.

The AOEL method

The appropriate N(L)OAEL for the exposure scenario under assessment must first be decided upon. The maximum exposure that a person should experience for that scenario, currently referred to as Acceptable Operator Exposure Level or AOEL, is determined by adjusting the no-effect level from the chosen animal test to be applicable to humans. This extrapolation is done by dividing the animal NOAEL by an assessment factor normally of 100 (Equation 4.3). The risk characterisation then compares this level with the estimated exposure for the use of the product. The comparison is usually done in terms of internal dose. Other limits (ADI) relevant to the consumption of residues via food or water may also be required to compare with estimated daily intakes.

$$\text{AOEL} = \frac{\text{NOAEL}}{100}$$

Equation 4.3 Estimation of Acceptable Operator Exposure Level (AOEL) by dividing no observed effect level (NOAEL) by an assessment factor normally of 100 (10×10 for inter-species and inter-individual variations).

The MOE method

The Margin of Exposure (MOE) method of risk characterisation can be applied to all exposure scenarios and all exposed populations for toxicological endpoints with a threshold mechanism. A MOE is calculated by dividing the NOAEL (or LOAEL if necessary) by the estimated human exposure (Equation 4.4). The acceptability of the resulting ratio is decided upon in the same way as for the assessment factors described for the AOEL. The default value required is 100, but another value may be required as a minimum if circumstances justify it (such as when the NOAEL is based on a steep dose-response curve). MOEs can be calculated for exposure via a specific route if this is appropriate and the necessary date are available. MOEs can also be calculated for total exposure. Route-to-route adjustments can be made based on absorption data, and the comparison can be made on an internal dose basis if appropriate.

$$\text{MOE} = \frac{\text{N(L)OAEL}}{\text{human exposure}}$$

Equation 4.4 Calculation of the Margin of Exposure (MOE) by dividing the No Observed Adverse Effect Level (NOAEL) or the Lowest Observed Adverse Effect Level (LOAEL) by the exposure level of the human population.

Approaches in the risk characterisation of biocidal active substances

The Biocidal Products Directive states that when deciding on an appropriate/adequate margin of exposure this should typically be 100 (equivalent to the assessment factors used for setting a health-based exposure limit which means a factor of 10 to account for intra-species (inter-individual) differences multiplied by a factor of 10 for inter-species extrapolation). A value higher or lower than 100 may be appropriate depending, amongst other things, on the nature of the critical toxicological effect (Biocidal Products Directive Annex VI, 70-71 (EC 1998).

When deriving a health-based exposure limit, for example an AOEL, ADI or TDI, the chosen assessment factor is already built into these values and they are directly comparable to the estimated internal exposure that should never exceed these limits. The conventional default assessment factor of 100 is usually applied

The AOEL is derived by choosing the most relevant NOAEL (or LOAEL if necessary) and appropriate assessment factors are applied. Further guidance in deriving the AOEL is provided in a draft guidance document for plant protection products. It should be noted that, overall, biocidal products are represented by more varied exposure scenarios than other groups of products such as plant protection products. The use of the AOEL as a method for risk characterisation should therefore allow for flexibility in order to provide risk characterisations that represent the wider variety of exposure scenarios when the data that allow this are provided.

Choosing the AOEL or MOE concept, or both?

Both approaches are mentioned in the Biocidal Products Directive. It is stated that for an *active substance* to be included in a positive list, health-based exposure limits such as AOEL, ADI or MRL (maximum residue level) are to be derived when relevant. The Biocidal Products Directive also states that the Member States, when making an authorisation decision for the biocidal product, shall decide on an appropriate MOE.

After profound discussions in the biocide working group for the TNsG as to whether one method should be preferred to the other, a consensus has been reached that the two methods should be used in parallel during an initial period of the review programme for existing biocidal active substances to build up experience in the practicability of the respective methods in the biocides process to come.

It could be argued that, as long as the assessment factors used in the process are well-reasoned, transparent and consistent, it may be of less importance which methodology of risk characterisation (i.e. whether the risk characterisation be based on a health-based exposure limit or on the MOE concept) is eventually preferred.

5 Risk assessment factors

The summary of “Risk assessment factors” is found in Section 1.1.2 (in English) and Section 2.1.2 (in Swedish)

5.1 Historical and current use of risk assessment factors

In traditional risk assessment, it is common practice to use an assessment factor to convert experimental animal or epidemiological data into a human exposure level considered to be of no concern for humans. This is done to take into account the fact that human beings might be more sensitive than experimental animals, the fact that certain individuals are more sensitive than the average population, and other uncertainties in evaluation of the database, the nature of effect, route-to-route extrapolation and dose-response relationships. This attempt to be prudent and cautious in assessing a risk due to limited knowledge should not be confused with the precautionary principle (see Section 4.1.1).

This Chapter (5.1) is a review of the different approaches in using assessment factors, historically and currently. The review does not cover all publications in this field. In most cases, we have chosen not to take a definite position on the different assessment factors in this section. Instead, it is the authors' own view that is presented and any comparison and discussion are found in Section 5.2.

A number of different factors are used in risk assessment, and these have been termed “safety factors” or “uncertainty factors”. Neither term is ideal because the term safety factor has implications of absolute safety, whereas uncertainty factors may be interpreted differently in different languages. Terms such as “safety factor”, “uncertainty factor” as well as “extrapolation factor” all appear as equivalent to “assessment factor”, however, often without any clear distinction being made between them. Some of the factors could be placed in a subgroup, for example inter-species and inter-individual factors can be referred to as extrapolation factors, while factors correcting for a LOAEL instead of a NOAEL, sub-chronic exposure to chronic or adequacy of the database may be referred to as database factors (EC 2000c). However, it is not always obvious how to place the factors. In this report, the term *assessment factor* will be used to cover all factors designated as safety factor, uncertainty factor, extrapolation factor, adjustment factor, conversion factor and the composites thereof, except in the description of the different risk assessment approaches.

When regulatory agencies first adopted the approach of setting acceptable levels of exposure to potentially risky substances, those levels were derived by dividing the dose at which no adverse effect was seen in animal studies by “safety factors”. The safety factors, or assessment factors, were designed to account for, among other things, differences between animals and humans and differences among humans.

The use of an assessment factor is a therefore a key part of risk assessment, even though the scientific basis of their original derivation is not clear. A default assessment factor of 100, but also other factors, is often in use for example in the risk assessment of plant protection products (see Section 4.3.1). If fixed default values are not used, the risk assessment is carried out by expert judgement as for new and existing industrial substances (see Section 4.3.2), where the database is evaluated in a case-by-case manner and a margin of safety or a margin of exposure is calculated by

dividing the NOAEL by the actual exposure. Both the method of using fixed default values and expert judgement are applied in the risk assessment of biocidal products (see Section 4.3.3).

The overall effect assessment factor consists of several factors. The most important are derived for: evaluation of the toxicological database, nature of effect, duration of exposure, route-to-route extrapolation, dose-response relationship, and inter-species and inter-individual variations. These are described and evaluated in Section 5.2. In connection with the scientific evaluation of the assessment factors, a brief review of the historical background and the present requirements for the use of assessment factors in human health risk assessment are discussed.

Assessment factors to date have only been used to compensate for uncertainties in the effect assessment. The use of assessment factors in exposure assessment might also be discussed, but this is not within the scope of this document.

5.1.1 Default assessment factors

Historically, the safety factor of 100 was introduced in 1954 in the U.S. in response to the legislative guideline needs in the area of food additives (Lehman and Fitzhugh 1954). This approach proposed that a safe level of food additives or contaminants could be derived from a chronic NOAEL from orally administered animals, divided by a 100-fold safety factor (Equation 5.1). Initially, Lehman and Fitzhugh reasoned that the safety factor of 100 accounts for inter-individual (human-to-human) variability, inter-species (animal-to-human) variability, allowance for sensitive human populations due to illness when compared with healthy experimental animals, and possible synergistic action of the many intentional and unintentional food additives or contaminants.

$$\text{ADI} = \frac{\text{NOAEL}}{100}$$

Equation 5.1 Calculation of Acceptable Daily Intake (ADI) by dividing no observed effect level (NOAEL) by the safety factor of 100

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) adopted the proposal by Lehman and Fitzhugh in a slightly modified form. The Joint Meeting of Experts on Pesticide Residues (JMPR) of the FAO/WHO in 1961 called the safe level the Acceptable Daily Intake (ADI), expressed in mg/kg bodyweight per day. The ADI is now widely accepted, as well as the tolerable daily intake (TDI) approach for contaminants. The procedures adopted by JECFA and JMPR do not generate a clear justification for deviation from the factor 100. However, in some individual cases an expert explanation is given for the use of factors other than 100.

It is apparent that the factor of 100 has no quantitative basis, and the choice of the value 100 is more or less arbitrary. Retrospectively, some attempts have been made to support a 100-fold safety factor: Bigwood (1973), associated with the WHO/FAO, and Lu (1979), associated with the WHO Expert Committee for Pesticide Residues, justified the 100-fold factor on the basis of differences in: body size of the laboratory animals versus that of human, water balance of exchange between the body and its environment among species, and susceptibility to the toxic effect of a given contaminant among species. Vettorazzi (1977) justified the use of the 100-fold factor on the basis of differences in susceptibility between animals and humans, variations

in sensitivities in the human population, the fact that the number of animals tested is small compared to the human population that may be exposed, the difficulty in estimating human intake, and the possibility of synergistic action among chemicals. Although the specific areas of uncertainty described by these authors to support a 100-fold factor differ somewhat, they can generally be viewed as due to inter-individual or inter-species variability. Consequently, it has been suggested that two 10-fold factors, one for each type of variability, could be used to describe the 100-fold factor.

In 1988, the United States EPA adopted the ADI approach in its regulatory measures against environmental pollution with a number of modifications (U.S.EPA, 1988, 1993). Instead of the terms ADI and safety factor, the terms reference dose (RfD)/Reference concentration (RfC) and uncertainty factor (uf) are used. The RfD is derived from the NOAEL by the consistent application of an uncertainty factor. The overall uncertainty factor generally consists of 10-fold factors for each of the following: human variation in sensitivity, inter-species variation, use of the NOAEL obtained from a less than lifetime study, use of LOAEL in the absence of NOAEL and adequacy of the total database. In addition, a "modifying factor" ranging from 1 up to 10 is applied (default = 1). The modifying factor is to take account for example of the quality of the study and completeness of the database. A modifying factor higher than 1 is used e.g. when the database includes a very small number of animals per dose level. Altogether, this might imply a total factor of several orders of magnitude (maximum value 100 000).

The uncertainty factors of 10 for inter-species (animal-to-human) and 10 for inter-individual (human-to-human) variation as used by the U.S.EPA were also proposed by Calabrese and Gilbert (1993). They suggested modifications of the uncertainty factors due to the lack of total independence of these factors. The inter-species (animal-to-human) uncertainty factor is generally recognised as providing an extrapolation from the average animal to an average individual assuming that humans may be 10-fold more sensitive. They assume that most human responses fall within approximately a 10-fold range, and that an uncertainty factor of 5 from the average individual would be expected to protect most humans.

The approach proposed by Renwick (1991, 1993) is also based on the 100-fold factor. It attempts to give a scientific basis to the subdivision of the uncertainty factors of 10 for inter-species and 10 for inter-individual (human) variation. The main feature of this approach is that inter-species and inter-individual differences are distinguished in kinetic and dynamic aspects. This offers the possibility of incorporating mechanistic information on these aspects in the establishment of the factors as shown for pharmaceuticals by Naumann *et al* (1997), provided sufficient data are available. The initial proposal by Renwick was that the 10-fold uncertainty factors should by default be divided into 4 for kinetics and 2.5 for dynamics. It should be noted that the proposed default values are derived from limited data, especially on pharmacodynamics, and that the data according to the author relate to "normal healthy adults" (see also Section 5.2.7).

The International Programme on Chemical Safety (WHO/IPCS 1994) has adopted the principles set forth by Renwick, but has suggested that while the uncertainty factors for inter-species extrapolation is subdivided 4- and 2.5-fold respectively, the uncertainty factors for inter-individual (human-to-human) extrapolation is subdivided evenly into 3.16 for both kinetics and dynamics (Figure 5.1). The reason for this

discrepancy from Renwick's initial suggestion was that the IPCS did not think there was enough information on inter-individual data indicating that toxicokinetic differences are greater than toxicodynamic differences.

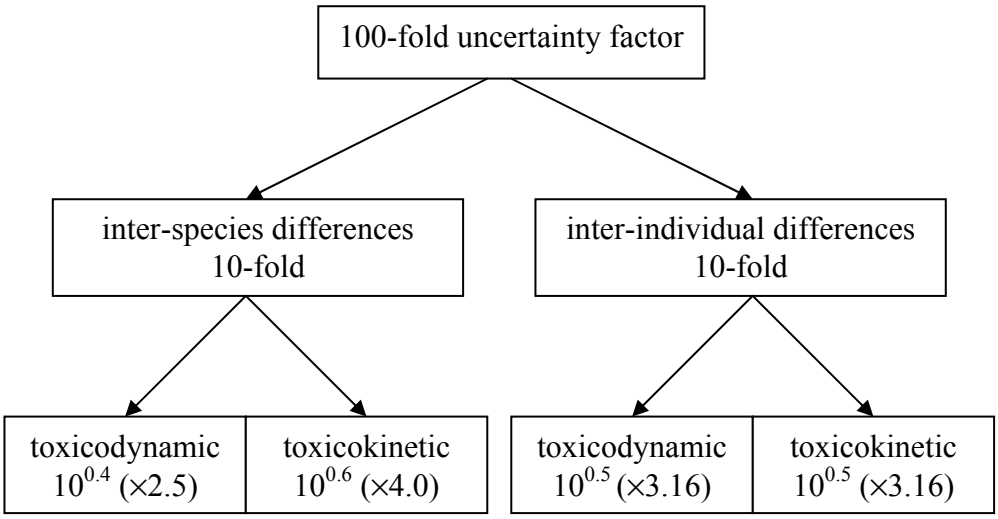

Figure 5.1 Division of the 100-fold factor according to the IPCS. The kinetic and dynamic aspects are differentiated into inter-species (animal-to-human) and inter-individual (human-to-human) differences.

Other approaches aim to discriminate factors to a larger extent in order to generate a rational choice and greater clarity. However, in practice it will not be possible to distinguish all these factors, and one should be aware that some of these might not be independent of each other.

In 1990, Lewis, Lynch and Nikiforov (1990) undertook the revision of the long-established practices, with the goal of introducing flexibility such that both new information and expert judgement could be readily incorporated. The Lewis, Lynch and Nikiforov method guides the data evaluator to adjust experimentally determined 'no effect' (or minimum effect) levels from animal experiments taking into account inter-species differences (animal-to-human), differences between experimental conditions and actual or anticipated human exposures, inter-individual differences (between humans), weight of evidence indicating an actual health hazard, quality of the experimental information base, uncertainties in extrapolating from animals to humans and potency of the toxic agent. An aggregate adjustment factor of 250 is typical, but the theoretical maximum adjustment value is 100,000.

The method used by TNO (Netherlands Organisation for Applied Scientific Research) regarding the risk assessment of workers to new and existing chemicals is developed using literature, supplemented by information from studies by Stevenson *et al* (1995a, 1995b), and by a guidance document for setting acceptable operator exposure levels for plant protection products (Anonymous 1995, quoted in Vermeire *et al* 1999). The method is used for setting health-based occupational reference values (HBORVs) for existing substances (Hakkert *et al* 1996). Assessment factors compensating for uncertainties inherent to extrapolation of experimental (animal) data to a given human situation and for uncertainties in the toxicological database have to be applied. If no conclusion can be drawn, a default factor is used. The factor for inter-species difference is a calculated adjustment factor, allowing for differences

in basal metabolic rate (proportional to the 0.75 power of body weight). An inter-individual factor of 3 is used for workers and a factor of 10 for the general population. For differences between experimental conditions and exposure pattern for humans, factors of 10 each are used for sub-acute to sub-chronic and sub-chronic to chronic respectively. If no relevant data on toxicokinetics and metabolism are available concerning route-to-route administration, worst-case assumptions with respect to % absorption have to be made.

The approach recommended by the ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) is to derive the best scientific estimate of a human no adverse effect level, which is referred to in this report as the Predicted No Adverse Effect Level (PNAEL)[6] (ECETOC 1995). This distinguishes three stages: first, application of an "adjustment factor" to the NOAEL/LOAEL of the critical effect, taking into account: experimental exposure in relation to the expected human exposure, extrapolation from LOAEL to NOAEL, route-to-route extrapolation, inter-species and intra-individual extrapolation. Second, application of an "uncertainty factor" to the PNAEL to take into account the degree of scientific uncertainty involved. The following degrees of confidence in the human PNAEL are suggested: high=1, medium=2 and low=larger uncertainty factor. Third, application of a non-scientifically based "safety factor" taking into account political, socio-economic, or risk perception factors. This method provides guidance for setting occupational and non-occupational limit values.

Kalberlah and Schneider (1998) have written a report on a research project presented and discussed in detail at joint discussions between experts from the German Federal Institute for Occupational Safety and Health (BauA) and the German Federal Environmental Agency (UBA). In this report an inter-individual factor of 25, consisting of sub-factors of 8 and 3 for toxicokinetics and toxicodynamics respectively, is suggested. The inter-species factor would be 14 - 21 for the mouse and 8 - 12 for the rat. This is based on scaling for caloric demand and multiplication by a factor of 2 - 3 in order to cover 95 percent of the substances examined. They state that the factor of 10 used for extrapolation of inter-species differences covers approximately 75% of the examined substances, based on probability distributions, but that uncertainties attach to this statement (see also Section 5.2.6). If sufficient data are available, substance-specific physiologically based pharmacokinetic modelling (PBPK) should always be given preference over the use of scaling factors. The benchmark dose is proposed instead of an extrapolation factor from LOAEL to NOAEL (see Section 5.2.5), but if an extrapolation factor has to be used a factor of 10 is mentioned as suitable. Concerning route-to-route extrapolation, their opinion is that it is necessary in each individual case to examine whether extrapolation appears justified.

In a recent publication from the Netherlands, it is stated that probabilistic distributions of default assessment factors will be applied in future risk assessments produced at RIVM and TNO (Vermeire *et al* 2001). The data-derived distributions of the inter-species assessment factor and the exposure duration factor are the same as discussed below in Sections 5.2.6 and 5.2.3. Since it is concluded that no database-derived distribution of the inter-individual factor can be made at present, a theoretical

[6] The Human Predicted No Adverse Effect Level (PNAEL) represents the best scientific estimate of the dose or exposure concentration, which will not lead to adverse effects in humans exposed by the route and under the regime for which the PNAEL was derived.

distribution based on the default value of 10, taking this value as the 99th percentile, is being considered for use by RIVM and TNO. When this theoretical inter-individual distribution was combined with the inter-species distribution, and adjusted by inter-species allometric scaling, the resulting 95th percentile value for the distribution of the combined assessment factor was 212 for rat experiments and 317 for mouse experiments (Vermeire *et al* 2001). It should be noted that the traditional deterministic approach (10 for inter-species × 10 for inter-individual) corresponds to the 88th percentile of the distribution. For the use of the probabilistic method in human risk assessment, see also Annex 2.

The European Commission has established an expert group (the Scientific Committee on Occupational Exposure Limits, SCOEL) with the task of recommending health-based occupational exposure limits. In its key document from 1999 (SCOEL 1999), the SCOEL states that there is no generally agreed approach on how to apply uncertainty factors in establishing health-based occupational exposure limits. The SCOEL further argues that it is often appropriate to apply lower uncertainty factors for occupational exposure limits than for limit values for the general population. The reasons are that the working population is less heterogeneous than the general population, the working population is only exposed during working hours, in contrast to the life-long exposure of the general population, and that in EU countries health surveillance and monitoring of workers may be performed. The SCOEL has agreed to use the following framework for uncertainty factors: uncertainty factors are only used when the effects follow a conventional threshold toxicological model (i.e. not used for genotoxic carcinogens), uncertainty factors are established on a case-by-case basis, and the confidence in the database will determine the magnitude of the uncertainty factor. The level of the database may range from high confidence (the critical effect has been observed in several studies and several species including man, the studies follow well described and accepted methodologies, a NOAEL can be defined with considerable confidence, all toxicological endpoints are well characterised) resulting in a low uncertainty factor, to low confidence (the database falls short in some relevant aspects, a NOAEL cannot be identified), resulting in a high uncertainty factor. The choice of uncertainty factor will be justified in the recommendation for an occupational exposure limit.

5.1.2 Chemical-specific assessment factors

Because there is a general lack of knowledge about differences in the sensitivity to toxic substances between species and between individuals, risk assessment often has to be based on the use of uncertainty (assessment) factors to compensate for this lack of knowledge. Any scientific data that might help to elucidate this question of differences in sensitivity should thus be utilised in risk assessment. The use of assessment factors is intended to make up for the lack of knowledge of a specific chemical. If this knowledge is available, it can be used in order to modify the default values in a substance-specific manner. A recent IPCS report (WHO/IPCS 2001) proposes using chemical-specific toxicological data instead of default assessment factors, when possible. The framework is the factor of 100 divided into 10x10 for inter- and intra-species differences. The default assessment factors are those proposed earlier by Renwick and IPCS, that is a 10-fold factor for inter-species differences, divided into the factors of 2.5 for toxicodynamics and 4 for toxicokinetics; and a 10-fold factor for inter-individual differences, divided into two equal factors of 3.16 each (Renwick 1993; WHO/IPCS 1994). The basic idea is to

replace one or more of these subfactors with chemical-specific factors that can be derived from studies that demonstrate differences in toxicokinetics or toxicodynamics. The assessment factor may both increase and decrease, and the differences may be considerable.

Many basic issues are discussed in the IPCS report. Before comparing toxicokinetic data, an important question is whether it is the parent compound or a metabolite that causes the critical effect(s). Another important factor is whether the effect is related to a maximal concentration of the compound/metabolite in the target organ or to the overall exposure. Other important factors are the relevance of the human study group, the exposure route, the dose levels and the number of subjects and samples (statistics).

