EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
Chemical Assessment and Testing

Ispra, 05/03/2010

Risk Characterisation of local effects

Guidance based on:

Risk Characterisation of local effects in the absence of systemic effects, published 27/05/2009

Risk Characterisation of local effects, COM note of 18/11/2009

Discussions in TM IV 2009, TM I 2010

This document is a working document which can be used for the risk characterization of local effects. Following experience with applying this document, it will be re-evaluated after approximately one year.

CONTENTS

Joint Research Centre · I-21027 Ispra (VA), Italy · TP 582
Telephone: direct line (+39)0332/……, · Telefax: (+39)0332/789963
E-mail: ……@ec.europa.eu
http://ihcp.jrc.ec.europa.eu

1. Background

The first version of this document (*Risk Characterisation of local effects in the absence of systemic effects*) was drafted by an e-mail working group formed by FR, UK, DE, NL and COM. It was endorsed in TM I 2009 and then finalised and published on 27/05/2009. Based on COM note of 18/11/2009, the RC of local effects was discussed again in TM IV 2009, where a second e-mail working group was formed. This group consisted of AT, DE, FI, FR, NL, NO, PT, SI, UK and CEFIC.

Several active substances which have been notified under the Biocidal Products Directive are, due to their inherent properties, more likely to induce local effects than systemic effects. In an extreme case, there may be local effects but no systemic effects at all. There is a need to harmonise the methodology used in the risk characterisation of local effects. This document intends to establish a common approach to risk characterisation of local effects following repeated exposure by extending the guidance in Chapter 4.1 of the *TNsG on Annex I Inclusion*. This document does not form a part of the TNsG, but is regarded as a TM decision on a workable approach towards risk assessment that is based on local effects. It was agreed in TM I 2010 that once sufficient experience has been gained of using this document, it will be reviewed and modified if needed, and it should then be included in the TNsG on Annex I inclusion.

Chapter 4.1.6 of the *TNsG on Annex I Inclusion* refers to an external reference value (AEC) for local effects, derived as local concentration in mg/m^3 air or mg/cm^2. The external reference values for different routes of exposure are named here as AEC_{dermal}, $AEC_{inhalation}$ and AEC_{oral}. These local AECs refer to external exposure, and therefore, absorption rates are not taken into account when calculating them.

This document was drafted for use in the Review Programme, but the principles should be usable at the product authorisation stage as well, *mutatis mutandis*.

2. Local effects

A local effect is an effect that is observed at the site of first contact, caused irrespective of whether a substance is systemically available. Local effects considered in this document are irritation and corrosion occurring on the skin, on the eyes, on the respiratory tract or on the GI tract.

Observed systemic effects that are clearly secondary to causative local effects should be considered as part of the local effects, and not as primary (“true”) systemic effects.

2.1. Minor irritant effects

Minor irritant effects are reversible effects (redness, oedema, but no clear cytotoxic effects or destruction of external membranes) observed in repeated dose studies in animals or humans that are on any time scale less severe than those that would warrant classification in an acute irritation study.

2.2. Local effects leading to classification

Irritative or corrosive effects that lead to classification are usually the result of single-dose studies which do not provide suitable dose-response information for quantitative RC. For such substances, repeated dose dermal and/or inhalation studies are usually not available, and consequently the basis for setting AECs is lacking. A qualitative RC is performed assuming that the effects leading to classification will also occur in repeated exposure and at lower concentrations/area doses, and the effects will be managed by means of CLP, RMMs and PPE.

2.3. Other local effects

For this document, *other local effects* will cover any local effects that do not fall under Chapters 2.1 and 2.2 above. Such effects could be e.g. cytotoxic effects, or effects for which reversibility cannot be assumed that are observed in repeated dose studies.

Sensitisation is not considered in this document. At present it is usually not possible to determine a reliable threshold for sensitisation, and consequently a quantitative RC for sensitising effects is not possible. As a general rule, sensitisation effects should be covered by classification and labelling.

3. Time frame and studies

Quantitative RC of local effects can be performed when local effects at the port of entry are seen in repeated dose studies that usually contain dose-response information:

- Local dermal effects in a repeated dose dermal study
- Local respiratory effects in a repeated dose inhalation study
- Local effects in the GI tract in a repeated dose oral study

The RC of local effects will be performed based on anticipated medium and/or long term human exposure, while acute exposure cannot be considered in a quantitative manner. Acute exposure of humans to irritant substances is dealt with in a qualitative assessment which aims at avoiding or minimising exposure by means of CLP, RMMs and PPE.