In order to obtain quantitative toxicokinetic data for comparisons between individuals or between animals and humans, human data are needed. These can be derived from *in vivo* experimentation in human volunteers using parameters such as clearance and/or area under the plasma concentration-time curve (AUC). The doses should preferably be close to the NOAEL in the animal experiments, and similar to anticipated human exposure levels in the human studies. *In vitro* measurements of critical processes, e.g. enzyme activity, can also be used.

Quantitative toxicodynamic data may be derived from comparative response data for the toxic effect itself or for a point in the chain of events that is considered critical to the toxic response (i.e. a relevant endpoint, sometimes referred to as biomarker of effect). This has to be based on understanding of the mode of action, under experimental conditions where toxicokinetic variations have been precluded. A surrogate marker for toxic effects should be validated as being representative, both qualitatively and quantitatively, for the critical toxic endpoint. Data related to the toxicodynamics in humans are needed, and may be derived from *in vivo* studies measuring the actual toxic response or a surrogate endpoint, or from *in vitro* studies in human tissues. *In vitro* studies are generally inadequate for the assessment of human variability. In practice there will be a lack of human *in vivo* data, and comparisons between animal and human toxicodynamics will have to be based on parallel *in vitro* dose-response studies with animal and human tissue samples. It is crucial that the *in vitro* system is representative of what happens *in vivo*, and that the endpoint measured is either the critical toxic effect or closely linked to the critical effect.

Discussion

The chemical-specific approach is attractive because it attempts to use scientific data. Although sound in principle, the proposed approach has limitations. It would, if applied, rely very heavily on scarce data that are not regularly investigated for. In most cases the experimental data will be weak or non-existent (especially toxicodynamic data). The approach might nevertheless be applied in those instances where the required quantitative data may be derived. In such cases the quality of the underlying studies must be carefully scrutinised, and criteria must be established. The most critical questions are whether the study population is relevant and representative, the number of subjects/samples is adequate and the dose-response data are adequate. The relevance of route and dose is, of course, also important. If *in vitro* data are used, there must be a clear link to the mechanism of toxicity.

Considering the far-reaching influence of a single experiment, the risk assessor must be extra cautious if the data would lead to low chemical-specific assessment subfactors implying that humans are less sensitive than the experimental animal, especially in the case of *in vitro* data.

The chemical-specific approach requires that human studies and studies using human tissue samples must be used. From ethical reasons, such studies may be controversial.

5.1.3 Children-specific assessment factor

Concern has been raised about pesticide residues in children's food and susceptibility of children and infants to these chemicals. The Food Quality Protection Act (FQPA) was signed into law in 1996 (U.S.EPA 1996). Among other changes it directed the U.S.EPA to apply an extra safety factor of 10 in assessing the risks to infants and children. The concepts for the children's health components of the law came from the 1993 National Research Council (NRC 1993) report on "Pesticides in the Diets of Infants and Children". In the report it was recognised that maturing organ systems of infants and children may be susceptible to injury by chemicals. There may be developmental periods (i.e. windows of vulnerability) when the endocrine, reproductive, immune, visual or nervous systems are particularly sensitive to certain chemicals. The U.S.EPA Toxicology Working Group advises the U.S.EPA Office of pesticide programmes to augment pesticide testing with new and improved guidelines for acute neurotoxicity, developmental neurotoxicity and two immunotoxicity studies: one in adult rats and one in an *in vitro* system (U.S.EPA 1999a). In addition, the group recommends that only in cases where data are complete and the age group or window of vulnerability during development has been clearly delineated, preferably based on human data or on animal data with supporting human data, should there be consideration of reducing the extra 10-fold inter-individual factor (U.S.EPA 1999a). However, when data are missing or inadequate, application of the extra uncertainty factor in addition to the 10-fold inter-individual variability factor is considered appropriate. Although the U.S.EPA Office of Pesticide Programs intends to expand its data requirements to include additional types of studies, the absence of these additional studies will not automatically be the basis for the imposition of a database uncertainty factor (U.S.EPA 1999a). In practice, factors of 3 and 10 have been used and the factor has also been used in cases when data have been sufficient but when there were reasons for concern.

A reference dose modified by an extra FQPA factor is referred to as a population-adjusted dose (U.S.EPA 1999a). There has been argument over whether available data generally do provide a scientific rationale for an extra factor for children or not, and there is no overall agreement on this. (Read more about differences between children and adults in Section 5.2.7, and about the adequacy of the database in Section 5.2.1) (NRC 1993; Renwick 1998; U.S.EPA 1999a; Renwick *et al* 2000; Bruckner 2000; Scheuplein 2000).

5.2 Evaluation of and recommendations for assessment factors

5.2.1 Adequacy of the toxicological database

It is important in the effect assessment to evaluate the toxicological database with regard to its adequacy. The adequacy of a study includes its validity and its relevance. The relevance refers to what has been studied (connected with what is being discussed or suspected, for example a specific endpoint or a target organ) and the validity refers to how the study was performed (if done with the correct formalities, for example in line with a current recommended guideline or other appropriate methodology). The validity and the relevance of a study or a whole database must be considered in relation to whether they might be relied or depended upon, i.e. the reliability has to be judged.

To ensure the adequacy of the database, formal requirements for the completeness of the data have been set up for plant protection products, for new and existing substances, and for biocides (for details, see Section 4.3).

Any tests carried out for the purpose of risk assessment within the Directive for Plant Protection Products, 91/414/EC (EC 1991), the Directive for New Substances, EC 67/548 (EC 1967), the Regulation on Existing Substances, EEC 793/93 (EC 1993a), and in the Directive for Biocides, 98/8/EC (EC 1998), should be conducted in compliance with Good Laboratory Practice (GLP) as set out in Directive 87/18/EEC (EC 1987). Tests should also be performed according to specifically laid-down methods. However, it is possible that other test data already exist which have been generated by other test methodologies, which do or do not apply directly to current GLP standards. The adequacy of such data and possible requests for new tests produced by the manufacturers and importers must be decided upon on a case-by-case basis. The need to minimise animal testing should be considered in this process.

Suggestions have been made to use an assessment factor for the confidence in the database, such as a proposal to distinguish between a high, medium and low degree of confidence (ECETOC 1995). WHO/IPCS (1999) and U.S.EPA (1993) also include assessment factors (1-10) for the total database quality. However, as no systematic approach to the basis for these assessment factors has been found, transparent expert judgement of the adequacy of the database in a case-by-case manner is at present the most useful tool in considering the database. Based on other organisations' default values, for practical reasons a factor of 1 – 5 could be chosen when judging the quality of the database.

An incomplete database (excluding those with exemption from complete testing requirements, see Section 4.3) under current regulations for plant protection products, new and existing substances and biocides are not to be considered for risk characterisation. Missing or additional test(s) must be asked for from manufacturers and importers.

5.2.1.1 Use of an extra assessment factor in the risk assessment of children

In the FQPA (Food Quality Protection Act) of 1996, the U.S.EPA is directed to apply an extra safety factor of 10 in assessing the risks of pesticides to infants and children (U.S.EPA 1996). In the interpretation of the law, the U.S. EPA's Toxicology Working Group for pesticides (U.S.EPA 1999b) assumes that the default inter-

individual 10-fold uncertainty factor will be adequate in the majority of cases for protecting children's health when a complete developmental toxicity database is available. However, when data specific to children's health are missing or inadequate for a particular pesticide, application of an extra safety factor in addition to the default inter-individual 10-fold uncertainty factor is considered appropriate to account for the possibility that children may be significantly more sensitive than adults. The size of the database uncertainty factor will depend on other information available in the database and how much impact the missing data may have in determining the potential toxicity of the pesticide for children. In practice, factors of 3 and 10 have been used and the factor has also been used in cases where data have been sufficient but where there were reasons for concern.

The opinion of Renwick and co-authors (Renwick *et al* 2000) is that available data do not provide a scientific rationale for an extra factor for children due to the inadequacy of inter-species and/or inter-individual uncertainty factors. Justification for the factor must relate to the adequacy and sensitivity of current methods or concern about irreversible effects in the developing organism. In addition, they point out that when adequate reproduction, multigenerational or developmental studies are conducted there will be no need for an additional 10-fold factor.

In a report from the Danish Environmental Protection Agency (Danish EPA 2001), it is strongly recommended that child-specific risk assessments should be performed for chemical substances intended for children (e.g. toys, cosmetics, child-care products, food additives in preferred products and pesticide residues in processed baby foods and infant formulas). In the risk assessment of chemical substances in use categories other than those mentioned above, it is recommended that the focus should be specifically on children, including the unborn child, if a potential exposure to a given substance may occur in these age groups. Furthermore, it is recommended that the risk assessment should be performed on a case-by-case basis. In cases where the data are insufficient to evaluate the susceptibility of children, including the unborn child, it is strongly recommended that additional safety measures (choice of safety factors) should be considered when acceptable or tolerable daily intakes, or health-based limit values, are established for chemical substances in products and foods intended for children.

In an unpublished report from the Norwegian Institute of Public Health (Lindemann *et al* 1999), it is concluded that the greatest differences compared with adults occur in the youngest children, neonates and early post-natal infants. As potentially vulnerable systems in infants and young children include the endocrine, reproductive (slow maturation reaching a peak immediately prior to adulthood), immune (develops postpartum), and nervous systems (develops either late during pregnancy or in the neonate), there is a need for extra guidance in assessing the risks of chemicals for children. As individuals during the embryo/foetal period, infancy and childhood can be identified as a group to whom special attention needs to be paid in the risk assessment (see Section 5.2.7), a deficiency in the overall database of data for children should be reflected. In harmony with the US legislation, a factor of 10 could be considered for such lack of data.

Suggestion for an assessment factor to be used for adequacy of the toxicological database

Transparent expert judgement of the adequacy of the database in a case-by-case manner is at present the most useful tool in considerations of the toxicological database. One recommendation is to be extra prudent in the risk characterisation when dealing with a poor database. Based on other organisations' default values, one could, for practical reasons, choose a factor of 1 – 5 when judging the quality of the database.

In the special case of children as a target group for the risk assessment and if there is a shortage of data for children, a larger factor of 1 – 10 is proposed to compensate for a poor database in this respect. The assessment factor is based on the severity of the effect and uncertainty of the difference in the sensitivity between young and adult individuals for the particular effect.

5.2.2 Nature of the effect

The nature of an effect includes the adversity of the toxicity expressed as the level of and the basis for N(L)OAEL values and the severity of the specific endpoint or key event (for example judging skin irritation less severe than teratogenicity). The potency, that is the strength of the toxic effect, should also, if possible, be considered.

Adversity: A commonly used definition of an adverse effect is: "changes in morphology, physiology, growth, development or lifespan of an organism which result in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences "

Although the above definition of adversity is adopted by many organisations (WHO/IPCS 1994, EC 1996), it is too unspecific for most purposes to serve as a tool in basic hazard assessment work. Attempts to identify adversity and non-adversity are found in the Global System for Harmonisation for Target Organ Systemic Toxicity (OECD 2001), in the EU classification and labelling system (EC 1967) and by the U.S.EPA (1986) and compiled in Table 5.1. As can seen from the table, the OECD and the EU judge adversity to be: "consistent and significant adverse changes…" (the borderline is marked with a bold line in the table). The U.S.EPA has not drawn a line but states that the borderline between non-adverse and adverse effects should be in the upper part of the table. For the purpose of risk assessment, we draw the conclusion in the present document that the borderline between adversity and non-adversity is found in shaded areas in Table 5.1. Furthermore, very slight effects might also be of relevance for risk assessment, for example in cases where various slight effects might go together to make up a syndrome (such as chloracne) and when very slight effects may be related to more adverse effects in the same target organ.

Areas in connection with the estimation of the adversity of an effect, where extra guidance is often required, are reversibility and irreversibility and adaptation to an exposure. Irreversible effects are always of great concern. Reversible effects may also be of great concern depending on the nature of the effect and on the setting in which they occur. It cannot be ruled out that a permanent lesion may have occurred even if the overt effect is transient. Furthermore, when there is a more or less

continuous exposure to a substance, the question of reversibility is not relevant because adaptation systems will be counteracted by new insults. In many cases it is not possible to draw any conclusion on whether an effect is reversible or not as such experimental data are very rare and all significant health effects that can impair function, both reversible and irreversible, should therefore be considered in the risk assessment.

An adaptation, where an organism stabilises its physiological condition after exposure to a chemical, without any irreversible disruption of a biological system and without exceeding the normal capacities of its response, should be considered as an early, not yet adverse, effect which later on might lead to an adverse effect and thus needs expert judgment in a case-by-case manner.

Severity: In the EU, the endpoints carcinogenicity, mutagenicity and reproductive toxicity (CMR) are considered as more severe than other endpoints as the substances classified in category 1 or 2 for one of the CMR effects, according to Directive 67/548/EEC, will automatically be noted under the Directive for Restrictions on the Marketing and Use of Certain Dangerous Substances (Directive 76/769/EEC) (EC 1976a). However, depending on criteria not yet harmonised, in Sweden all substances and preparations labelled as "Toxic" or "Very toxic" for acute or chronic toxicity or in category 1 or 2 for one of the CMR effects are prohibited for use in consumer products.

The proposal for an extra assessment factor in the case of high severity is included as a recommendation in the guidance document to the Biocidal Products Directive (EC 1998). It is also recommended in the Technical Guidance Document (EC 1996) in the risk assessment for new and existing industrial chemicals to be extra prudent when dealing with endpoints of high severity. A practice of being extra-cautious has developed in the risk assessment of severe effects caused by plant protection products.

A number of bodies, including the WHO and FAO Joint Expert Committee on Food Additives (WHO/IPCS 1987) and the Joint Meeting on Pesticide Residues (WHO/IPCS 1990), have additionally incorporated an additional "safety factor" of up to 10 in cases where the NOAEL is derived for an effect which is of a high degree of severity, especially if associated with a shallow dose-response relationship. This additional factor has been applied in such cases to provide a greater margin of safety between intake/exposure of any susceptible humans and the dose-response curve for such toxicity in animals.

Potency: If possible, the potency of a toxic substance should also be considered in the risk assessment for extrapolation reasons between experimental animals and humans. The estimation of the potency allows for relative comparisons of toxicity between different chemicals. The dose that induces a well-defined effect in a certain percentage of the experimental animals is calculated in the potency approach. The two most common potency measures are the LD_{50} for acute lethal toxicity (although now replaced by other guideline tests) and the TD_x (Tumour Dose) for the induction of tumours.

Table 5.1 Adverse and non-adverse systemic effects as identified by various organisations basically for classification and labelling purposes (U.S.EPA 1986; EC 1967; OECD 2001). OECD and EU judge adversity to be: " consistent and significant adverse changes…" (borderlines are marked with a bold line). The U.S.EPA states that the borderline between non-adverse and adverse effects should be in the upper part of the table. Our recommendation is that the effects found in the grey shaded areas indicate where the borderline between adverse and non-adverse effects might be drawn.

OECD	EU	U.S.EPA
Clinical observations or small changes in bodyweight gain, food consumption or water intake that may have some toxicological importance but that do not, by themselves, indicate "significant" toxicity.	Clinical observations or changes in bodyweight gain, food consumption or water intake, which may have some toxicological importance but which do not, by themselves, indicate "serious damage".	Biochemical/haematological change with no pathological change and no change in organ weight; or a change in organ weight with no pathological and biochemical/haematological change
Small changes in clinical biochemistry, haematology or urinalysis parameters and /or transient effects, when such changes or effects are of doubtful or minimal toxicological importance.	Small changes in clinical biochemistry, haematology or urinalysis parameters which are of doubtful or minimal toxicological importance	Biochemical/haematological change with no pathological change and with a change in organ weight
Changes in organ weights with no evidence or organ dysfunction.	Changes in organ weights with no evidence of organ dysfunction	Enzyme induction and subcellular proliferation or other changes in organelles but no other apparent effects
Adaptive responses that are not considered toxicologically relevant	Adaptive responses (e.g. macrophage migration in the lung, liver hypertrophy and enzyme induction, hyperplastic responses to irritants).	Biochemical/haematological change with slight pathological changes
Substance-induced species-specific mechanisms of toxicity, i.e. demonstrated with reasonable certainty to be not relevant for human health, should not justify classification.	Where a species-specific mechanism of toxicity (e.g. specific metabolic pathways) has been demonstrated.	Hyperplasia, hypertrophy or atrophy with a change in organ weight
Any consistent and significant adverse change in clinical biochemistry, haematology or urinalysis parameters	Any consistent changes in clinical biochemistry, haematology or urinalysis parameters, which indicate severe organ dysfunction. Haematological disturbances are considered to be particularly important if the evidence suggests that they are due to decreased bone marrow production of blood cell	Reversible cellular changes: cloudy swelling, hydropic change or fatty changes

cont.

OECD	EU	U.S.EPA
Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver).		Necrosis, or metaplasia with no apparent reduction in organ function; any neuropathy without apparent behavioral, sensory, or physiological changes
Significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g., sight, hearing and sense of smell).	Major functional changes in the central or peripheral nervous system, including sight, hearing and the sense of smell, assessed by clinical observations or other appropriate methods (e.g. electrophysiology)	Necrosis, atrophy, hypertrophy, or metaplasia with a detectable reduction in organ functions; any neuropathy with a measurable change in behavioral, sensory, or physiological activity; reduced body weight gain; clinical symptoms
Significant organ damage that may be noted at necropsy and/or subsequently seen or confirmed at microscopic examination.	Major functional changes in other organ systems (for example the lung)	Necrosis, atrophy, hypertrophy, or metaplasia with definitive organ dysfunction; any neuropathy with gross changes in behaviour, sensory or motor performance
Multifocal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity.	Widespread or severe necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity (e.g. liver)	Pronounced pathological changes with severe organ dysfunction; any neuropathy with loss of behavioral or motor control or loss of sensory ability
Evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.	Evidence of appreciable death in vital organs incapable of regeneration (e.g. fibrosis of the myocardium or dying back of a nerve) or in stem cell populations (e.g. aplasia or hypoplasia of the bone marrow)	Death or pronounced life-shortening
Morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses or concentrations, due to bioaccumulation of the substance or its metabolites, or accumulation of effect owing to the ability of the detoxification process becoming overwhelmed by repeated exposure to the substance or its metabolites.	Substance-related deaths	

Taken together, on the basis of the intricate pattern of the unlimited combinations of adversity, severity and potency that can be formed, it is extremely difficult to set forth other than very rough guidelines on how to assess the nature of the effect. In comparison with other organisations' default values for severity, for practical reasons a factor of 1 – 10 could be chosen when judging the nature of the effect.

Suggestion for an assessment factor to be used for the nature of effect

The recommendation is that the nature of effect should be taken into account. The adversity, potency and severity of a toxic substance should be considered. Depending on the complexity of the nature of effect, a case-by-case expert judgement appears at present to be the most appropriate method to use. In comparison with other organisations, we suggest a factor of 1-10 when judging the nature of effect.

5.2.3 Duration of exposure

The most relevant study to base a risk assessment upon is a study that mimics the human exposure situation as well as possible. Such a study is, however, difficult to design in practice. In many cases a lifelong exposure is the most relevant exposure scenario for humans. In other cases a lifelong exposure is assumed for conservative reasons. In these cases, a lifetime animal study (in practice a chronic study) is the most relevant study on which to base the risk assessment. However, in cases of short-term actual human exposure (e.g. in certain exposure situations with plant protection products) it might be more appropriate to base the risk assessment on a shorter than lifetime study (see Section 4.3.1.4)

According to OECD guidelines dosing periods in rodents lying between the single dose and 10% of life-span dosage are often called "subacute". They are also (more correctly) called "short-term repeated dose studies" (14, 21 and 28 days). In rodents, a "subchronic" study according to OECD guidelines has the duration of 90 days. An exposure duration longer than three months (most often 12-24 months) is called chronic exposure.

For numerous substances, data are only available from acute, subacute or subchronic animal experiments. This raises the question of what statements these findings can provide for longer-term (chronic/life-time) exposure for humans. It can be assumed that in most cases an increase in the duration of exposure leads to a lower NOAEL (the highest dose at which effects are not observed). The main issue is the size of this difference in NOAEL.

There might be various reasons behind the fact that a longer duration leads to a lower NOAEL. It might be due to the fact that some substances bioaccumulate and consequently a critical threshold in the body, at which adverse effects occur, may only be reached after a longer period of time. In addition, there might be a long period of latency before damage becomes apparent. It is also necessary to take into account the possibility that a study of longer duration may reveal a target tissue/organ/system that was not affected in a relatively short-term study (EU 1996). Moreover, in subchronic toxicological studies fewer animals are usually used per group than in chronic studies. It may thus, for statistical reasons, be expected that NOAELs from subchronic studies tend to be higher than NOAELs from chronic studies, even if the dose-relationships in both studies were identical.

The U.S.EPA (1993) suggests a factor of 10 for extrapolation from subchronic to chronic exposure. ECETOC (1995) suggests an assessment factor of 2-3. The EU (1996) suggests no default value for duration extrapolation but a value based on a case-by-case determination (expert judgement of scientific information). The International Conference on Harmonisation (ICH) has a Consensus Guideline for residual solvents as impurities in pharmaceuticals (EMEA/ICH 1997). Their default assessment factors for duration is 2 for a 6-month study in rodents, 5 for a 3-month study in rodents and 10 for studies of shorter duration. For classification and labelling of chemicals within the European Union (Directive 67/548/EEC) (EC 1967), a factor of 3 is used for extrapolation of target-organ toxicity from subacute to subchronic exposure (the classification and labelling are to be based on subchronic data). If chronic data are available, extrapolation should be done in a case-by-case manner. For comparison, if the cumulative dose is the decisive aspect and only the exposure time is considered, the following factors result: a factor of 3 from subacute (1 month) to subchronic (3 months) exposure and a factor of 8 from subchronic (3 months) to chronic (24 months) exposure in rodents.