Only qualitative RC is generally possible for local effects that are observed in single dose studies. These studies do not usually provide suitable dose-response information for quantitative assessment. Nevertheless, in some cases relevant information may be available only from these studies and could be taken into account in a quantitative RC. As a general rule, these effects are covered by a qualitative RC and are managed by means of assignment of R- and S-phrases, or H and P statements in the CLP regulation, and by applying appropriate RMMs and PPE:

- Irritation in an acute toxicity test (oral, dermal, inhalation)
- Irritation in an acute skin/eye irritation test

4. Deciding on systemic or local RC

The RC should be provided for the most critical effects, which can be either systemic or local. In order to decide which one of these is more critical, a comparison between the systemic NOAELs and local NOAECs will not always be sufficient. If the need for either the local or systemic RC cannot be clearly excluded, it will be necessary to derive both systemic AELs and local AECs. AELs and AECs are not directly comparable, and therefore a conversion is necessary as described in Chapter 6.

- If the local effects occur at concentrations/area doses that are above the overall systemic NOAEL in the same species, it will not be necessary to derive AECs or perform a local RC. This is because it is not expected that the assessment factors (AF) used for local effects will be larger than those for systemic effects, and the systemic assessment is thus sufficient to cover the local effects as well.
- If the local effects occur at lower concentrations/area doses than systemic effects, both AELs and AECs should be derived because it will often not be possible to unequivocally compare the local NOAECs and systemic NOAELs. Whether the AECs or AELs are more critical will also

depend on the product composition and the intended use. The most critical values (AECs or AELs) will then be used in the RC, and the respective reasoning shall be included.

- If local and systemic effects occur at the same range of concentrations/area doses, both local and systemic RC should be carried out, and the most critical values (AECs and AELs) will be used in the RC. Local RC can in such cases still be deemed unnecessary if it is concluded that due to different AFs the systemic RC will be clearly more critical. Such reasoning will be included in the assessment.

Reverse reference scenarios may be helpful in deciding if local or systemic effects are more critical (calculating the amount of product/formulation to which a human should be exposed to in order to achieve potentially critical systemic doses of the active substace).

When both local and systemic effects are observed and the local effects are regarded as minor irritant effects, systemic AELs should always be derived, even when local effects result in more critical AECs (lower MOEs). This is because AECs based on minor irritant effects would not have consequences on the Annex I inclusion decision (See chapter 8.1.2 below). Therefore, in such a case, the Annex I inclusion decision is made based on the RC using systemic AELs that may be higher than local AECs (after conversion to same units).

It will be clearly indicated in the assessment whether the AECs or the AELs are more critical, and only the more critical ones will be used in the RC. The less critical AECs or AELs may be included in the assessment for transparency, clearly indicating the reasoning and calculations leading to the conclusion on which values are the most critical ones. When local and systemic effects occur at comparable dose/concentration levels, the decision on the more critical approach will often depend on the product/formulation and the intended use.

5. Quantitative or qualitative local RC

If it is concluded that local RC will be more critical than systemic RC, then quantitative local RC should be attempted. There are however many uncertainties involved, including the quality of the effect data and whether it is quantitative, the quality of human exposure information, and the available information on product formulation(s). The availability, quality and relevance of human exposure data will be a critical factor for determining the uncertainty associated with the quantitative local RC.

The uncertainties should be described in the assessment, and a conclusion be given on the feasibility of a quantitative local RC of sufficient quality. If it is concluded that a quantitative RC is not feasible, then a qualitative RC is performed and all available information will be included to facilitate a possible quantitative RC at the product authorisation stage.

6. Routes of exposure

6.1. Dermal route

Local dermal effects are considered when the NOAEL (in mg/kg bw/day) related to local effects is lower than the overall systemic NOAEL (converted to an external value) and the NOAEL related to systemic effects in the dermal study. Whether local effects are more critical than systemic effects will depend on the AFs used.

AEC_{dermal} is based on effects that are concentration dependent rather than dose dependent, and should be given as a concentration (mg/L) or percentage and, if available, in mg/cm^2. These units are also appropriate for monitoring purposes. The choice of unit can be made based on a known mechanism of action: while mg/L or a percentage may be more appropriate for pH-dependent effects, mg/cm^2 might be more suitable for other forms of reactivity.

AEC_{dermal} can be derived from a NOAEL value by first converting the NOAEL (mg/kg bw) into a NOAEC (mg/cm²) as shown below:

$$NOAEC\ in\ mg/cm^2 = \frac{Total\ dose\ applied\ in\ mg}{Treated\ surface\ in\ cm^2} = \frac{(average\ animal\ weight\ in\ kg) \times (dose\ in\ mg/kg\ bw)}{Treated\ surface\ in\ cm^2}$$

If the contact surface area is not available, it should be considered whether the area can be derived from the default body surface values given in the *TGD on Risk Assessment*. These values can be used e.g. when the study report provides the contact area as a percentage of the total body surface, as in OECD guidelines 410 and 411.

Repeated dose dermal toxicity studies are required in the dossier under Directive 98/8/EC only when potential dermal exposure is significant and route-to-route extrapolation is not possible. A dermal study may be necessary when dermal route is more relevant than other routes, or when specific effects of concern are different from the effects seen in the studies by other routes.

The exposure of laboratory animals in the repeated dose dermal toxicity study is not directly comparable to typical human exposure, mainly because the test animals are exposed under (semi-) occlusion (bear in mind that e.g. the area dose and the type of formulation versus active substance could be important for non-comparability), whereas humans will normally be exposed to bare skin (an exception is exposure on hands under reused gloves, which may be considered as occlusive conditions – gloves only provide enhanced barrier properties when new and replaced regularly). Although such differences in exposure conditions are not limited to the dermal route, this can make it difficult to use the results of animal studies for human risk assessment.

6.2. Inhalation route

Local respiratory effects are considered when the NOAEC related to local effects is lower than the systemic NOAEC in the inhalation study or lower than the (overall) systemic NOAEL (after having converted the NOAEC related to local effects into mg/kg bw). Whether local or systemic effects are more critical will depend on the AFs used.

If critical local effects are observed in inhalation toxicity studies, the highest non-irritating concentration in animal studies (respiratory NOAEC) should be used to calculate $AEC_{inhalation}$. This value is then compared with the concentrations that humans are expected to be exposed to. Both NOAEC and $AEC_{inhalation}$ are usually expressed in mg/m^3.

Repeated dose inhalation toxicity studies are usually required in the dossier under Directive 98/8/EC only for volatile substances (vapour pressure > 10^{-2} Pascal) or in cases where potential inhalation exposure is significant. In some other cases (e.g. aerosols and dusts/particulate matter), studies by inhalation route should also be required in addition to studies by the oral route.

Substantial uncertainty may result from the difference between the formulation and the active substance. This might reduce the usability of the active substance data for the assessment of the (representative) product.

6.3. Oral route

Chapter 4.1 of the *TNsG on Annex I Inclusion* does not indicate how to derive an external reference value when an active substance induces local effects on the GI tract. It seems unlikely that an AEC_{oral} would need to be derived for a biocidal substance, but an approach is suggested for completeness.

Local oral effects are considered when the NOAEL (in mg/kg bw/d) related to local effects is lower than the (overall) systemic NOAEL and the systemic NOAEL in the oral study. Observed systemic effects secondary to causative local GI tract effects should be considered as part of the local effects. Whether local or systemic effects are more critical will depend on the AFs used.

If critical local effects are observed in oral toxicity studies, the highest concentration with no local effects in animal studies (oral NOAEC) should be used to calculate AEC_{oral}. This value is then compared to the concentrations that humans are expected to be exposed to. AEC_{oral} is expressed as a concentration (mg/L), as a percentage, or as ppm. If the NOAEL is expressed in mg/kg bw/day, this should be converted to a concentration by using information on the administration method usually available in the study report.

Substantial uncertainty may result from the difference between the formulation and the active substance. This might reduce the usability of the active substance data for the assessment of the (representative) product.

7. Assessment factors (AF)

It is recommended as the first option to apply the AFs as suggested in the REACH guidance[1] where applicable, with the exception that the same AF shall be applied for professionals and non-professionals (referred to as “workers” and “general population” in REACH).

It is acknowledged that the empirical data basis for these AFs is very weak, and they should therefore be applied with caution. The AF values will be considered on a case-by-case basis, and the reasoning/justifications will always be given. New literature should be taken into account where possible.