In several studies, the relationship between NOAELs from semichronic (around 90 days in rodents, i.e.subchronic) and chronic oral studies have been examined (reviewed by Vermeire *et al* 1999). NOAELs for agrochemicals, Natinal Toxicological Program (NTP) studies etc. were used. The most likely distribution of the NOAEL ratios (NOAELsubchronic/NOAELchronic) was considered to be log-normal; the geometric mean and geometric standard deviation was thus estimated. Taken together, the geometric means for the ten distributions of oral NOAEL ratios reviewed by Vermeire *et al* (1999, 2001) were similar, approx. 2. This means that NOAELs were on average twice as high in subchronic compared to chronic studies for the chemicals evaluated. A geometric standard deviation of 3.5 was proposed on the basis of the different data sets (Vermeire *et al* 2001). If the 90^{th}, 95^{th} and 99^{th} percentiles are calculated from such a proposed distribution of NOAEL ratios (GM 2; GSD 3.5), the default values would be 10, 16 and 37 respectively (Figure 5.2, Table 5.2). The commonly used assessment factor of 10 would thus coincide with 90% of the variation among different substances. Whether these distributions also apply to inhalatory and dermal subchronic-to-chronic ratios is questionable. (The reader is referred to Annex 2 for more information about probabilistic approaches in risk assessment.)

Vermeire *et al* (1999) concluded that the most relevant NOAEL ratios (subchronic/chronic) were those based on the same species, and that the most relevant distributions of NOAEL ratios are those that include a sufficient number of matched pairs of NOAELs from various species. Unfortunately, the available distributions also included inter-species variation. However, in the largest study reviewed (n=149) the variance did not decrease when calculations were based only on studies in rats (n=70; Pieters *et al* 1998).

A similar distribution was calculated from studies examining extrapolation from oral subacute to chronic exposure (Table 5.2; Vermeire *et al* 2001). Based on these studies, the geometric mean of the subacute-to-chronic ratios was approximately 5 (significantly higher than that of the subchronic-to-chronic ratios) and the geometric standard deviation was 3.5. Based on such a proposed distribution, the default values for the 90^{th}, 95^{th} and 99^{th} percentiles were calculated to be 25, 39 and 92 respectively.

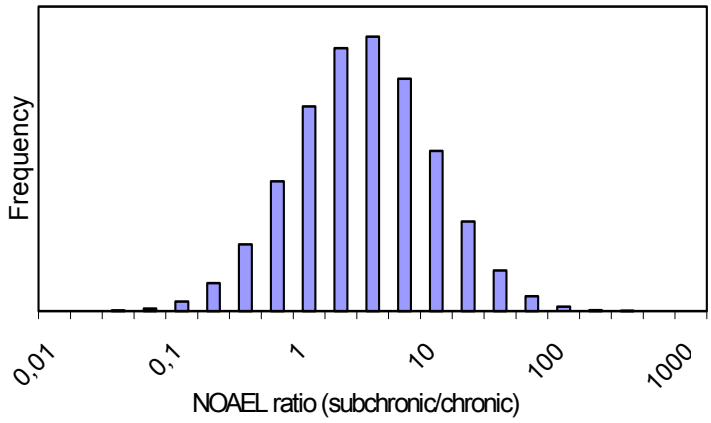

Figure 5.2 Distribution of subchronic (semichronic) to chronic assessment factor (recalculated from Vermeire *et al* 1999 and Vermeire *et al* 2001)

Based partly on the same studies, but also including inhalation studies, Kalberlah and Schneider (1998) concluded that the factor of 10 which is traditionally used for extrapolation from subchronic to chronic exposure presumably covers approximately a 75th percentile of the cases. A value of 2-3 covers the geometric mean of the distribution (Table 5.2). The average 90th percentile based on several distributions reviewed was 23. If an outlier was excluded, the average 90th percentile was instead 10, which is similar to the value reported by Vermeire. In the case of subacute to chronic exposure duration, a time assessment factor of 6 appeared to be justified when the geometric mean of the distribution was taken as the basis for calculations, but the width of the distribution was considerable (Table 5.2).

Table 5.2 Geometric mean (GM), geometric standard deviation (GSD) and 90th, 95th and 99th percentiles for distributions of NOAEL ratios obtained from animal experiments with different chemicals (subchronic/chronic and subacute/chronic, respectively) reported by Vermeire *et al* (2001).

	GM	GSD	90th	95th	99th
Subchronic/chronic	2	3.5	10	16	37
Subacute/chronic	5	3.5	25	39	92

An important aspect of the differences in exposure between humans and experimental animals is the impact of a peak exposure or a more continuous exposure for the toxic effect. In oral rodent studies, the animals are exposed either via gavage or via the diet. A gavage exposure once a day leads to a relatively high peak exposure resulting in a higher C_{max} than exposure via the diet where the animals consume the test substance during a longer period of the day. Exposure via the diet will result in a lower C_{max} and an AUC that is similar or maybe higher than the AUC in gavage-treated animals. A lower AUC in gavage-treated animals may be a consequence for example of lower absorption. In addition, the metabolism of the test substance may differ between gavage- and diet-treated animals due to induction or inhibition of enzymes or capacity limits. Another important issue concerns the mechanism of action – is the effect(s) elicited particularly by a high C_{max} or a high AUC? These aspects should, if possible, be considered in a case-by-case manner, comparing the exposure of the animals with the exposure situation(s) known or estimated for humans. However, unfortunately sufficient data on kinetics and mechanism of action are rarely available for such a consideration.

Suggestion for an assessment factor to be used for the duration of exposure

Extrapolation from studies with shorter duration to long-term exposure is sometimes necessary due to lack of relevant data for risk assessment of chronic exposure in humans. It is assumed that the NOAEL is lower in chronic studies than in shorter studies. This may be due to statistical reasons (smaller group size in shorter studies), concentrations of the chemical increasing with time or adverse effects taking time to develop and become observable. In addition, different critical effects are sometimes identified in subchronic and chronic studies. Since data on chronic exposure are often not available for the risk assessment of chemicals, a factor is needed to extrapolate to chronic exposure in cases when this is assumed as the actual exposure situation for humans. Default factors ranging from 2-10 have been proposed by different organisations. A ratio of 2-3 has been reported from studies examining the actual relationship between NOAELs from subchronic and chronic studies (geometric mean; Vermeire et al 1999, 2001, Kalberlah and Schneider 1998). Taking 95% of the substances compared into consideration would result in a factor of 16 (95th percentile calculated from one of these distributions; Vermeire et al 2001), which is higher than the default factors generally used.

We suggest that, if necessary, extrapolation can be performed from subchronic to chronic exposure. It is suggested that such an extrapolation should be based on the distribution of NOAEL ratios reported by Vermeire et al (2001). If the level of 95% is chosen (covering 95% of the substances compared,) the corresponding assessment factor is 16. Extrapolation from subacute to chronic exposure should preferably not be performed, but if it is necessary a similar approach is suggested. For this extrapolation, an assessment factor of 39 corresponds to the 95% level (based on the log-normal distribution of NOAEL ratios from subacute and chronic exposure studies; Vermeire et al 2001).

5.2.4 Route-to-route extrapolation

Most toxicity studies are performed using oral exposure to the test chemical. However, for most chemicals, besides residues or contaminants in food and drinking water, the predominant routes of exposure in humans are via the skin or via inhalation.

In cases where relevant data are lacking on exposure routes of interest, route-to-route extrapolation is used in risk assessment. However, in general, route-to-route extrapolation is thought to be a poor substitute for toxicity data obtained using the appropriate route of exposure and can only be used for substances that produce systemic toxicity (EU 1996). A route-to-route extrapolation includes examination of whether knowledge resulting from studies concerning one route of application can be transferred to another route of application. In the case of systemically acting substances, there are limited possibilities for extrapolation from one exposure route to another. The main obstacles are differences in the degrees and rates of absorption by different exposure routes and differences in biotransformation, particularly in the case of first-pass metabolism (EU 1996). In the absence of adequate toxicokinetic data and in order to account for differences between routes of exposure, data on acute toxicity or physical and chemical properties can be used, if available. However, these methodologies should be used with caution since they are based on broad

assumptions. It should be noted that there exist route-specific effects, e.g. due to accumulation in certain organs such as the brain. The degree of absorption through the skin and in the lung depends on several factors besides the identity of the chemical. For example, vehicle, dose level, physical and chemical properties (gas/particle-bound, lipophilicity etc.), temperature, humidity, as well as the quality of the skin (thickness, age, presence of eczema etc.) can influence the dermal absorption. The degree of absorption in the lung may depend on factors such as particle size, lipophilicity/hydrophilicity and gas-phase or particle-bound state.

Estimated dermal NOAEL from oral NOAEL

Unless there are data to the contrary, it is assumed by the EU(1996) that the NOAEL for repeated dose studies is the same for both routes on a body weight per day basis. Dermal absorption is almost always likely to be less than, or no more than equal to, oral absorption, and dermal resorption is generally slower than oral resorption. A case-by-case estimation of dermal absorption and dermal NOAEL is used in the risk assessment of existing chemicals within the EU. If rates of dermal absorption have been determined, these can be used to derive a dermal NOAEL from an oral NOAEL.

Estimated inhalation NOAEL from oral NOAEL

One of the commonest problems in route-to-route extrapolation relates to inhalation exposure of humans where there is lack of toxicity data for this route. If there are inhalation LC_{50} data, the EU (1996) suggests that these data can be calculated from the equivalent inhalation LD_{50} (dose absorbed) by assuming a percentage value for absorption via the lungs (75% and 100% are commonly used) and taking into account the respiratory rate and body weight. The ratio of the calculated LD_{50} value to the measured oral LD_{50} value can then be used to estimate the inhalation NOAEL from the oral NOAEL. In the absence of an LC_{50}, an oral repeated NOAEL can be converted to an approximate inhalation NOAEL using the physiological parameters above.

Vermeire *et al* 1999, EU(1996) and ECETOC (1995) suggest no default value for route-to-route extrapolation, but a value based on a case-by-case determination (expert judgement and scientific information). Others (WHO/IPCS 1987, WHO/IPCS 1990, U.S.EPA 1993, WHO/IPCS 1994) do not account for route-to-route extrapolation

The currently applied route-to-route extrapolation methodology is an easy, straightforward way to determine a dermal or inhalation NAEL (No Adverse Exposure Level, i.e. an estimated value) based on an oral NOAEL. However, these methods have not been validated. Wilschut *et al* (1998) have performed a study aimed at evaluating route-to-route extrapolation on the basis of absorption or acute toxicity data. They compared estimated NAELs with repeated-dose toxicity data. In extrapolation from oral to respiratory route (n=28), they found that the predicted respiratory NAEL was often overestimated, i.e. the substance was considered less toxic than it actually was. For oral to dermal extrapolation (n=25), the predicted dermal NAEL was often underestimated, i.e. the substance was considered to be more toxic after extrapolation compared to actual experimental observations. However, a drawback of this study is that, in order to collect enough data, comparisons between different species were included and inter-species extrapolation was applied on the basis of caloric demand (see 6.7). In addition, in the case of

missing NOAEL values, Withschut *et al* (1998) used a factor of 3 for an extrapolation from LOAEL to NOAEL. Based on the results, the authors suggest that an uncertainty factor should be applied in the case of route-to-route extrapolation. The authors conclude that unknown factors (other than absorption) may be involved in the differences in systemic toxicity between exposure routes. However, they also state that the reliability of the data is questionable, because the influence of the several assumptions made in order to derive comparable data on the ratio of the predicted NAEL and the NOAEL is unknown.

When performing route-to-route extrapolations, it is important to keep in mind that in the oral study the degree of absorption might be substantially lower than 100%. This means that an assumed degree of absorption of 100% via inhalation (compared to the oral absorption) may not always be a conservative assumption. There are cases where the degree of absorption via inhalation is much higher than the oral absorption, e.g. for cadmium.

It should also be kept in mind that local toxicity in the airways in certain cases may be a sensitive and important effect that cannot be identified on the basis of oral toxicity studies.

Suggestion for an assessment factor to be used for route-to-route extrapolation

Route-to-route extrapolation is used in risk assessment when relevant data are lacking on the exposure route of interest for human exposure. If kinetic data are available these should be used for comparisons. However, extrapolations are often based on crude assumptions of only the degree of absorption at the different exposure sites. It should be noted that an assumed degree of absorption of 100% (i.e. 100% of oral absorption) is probably conservative for dermal absorption. However, in the case of inhalation such an assumption may represent an underestimation of the actual absorption since the degree of absorption via inhalation may be higher than via the oral route. In addition, route-to-route extrapolation can only be performed in the case of systemic toxicity and possible local toxicity in the airways cannot be detected. Not only the degree of absorption but also metabolism should be considered, e.g. compounds may be highly metabolised in the liver due to first-pass effect in the case of oral exposure but much less metabolised in the case of other routes of exposure.

There are databased distributions of NOAEL ratios from different routes of exposure. However, the size and reliability of this database are limited and we therefore suggest that these distributions should not be used.

We suggest that kinetic data are required if possible and that route-to-route extrapolation should be performed in a case-by-case manner based on expert judgement of scientific information. In cases where no data are available to base the extrapolation upon, we suggest that 100% should be used as the default degree of absorption. It should be kept in mind that this default level is generally very conservative in the case of dermal exposure, while in the case of inhalation exposure this default level may not be conservative at all, and may even be the opposite.

5.2.5 Dose-response curve and extrapolation from LOAEL to NOAEL

Dose-effect is a graded measure of the increase or decrease in effect with dose (e.g. liver weight), whereas dose-response is a measure of the increased risk with dose in a population (e.g. incidence of tumours, which are either present or not). The distinction is necessary in order to determine an appropriate mathematical or statistical model for analysis. However, this is not the main objective of this report and the term effect is sometimes used interchangeably with response.

Empirical observations have generally revealed that, as the dose of a toxicant increases, the toxic response (in terms of adversity or incidence of response) also increases. A problem for the risk estimation procedures is that low doses in experimental animals sometimes produce a response that is statistically significantly less than the background incidence in untreated animals, a phenomenon known as hormesis (Calabrese and Baldwin, 1998). Although hormesis remains controversial, it is not without biological plausibility (Sielken *et al* 1995), because a low exposure may serve to stimulate protective and homeostatic processes in excess of the amount of added insult to the system. Hormesis will not be discussed in greater detail in this document.

Subdivision of toxic effects into threshold and non-threshold has been the basis for risk assessment for the past 30-40 years (WHO/IPCS 1999). The proof of absence or presence of a threshold remains a matter for debate in risk assessment. However, for most types of toxic effects (i.e. organ-specific toxic effects, non-genotoxic carcinogenesis, neurological/behavioural, reproductive or developmental effects), it is generally considered that there is a dose or concentration below which adverse effects will not occur (i.e. a threshold). The dose that can be considered as a first approximation of the threshold is therefore critical. One such estimate of the threshold is the No Observed Adverse Effect Level (NOAEL). For an illustration of dose-response and NOAEL, see Figure 5.3.

A NOAEL for an experiment is defined as the greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development or life span of the target organism under defined conditions of exposure (WHO/IPCS 1994), for further details see Section 5.2.2. However, it is important to remember that the NOAEL is an estimated value and, necessarily, not the same as the “true” no adverse effect level. Although the NOAEL could be considered an estimate of the true no adverse effect level, the quality of the estimate cannot be assessed. The term NOEL (no observed effect level) is used for all kinds of biological effects, including biological effects that are not considered as adverse. In the context of JECFA evaluations, NOAEL and NOEL are interchangeable since adversity is also included in the concept of NOEL (WHO/IPCS 1987). However, the general opinion in the EU risk assessment processes of existing chemicals, plant protection products and biocides is that not all biological effects are signs of toxicity and that these effects should therefore not be the basis for quantitative risk assessment. The term NOAEL has thus tended to be used more in recent years, and is used in this report.

One of the most evident limitations of the NOAEL approach is that it does not take into account the slope of the dose-response curve for the particular response of interest. NOAEL is by definition one of the doses tested, and apart from ensuring that the number and spacing of data points is adequate to provide a reasonable

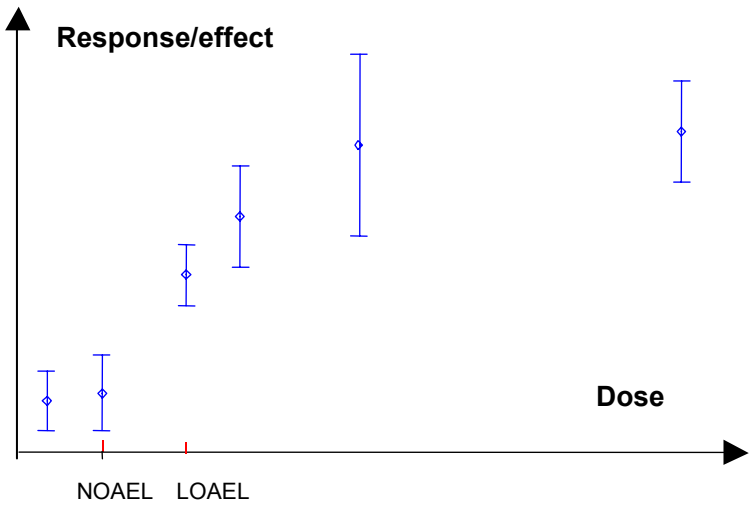

Figure 5.3 Dose response relationship showing the NOAEL (No Observed Adverse Effect Level) and LOAEL (Lowest Observed Adverse Effect Level)

estimate of the NOAEL all other data points are ignored. This implies that guideline values are set at levels dictated by experimental design, not by biological relevance. An additional limitation of the NOAEL is that the approach encourages poorly designed studies, since a study with a small number of animals tends to give a higher NOAEL. These and other limitations of the NOAEL approach have prompted alternatives. One proposed alternative to the NOAEL approach is the benchmark dose method (Crump 1984). In the benchmark dose method, a mathematical model is fitted to the entire dose response data within a study, and more biological information is thus incorporated in the resulting estimates of human limit values (e.g. ADIs) (for more information about the benchmark dose method, see Annex 1). The benchmark dose method is already in use in risk assessments by the U.S.EPA, but in Europe the method is still under evaluation and consideration.

It is sometimes not possible to find a dose level not giving rise to effect/response. In traditional risk assessment, an LOAEL (Lowest Observed Adverse Effect Level) is then set and extrapolated to a NOAEL. The extrapolation from a LOAEL to a NOAEL may be regarded as part of the dose response analysis. Analysis of several databases does support the statement that a factor of 10 or lower is adequate for this extrapolation from LOAEL to NOAEL (Dourson *et al* 1996; Dourson and Stara, 1983; Kadry *et al* 1995). However, the use of historical LOAEL/NOAEL ratios to estimate NOAEL from LOAEL may be questionable, since doses in toxicological tests are usually spaced at fixed intervals, and the observed distribution of LOAEL/NOAEL ratios primarily reflects the historical use of various dose spacing. In risk assessment reports of existing substances within the European Union, extrapolation factors of between 3-5 are used without any scientific basis. Similarly, for plant protective products a factor of 2-5 is often used. No factor has been agreed upon in the EU biocides area. The suggestion of using a default factor of up to 10 is supported by the fact that, according to OECD guidelines for repeated dose toxicity, the interval between doses should be 2-4, with a maximum of 10 in a 90-day study. Kalberlah and Schneider (1998) suggest that extrapolation from LOAEL to a no

adverse effect level should not be performed. Instead the benchmark dose method is suggested when possible, and when this is not possible the authors suggest the use of an extrapolation factor of 10.

The slope of the dose-response curve always has to be considered in a risk assessment. A moderate assessment factor may provide an adequate margin of safety if the dose response relationship is relatively steep but may not be sufficiently conservative if the dose-response curve is relatively shallow (Figure 5.4).

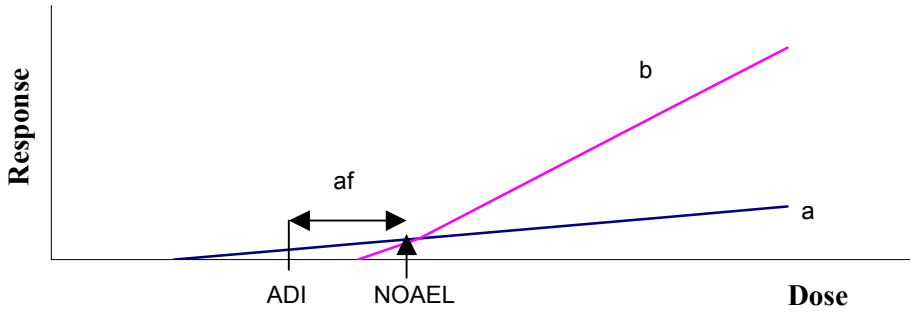

Figure 5.4 Schematic illustration of the traditional setting of an acceptable level of exposure (ADI) by dividing the dose at which no adverse effect is seen in an animal study (NOAEL) by a safety factor (af). The two hypothetical dose-response curves have identical NOAEL. If a uniform safety factor is applied, there will be an adequate margin of safety at the ADI for function “b” but not for ”a”.

The importance of the slope of the dose-response curve and the overlapping with different assessment factors such as the inter-individual assessment factor has been discussed in the literature (Clewell and Jarnot 1994; Kalberlah and Schneider 1998). In the benchmark dose (BMD) approach, the entire dose response data from a study is considered, which is one of the main advantages of this method compared to the NOAEL method, and the BMD method has therefore been suggested for instance by Kalberlah and Schneider (1998). A number of bodies, including the WHO and FAO Joint Expert Committee on Food Additives (WHO/IPCS 1987) and the Joint Meeting on Pesticide Residues (WHO/IPCS 1990), have incorporated an additional “safety factor” of up to 10 when the NOAEL is derived for an effect which is of a high degree of severity, especially if associated with a shallow dose-response relationship.

In evaluating dose-response relationships, certain difficulties arise, such as how much of the external dose is absorbed and how much reaches the target organ. The internal exposure, and especially the exposure at the target site, is more relevant than the external exposure, but we rarely have this knowledge. If the uptake is known, the total body burden could be calculated and a systemic NOAEL could be estimated. It is therefore important to include more information on the toxicokinetics of the substance of interest in order to be able to make more adequate risk assessments. One possible method to study this is (physiologically based) pharmacokinetic modelling.