When the principles given in the REACH guidance are not considered applicable, the TM considerations given in Appendix 1 should be taken into account. This is supplementary information that can be used in expert judgment.

8. Outcome of the assessment

8.1. General considerations

When both systemic and local RC are considered, the results of the more critical approach will be given in detail, and the reasoning by which it was concluded to be more critical should be included for transparency. When both AELs and AECs are derived, both will be included to justify the choice of the most critical effect.

Only when it cannot be concluded which approach (local or systemic) would be more critical, both local and systemic RC should be carried out. In this case both approaches are documented in detail.

In the RC of local effects, the nature of the effects needs to be considered. When the effects are considered as “minor irritant effects” (as defined in Chapter 2.1), this may result in different conclusions than if the effects were considered as “local effects leading to classification” or “other local effects” (as defined in Chapters 2.2 and 2.3).

The nature of the effect is dependent on the concentration and the dose of the active substance. The same substance may, at a low concentration and a high dose, cause only minor irritant effects while causing severe systemic effects, whereas more severe irritative/corrosive effects with no systemic effects may occur at higher concentrations and a low dose. It is thus necessary to consider the effects

[1] Guidance for the implementation of REACH (2008), Guidance on information requirements and chemical safety assessment, Chapter R.8, p. 119-120, Application of assessment factors to obtain the DNEL

at the concentration and at the dose level that are foreseen for humans in the RC. The assessment will concern actual exposure, after having taken into account appropriate RMMs and PPE (e.g. exposure under the gloves).

8.2. Minor irritant effects

8.2.1. Exposure < AEC

If the result of the RC of local effects is that human exposure does not exceed AEC, no further measures are necessary. Information on the possibly required PPE and RMM will be included.

8.2.2. Exposure > AEC

When the only local effects are minor irritant effects (see Chapter 2.1 above), these will not be sufficient to justify an Annex I non-inclusion of the active substance even when exposure exceeds the AEC(s) (after taking into account all the appropriate RMMs and PPE). This is because:

a. Minor irritant effects would not constitute an unacceptable risk to humans, which is the BPD requirement for Annex I non-inclusion.

b. Such effects will be heavily influenced by the formulation and the use patterns, and it is therefore an issue to be controlled at the product authorisation stage. Repeated dose studies may, however, be available only for the active substance dossier, and therefore all relevant information needs to be included in the CAR to allow informed decision making at product authorisation.

c. The effects are assessed taking into account the foreseen human exposure level, and ensuring that the exposure level in the studies was sufficient to cover possible human effects (see below in this chapter, and chapter 8.1). The assessment will only be valid if it can be assumed that the actual effects in humans will be minor irritant effects. Ultimately this will be controlled at the product authorisation stage, where all the necessary information is available, including the solvent, coformulants, pH and use patterns.

The conclusion that the risk is acceptable requires that 1) reversibility of the effect can be assumed, 2) the intervals of exposure allow complete healing before further exposure occurs and/or exposed individuals would be able to take measures when irritation occurs. Consideration should be given to whether exposure is primary or secondary, as in the latter case the exposed persons might not be aware of the possibility of exposure.

When exposure exceeds the AEC even after all the appropriate RMMs and PPE have been taken into consideration, the conclusions of the RC of local effects will be included in Doc I, *Elements to be taken into account by Member States when authorising products*. At the product authorisation stage, PPE should be recommended and appropriate warning should be given to the user of the possibility of reversible irritant effects.

If systemic effects are observed as well as minor irritant effects, a systemic RC will always be carried out (even when local effects result in more critical AECs and a lower MOE).

Since the severity of the effects will depend on the concentrations used, it will need to be ascertained that the exposure level in the studies was sufficient to cover possible human effects at the foreseen human exposure level (considering both concentration and quantity). It will need to be ascertained that the actual human exposure levels will not be orders of magnitude higher than the AEC. This is because with rising concentrations/area doses, the effects tend to become more severe. Generally, exceeding the AEC up to 10-fold might be considered acceptable depending on the nature

and frequency of exposure. However, if the dose-response curve is steep, the excess above the AEC should be smaller. This will need to be decided on a case-by-case basis.

8.3. Local effects other than minor irritant effects

8.3.1. Exposure < AEC

If the result of the RC of local effects is that human exposure does not exceed AEC, no further measures are necessary. Information on the possibly required PPE and RMM will be included.