Suggestion for an assessment factor to be used for the extrapolation from a LOAEL to a NOAEL

It is recommended that as much information from the studies as possible should be used. The data and the shape of the dose response curve have to be considered to estimate a dose for humans representing an acceptable safe exposure level. If data admit the use of the benchmark dose, this (in comparison to NOAEL) implies that biological information is incorporated in the resulting estimates of human limit values. However, until more endpoint-specific information is available or a consensus on risk levels is reached, both the benchmark dose approach and the traditional NOAEL approach should be used.

In general, extrapolation using the historical LOAEL/NOAEL ratio should not be undertaken in order to arrive at the dose without adverse effect from the LOAEL. The benchmark dose approach represents a more scientifically credible way of dealing with risk assessment for databases, which do not allow determination of a NOAEL. However, if it is not possible to use the benchmark dose approach, or to set a NOAEL, an extrapolation factor of 3-10, depending on the shape of the curve, is suggested for extrapolation from LOAEL to NOAEL. A LOAEL should preferably only be used in the case of a steep dose-response curve, and there is no guarantee that extrapolation of a LOAEL with any factor will yield an estimate of the NOAEL.

Inclusion of more information on the toxicokinetics of the substances of interest is also required to be able to calculate the total body burden and to make more adequate risk assessments.

5.2.6 Inter-species (animal-to-human) extrapolation

An inter-species assessment factor is necessary due to the lack of a NOAEL based on human data. When a risk assessment is based on animal data, an inter-species assessment factor should therefore be used. Although clear differences for example in the size, shape and markedness of particular functions exist between various mammalian species (including man), there are also fundamental similarities in the structure of body cells, in energy turnover and in the genetic information etc. which justify the assumption of the fundamental comparability of biological processes. The cellular makeup in terms of cellular energy supply, synthesis, cell proliferation and apoptosis as well as DNA repair mechanisms appears to be very similar in all mammalian species, including humans (Knudsen 1999).

However, it should be noted that there are species-specific differences in metabolic patterns that makes predictions between species difficult.

Account should be taken of species-specific differences between animals and humans for the extrapolation of data from animal studies to humans. The inter-species assessment factor is generally recognised as providing an extrapolation from the average animal studied to an average human being, assuming that humans might be 10-fold more sensitive than experimental animals. Species differences result from metabolic, functional and structural variations. In comparison with rodent species, the administration of the same quantities of harmful substance per kg body weight frequently results in more severe toxic effects among larger animals and man (Kalberlah and Schneider 1998). This difference in sensitivity between species is

mainly due to the fact that the body weight does not correlate with many physiological functions as well as the metabolic rate or caloric demand do (see below). The average sensitivity of man (after scaling for caloric demand) is comparable to other species. However, an extra assessment factor is needed to account for inter-species variability such as the specific cases when man is more sensitive than animals. Some examples of the many differences between humans and experimental animals that may result in higher sensitivity in man might be the higher brain weight and oxygen consumption as well as the relatively low fertility (e.g. lower sperm count; Kalberlah and Schneider 1998).

Based on calculations on body weight, a factor of 10 for inter-species variation has been suggested by WHO/IPCS (1987, 1990, 1994, 1999) and U.S.EPA (1993). Within the EU, different approaches in using assessment factors are applied in the risk assessment of plant protection products (EC 1991), new and existing industrial chemicals (EC 1967, 1993a) and biocidal products (EC 1998) (see Section 4.3).

Renwick (1993) suggested that the traditional inter-species assessment factor of 10 should be divided into 4 for differences in kinetics and 2.5 for toxicodynamic differences. The suggested factor of 4 for differences in kinetics is largely based on the extent of absorption and the rate of elimination or clearance in different experimental animals. The suggested factor of 2.5 for toxicodynamic differences is not scientifically based, but mainly the remaining value to fit the traditional total inter-species factor of 10. The toxicodynamic factor should also include possible differences in metabolic activation within the target organ because this would not be allowed for under toxicokinetic measurements. WHO/IPCS (1994, 1999) has adopted this system.

Three methods are used in practice to account for differences in metabolic size: extrapolation based on body weight, surface area and caloric demand. These methods can be described by an allometric equation, and for that purpose body weight has to be raised to the power 1, 0.67 and 0.75 respectively. Based on theoretical grounds (e.g. more rapid metabolism in smaller animals compared to larger ones), scaling on the basis of surface area or caloric demand can be considered more appropriate than extrapolation based on body weight (Vermeire *et al* 1999). Assessment factors are calculated to express the dose in mg/kg body weight. Using the factor for caloric demand ($bw^{0.75}$), these factors are: 7 for mice, 4 for rats, 2.4 for rabbits and 1.4 for dogs (for extrapolation to humans). Similarly, using the factor for surface area ($bw^{0.67}$), these factors are: 14 for mice, 6 for rats, 3 for rabbits and 1.7 for dogs. In the case of inhalation NOAELs for systemic effects, no correction has to be made for differences in metabolic size, because extrapolation is already based on the toxicological equivalence of a concentration of a substance in the air and animals and humans breathe at a rate depending on their caloric requirements.

TNO (cited in Vermeire *et al* 1999) suggested a scaling factor for metabolic size for oral studies (depending on species) and a factor of 3 for remaining variability. For inhalation, no toxicokinetic scaling factor should be used (only 3 for remaining variability). ECETOC (1995) suggested a factor of 4 for oral exposure (based on caloric demand and in the case of a rat study) and a factor of 1 for inhalation. For toxicodynamics, ECETOC (1995) suggests a factor >1 only when humans are considered to be more sensitive than the most sensitive species, otherwise no factor. The International Conference on Harmonisation recommends different factors for extrapolation from different experimental species, e.g. 5 from rats, 12 from mice, 2

from dogs, 2.5 from rabbits and 3 from monkeys. These factors are based on comparisons of surface area between experimental species and humans (EMEA/ICH 1997).

Kalberlah and Schneider (1998) concluded that wide deviations from the allometric predictions are possible in individual cases (i.e. variation in toxicodynamics). In the majority of cases the differences in both directions do not exceed a factor of 2 or 3. To the factor of 2-3, the metabolic scaling should be "added", which is a factor of 4 for a rat study and a factor of 7 for a mouse study. The total inter-species assessment factor would be 8-12 for a rat study and 14-21 for a mouse study.

In order to estimate the inter-species variability, Vermeire *et al* (1999) compared NOAELs for 184 substances (including pesticide dossiers, existing chemical dossiers, IPCS Environmental Health Criteria documents, JMPR evaluations and public literature), where mice, rats and dogs were exposed to the same compound via the same route and with the same duration of exposure. NOAELs based on carcinogenicity were left out of consideration. Two categories of exposure duration were defined: subacute and subchronic/chronic. These comparisons between experimental animals were made due to lack of human data. The actual uncertainty in extrapolating from animals to humans is likely to be at least as large as the uncertainty in extrapolating among mice, rats and dogs. The oral NOAELs were adjusted to account for differences in metabolic size. After adjustment for metabolic size with an allometric scaling factor, the remaining variability includes differences in toxicodynamics but also species-specific toxicokinetics such as different expression of metabolising enzymes. The distribution of the NOAEL ratios was described by a log-normal distribution. The geometric means (GM) of the adjusted NOAEL ratios were on average 1 ($NOAEL_{mouse}/NOAEL_{rat} = 2.4$, $NOAEL_{mouse}/NOAEL_{dog} = 1.3$, $NOAEL_{rat}/NOAEL_{dog} = 0.5$), and the geometric standard deviations (GSD) of the NOAEL ratios were on average 6 (5.7, 6.1 and 5.1 respectively). In the absence of equivalent human NOAEL data, it was suggested that this distribution (GM 1; GSD 6) would also characterise inter-species differences between animals and humans. A particular percentile of this distribution was proposed for use in establishing a default value for the assessment factor in the extrapolation from animals to humans that accounts for the remaining variability (Vermeire *et al* 1999). A re-analysis and extension of this database led to a lower standard deviation (GSD 4.5) (Rennen *et al* 2001; Vermeire *et al* 2001).

If the 90^{th}, 95^{th} and 99^{th} percentiles are calculated from such a distribution of remaining variability (probably in the main toxicodynamics), the default values would be 7, 12 and 33 respectively (Table 5.3; caloric demand not included). For extrapolation from the rat to human, the traditional factor of 10 coincides with the 73^{rd} percentile (Figure 5.5; caloric demand included). The authors discuss the possibility that the probably large noise in the NOAELs will itself boost the dispersion of the observed distributions.

An approach to study the validity of the inter-species assessment factor of 4 for toxicokinetics, as recommended by Renwick (1993) and WHO/IPCS (1994, 1999), has been undertaken by Renwick's research group by compiling published toxicokinetic data from different animal species as well as humans. A group of compounds that are metabolised by one of the cytochrome P450 isoenzymes, CYP1A2 was used in a paper by Walton *et al* (2001a). The studied compounds were caffeine, theobromine, theophylline and paraxantine, and the main common

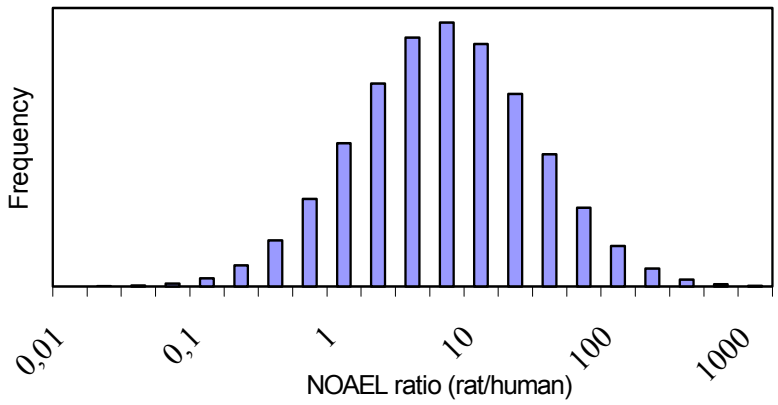

Figure 5.5 Distribution of the inter-species variability including an allometric scaling factor for differences in metabolic size (in this case 4 for extrapolation from a rat study (recalculated from Vermeire *et al* 1999, 2001).

pharmacokinetic parameter recorded for the different species was total clearance adjusted to body weight (ml/min/kg). The absorption, bioavailability and route of excretion were generally similar between humans and the animal species. The ratio of the mean clearance values for the different compounds (not all investigated in all species) compared to the human data were 10.6 for the mouse, 5.4 for the rat, 2.6 for the rabbit and 1.6 for the dog. Thus, the 4.0-fold default factor was exceeded for both the rat and the mouse.

Table 5.3 Geometric mean (GM), geometric standard deviation (GSD) and 90th, 95th and 99th percentiles for a log-normal distribution of variability in toxicodynamics[1)] based on NOAEL ratios in studies with mice, rats and dogs (calculated using the distribution parameters given in Vermeire *et al* 2001).

	GM	GSD	73rd	90th	95th	99th
NOAEL ratios for different species	1	4.5	2.5[2)]	7	12	33

1) More correctly variability other than in metabolic size

2) Corresponds to the traditional default factor for toxicodynamic variation

In a further study (Walton *et al* 2001b), pharmacokinetic data were compiled for a number of drugs that undergo extensive glucuronidation either in humans or in one of the animal test species. As in the previous study, the primary pharmacokinetic parameter used to estimate the internal dose was clearance adjusted to body weight (ml/min/kg). Oral clearance was the preferred parameter. There were major inter-species differences in the nature of biological processes which influences the internal dose, including the route of metabolism, the extent of presystemic metabolism and enterohepatic recirculation. There was also a wide variability in the magnitude of differences in the internal dose for all of the test species. When all data were considered together, the mean values for the oral clearance ratios compared to humans were 4.5 for the mouse, 9.1 for the rat, 8.7 for the rabbit and 9.7 for the dog. Thus, the 4-fold default factor was exceeded for all test species. According to the authors, the highly variable database for the glucuronidation metabolic pathway cannot support the determination of a pathway-related default value for a specific test species to replace the toxicokinetic default of 4.0.

Suggestion for an assessment factor to be used for the inter-species extrapolation

An inter-species assessment factor is needed when a risk assessment is based on animal data. This factor has to account for differences in metabolic size (the major part of toxicokinetics that can be described with an allometric scaling factor) and also allow for cases where man is more sensitive than the experimental animal studied (mostly toxicodynamics, but also including qualitative differences in toxicokinetics). The size of the scaling factor depends on the size of the animal species on which the risk assessment is based. A scaling factor of 4 (the same value as the traditional default factor for toxicokinetics) is appropriate for rats, but should be modified if the risk assessment is based on studies in other species. The traditional factor of 2.5 for toxicodynamics, which is not scientifically based but instead set to fit into the total factor of 10, has been estimated to account for 73 % of the remaining variability (not including allometric scaling for metabolic size) among different substances. On average, no difference was found between species after adjustment for metabolic size from studies examining the actual relationship between NOAELs in rats, mice and dogs (geometric mean = 1; Vermeire et al 1999, 2001). This distribution thus only concerns differences in toxicodynamics (and qualitative differences in toxicokinetics). Taking 95% of the substances compared into account would result in an assessment factor of 12 (95th percentile calculated from the log-normal distribution).

The data on comparative pharmacokinetics of drugs in humans and different animal species might be used to create species- and pathway-related default assessment factors (Walton et al, 2001a;b). However, the highly variable data on compounds that are cleared by glucuronidation did not support this approach (Walton et al 2001b). The data collected by Walton et al indicate that the default inter-species toxicokinetic factor of 4 does not adequately cover the recorded inter-species differences.

We suggest that a species-specific default factor should be used for inter-species extrapolation regarding metabolic size. This factor should be based on differences in caloric demand and is thus for extrapolation from rats 4, from mice 7, from guinea pigs 3, from rabbits 2.4 and from dogs 1.4. The remaining variability can be described by a distribution. Which percentile of the distribution (i.e. percent of substances to be covered by the factor) should be chosen is a matter of judgement. The composite inter-species assessment factor would be 48 (12x4) if the 95th percentile is chosen, and in the case of extrapolation from a study on rats. The traditional default factor of 10 only covers the 73rd percentile of the distribution of experimental animals.

5.2.7 Inter-individual (human-to-human) variations in sensitivity

In comparison with the genetically relatively homogenous strains of laboratory animals, considerably more individual differences in the reaction to harmful substances occur in humans. Risk assessments are usually based on data from studies in similar animals of the same age. In addition, the animals are initially healthy, and are fed with the same food, etc. The animal NOAEL value is extrapolated to an exposure level that is considered to be without appreciable health risk for the general population. However, susceptibility to chemicals may depend on a number of determinants, including for example the genotype and gender of the individual, age, physiology, health status and lifestyle factors (Figure 5.6). This raises the questions whether it is possible to generalise to the average population or whether there is any particular vulnerable sub-population that should be taken into consideration in the risk assessment, and which is supposed to be protected by the risk assessment. In this section, some important factors that might influence inter-individual variation are evaluated and quantitative assessments of the possible variations are made where possible.

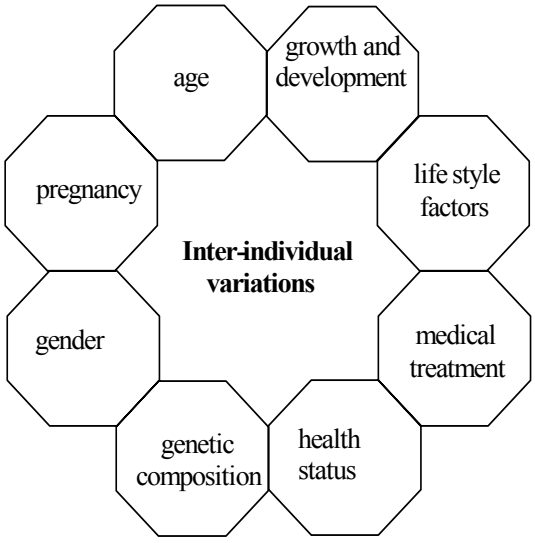

Figure 5.6 Hereditary characteristics and different conditions that may affect the inter-individual difference between humans

Some organ systems show specific vulnerability to chemical toxicity during development, as organ maturation is an ongoing process. The process is ongoing throughout the embryo/foetal period, infancy and childhood and during the pubertal period and adolescence and will not be completed until adulthood. More specific information on developmental windows of exposure will improve risk assessment by identifying the most sensitive windows. For example, the *reproductive* and *endocrine* systems mature slowly, reaching a peak immediately prior to adulthood, the *immune* system develops both pre- and postpartum, the *nervous* system develops both late during pregnancy and in the infant and child. Moreover, effects on any of these organ systems may affect the outcome of a toxic response on the other organ(s).

The central *nervous system* in particular should be considered a major target organ as its development is very complex and the developmental period is longer than for other organs. The locations and numbers of cell receptors are different in the

immature and adult individual. Brain growth in children occurs rapidly during the first two years of life, and at the age of two about three-quarters of the total number of cells in the brain is present. Subsequent growth after the age of two continues more slowly. Brain growth after birth is due to myelination of white matter, maturation of axonal and dendritic outgrowth, and multiplication of glial cells. The higher relative cerebral blood flow and immaturity of the blood-brain barrier (the blood-brain barrier is gradually developed during gestation and is not complete until around 6 months after birth) may result in increased exposure of the infant central nervous system to potential toxic substances. Furthermore, the high lipid content in the central nervous system and its relatively greater mass in children may influence the distribution and storage of xenobiotics in infants. In contrast to humans, the brain growth spurt in rats and mice is neonatal, spanning the first three or four weeks of life. This knowledge is relevant in relation to the testing of chemical substances.

In the immune system, the lymphoid organs are formed during the foetal stage and are fully developed at birth while the development of immune cells continues after birth and throughout life. The maturation of the immune system and thus the production of activated and memory T- and B cells starts after birth and is also a process that continues throughout life, driven by the exposure to antigens, e.g. infectious agents or vaccines, in combination with the genetic background of the individual. Both the foetal period of immune system development and the subsequent development and maturation after birth have been shown to be very sensitive to exposure to xenobiotics, although little is known about the mechanisms. Perturbation of the immune system by xenobiotics can thus occur at different stages in life leading to immunosuppression (decreased resistance to infections) or immunopotentiation (allergic reactions or autoimmunity).

The endocrine system is a complex network of glands that produces hormones and releases them into the bloodstream so that they can influence tissues throughout the body. These hormones are chemical messengers that affect every organ in the body. Endocrine glands also send signals to other endocrine glands, resulting in complex interactions. The major endocrine glands include the hypothalamus, pituitary gland, thyroid gland, parathyroid glands, adrenal glands, pineal gland, thymus gland, pancreas, testes and ovaries. By secreting various hormones, the hypothalamus and the pituitary gland control many normal functions, including sleep, appetite, temperature, sexual maturation and reproduction. Substances that interfere with hormonal systems may influence the development of an organism. It has been shown that a number of the chemical substances present in the environment may exert oestrogenic, anti-oestrogenic anti-androgenic, or related activities on reproduction in laboratory animals and in animals in the wild. Similar effects on humans cannot be excluded. In addition, growth hormone promotes protein building in cells and other functions that stimulate muscle and bone growth through childhood and adolescence. The thyroid gland’s main function is to convert the amino acid tyrosine and iodine into the hormones thyroxine and triiodothyronine, which regulate the body's metabolism. Thyroxine, the more abundant of the two, is necessary for normal development through infancy and childhood.

It should also be noted that internationally agreed test guidelines are at present not available for investigating effects from endocrine disruptors and immunotoxic substances.

5.2.7.1 Age and development

A human being changes, anatomically, biochemically and physiologically, during its lifetime from the conception to its death, and there may be windows of increased vulnerability or periods of human development when chemical exposures may substantially alter organ structure or function. The available guidelines appear to be designed primarily for the assessment of toxicity in adult animals, although developmental studies are required for the detection of teratogenicity. For obvious ethical reasons, few *in vivo* response data are available in humans, and most reports relate to acute responses such as haematopoietic changes associated with chemotherapy and also from accidents and misuse. Consequently, most of our knowledge is about adults and we know very little about susceptibility of elderly people, teenagers, children and the unborn child. Children, as well as the unborn child, have in some cases appeared to be uniquely vulnerable to chemical toxicants because of their biological growth and development (for example an immature blood-brain barrier). Accordingly, there has been a great deal of concern over whether children are a vulnerable sub-population and whether there should be an extra assessment factor for the difference between children and adults (see Section 5.2.1.1). The concept that infants and children may be a sensitive sub-group relates to their relative immaturity compared to adults. It should also be noted that children's exposure pattern differs from that in adults; however, exposure will not be discussed in more detail in this document.

It is important to have knowledge about normal human developmental stages to be able to understand developmental toxic effects and at what stage in life a human being may be most vulnerable to different damages. Many complex biological changes occur during development that can have profound consequences on sensitivity to the effects of exogenous chemicals. The outline below is incomplete, but is meant to illustrate one important point: the developing foetus, new-born, infant and child are not just small adults but unique populations for health risk assessment. The different stages in human development and the corresponding time periods are summarised in Table 5.4.

In female foetuses, the *germ cells* divide during foetal growth and females are born with their total number of oocytes. The primary oocyte is arrested in the first meiotic division and the second meiotic division does not take place until the time of ovulation during the adult female's reproductive life. In contrast, male germ cell formation and subsequent maturation do not start until puberty and continue throughout life. As cells are particularly sensitive to exogenous substances when dividing, this difference between men and women may indicate that the highest risk of causing mutations in germ cells occurs in the fertile period for both genders, but the female foetus may also be at increased risk during the foetal period (LaBarbera 1997; LST 1989; Johnson *et al* 1997; *all quoted in Danish EPA 2001*)

During the *preimplantation* period, the conceptus is generally considered insensitive to exogenous insults in terms of classical malformation, but exposure to certain substances may have potential for inducing embryonic death and/or latent developmental defects (e.g. DDT, nicotine). A reasonable explanation for this is that a massive insult during this developmental stage will kill the conceptus, while if only a few cells are damaged they will be replaced by other cells as the cells of embryoblast still maintain a high degree of pluripotency. (Dencker and Eriksson 1998; LST 1989; Rogers and Kavlock 1996; *all quoted in Danish EPA 2001*)

Table 5.4 Summary of stages in human development (modified from Larsen and Pascal 1998; Scheuplein *et al* 2002)

Developmental stages	Time period
Preimplantation stage	First 2 week of pregnancy
Embryonic stage	2-8 weeks of pregnancy
Foetal stage	from 9 weeks of pregnancy until birth
Pre-term birth	24-37 weeks of pregnancy
Full-term birth	38-42 weeks of pregnancy
Perinatal stage	from 39 weeks of pregnancy until 1 week after birth
Neonatal stage	Birth-4 weeks
Post-natal	After birth
Infancy	Birth-12 months (young: 0-4 months; older: 4-12 months)
Childhood	1 -12 years (from infancy to puberty) (young: 1-4 years; older: 4-12 years)
Puberty	period during which the secondary gender characteristic begins to develop and the capability of sexual reproduction is attained.
Adolescence, Teenager	12-18 years
Adulthood	>19 years (having attained full growth of maturity)

The *placenta* is the interface between the mother and conceptus. Chemical substances, which pass maternal membranes, are likely also to pass the placental barrier. In general, the foetus is therefore not protected against xenobiotics that circulate in the maternal blood. Exposure during pregnancy may affect the placenta, which may in turn affect the developing embryo/foetus. Effects on the placenta may include alteration in blood flow and perfusion, metabolism, or in extreme cases, necrosis and separation from the uterine wall (Clarke1997; LST 1989; Hakkola *et al* 1998; *all quoted in Danish EPA 2001*; U.S.EPA 1999b).

In humans, *organogenesis* (used here with the same meaning as embryogenesis) occurs approximately between 2 weeks and 2 months of gestation. The period of organogenesis is considered to be the developmental phase most sensitive to exogenously induced classical malformations in single organs and/or syndromes of malformations. Differentiating cells in various organ precursors start to develop susceptibility e.g. to xenobiotics affecting specific receptors. Lost cells can no longer be replaced by surrounding cells, and disturbances will result in permanent damage.

Foetogenesis, the period from the 9th week of pregnancy on, is characterised by tissue differentiation, growth and physiological maturation. During this period the conceptus becomes increasingly resistant to the actions of teratogens. Exposure to xenobiotics during this period is most likely to result in effects on growth and functional maturation and not effects resulting in major morphological malformations. Receptors and other molecular targets for substances affecting future functions are continuously developing, so that the foetus may be even more sensitive than the embryo to some pharmacological effects. Functional abnormalities of the central nervous system and reproductive organs, including behavioural, mental and motor deficits and decreases in fertility are among the possible adverse outcomes. These manifestations are not necessarily apparent at birth. In the 36th week of pregnancy, organ differentiation is more or less complete and the foetus is described as being fully developed and during the final weeks of foetogenesis, growth occurs. Birth normally occurs in the 40th week of pregnancy (Dencker and Eriksson 1998; LST 1989; Rogers and Kavlock 1996; *all quoted in Danish EPA 2001*).

The *perinatal* period includes the time shortly before and after birth. Parturition creates a new situation for the offspring with respect to many physiological systems. Changes in heart rate, peripheral vascular resistance and a redistribution of blood flow occur. At birth, elimination of substances across the placenta ceases and, with the collapse of the placental circulation, hepatic blood flow and oxygen supply dramatically decrease.

Infancy occurs from birth up to 12 months of age. During this year, the individual changes from a helpless breastfeeding infant to a small child that is more and more like an adult, in relation to both exposure and development. During the lactation period, the human xenobiotic metabolising systems are still immature, but the newborn is no longer protected by the maternal metabolic system. The infant may thus be highly susceptible to xenobiotics in this period. However, to some extent the newborn is still protected as the breast milk has been subject to the maternal metabolic system. Of special concern for neonatal exposure are lipid-soluble toxicants that had previously accumulated in maternal fat stores before and during pregnancy. As the body fat content returns to non-pregnant levels, the concentration of lipophilic toxicants in the rest of the body increases and this may cause enhanced potential for lactational transfer of harmful substances

.

In general, the most prominent differences in toxicokinetics are found in children less than one year of age and especially in the first few days and weeks of life (Table 5.5). By the age of 2 years, most of the biochemical and physiological parameters that affect toxicokinetics have reached maturation, although differences still exist (Lindemann *et al* 1999).

Although not proven, it is generally assumed that children, in comparison with adults, have more sensitive skin, develop contact allergy more easily, and are more sensitive to skin irritants.

During *adolescence* there are highly fluctuating hormone levels and the body is finalising its growing and developing of different organ systems. The oestradiol, progesterone and testosterone levels increase. In addition, the development is influenced by glucocorticosteroids and thyroid and growth hormones. Little is known about the variability in sensitivity of teenagers between 13 and 20. This group, for example, is not included in the clinical trials of medical substances. The maturation of the reproductive system and late maturation of the immunosystem in particular might make this sub-population especially vulnerable to exposure to chemical substances.

From about the age of 20 until death, a human being is considered to be an *adult*. This is what in risk assessment is traditionally considered to be the general population. Irrespective of other differences there might be different conditions during this adulthood, such as *pregnancy*, that might change the susceptibility to exposure to chemicals. During pregnancy, many physiological changes occur in the maternal organ system as a consequence of, and in order to support, the rapid growth of the foetus and reproductive tissues. These changes may in different ways influence

Table 5.5 Some important differences in toxicokinetics between neonates/infants and adults (Lindemann *et al* 1999, Scheuplein *et al* 2002).

Absorption	
Skin	Higher permeability in newborns compared to adults due to low keratinisation in newborns (especially pre-term neonates). Infants and young children have higher surface area to body weight ratio
Lung	Lung volume is smaller in neonates than in adults in relation to body weight. Alveolar surface increases markedly during the first 2 years of life The number of alveoli continues to increase until about age 8, after which they increase in size instead. Lung growth continues throughout childhood into early adulthood, reaching a plateau and then decreasing with age. Pulmonary vascularisation is less extensive in neonates than in adults Breathing rate varies with both age and physical activity
Gastro-intestinal (GI) tract	Neonates have a higher gastric pH, delayed gastric emptying, and a reduced and more irregular GI motility than adults. Neonates have lower pancreatic enzyme function and decreased bile secretion The GI tract is rapidly colonised after birth and thereafter gradually developed to adult conditions.
Distribution	
Total body water	Proportionally higher in neonates and infants.
Total body fat	Increase from 1-3% body weight in mid-gestation to ~16% at term, and peaks at 25% by 6-9 months postnatal. Decreases as percent of body weight over 6-7 years of childhood to adult values.
Plasma proteins	Lower xenobiotic-binding capacity in neonates and infants than in adults.
Blood-brain barrier	The blood-brain barrier reaches adult capacity at about 6 months of age
Metabolism	
Metabolism	Overall liver capacity is about 1/3 of adult capacity 3 months after birth and reaches or temporary exceeds adult capacity at the age of 2 years. The individual biotransforming enzymes mature at different times (from full capacity at birth to maturation at 3 to 4 years of age) Total cytochrome P450 levels are lower in neonates and infants than in adults (phase I) Newborns have a very low overall glucuronidation capacity. The capacity reaches adult levels at 3-4 years of age (Phase II). Maturation of glutathione metabolic capacity is complex. The isoenzymes have overlapping substrate specificities and mature at different rates.
Elimination	
Kidney function	Exceeds adult capacity in young children, reaches adult capacity within 1 year. Glomerular filtration rate is low in neonates and increases rapidly during the first year. Tubular secretion is low in neonates and reaches adult levels at 1 year.
Bile acid secretion	Bile acid secretion is low in neonates and reaches adult levels by 6 months.

the intake, absorption, distribution, metabolism and elimination of xenobiotics, and may involve the gastrointestinal tract, cardiovascular system, excretory system and respiratory system. Finally, there are signs of metabolic changes and increasing activity in certain endocrine organs.

In the case of the *ageing organism*, various metabolic processes change in comparison with the efficiency observable in the early adult stage of development. The slowing down of the metabolic and elimination processes may lead to reduced elimination of toxic substances. This results in a higher sensitivity to these substances (Calabrese, 1986). The amount of data available for the purpose of performing a quantitative estimation of differences between old people and other adults is sparse. The differences are presumably less pronounced than those between infants and adults. The following is known from animal experiments: the distribution rate in the body decreases in circulatory efficiency, the plasma-protein binding decreases, certain metabolic activities are reduced, the elimination of substances via the gall bladder is reduced and the renal clearance is reduced. These changes may produce a situation in which the elimination is reduced and as a consequence the internal exposure is increased. A higher amount of fat as a proportion of the total body weight may also increase the storage of lipophilic substances in the body (WHO/IPCS 1993).

In addition, various organ systems alter functionally with increasing age (WHO/IPCS 1993; Kalberlah and Schneider 1998). In the nervous system the density of neurons and dendrites is reduced, the receptor densities on the cell surfaces are reduced, the immune system reacts more slowly to the invasion of germs, the cardiovascular system is limited functionally as a result of arteriosclerotic symptoms and the respiratory tract has a smaller area for gas exchange. It is considered likely, although not shown, that the skin of old people, in comparison with younger persons, is more sensitive to skin irritants and that the skin is more permeable to different substances. However, adults and elderly people do not experience a reduced risk of developing contact allergy compared with young persons (Weltfriend *et al* 1996).

Suggestion for an assessment factor to be used for age and development

Most of our knowledge about vulnerability to chemicals comes from studies on adult animals, patients or in some cases healthy human volunteers, and we know very little about the susceptibility of elderly people, teenagers, children and the unborn child. However, children as well as the unborn child have in some cases appeared to be uniquely vulnerable to chemical toxicants because of their biological growth and development. In addition, there may be windows of vulnerability or periods of human development when chemical exposures may substantially alter organ structure or function. The greatest differences compared with adults occur in neonates, infants and the youngest children. Potentially vulnerable systems in infants and young children include the endocrine, reproductive (slow maturation reaching a peak immediately prior to adulthood), immune (develops postpartum) and nervous systems (develops both late during pregnancy and in the child). A proposal for an assessment factor of 1 – 10 in case of an inadequate database for risk assessment of children is made in Section 5.2.1.

5.2.7.2 Genetic polymorphism

Genetic factors participate to a varying degree in the complex interplay between the environment and an individual. These factors range from rare, highly penetrant, dominant mutations to common genetic variants that modulate an individual's response to exposure to chemicals in the environment. Examples of rare inherited disorders are xeroderma pigmentosum (XP) and ataxia telangiectasia (AT), which are associated with defective DNA repair, inefficient DNA replication or chromosomal instability. It may be pointed out that although a disorder is very rare, the heterozygous genotype (individuals having one defective allele) may not be so uncommon.

Many of the enzymes involved in the biotransformation of xenobiotics are polymorphically distributed in the human population. Genetic polymorphism is defined as the existence of at least two different alleles, with allele frequencies exceeding 1 %, at a particular genetic locus. The allelic variants include point mutations, which may or may not lead to an amino acid shift, as well as deletions and insertions. Deletion of the whole gene (null allele) has been described for the glutathione transferases (GSTM1 and GSTT1), and genetic variants involving duplication of the whole gene have been found for cytochrome P450 (CYP) 2D6, for example. Genetic variants in the non-coding regions of various genes have also been found, which may modulate the basal or induced levels of the corresponding protein. The phenotype, corresponding to a particular genotype, is in many cases unknown. An amino acid shift may cause an increase, a decrease, or no change in enzymatic activity. Furthermore, the change may depend on the substrate of the enzyme.

Toxicokinetics

There are many examples in the literature showing a more than tenfold variation in metabolic capacity depending on genetic polymorphism in biotransforming enzymes (Kalberlah and Schneider 1998; Tables 5.6 and 5.7). The effects of genetic polymorphisms are highly dependent on the compound being metabolised as well as on which enzymes are involved. This may vary in different tissues and cells. As an example, the amino acid substitution at residue 104 of glutathione transferase P1 results in a decreased activity towards 1-chloro-2,4-dinitrobenzene, whereas the glutathione conjugation of the benzo(a)pyrene metabolite BPDE is increased. Furthermore, a particular genetic variant may be associated with an increased risk of one effect and a decreased risk of another effect of the same compound. Lack of GSTT1 (homozygosity for the null allele) thus appeared to increase the formation of protein adducts and decrease the neurotoxic effects caused by high exposure to methyl bromide (Garnier et al 1996).

The inter-individual differences in the genetics of drug metabolising enzymes affect the outcome of drug therapy in a pronounced manner. The effective dose to yield the same plasma level, for example, of the antidepressant nortriptyline should therefore be 20 mg for patients lacking a functional enzyme that metabolises the drug (CYP2D6) as compared to 500 mg to patients having several copies of the gene encoding CYP2D6. Similarly, the right dose of warfarin differs about tenfold between patients having or lacking functional CYP2C9, and it is anticipated that about 10-20 % of all adverse drug reactions could be avoided by predictive genotyping.

Table 5.6 Examples of inter-individual variation in enzymatic activities in human liver microsomes (n=14).

Probe substrate	Enzyme	Fold variation	Ref
7-Ethoxyresorufin	CYP1A2	20	Bogaards *et al* 2000
Coumarin	CYP2A6	34	Bogaards *et al* 2000
Chlorzoxazone	CYP2E1	4.5	Bogaards *et al* 2000

Table 5.7 Examples of inter-individual variation in *in vivo* phenotyping.

Probe substrate	n	Enzyme	Fold variation	Ref
p-Aminosalicylate	88	NAT1	7	Vaziri *et al* 2001
Caffeine	95	CYP1A2	10	Vaziri *et al* 2001
Dextromethorphan	24	CYP2D6	4600	Labbé *et al* 2000
Chlorzoxazone	95	CYP2E1	10-15	Lucas *et al* 1999
Chlorzoxazone	145[1)]	CYP2E1	Approx. 100	Lucas *et al* 1999

[1)] incl alcoholics

It is also important to acknowledge that many compounds are metabolised in more than one step, or by different competing pathways, involving several different enzymes, which all may vary between individuals. Benzene poisoning, for example, has been shown to be dependent on the individual activity of two biotransformation enzymes, CYP2E1 and NAD(P)H: quinone oxidoreductase (NQO1). Individuals having high CYP2E1 activity (probably partly dependent on genetic factors) and the low activity NQO1 genotype were found to have a seven-fold increased risk of hematotoxicity compared to individuals with opposite features of these enzymes (Rothman *et al* 1997).

Toxicodynamics

In addition to polymorphisms in biotransformation enzymes, which may affect the toxicokinetics of a chemical, some genetically determined variations in toxicodynamic processes have also been described. Genetic polymorphisms are present in many receptor genes, for example the D2 dopamine receptor or the β_2-adrenoceptor, but the functional significance of these variations has been less studied, and the importance of this genetic variation for cell signalling is consequently uncertain.

Humans also display marked inter-individual variability in capacity to repair damaged DNA. This variation is partly due to genetic factors, and genetic polymorphisms have been found in several proteins involved in DNA repair (Duell *et al* 2000; Kohno *et al* 1998; Lunn *et al* 2000)

Ethnic differences

The polymorphisms in genes encoding drug- and xenobiotic metabolising enzymes are far more prominent than those seen in genes encoding enzymes of endogenous importance with defined physiological functions. The basis is inherent in the fact that the former have evolved because of adaptation to the environment, with emphasis on the particular diet. Because of genetic drift, where a small population has migrated and then expanded, the inter-ethnic differences in the distribution of the genes encoding xenobiotic metabolising enzymes are sometimes large. As an example, the frequency of the GSTT1 null genotype is 10-15 % in northern Europe and 50–60 % in China. There are also examples of alleles that are only found in some populations, such as the African-specific [191]NAT2 (*14) allele. Drug metabolism, as well as

perhaps sensitivity to xenobiotics, is therefore remarkably different in different parts of the world. It should be noted that, within a geographical area, a population may consist of several ethnic groups.

Interplay with physiological and environmental factors

In addition to genetic factors, the variation between individuals in the enzymatic activity of an enzyme also depends on physiological and environmental factors, such as diet. The interplay between genetic factors, chemical exposure and dietary components is very complex. It has, for example, been shown that the inhibitory effect of isothiocyanates, found in vegetables, on lung cancer development depends on the genotypes of GSTM1 and GSTT1, which metabolise this food component as well as many substances found in tobacco smoke (London *et al* 2000).

Individual versus population risk

Although the risk to an individual in many cases appears to be only slightly modulated by a single polymorphism in the gene for a xenobiotic-metabolising enzyme, this may strongly affect the risk in a whole population. As an example, the GSTM1 deficiency is a moderate risk factor for lung cancer development, and yet it was calculated that GSTM1 deficiency accounts for approximately 17 % of lung cancer cases because of the high prevalence of GSTM1 null (approx 50 %) (McWilliams *et al* 1995). In addition, it has been estimated that for a rare inherited disorder, ataxia telangiectasia, the heterozygous genotype, which occurs in about 1 % of the U.S. population, may be responsible for more than 7 % of breast cancer cases in the United States (Perera 1996).

Suggestion for an assessment factor to be used for genetic composition

There are many examples in the literature showing a more than tenfold difference in metabolic capacity depending on genetic polymorphisms in biotransformation enzymes. It is also important to note that many chemicals are metabolised in several steps by more than one enzyme, and that the total inter-individual variation is dependent on the variability in each step. A large variation in metabolic capacity does not necessarily correspond to an equal variation in toxicity. Knowledge concerning genetic polymorphism in toxicodynamics is limited. It is not possible to suggest a default assessment factor for genetic composition, but it must be kept in mind that inter-individual differences in sensitivity due to genetic composition may be substantial.

5.2.7.3 Gender

Women and men differ from each other in some constitutive and physiological parameters. For example, weight, tidal volume and the water and fat content of the body differ between genders. However, the differences become smaller when normalised according to body weight and surface area. There is little information on environmentally related health effects that are manifested differently in women compared to men. Both biological events and non-biological gender factors in women's lives can affect the exposure to chemicals, as well as the kinetics and toxicity. For example, a recent study on Swedish twins revealed that blood concentrations of lead and cadmium are governed by genetic factors far more in women than in men (Björkman *et al* 2000).

Physiological factors that may influence kinetics and toxicity of chemicals within a woman's body include changes in relation to the onset of menstruation, pregnancy,

lactation and menopause (Silbergeld and Flaws 1999). For example, the blood output from the heart increases during the course of pregnancy by 50%. According to Kalberlah and Schneider (1998), differences in physiological parameters that may influence the toxicokinetics of harmful substances are, as a rule, below a factor of 2. On the basis of animal data, Krasovskii (1976) states that in the case of rats, gender-specific differences do not exceed a factor of 2-3 in sensitivity in tests performed on 149 substances. Only with regard to phosphate esters were female animals approximately 3-4 times more sensitive (no information on the endpoint).

Human epidemiological data, as well as animal data, indicate that females are more susceptible than males to the toxic effects of ethanol (*quoted in Danish EPA 2001*). It is also generally assumed that women have a, genetically determined, more sensitive skin than men have; however, this has not been proven. Women have hand eczema and nickel allergy more commonly than men. This is due to the fact that women are more exposed to wet work both at work and in leisure, and also to contact with nickel (Weltfriend *et al* 1996, Meding 2000).

An example of differences in susceptibility between genders is cadmium-induced toxicity. In contrast to other toxic metals, the internal dose of cadmium is generally higher in women than in men (Järup *et al* 1998). A likely explanation is that low iron status, which is prevalent in women of childbearing age and pregnancy, leads to increased cadmium absorption from the diet which is the main source of exposure besides smoking (Åkesson *et al* 2002). Cadmium accumulates in the kidney cortex, and the critical effect is renal tubular damage, which is already detected at present levels of environmental exposure (Hellström *et al* 2001). Although a neglected research area, a few studies indicate an increased risk of cadmium-induced renal damage in women (Yamanaha *et al* 1998; Oo *et al* 2000, Hellström *et al* 2001). Whether this is due to a higher internal dose of cadmium in women compared to men (due to higher absorption) or to an increased susceptibly to cadmium-induced kidney damage is not known. Cadmium may also cause bone damage, which has occurred more frequently in women than in men. The most advanced form of chronic cadmium intoxication (Itai-itai disease, a combination of severe osteomalacia and osteoporosis) almost exclusively struck elderly Japanese women consuming contaminated rice (Kjellström 1986).

Another example is the recently indicated decline in male birth ratio associated with male foetal death due to methyl mercury exposure in Japan (Sakamoto *et al* 2001). Similarly, maternal smoking was shown to affect foetal growth more in the male foetus than in the female (Zarén *et al* 2000), while both maternal and paternal smoking at the time of conception decreased the male:female sex ratio of the newborn infants (Fukuda *et al* 2002). The male:female offspring ratio also declined following exposure to dioxin in Seveso (Mocarelli *et al* 2000). Stress may have a similar effect as chemicals. An abrupt reduction in sperm mobility was observed after the Kobe earthquake, and nine months later there was a significant decline in the male:female sex ratio of the newborns (Fukuda *et al* 1998).

Great differences are, of course, also to be expected if harmful substances have specific mechanism(s) of action that affect gender-specific differences, i.e. as a result of interaction with hormonal regulation, specific damage to the sex organs or adverse effects on organs in the development of the infantile organism, since fundamentally different mechanisms of adverse effect are frequently present here.

Suggestion for an assessment factor to be used for gender differences

There is little information on environmentally related health effects that are manifested differently in women in comparison with men. Both biological events and non-biological gender factors can affect the exposure to chemicals, as well as the kinetics and toxicity. There is at present no reason to believe that a default value could be used for gender-related differences in toxic response. Instead, a case-by-case expert judgement has to be made for each scenario and population studied.

5.2.7.4 Disease

Limited information is available on pre-existing diseases as a general risk factor for increased sensitivity to chemicals. However, in general a diseased state can be expected to influence the sensitivity to harmful substances. This is of special concern when the target organ of the toxic effect is affected by the disease or when the metabolic conversion is disturbed. In addition to obvious diseases of important organs such as the lungs, the liver or the kidneys, hereditary or acquired characteristics, such as acatalasemia, glucose-6-phosphate dehydrogenase deficiency, immunodeficiency, hypersensitivity, ataxia teleangiectatica and xeroderma pigmentosum may also influence sensitivity to foreign substances. Risk factors for widespread diseases such as cardiovascular diseases, diabetes and neurological diseases are also of importance.

A few examples of the influence of diseases on the risk assessment of chemicals are given below.

As a result of reduced elimination*, liver diseases* may also have an effect on the metabolism of harmful substances. In the case of nicardipine, a calcium blocker, the AUC was approximately 4.5 times greater among persons with liver diseases. The effect on blood pressure was much more marked in these cases (Renwick 1993). In a similar way, the toxicokinetics of acetaldehyde (metabolite of ethanol) is influenced by previous damage to the liver caused by alcohol. The maximum blood concentration and AUC differ between persons with a liver disease caused by alcohol and control persons by a factor of 3.3 and 2.8 respectively (Wicht *et al* 1995).

Persons with *lung diseases* such as asthma and chronic obstructive lung disease, children and the elderly are generally regarded as sensitive groups with regard to air pollutants. Controlled inhalation studies with nitrogen dioxide have demonstrated that persons with mild asthma react with an increased airway responsiveness at about ten times lower concentrations than normal subjects do, when compared as group means. The inter-individual variation can be much higher. Decreased lung function also appears at lower concentrations in patients with asthma and chronic bronchitis compared to normal subjects. The same is also true for other pollutants such as sulphur dioxide. In contrast, the effect of ozone on lung function and bronchial responsiveness is generally similar in patients with asthma as in normal persons. However, asthmatic subjects are more sensitive to inflammatory reactions in the airways caused by ozone than normal persons are. In epidemiological studies, effects on lung function in children have been observed at lower concentrations than in adults (Berglund *et al* 1993; Bylin *et al* 1996).

An atopic is an individual with *immunologically mediated allergy* (an IgE-dependent allergy) for a particular allergen such as pollen, food constituents or chemicals.

Atopics may develop life-threatening reactions at an exposure level that is insignificant for the population in general. The most dangerous example of such reactions is the anaphylactic response, and several deaths have been reported. Other symptoms are asthma and rhinitis.

Most subjects with atopic diseases have, by definition, an abnormal propensity to produce IgE antibodies and become sensitised to any kind of respiratory allergens. Consequently individuals, although currently not expressing sensitivity for example to a new occupational allergen, have a significantly increased risk in environments with strong allergens of developing IgE responses and as a consequence a risk of developing diseases such as asthma. In individuals with non-immunological hypersensitivity (i.e. not IgE-mediated) asthma and rhinitis but not an anaphylactic shock may be elicited by the allergen.

Respiratory hypersensitivity is induced by the inhalation of antigens present in our environment (e.g. pollen, foodstuffs, chemicals). In addition, there might be a genetic component making certain individuals more and others less prone to allergenic and non-allergenic stimuli resulting in allergy and other hypersensitivities. It is also suggested that environmental factors such as diesel exhaust particles may act as adjuvant factors, enhancing the sensitisation response to common airborne allergens. Such interactions may also contribute to the phenomenon of bronchial hyper-responsiveness. Many individuals with allergy (exclusively IgE-mediated) and other hypersensitivity diseases (immunological as well as non-immunologically mediated) are in addition hyper-responsive to exposure to a wide diversity of environmental risk factors, factors that by themselves do not cause allergic reactions. This is particularly prominent in the airways for asthmatics and is often termed bronchial hyper-responsiveness.

15-20% of adults in the general population are *contact allergic* to one or more substances. Nickel, chromate, preservatives, perfumes, colophonium (rosin), rubber chemicals and plastics are among the most prominent contact allergens. Nickel holds an exceptional position as 15% of women and 2-5% of men are allergic to nickel, and 30-40% of nickel-sensitive people develop hand eczema. More than 3,700 different chemical substances have been identified as contact allergens. Allergy to proteins in natural rubber latex (latex allergy) is an increasing problem due to sensitisation among health-care workers caused by protective rubber gloves, and among some groups of patients. Up to 10% of operating-room staff are allergic to latex. A person with latex allergy may also develop mild or severe symptoms (contact urticaria, allergic rhinitis, asthma and anaphylactic shock) after contact with balloons, condoms, other rubber products and some fruits and vegetables.

10% of the adult general population have *hand eczema*. The most important causes and background factors are atopic dermatitis in childhood (see below), wet work and contact allergy, primarily nickel allergy. Several factors act together. Hand eczema is most common in young women. The skin of the hands, in people with ongoing hand eczema, has increased sensitivity to aggravating factors (allergens and irritants). This increased sensitivity also persists for months after the dermatitis has healed, due to an injured skin barrier function.

Atopic dermatitis is becoming more prevalent. The reason for the increase is not fully understood, but environmental factors are thought to play a role. Today, 20% of schoolchildren have, or have had, atopic dermatitis. This dermatitis often heals

before adulthood, but it does also often recur as hand eczema. People who have or have had atopic dermatitis have increased susceptibility to skin irritants such as water, cleaning agents, surfactants, organic solvents, alkaline and hygroscopic substances, mineral fibres and friction. The skin is also dry, itchy and prone to develop eczema after the dermatitis has healed, and is more sensitive to skin irritants than in other persons. This explains why atopic dermatitis in childhood is one of the most important causes of hand eczema in adults.

There is a general assumption that persons with atopy have a genetically determined increased risk of also developing contact allergy. However, atopics are not affected more than others by contact allergy, only to substances in topical medicines to which they are exposed (topical steroids, preservatives etc).

Suggestion for an assessment factor to be used for diseases

A diseased state can be expected to influence the sensitivity to harmful substances, especially when the target organ of the toxic effect is affected by the disease or when the metabolic conversion is disturbed. However, there is no proposal at present for the use of a default assessment value. Instead, a case-by-case expert judgement has to be made for each scenario and population studied.

The assessment of risks with respect to allergy and other hypersensitivities includes several aspects that differ from the assessment of toxic agents in general. For example, based on current knowledge, it is generally not feasible to set threshold values. The EU criteria for classification and labelling are aimed at classifying and labelling chemical products that can induce – not elicitate - inhalation and dermal sensitisation. However, the general classification limit (1% for the substance and products containing 0.1% of a classified allergen respectively) is purely administrative, and sensitisation as well as elicitation to many substances can occur at levels far lower. There are limited ways at present of protecting against induction, and to a certain degree elicitation, of contact allergy; one example is through legislation as in the Nickel Directive (EC 1994c) and also the Cosmetics Directive (EC 1976b).

5.2.7.5 Life-style

The toxic effects of alcohol, tobacco smoke and drugs often far exceed those from environmental toxins and may also modify their toxic response. For example, ethanol can cause elevated levels of many enzymes involved in biotransformation. A critical issue is ethanol-inducible cytochrome P450 2E1 (CYP2E1), which is the major enzyme responsible for the metabolism of organic solvents and some pre-carcinogens. Prolonged ethanol intake causes irreversible damage in the central nervous system and in the liver, resulting in marked decreased capacity for detoxification and increased sensitivity to xenobiotics. Tobacco smoke is considered to be one of the more severe confounders in epidemiological studies, due for example, to its ability to affect enzyme activities and to cause various health effects. One of the most flagrant effects of tobacco smoke is its ability to increase asbestosis mortality and the risk of lung cancer in persons exposed to asbestos. Commonly used analgesics such as paracetamol may also influence the toxicokinetics of organic solvents. Nutritional factors such as a poor or an unbalanced diet may influence the intake and probably also the biotransformation of food pollutants. Ethnic differences due to variations in habits (e.g. dietary), genetic composition etc. might also affect the outcome of a risk assessment.

Suggestion for an assessment factor to be used for life-style variations

Although the effects of various "life-style factors" on risk assessment are obvious, there is at present no proposal for the use of a default value. Instead, a case-by-case expert judgement has to be made for each scenario.

5.2.7.6 Literature review on the inter-individual assessment factor

Calabrese (1985) found considerable differences among human subjects in their capacity to metabolise foreign substances. The range of responses varied widely, depending on the substance, enzyme and organ considered. Furthermore, it was apparent that human variation might range up to two or three orders of magnitude, indicating that human variation in the metabolism of various xenobiotics can exceed a factor of 10. The author's conclusion was that a factor of ten would be sufficient to protect the majority (80-95%) of the human population against adverse health effects in most cases. The remaining 5-20% of the population exhibit responses outside of the 10-fold range of variation, but whether and to what extent these individuals may be at increased risk is not generally known.

On the basis of 101 datasets relating to 49 substances (mostly drugs), Hattis *et al* (1987) examined the variability of various toxicokinetic parameters in healthy adults. In the case of parameters for internal exposure (area under the curve as well as maximum plasma concentration) the variability with the dose was slightly higher than for the half-life. For the median chemical, a tenfold difference in these pharmacokinetic parameters would correspond to 7-9 standard deviations in populations of "normal healthy adults", while for one relatively lipophilic chemical a tenfold difference would correspond to 2.5 standard deviations in the population. Thus, according to Kalberlah and Schneider (1998), a factor of 10 ensures protection of >>99% of the population if the average behaviour of the substances is taken as the basis. In the case of the substance for which a high degree of variability was observed, a factor of 10 (for toxicokinetic variability only) signifies protection of about 99% of the studied populations. In order to achieve a level of statistical certainty amounting to approximately 95% a factor of 4.5 would be required for such substance. Re-analysis of the data of Hattis *et al* (1987) showed that the variation between individuals for the plasma half-life was quite small (Schaddelee 1997*, quoted in Vermeire et al 2001*). Defining the inter-individual factor as the ratio of the P_{50} and P_{05} resulted in a factor of 1.4. It should be noted that only data on healthy adults were available, and it should also be taken into account that variation exists in pharmacodynamics. The human variation in sensitivity is thus underestimated.

Renwick (1991) discussed toxicokinetics and toxicodynamics variability separately. In justifying a toxicokinetic sub-factor, he takes as his starting point 7 toxicokinetic studies on 8 substances in healthy adults (Renwick1993). In justifying a toxicodynamic sub-factor, he used 8 toxicodynamic studies on 6 substances. The toxicokinetic coefficients of variation within the study groups were 36, 456, and 113 %, for the substances min, max and mean respectively. The toxicodynamic coefficients of variation for the substances within the study groups were 7, 73, and 40 % for min, max and mean respectively. The data indicated that toxicokinetic differences were generally greater than toxicodynamic differences. With one exception, the ratios between the maximum and mean value for a substance toxicokinetic parameter in these investigations amounted to approximately a factor of 4. Renwick concluded that a factor of 3-4 was sufficient to consider toxicokinetic

differences for 99% of the healthy, adult population and for 80% of the substances. As a result, Renwick proposed subdividing the inter-individual factor of 10 into one toxicokinetic factor of 4 and one toxicodynamic factor of 2.5. Re-analysis of the data using distributions instead of max/mean ratios produced comparable results (Schaddelee 1997, *quoted in Vermeire et al 2001*).

The IPCS (WHO/IPCS 1994) has to a large extent followed the proposal made by Renwick, with the exception that the 10-fold factor is equally divided into 3.16 for both toxicokinetics and toxicodynamics. This is based on the assumption that the slightly greater variability in the kinetic data compared with dynamics is not sufficient to warrant unequal subdivision of the 10-fold factor into a toxicokinetic factor (4) and a toxicodynamic factor (2.5).

Kalberlah and Schneider (1998) proposed an inter-individual factor of 25 consisting of a sub-factor of 8 and 3 for toxicokinetics and toxicodynamics respectively. The toxicokinetic factor of 8 is based on a factor of 5, from toxicokinetic evaluations performed by Hattis *et al* (1987) and Renwick (1993) relating to investigations with healthy adult test persons, and a factor of 3 for enzyme polymorphisms, based on individual data in the range of 3 - 10. According to the authors, these data were dependent and thus not multiplicative but additive (5+3=8). Since toxicokinetics and toxicodynamics were regarded by the authors as independent parameters, these two factors are multiplicative (3×8=24~25). The toxicodynamic database is limited in relation to toxicodynamic differences in the general population. Evaluations on the basis of a few examples indicate a range of up to sevenfold difference in sensitivity, while the majority of substances can be considered using a factor of 3. Kalberlah and Schneider are not of the opinion that it is necessary to regard children or old people as being generally more sensitive than adults. However, in particular cases children may show higher sensitivity due to specific differences in toxicokinetics and toxicodynamics, while data on older people are rare and require further validation.

Silverman et al (1999) used data from clinical trials on six pharmaceutical compounds to study the variability in pharmacokinetic parameters (AUC and C_{max}). The mean values and standard deviations for each study were used to create log-normal probability distributions. The ratio of the upper 95^{th} confidence limit to the mean value was used for data-derived assessment factors. Of the 15 possible data-derived assessment factors calculated, 3 exceeded the default factor of 3.16 for toxicokinetics.

Renwick and Lazarus (1998) analysed the default uncertainty factor for human variability, based on the evaluation of a relatively extensive database, especially on variability in toxicokinetics. Data for the kinetics of 60 compounds were identified, representing a range of pathways of metabolism or clearance. The mean coefficient of variation was 38 % (range 9-114 %). It is not clear whether the studies are representative of the whole population, since it is not stated how many of the studies are performed on “healthy adults”. In addition, about one quarter of the studies contained small numbers of subjects (n<10). Data for dynamics were identified for 49 compound related effects; the majority were short-term changes in cardiovascular or CNS measurements. The mean coefficient of variation was 51 % (range 8-137 %). These data indicate that variability in toxicodynamics is greater than variability in toxicokinetics. However, according to the authors this difference in variability is not sufficient to warrant an unequal subdivision in favour of dynamics since the data

included a number of receptor-mediated clinical responses in patients. Factors such as aging and disease processes may have contributed to the greater variability in dynamics compared to kinetics, since unlike the kinetic data much of the dynamic data was for the clinical treatment of patients. In the study, the proportion of a population not covered by the default factor was estimated by multiplication of the proportions for each of the 3.16-fold sub-factors. According to the author, the data and analysis performed indicated that the 10-fold factor due to variability between individuals is an appropriate default value and would cover the vast majority of the population (99.9%) based on the *mean* coefficients of variation. A number of factors to be considered in the interpretation of population distribution analysis were identified, e.g. not all data are normally or log-normally distributed as was assumed here, the subjects should be representative of the group as a whole but here most data refer to healthy young adults, factors such as aging an disease processes may add to the variability estimation and this estimation should be most important for hepatic and renal changes since these would affect the fate of most foreign compounds, the variability considered in this paper is derived from single exposures while repeated exposure would be more relevant for chronic exposure. In addition, genetic polymorphism can have a profound influence on the validity of a 3.16-fold default factor for kinetics, but does not automatically invalidate the default values. Genetically determined differences are of greatest relevance to risk assessment when the polymorphic pathway represents the major route of elimination.

Renwick and his group are setting up a database on compounds identified from ongoing literature searches (Renwick and Lazarus 1998; Renwick *et al* 2000; Renwick 2001). The validity of the default factor of 3.16 for human variability in toxicokinetics was analysed (Dorne *et al* 2001a,b; 2002), using kinetic data mainly obtained from human drug trials. In the paper by Dorne *et al* (2001a), the human variability in the kinetics of substrates for CYP1A2, which is a major human cytochrome P450, was studied using published kinetic data on caffeine, theophylline, paraxanthine and theobromine. The variability in clearance and AUC (area under the plasma concentration – time curve) after oral or intra venous dosage ranged from 34 to 42% (coefficient of variation, CV) in healthy adult volunteers when the results with the different substrates were pooled. The pooled results for the different substrates allowed a calculation of the percentage of a population covered by the 3.16 default factor. At least 99 % of the healthy adult population would be covered, assuming a log-normal distribution. Depending on the route and the substrate, pregnant women, the elderly, infants and patients with liver disease would not be covered by the 3.16 factor. In addition, the default factor would be totally inadequate for neonates (99 – 100 % not covered) and pregnant women at term (approximately 50 % not covered). The authors (Dorne *et al* 2001a) suggest an additional assessment factor for risk assessment of CYP1A2 substrates in neonates as previously mentioned by Renwick (1998) and Renwick *et al* (2000).

Data on the pharmacokinetics of drugs that are metabolised by the polymorfic enzyme CYP2D6, were compiled by Dorne *et al* (2002). The pooled results from a large number of studies with different drugs allowed comparisons between the geometric mean of clearance and AUC in healthy adults and different subgroups ((poor and extensive metabolisers; children (only one substance but including 173 children); and the elderly)). According to this pooled analysis, most subgroups would not be covered by the kinetic default factor of 3.16. CYP2D6-related factors necessary to cover 95-99 % of each subpopulation ranged from 2.6 to 4.1 in non-

phenotyped and extensive metabolisers to 15 - 18 in poor metabolisers, 5.0 – 8.4 in the elderly and 22 – 45 in children.

The appropriateness of the default factor has also been investigated for glucuronidation using an extensive database of substrates metabolised primarily by this pathway (Dorne *et al* 2001b). Low inter-individual variability (about 30 – 35 %) was found for the kinetic parameters in healthy adults, indicating that more than 99 % of this group would be covered by the default kinetic factor of 3.16. A pooled analysis identified neonates as the most susceptible subgroup, although 99 % of children, infants and the elderly would be protected. The 3.16 factor was also insufficient for patients with liver or kidney disease.

Similarly, Hattis and co-workers are involved in a U.S.EPA-funded effort to analyse existing information on human variability in susceptibility to non-cancer effects (Hattis *et al* 1999b). For instance, a children's pharmacokinetic database has been compiled by Hattis group, which enables comparison of pharmacokinetic parameters between children and adults, using published literature data for 45 drugs. The main common pharmacokinetic parameter was the half-life of the drug. The analysis indicated that premature and full-time neonates tend to have 3 to 9 times longer half-live than adults. This difference disappears by 2-6 months of age. Beyond this age, half-life can be shorter than in adults for specific drugs and pathways. As the range of neonate/adult half-life ratios exceeds the default assessment factor of 3.16, this factor may not be adequate in the early postnatal period (Ginsberg *et al* 2002).

Hattis and co-workers have also investigated differences in toxicodynamics (Hattis *et al* 1999a). Data were gathered for both local effects (respiratory effects and eye, nose and skin irritation) and some systemic effects. The variability in these parameters was higher than the toxicokinetic variability reported earlier. Based on these distributions, Hattis *el al* (1999a) attempted to estimate the incidence of effects that would be expected for a tenfold lowering of exposure from a 5% incidence level (approximately equivalent to a NOAEL) if the population distribution of susceptibility is also log-normally distributed in the extreme tails. The results indicated that a tenfold reduction in dose would correspond to effect incidences ranging from slightly less than one in 10,000 for a median chemical or median response, to a few per 1000 for chemicals and responses that have greater human inter-individual variability. According to the authors, the use of the traditional 10-fold assessment factor may allow appreciable incidences of responses in some cases. Thus, according to the author, if the underlying estimates of the extent of log-normal response variability are approximately correct, use of the traditional tenfold assessment factor, without any other protective factors, would appear to run the risk of response incidences that are high enough to be of some concern.

Some probabilistic distributions have also been proposed (Slob and Pieters 1998; Baird *et al* 1996; Swartout *et al* 1998; Price *et al* 1997). Baird *et al* (1996) proposed a distribution on the basis of acute toxicity data on heterogeneity in rats and on the basis of assumptions on the unknown heterogeneity between rats and humans. This approach has been considered for instance by Vermeire *et al* not to be relevant for humans (Vermeire *et al* 2001). Slob and Pieters 1998; Swartout *et al* 1998; Price *et al* 1997 proposed a theoretical distribution which they considered to be consistent with the current use of the default value of 10. Slob and Pieters (1998) assumed the default 10-fold inter-individual factor to be conservative and to correspond to the 99th percentile of a log-normal distribution. Since the factor cannot be lower than 1, the

geometric mean of the distribution is 4 (1+3) with a geometric standard deviation of 1.6. (For more information on the probabilistic risk assessment, see Annex 2).

Suggestion for an inter-individual assessment factor derived from a literature review

To account for variability between individuals, a factor of 10 has generally been assumed to be appropriate for deriving health-based limit values. Although the 10-fold factor might be reasonable in many cases, it is quite easy to find cases where it is not. It should be borne in mind, for instance, that variation exists in toxicodynamics, which is not always considered, and when genetically determined differences may be involved the variation may be several orders of magnitude. Furthermore, the data sets provided on toxicokinetics are mostly from studies on healthy adults or patients and not reflecting the general population. Circumstances that may affect this variation are, for example, age, health status, gender, medical treatment and pregnancy.

On the basis of the toxicokinetic evaluations performed by Hattis et al (1987) and Renwick (1993) relating to investigations with healthy adult test subjects, a factor of 4-5 for toxicokinetic differences seems appropriate to protect the corresponding population, i.e. healthy adults (Kalberlah and Schneider 1998). According to Renwick and Lazarus (1998) the 3.16-fold inter-individual factor for toxicokinetics previously suggested (WHO/IPCS 1994) is sufficient to protect the vast majority of the population. However, this conclusion was based on the mean coefficient of variation within the studied groups of the substances investigated. In comparison, in the study by Hattis et al (1987) a factor of 10 (for kinetics) ensures protection of >99% of the population if the average behaviour of the substance is taken as the basis. According to an interpretation of these data by Kalberlah and Schneider, a factor of 4.5 (for kinetics) would be required for a chemical that shows a higher degree of biological variability in order to protect 95 % of the population. Studies by Dorne et al (2001a,b; 2002) indicated that at least 99% of the healthy adult population would be covered by the toxicokinetic default factor of 3.16, but that this factor would not be adequate for certain subgroups of the population. We suggest that for healthy adults a factor of 3-5 may be considered for the toxicokinetic parameter, while for the general population we do not find this factor protective enough. However, we do not consider that it is possible, based on present-day knowledge of inter-individual variability, to suggest an alternative default value for toxicokinetics for the general population.

Scientists are all in agreement, that genetic polymorphism is important for the inter-individual variability, especially if the polymorphic pathway represents the major route of elimination. In the report by Kalberlah and Schneider (1998), a factor of 3 is used for inter-individual variation due to genetic polymorphism based on limited data. This factor of 3 for genetic polymorphism is, according to the authors, additive to the toxicokinetic factor of 5. Since the genetic variation may be several orders of magnitude (as described previously in Section 5.2.7.2), we do not consider this factor of 3 to be sufficiently protective. It is not possible to suggest a default assessment factor for genetic composition, but it must be kept in mind that genetic composition really has to be taken into consideration.

The possibility of obtaining metabolic pathway-related toxicokinetic defaults, as investigated by Dorne et al (2001a,b; 2002), might be a promising way forward, but the results as of to-day no not seem valid enough to propose such default assessment factors.

Toxicodynamic data are still limited and in many cases also based on short-term changes after medical treatment at therapeutic doses. There are data indicating that variability in toxicodynamics is greater than variability in toxicokinetics. However, factors such as aging and disease processes may have contributed to the greater variability in dynamics compared to kinetics, since unlike the kinetic data much of the dynamic data was for the clinical treatment of patients. We do not consider that it is possible, based on present-day knowledge of inter-individual variability, to suggest an alternative to the default value of 3.2 for toxicodynamics for the general population.

Some probabilistic distributions have also been proposed, but at present there is no database-derived distribution of the inter-individual factor. We question the use of a theoretical distribution based on the default value of 10. Insufficient data are available for the assumptions made. Further research on the basis of a larger database is recommended to determine a data-derived distribution instead.

5.2.7.7 Summary of inter-individual variations

Some literature data indicate that a factor of 10 is sufficient to protect the vast majority of individuals. However, this is based on the mean coefficient of variability within the studied population and is not sufficient to protect against substances showing a higher degree of variability in biological response. We suggest that an inter-individual factor of 3-5 might be sufficient to reflect the toxicokinetic variability between healthy adults. The data available on toxicodynamics that were used to draw conclusions about differences between individuals are limited, and we do not consider that it is possible, based on present-day knowledge of inter-individual variability, to suggest an alternative to the default value of 3.2 for toxicodynamics for the general population.

In view of the state of current knowledge, a factor of 10-16 (3-5 times 3.2) is suggested as a minimum. No attempt is made to provide a factor covering the inter-individual differences in sensitivity with the inclusion of all of the various risk groups. In some parts such as genetic composition, data indicate that differences between individuals may be several orders of magnitude, and in many cases there is not enough information on toxicodynamics or toxicokinetics, or on the effect of exposure for the most susceptible sub-population. For other factors such as life-style and illness, knowledge is even more limited. In these cases, it is impossible to propose a default value for risk assessment purposes. Instead, it is important to make a qualitative case-by-case expert judgement, depending on the effect studied, the mechanism of action and the exposure of concern.

The timing of the exposure may be as important as the dose in determining the potential toxicity of a chemical. Due to the occurrence of critical windows, susceptibility to chemicals has to be considered to be particularly high during the pre-conception, prenatal and postnatal periods, including childhood and adolescence. It is also recommended that special attention should be paid to the protection of pregnant women. Some organ systems show specific vulnerability to chemical

toxicity during development, as organ maturation is an ongoing process until adulthood. If effects on the endocrine, reproductive, immune and nervous systems are suspected or indicated, studies on the specific organ systems in the appropriate test systems are necessary to make an adequate risk assessment. Concerning immunotoxicity, very limited information is available, but since these organ systems mature slowly (for instance the nervous or reproductive systems), we consider there are reasons for extra prudence in the risk assessment.

It is recommended that an attempt should be made to perform foetus-, child- and teenager-specific risk assessments for chemical substances and products to which they are likely to be exposed. A complete test battery including appropriate evaluation of animal reproduction and developmental as well as evaluation of neurotoxicity on adult animals could be complemented by more refined studies possibly on younger animals for chemical substances in products and foods intended for children. Furthermore, it is recommended that the risk assessment should be performed by experts on a case-by-case basis for each substance and for each exposure scenario. In the case where the data are insufficient to evaluate the susceptibility of children, including the unborn child, it is suggested that an additional safety factor (1-10 for an inadequate database) should be considered (see also Section 5.2.1).

5.2.8 Derivation of an overall assessment factor

In traditional risk assessment it is common to use an assessment factor to convert experimental animal data or epidemiological data into a human exposure level considered to be of no concern. The overall effect assessment factor consists of several factors that are described above in Sections 5.2.1 - 5.2.7. The most important include: adequacy of the database, nature of effect, duration of exposure, route-to-route extrapolation, dose-response curve consideration, inter-species extrapolation and inter-individual variations.

The point estimates of various assessment factors are multiplied in the standard procedure to obtain an overall assessment factor. However, the more extrapolation steps are taken into account, the higher the level of conservatism. Since the different assessment factors are not always independent of each other, this might lead to over-conservatism and a loss of confidence in the health-based limit value.

It has been suggested by Vermeire *et al* (1999) that this over-conservatism and piling up of worst-case assumptions can be avoided by using probability distributions. Each assessment factor is considered uncertain and characterised as a random variable with a log-normal distribution (with a geometric mean (GM) and a geometric standard deviation (GSD)). This method requires characterisation of the distribution of each assessment factor and of possible correlation between them. As a first approach, it is assumed that all the factors are independent.

If two distributions are combined, a new distribution is achieved with a new geometric mean and a new geometric standard deviation. A certain percentile can be chosen from this new distribution. It should be noted that multiplication of the percentiles should not be performed. It is also possible to combine a distribution with a point estimate. For instance, a chosen percentile can be combined with a point estimate by multiplication. Which percentile of a distribution should be chosen is a matter of policy.

Characterisation of a data-based distribution is only available at present for the inter-species extrapolation factor and the factor for duration of exposure. After correction for differences in metabolic size (between humans and the experimental animal used) the remaining inter-species factor can be described by such a distribution (GM=1, GSD=4.5; Section 5.2.6) (Vermeire *et al* 2001). If, for example, the 95th percentile of the remaining inter-species distribution is selected (=12) and this value is corrected for the differences in metabolic size between rat and human (=4), an inter-species assessment factor of 48 is achieved for rat experiments. According to Vermeire *et al* (2001), the factor for duration of exposure can also be described by such a distribution (GM=2, GSD =3.5; see Section 5.2.3).

A probabilistic distribution has also been proposed for the inter-individual factor based on the default value of 10 (Vermeire *et al* 2001), but we question the use of a theoretical distribution and suggest that the point estimate should be used instead for the inter-individual factor. In this report a value of 10-16 is suggested as a minimum for the inter-individual assessment factor.

Two of the most important assessment factors are those for inter-species and inter-individual variation. If, for example, the 95th percentile of the remaining inter-species distribution is selected, the combined inter-species and inter-individual assessment factor would become 48 times 10-16=500-800 for rat experiments. This factor is higher than was recently proposed by RIVM and TNO (220 based on a combined probabilistic distribution for inter-species variation and a theoretical inter-individual distribution), and higher than the traditional assessment factor of 100. For mouse experiments, the combined assessment factor would become higher (800-1300).

If two distributions are combined instead, a new distribution is achieved with a new geometric mean and a new geometric standard deviation. However, it is not possible to combine the percentiles of two distributions. If, for example, the distributions for inter-species extrapolation and duration of exposure (subchronic/chronic) were combined, the 95th percentile of the new distribution would be 50. This implies a combined assessment factor of 200 for rat experiments and 350 for mouse experiments after correction for differences in metabolic size.

For further information on probabilistic risk assessment see Annex 2.

Suggestion of how to determine an overall assessment factor

It is recommended that distributions of the assessment factors should be used in calculation of the overall assessment factor, if available. However, today such data are only available for the inter-species extrapolation factor and the factor for duration of exposure. When distributions of the assessment factors are not available, point estimates could be used. Distributions and point estimates can be used in parallel and combined when necessary. Which percentile of the distribution should be chosen is a matter of policy.

Two of the most important assessment factors are those for inter-species and inter-individual variation. If, for example, the 95th percentile of the inter-species distribution is selected, and combined with the point-estimate of the inter-individual assessment factor, the overall factor would become 500-800 for rat experiments. This can be compared with the traditional assessment factor of 100 for inter-species and inter-individual variation. If the distributions for inter-species extrapolation and duration of exposure (subchronic/chronic) are combined, the assessment factor will be 200 for rat experiments if the 95th percentile of the combined distribution is selected.

6 Acronyms and abbreviations

ADI	Acceptable Daily Intake
AF	Assessment Factor
AOEL	Acceptable Operator Exposure Levels
ARfD	Acute Reference Dose
BMD	Benchmark Dose
BMDL	Lower Confidence Limit of the Benchmark Dose
BMR	Benchmark Response
BP	Biocidal Products
CMR	Carcinogenic, Mutagenic or toxic to Reproduction
CSTEE	Scientific Committee on Toxicity, Ecotoxicity and the Environment
DG	Directorate General
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EEC	European Economic Community
EINECS	European INventory of Existing Commercial Substances
ELINCS	European List of Notified Chemical Substances
EPA	Environmental Protection Agency
ESR	Existing Substances Regulation
EU	European Union
ExS	Existing Industrial Substances
FAO	Food and Agriculture Organisation in the U.S.
FQPA	Food Quality Protection Act
GLP	Good Laboratory Practice
GM	Geometric Mean
GSD	Geometric Standard Deviation
HEDSET	Harmonised Electronic Data Set
HPVC	High Production Volume Chemical
ICH	International Conference on Harmonisation
IMM	Institute of Environmental Medicine, Karolinska Institutet, Sweden
IPCS	International Programme on Chemical Safety
IUCLID	International Uniform ChemicaL Information Database
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint Meeting of Experts on Pesticide Residues
KemI	National Chemicals Inspectorate, Sweden
LD	Lethal Dose
LOAEL	Lowest Observed Adverse Effect Level
LPVC	Low Production Volume Chemicals
MOE	Margin of Exposure
MOS	Margin of Safety
MRL	Maximum Residue Level
NAEL	No Adverse Exposure Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NRC	National Research Council
NS	New Industrial Substances
OECD	Organisation for Economic Co-operation and Development
OJ	Official Journal
PBPK	Physiologically Based Pharmacokinetic Modelling
PNAEL	Predicted No Adverse Effect Level
PPE	Personal Protective Equipment
PPP	Plant Protection Products
REACH	Registration, Evaluation and Authorisation of CHemicals

RfD	Acute Reference Dose
RIVM	National Institute of Public Health and the Environment, The Netherlands
RPE	Respiratory Protective Equipment
SCOEL	Scientific Committee on Occupational Exposure Limits
TDI	Tolerable Daily Intake
TDx	Tumour Dose
TGD	Technical Guidance Document
TNO	Netherlands Organisation for Applied Scientific Research
TNsG	Technical Notes for Guidance
WHO	World Health Organization

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Annexes

Annex 1

The benchmark dose method

Background

The use of the NOAEL (No Observed Adverse Effect Level) approach in health risk assessment has several limitations, some of which have been highlighted in the literature (Crump1984; Kimmel and Gaylor 1988; U.S.EPA 1995; Faustman 1996): First, the NOAEL is by definition one of the experimental doses and is highly dependent on the dose selection. Hence, the characteristic of the dose response relationship plays little role in determining the NOAEL. Guideline values are therefore set at varied levels dictated by experimental design, not by biological relevance. Second, experiments that have fewer animals tend to result in higher NOAELs. Poor designs are thus rewarded. Larger studies should provide greater accuracy and thus allow a higher ADI (Acceptable Daily Intake), not the opposite. Third, the calculation of a NOAEL generally utilises data that are categorised into distinct dose groups. However, in some studies (including most epidemiological studies) categorisation of subjects into dose groups is an arbitrary process. These and other limitations of the NOAEL approach in risk assessment have prompted development of an alternative, and one approach proposed is the benchmark dose method (Crump 1984).

Benchmark dose

The benchmark dose is defined as the dose that corresponds to a specified change in an adverse response compared to the response in untreated animals (Crump 1995). It is determined by modelling a dose response curve in the region of the dose response relationship where biologically observable data are feasible (Figure 1). To take account of the uncertainty of the data, the dose of interest is the lower confidence limit of the benchmark dose.

Since the terminology used in the benchmark dose literature is confusing and different authors use different terminology, some clarifications need to be made. In this document, the term benchmark response (BMR) is used for the predetermined (by the user) level of change in adverse response. The benchmark dose (BMD) represents the dose corresponding to the BMR. The lower confidence limit of the BMD will be called the BMDL. This terminology is consistent with the terminology used by Crump (1995) and with the terminology used by the U.S. EPA BMD software (BMDS). A similar method is described in a recent report from TNO (Dekkers *et al* 2000). However, in that report the term critical effect size (CES) is used for the predetermined increase of an effect (continuous data), and the term BMR is used for a predetermined increase in a risk (quantal data). Those increases correspond to a critical effect dose (CED), and the lower confidence bound on the CED is the benchmark dose (BMD).

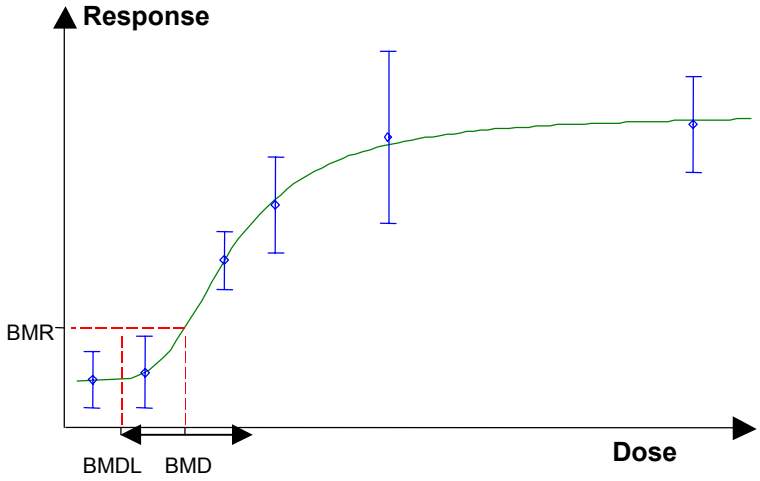

Figure 1. Illustration of the benchmark dose approach. The benchmark dose (BMD) corresponds to a predefined increased risk (BMR). The lower confidence limit of the dose that would result in the required response is the BMDL. The double-sided arrow is a symbol for the confidence interval of the benchmark dose.

Use

Internationally, the benchmark dose is already used by the U.S. EPA to derive health-based limit values. Within the OECD, the benchmark dose approach is often a point of discussion, but not yet implemented in regulatory toxicology. In a revision of the current technical guidelines for new and existing chemicals (EC 1996) within the EU, the benchmark dose approach is included as an alternative to the traditional NOAEL approach in health risk assessment. In Sweden, and in many other countries in Europe, the traditional NOAEL approach is still in use, although efforts are being made to investigate and evaluate the benchmark dose approach at the Institute of Environmental Medicine.

Several software packages are available today for use of the benchmark dose method. Some are commercially available, for example those developed by K.S. Crump (Howe 1990a; Howe 1990b). Others are freely available, for example the U.S. EPA benchmark dose software (U.S.EPA 2002)

The benchmark dose concept

The benchmark dose (BMD) method represents a more powerful statistical tool in comparison with the NOAEL approach. This greater complexity will result in higher demands on the risk assessor.

Form of data

Depending on the nature of the experiment, the resulting data can be divided into two different types: continuous or dichotomous data. Continuous data provide an exact numerical measure of some biological effect (e.g. liver weight), while dichotomous data only yield the rough two-valued judgement: response or not (e.g. tumours, which are either present or not). Dichotomous data are therefore more straightforward for the health-risk assessment of chemicals, since the judgement as to what is adverse or non-adverse has already been made when forming the dichotomous data and is not needed in setting a benchmark dose. With continuous data, there is a grading of the effect. However, it might be difficult to judge which

effect level should be regarded as adverse/non-adverse. There might be some additional information concerning the effect and it might consequently be known what level constitutes a health risk. But this information is not within the data and the risk assessor therefore has to make this judgement.

A way of interpreting continuous data utilises a cut-off value (some predetermined level of adverse effect). The data can be directly transformed into dichotomous format. Alternatively, a non-quantal approach can be applied given a model for the continuous dose-effect relationship and a certain assumption of the distribution of the effect (Gaylor and Slikker 1990). This latter method is sometimes referred to as the hybrid method, since it applies a dichotomous risk type on continuous data, and is preferred since less data is lost in the transformation (West and Kodell 1999). There is a similarity between the additional risk response type in the hybrid method and the scaled effect in the sense that these approaches can produce the same BMD and BMDL (Crump 1995, 2002). Use of the hybrid method can be an advantage when comparing different studies since the same definition of the benchmark response can be used for both dichotomous and continuous data (Nilsson 2001; Sand *et al*, 2002). However, there is still little practical experience in using this method.

Number of dose levels

It is not possible to set a benchmark dose if the numbers of dose levels available are not sufficient for the mathematical model. The numbers of dose levels are therefore critical for benchmark dose modelling. In OECD guidelines, for example, three dose groups and one control group are considered (OECD 1998). In many cases it will be possible to model these data but in the case where data are continuous and level off at higher or lower doses, for example, it will not be possible to fit a model to the data. Other data may have an even smaller number of dose levels, which means that fitting to a mathematical model is limited. It is obvious that with more information, in this case more dose levels, a better picture of the dose-response relationship is achieved.

It is important to remember that for ethical reasons it is important to minimise the number of animals in an experiment. It is possible that the incompatibility between a sufficient number of animals and a greater number of doses can be solved by increasing the number of dose groups at the cost of decreasing the number of animals in each dose group (Slob and Pieters 1997). This would make it possible to use the benchmark dose with the same amount of animals as in an ordinary guideline study. However, this has to be further evaluated and discussed since it implies changes in existing guidelines.

Model selection

Since many different mathematical dose response/effect models are available in using the benchmark dose method, the process of model selection represents an important aspect when decisions have to be made. However, for a given dose response/effect set it is often difficult to decide *a priori* what biologically based model to prefer. In this part of the process, statistical tools usually become very important. Different statistical tests can be used to assess the model fit to the data. In addition, another important rule when analysing model performance is to account for the model complexity (number of incorporated parameters).

Fitting

Models are commonly fit to data by using the maximum likelihood method (Crump 1984; Kupper *et al* 1986). This is a weighted least square method that considers the uncertainty of the data. The standard deviation of the response at each dose is taken into account when fitting the model. The absolute distance from model estimate to observed mean values is therefore allowed to be greater for dose groups with large standard deviations in the response.

Different types of statistical testing can be accommodated when validating the degree of model fit. Likelihood ratio tests, which are applicable to both dichotomous and continuous endpoints and the Pearson chi-square test (χ^2-test) for dichotomous endpoints are the ones utilised by the U.S. EPA in their BMD software.

Generally, within a family of models, as additional parameters are introduced the fit will improve. This behaviour is due solely to the increase in parameters and such tests cannot be applied to compare models from different families. Akaike's Information Criterion, AIC (Akaike 1973), on the other hand, can be used to compare different models using a similar fitting method.

Confidence level

The methods of confidence limit calculation and the choice of confidence limit is critical for the BMDL. By convention, the size of the statistical confidence limit ranges from 90-99 percent. For the benchmark dose method a one-sided 95-percentage confidence limit is reasonable and often used.

As an alternative approach, Slob and Pieters proposed finding the complete probabilistic uncertainty distribution of the BMD (Slob and Pieters 1998). Once the dose-response model has been fitted to the continuous data, Monte Carlo sampling is used to generate a large number of new data sets from the model, each time with the same number of data points per dose group as in the real experiment. For each generated data set the critical effect dose is re-estimated. Taking all these critical effect doses together results in the required distribution from which any desired confidence interval can be derived.

Risk level

In contrast to the NOAEL, the BMD corresponds to some predetermined change in adverse effect, the benchmark response (BMR). A crucial part of the benchmark dose method therefore consists in the determination of this predetermined level. This represents the major difficulty with the BMD approach, since there is still no general agreement regarding the adverse effect level that can be allowed. In theory, depending on the endpoint, different values of BMR should probably be employed.

The most common BMRs are 1%, 5% and 10% change in response for quantal endpoints (Faustman 1996). Research in this area has revealed that an increase of about 5-10 % will be comparable to a NOAEL in most cases (Haag-Grönlund *et al* 1995, Faustman 1996). For continuous data there is a grading of the effect, and it is difficult to judge what effect level should be regarded as adverse/non-adverse. It is therefore especially important to reach a consensus for continuous data.

Response type

The BMR can be defined in various ways and many different response types are available, e.g. increase in relation to background or absolute increase. The problem of selecting response type mainly concerns continuous data, and the type to prefer generally has to be determined in a case-by-case judgement. The response type together with the chosen risk level will affect the size of the benchmark dose. There is an alternative method for defining the benchmark response for continuous data; it involves a definition of a cut-off value above which effects are considered adverse. This formulation allows continuous data to be used with the dichotomous response types. This can be achieved through a definition of some abnormal level in the control group. Thereafter the dichotomous response types can be used. With this method it would be possible to compare between different effects. However, this method has not yet been fully validated and only limited programs are available on the market at present.

In summary

The benchmark dose (BMD) method represents a more powerful statistical tool in comparison with the NOAEL approach. Since the BMD method involves fitting of a mathematical model to the entire dose response data within a study, more biological information is incorporated in the resulting estimates of human limit values (e.g. ADIs). In contrast, ADIs retrieved from the NOAEL approach are only based on information collected at one of the experimental doses in the study. This greater complexity represents a step in the right direction for a more accurate risk assessment. However, since the benchmark dose method involves certain problems and decision-making, it will result in greater demands on the risk assessor. It is important that the decisions made are clearly described. If, for instance, more information is available and a revised risk assessment will be performed, it is easy to do if all the decisions are transparently described.

Crucial parts of the benchmark dose method constitute the determination of a predetermined level of effect/response on which to base the benchmark dose, the mathematical model and how to express the increased response/effect (in relation to background or otherwise). In addition, the number of doses versus the number of animals per dose-group is of importance for the benchmark dose method to fully replace the NOAEL approach.

The suggestion is that until a consensus is reached, both the benchmark dose approach and the traditional NOAEL approach should be used. A 5% change in response is suggested for quantal data as the first choice in risk assessment, since an increase in response of about 5-10 % for quantal data seems to be comparable to a NOAEL in many cases. Our own experience indicates that 5 % seems to be an appropriate choice, at least for developmental toxicity, since it is desirable to set a low change in response and yet be independent of the model selection. In cases where it is not possible to fit a model to the data, the traditional NOAEL approach should be used. The use of both approaches would also make it possible to validate the use of the benchmark dose method on different kinds of data.

For continuous data there is a grading of the effect, and it is difficult to judge what effect level should be regarded as adverse/non-adverse. It is therefore especially important to reach consensus for continuous data. Until more endpoint-specific information is available, a 5% change is suggested.

It is recommended that evaluations on different risk levels should be continued, especially for continuous data, and the possibility to apply dichotomous risk types on continuous data. In addition, it is important to know whether it is possible to increase the number of dose levels without increasing the number of animals in the experiments.

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Annex 2

Probabilistic methods for assessment of the health risk of chemicals

Introduction

A core element of risk is uncertainty represented by plural outcomes and their likelihood. There is no risk if the future outcome is uniquely known and hence guaranteed. The probability that we will die some day is equal to 1, so there would be no fatal risk if a sufficiently long time frame is assumed. Equally, rain risk does not exist if there was 100 % assurance of rain tomorrow, although there would be other risks induced by the rain. In a formal sense, any risk exists if, and only if, more than one outcome is expected at a future time interval.

In any practical risk assessment we have to deal with uncertainties associated with the possible outcomes. One way of dealing with the uncertainties is to be conservative in the assessments. For example, we may compare the maximal exposure to a chemical with a conservatively chosen reference value. In this case, if the exposure is below the reference value, it is possible to assure that the risk is low. Since single values are usually compared and this approach is commonly called the "deterministic". Its main advantage lies in its simplicity and in the fact that it requires minimum information. However, problems arise when the reference values are actually exceeded or might be exceeded, as in the case of potential exposures, and when the costs of attaining the reference values are high. In those cases, the lack of knowledge on the degree of conservatism involved impairs a rational weighing of the risks against other interests.

We here describe an alternative, more consistent, approach for dealing with uncertainties, known as probabilistic risk assessment. The essence of this approach consists in explicitly quantifying the uncertainties in terms of probabilities. Our first aim is to delineate the approach, in comparison with the deterministic approach, to define the entities involved in the assessment and their relationship. The second aim is to identify possible strategies for deriving and combining the probability distributions needed. Finally, we discuss the advantages and disadvantages of the probabilistic approach.

Probabilistic versus deterministic assessments

Let us consider the commonly applied risk quotient (RQ), which can be generally defined as the ratio between the exposure to a chemical and the reference value adopted for this chemical (Equation 1). In a deterministic assessment, single estimates are used for the exposure and the reference value. If these were conservatively chosen and a value below 1 was obtained for the RQ, then it can be assured that the probability of the exposure exceeding the reference value is low, i.e. the risk is low. Obviously, the exposure and reference levels should be expressed in the same units, for example in units of dose, intake rate or environmental concentration.

$$RQ = \frac{Exposure}{reference\ value} \qquad \textbf{(Equation 1)}$$

The essence of the probabilistic approach is to treat the *Exposure* and the *reference value* (Equation 1) as random variables. In this case, the RQ is also a random variable that can be described with a probability density function, commonly known as the "risk profile" (see Figure 1). A deterministic RQ is just one value among the universe of all values that the RQ can possibly take. The probability that the RQ is above 1 (indicated area in Figure 1) is a quantitative measure of the risk. In contrast, the deterministic approach only provides a qualitative risk estimate.

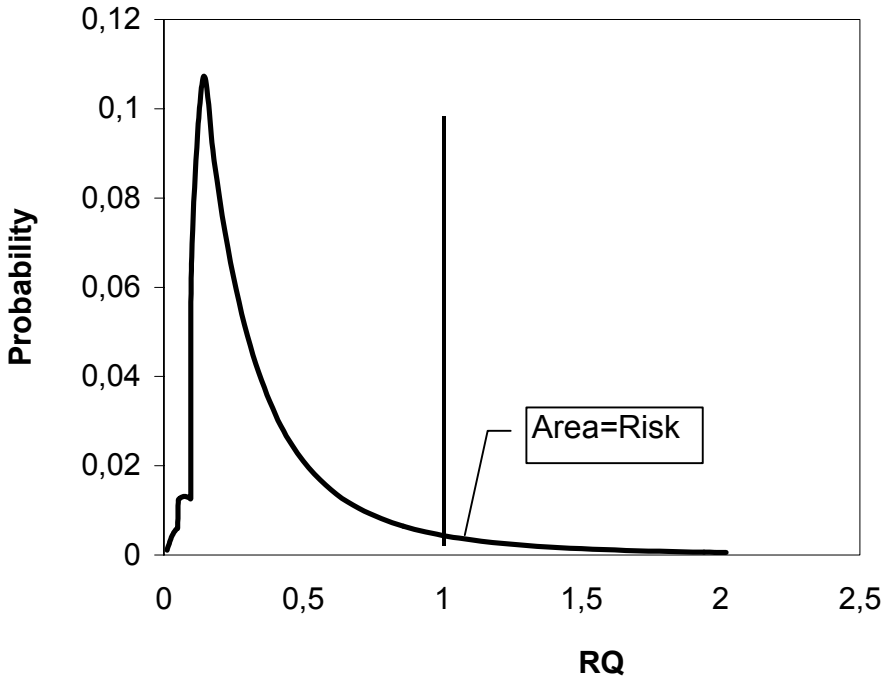

Figure 1. Example of probability density function corresponding to the risk quotient, commonly known as the "risk profile". The area under the curve for RQ>1 is a quantitative measure of the risk.

Another very informative risk communication tool is a curve with exposures (or effects) in the x-axis and probabilities in the y-axis (Figure 2). To estimate the probability that the exposure (effect) is above a particular level, simply draw a line up from the x-axis to the curve, and then draw a line to the y-axis. Such curves can be estimated for each scenario of concern, or in cases where the risks are additive, they can be integrated to estimate the total risk (Figure 2).

In the deterministic approach, normally, conservative values are used in Equation 1. Given the multiplicative nature of the model, a substantial magnification (positive bias) of the conservatism may take place. For this reason, values of RQ close to or above 1 will carry very little information about the risks. A common way to deal with this problem is to carry out assessments in tiers. This means that more realistic quotients are estimated whenever a conservative assessment yielded RQ > 1. This approach could be seen as a simplified version of a probabilistic approach. In any

case, the interpretation of the results would require knowledge about the distribution of the exposure and the reference value. For example, using mean values in Equation 1 is meaningful only if the magnitudes follow a normal distribution, which is rarely the case.

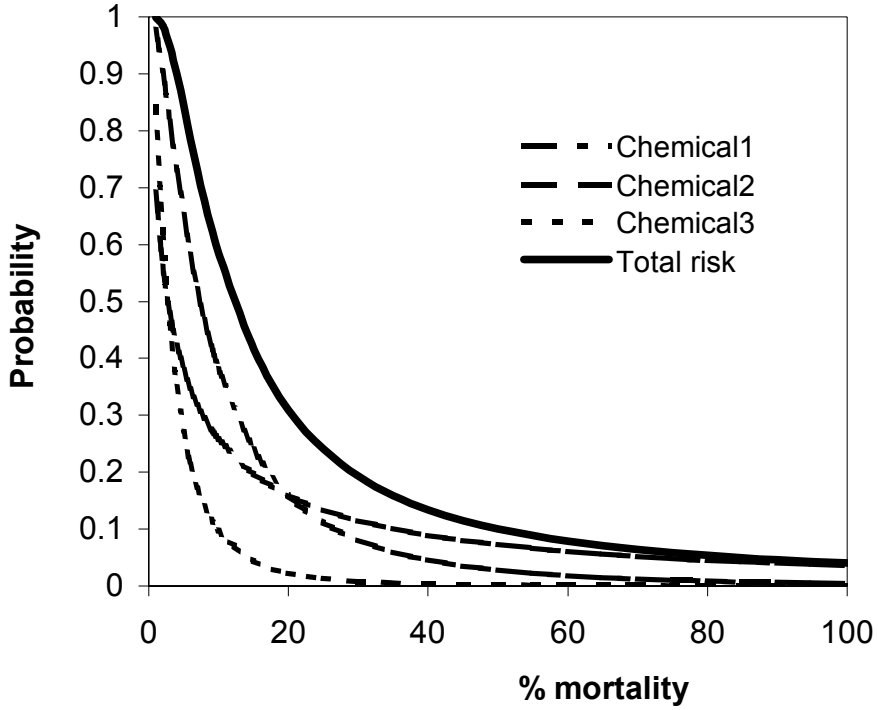

Figure 2. Inverse cumulative distribution of the mortality due to a chemical (in %) showing the probability that the mortality is above a certain value (risk).

Derivation of the probability distributions

The acceptance and practical application of the probabilistic approach hinges on sufficient support from data and knowledge to obtain the necessary probability distributions. A discussion on possible strategies for deriving the probability distributions of the exposure and the reference values follows below. It should be noted that the probabilistic approach could in principle be implemented gradually. This means that probability distributions could be incorporated in the RQ as they become available and be successively improved as new information and knowledge are obtained.

Exposure

When sufficient empirical data are available, the probability distribution for the exposure can be directly estimated using standard statistical techniques (Taylor 1993). This would be the case, for example, when the RQs are expressed in terms of environmental concentrations, which could be obtained by means of environmental monitoring. Models can also be used for indirect estimations of the exposure from available data. For example, doses to man can be estimated from the intake rates using metabolic and dosimetric models. Intake rates could be estimated from the environmental concentrations or release rates into the environment using migration and dispersion models. The indirect estimations require that propagation of the uncertainties is carried out. When the models are relatively simple, analytical methods such as variance propagation could be used (see for example Morgan and

Henrion (1990) and Hammonds *et al* (1994)). If there is a simple additive model and the input variables are independent, the mean value of the output distribution is the sum of the input variables means. Similarly, the variance of the output distribution is the sum of the variances of the input variables. The shape of the resulting output distribution will tend to be normal even if the distributions assigned to the inputs were not. A similar approach can be taken with multiplicative models after first converting the model to its additive form by logarithmically transforming the input variables. The output distribution of a multiplicative model will tend to be log-normal even when the input distributions were not. When the models are more complicated, the propagation of uncertainties can be carried out by means of Monte Carlo simulations (see below).

Reference values

The reference values are related to the health effects of the chemicals and are obtained from studies of dose-effect relationships. Examples of reference values are the No-Observed Adverse Effect Levels (NOAEL) and the Benchmark Doses (BMD). A correction is usually introduced to take account of inter-species and intra-species variation:

$$reference\ level = \frac{NOAEL}{EF_a * EF_b} \qquad \textbf{(Equation 2)}$$

where,

EF_a and EF_b are extrapolation factors used for taking into account variations between species and among individuals of a species, in this case humans.

Distributions for the NOAEL could be obtained directly from the experimental data. The NOAELs are, however, usually subject to large errors and it is only possible, in practice, to assess critical effect doses (CED) for those endpoints that have been measured. The NOAEL can be thought of as the lowest of all possible CEDs, including those that have not been measured. Furthermore, the most sensitive endpoint in the test animal may not be the same in the human being. In response to the above limitations in the NOAEL, the method of benchmark doses (BMD), described in detail in Appendix 1.1, has been developed. A probabilistic variant of this method would be to use the whole probability distribution derived from the dose-response curve as reference value, and not a single percentile (typically 1%, 5% or 10%) as is the case with the BMD (see an illustration of this method in Figure 3).

Probability distributions of the EF can also be derived from experimental studies as described by Slob and Pieters (1998). By combining these distributions with the distribution for the NOAEL, or the BMD, it is possible to obtain a distribution for the reference levels. The validity of this approach in the context of a probabilistic assessment could, however, be questioned. Indeed, the rationale of the extrapolation factors in Equation 2 is to guarantee that the reference values are conservative. The essence of the probabilistic approach is to consider explicitly the uncertainties and there is therefore no need to be conservative. The multiplication of several probability distributions may also result in "distortions" in the distribution of the reference levels. This procedure might, for example, yield distributions with very long tiles corresponding to outcomes resulting from combinations practically impossible in practice.

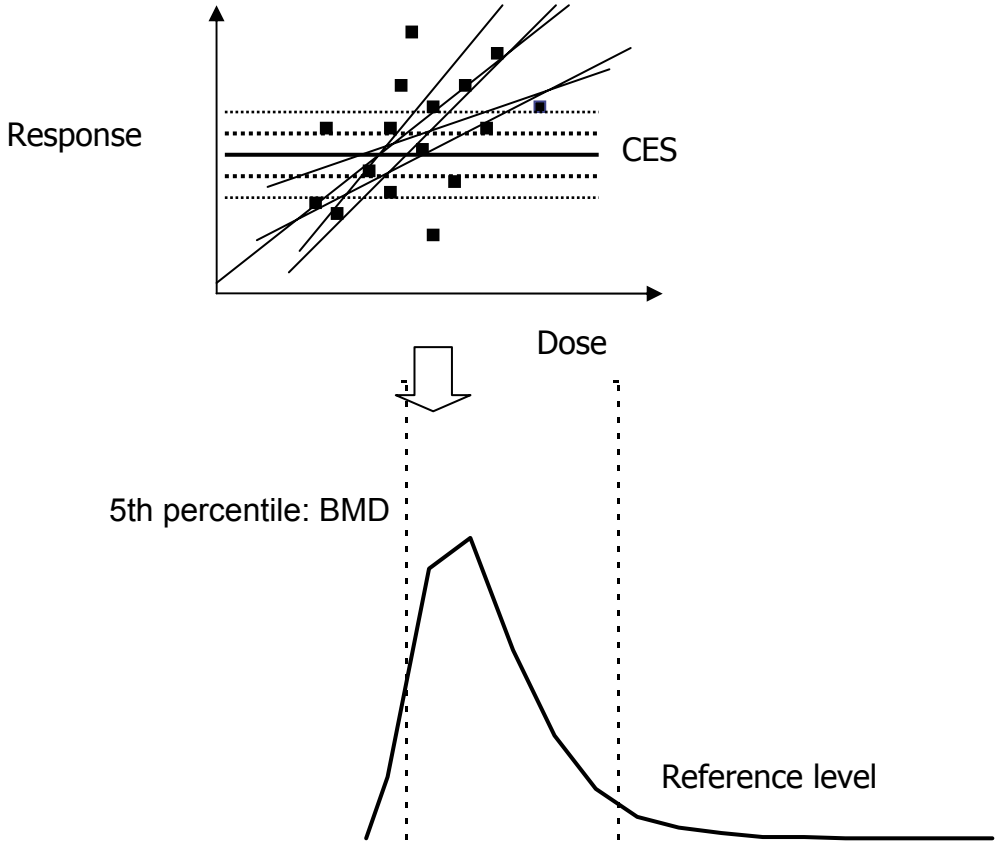

Figure 3. Probability distribution for the reference level obtained from the dose-response curve using the bootstrap method. The 5 % of the distribution corresponds to the benchmark dose (BMD) (Adapted from Slob and Pieters 1998).

An alternative to the extrapolation factors is to consider the CES as a random variable. If the method illustrated in Figure 3 is applied for deriving the reference values, then a probability distribution could be used for the CES (represented by several levels of CES in Figure 3). This will reflect uncertainties arising when extrapolating from other species to humans and from the variability among individuals.

Risk characterisation

A probabilistic characterisation of the risk consists in combining the probabilistic distributions of the *Exposure* and the *Reference Value* in Equation 1. When simple analytical expressions for the probability distribution are available, variance propagation can be applied to derive the risk profile (Morgan and Henrion 1990; Hammonds *et al* (1994). When analytical expressions of the distributions are not available, or when the distributions cannot be combined analytically, these can be combined using Monte Carlo analysis, the principle of which is described below.

Monte Carlo analysis

The basis for a Monte Carlo analysis is straightforward: point estimates in a model equation are replaced by probability distributions, samples are randomly taken from each distribution, and the results tallied, usually in the form of a probability density function or cumulative distribution. Several variations of the Monte Carlo technique for sampling from input distributions are available (Morgan and Henrion 1990). One

variation is importance sampling, where values of particular importance (usually the tails of the input distributions) are sampled more often and then given reduced weight to improve resolution in the tails of the output distribution. In stratified sampling, the input distributions are divided into intervals and input values obtained by random sampling from within each interval. The most popular version of stratified sampling is Latin hypercube sampling, which divides input distributions into intervals of equal probability. Latin hypercube sampling is more precise than conventional Monte Carlo sampling, because the entire range of the input distributions is sampled in a more even, consistent manner (Iman and Helton 1988).

Second-Moment Formulation

In many cases the probability distributions of the *Exposure (E)* and the *Reference Value (RV)* are not known. Often the available data are scarce and only estimates can be made on the first and second moments of the probability distributions.

Let us assume that estimates are available on mean values *M(E), M(RV)* and variances *Var(E)* and *Var(RV)* of the *Exposure* and the *Reference Value*, although the probability distribution laws might be unknown

The failure condition of the system may be expressed in terms of a performance index, for example the safety margin (*SM*), defined as the *Exposure* minus the *Reference Value*. The critical condition for failure of the system is when the *SM* equals nil. By introducing the reduced variables E^* and RV^* in the equation for the critical condition, the following limit-state equation for failure is obtained:

$$\sqrt{Var(RV)} \cdot RV^* - \sqrt{Var(E)} \cdot E^* + M(RV) - M(E) = 0 \qquad \textbf{(Equation 3)}$$

where,

$$E^* = \frac{E - M(E)}{\sqrt{Var(E)}}, \quad RV^* = \frac{RV - M(RV)}{\sqrt{Var(RV)}}$$

As shown in Figure 4 the geometrical representation of Equation 3 in the space of reduced variables E^* and RV^* is a straight line, which divides the plane into two parts; the upper part, where $SM < 0$, represents the failure of the system and the rest of the plane indicates safe conditions ($SM > 0$). All points on the straight line correspond to values of *E* and *RV* for which the failure or critical condition is valid (failure line). The distance *d* (Equation 4) between the origin and the failure line is a measure of the reliability of the system:

$$d = \frac{M(RV) - M(E)}{\sqrt{Var(RV) + Var(E)}} \qquad \textbf{(Equation 4)}$$

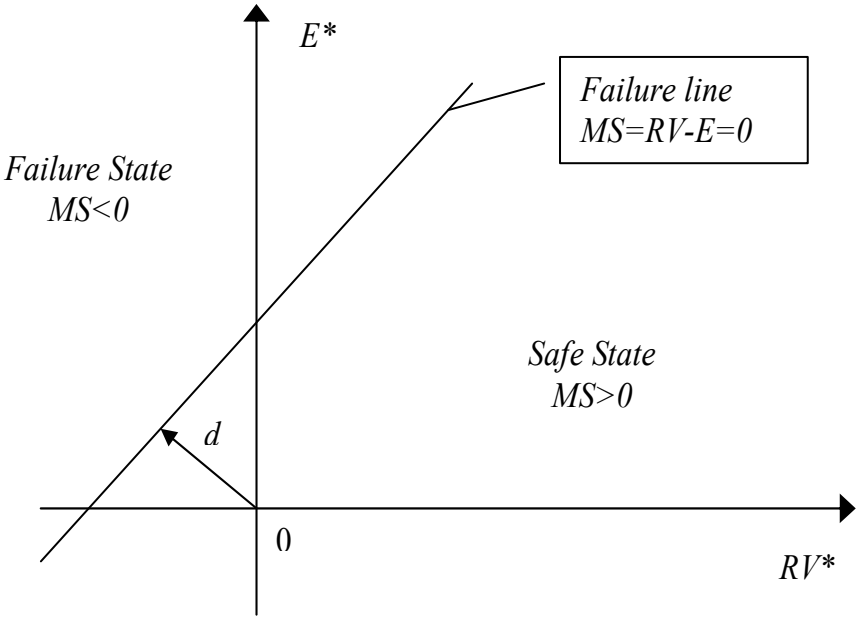

Figure 4. Reliability condition obtained by use of the second moment formulation (after Ganoulis 1994).

If E and RV are normal variables, the risk can be computed in terms of the distance d as $1-\Phi(d)$, where Φ is the cumulative normal function. Generalisations of this method for other types of distributions and when E and RV are functions of other variables of the system can be found in Ganoulis (1994).

Advantages and disadvantages of the probabilistic approach

The probabilistic approach provides a more complete quantitative characterisation of the uncertainties and is less likely to include bias than the more simple deterministic approach. Even with a tiered approach, each deterministic assessment provides single values for estimates of exposure from a given pathway. Such single-value risk estimates do not provide information on the variability and uncertainty that may be associated with an estimate.

When combined with sensitivity analyses, the probabilistic approach allows a more informative "what-if" assessment of the impact on the risk estimates of a change in an individual parameter or a group of parameters, thus providing a cost-effective tool for making risk management decisions.

The probabilistic analysis also permits more constructive comparisons of remedial alternatives when diverse attributes must be compared to systematically reduce the baseline risk. This includes comparing alternatives or intervening measures that could also cause remediation risks.

The main disadvantage of the probabilistic approach is that time and effort is required in order to set up the database and document the rationale for the probability density functions for individual parameters in the risk algorithm. The distribution patterns for some parameters are often not definitively known, requiring the use of credible professional judgment or costly site-specific studies or data collection

efforts. The impact of interdependencies between or among variables may also be difficult to quantify if their co-relations are not well known, which is often the case.

In view of the above discussion, the probabilistic approach appears to be most appropriate when the risks are not trivial, for example in sites where the risk is at or slightly below the acceptable level of risk or hazard, and where the remediation cost is potentially high.

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