8.3.2. Exposure > AEC

When local effects are observed in repeated dose studies and they cannot be considered as minor irritant effects, it is necessary to consider the reversibility of the effects, and possible more serious consequences of continued exposure.

Exceeding the calculated AEC(s) would result in an unacceptable scenario (i.e. possible Annex I non-inclusion) when 1) the local effects cannot be identified as reversible, or 2) a serious health effect is considered possible as a consequence of exposure. In the absence of such concerns, expert judgment should be used to decide whether the same principles can be applied as for minor irritant effects, and whether Annex I inclusion would be possible.

Irreversible effects should in general not be considered as acceptable. It is however not always clear whether an effect is reversible or not, because the study may not have been designed to answer such questions, and because the nature of the effect may depend on the concentration and the dose level. Furthermore, irritation (and corrosion) can be determined by various methodologies with different sensitivities to endpoints like heat, redness, swelling, pain and dysfunction. Expert judgment may be needed to evaluate whether the effect is of a reversible nature or not.

If it is foreseen that continued exposure may occur even after the onset of the observed effects, it is necessary to consider the possibility of development of respiratory diseases, cancer or any other serious health effects that might result. Depending on the effects, substances tested and possible formulations, either 1) additional RMMs and PPE should be considered (e.g. barrier creams, specialised gloves, frequent change/removal of gloves, coveralls, specialised respiratory equipment), 2) further data can be required to remove the concerns, or 3) the concerns need to be clearly communicated in the RC to allow these to be properly addressed at the product authorisation stage through a refined exposure assessment and/or RC.

For local effects in general, a low concentration/area dose will often result in minor irritant effects, and only with increasing concentration/area dose the effect will become more severe. Therefore the data must be assessed in its entirety to conclude whether exceeding the AEC should in fact be considered as resulting in effects that are more severe than minor irritant effects.

9. Concluding remarks

The studies to be used for the RC of local effects may be part of the core data set, but even as such, these are not always required by the RMS. Their availability will depend on e.g. the application of the substance/product, the route of exposure, the suitability of active substance data for evaluation of the representative product, the degree of dermal penetration, the results of the acute toxicity test and the physico-chemical properties of the substance. This is a problem because equal treatment of active substances cannot be guaranteed in a situation where restrictive decisions are made based on information that is not mandatory.

This guidance should be used with the necessary flexibility until more experience on RC of local effects has been gained. Expert judgment should be used to avoid the risk of overconservative and disproportionate results, taking always into consideration a weight-of-evidence approach and any realistic exposure scenarios.

10. ABBREVIATIONS

AEC	Acceptable Exposure Concentration
AEL	Acceptable Exposure Level
AF	Assessment Factor
BPD	Biocidal Products Directive
CLP	Classification, Labelling and Packaging
C&L	Classification and Labelling
GI	Gastrointestinal
IPCS	International Programme on Chemical Safety
LLNA	Local Lymph Node Assay
MOE	Margin of Exposure
NOAEC	No Observable Adverse Effect Concentration
NOAEL	No Observable Adverse Effect Level
PPE	Personal Protective Equipment
RC	Risk Characterisation
RMM	Risk Management Measures
RMS	Rapporteur Member State
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance

Appendix 1. TM considerations on AFs

This Appendix provides some TM considerations on AFs. This supplementary information can be used in expert judgment.

Interspecies AF

Interspecies AF can be divided into a toxicodynamic component and a toxicokinetic component. For rat to human interspecies extrapolation, these components are usually set at 2.5 for the toxicodynamic component and 4 for toxicokinetic differences. In the risk characterisation of local effects, both these components can in certain circumstances be reduced. The uncertainties on the AFs can be very high for local effects, and any adjustments should be done with caution. The value by which the AF is adjusted should be considered on a case-by-case basis, and the reasons should always be justified.

- Toxicokinetic AF 4

  When the mode of action is direct chemical/pH reactivity, the toxicokinetic component of 4 can be disregarded for local effects as such effects do not involve kinetic and metabolic processes. It may be necessary to consider whether the mode of action may involve other than direct chemical/pH reactivity, resulting in a need to apply an AF for toxicokinetic differences.

  > *For local effect at the port of entry (skin, eye, G.I. tract) it is sometimes justified to assume that either toxicokinetics or –dynamics (or both) do not contribute significantly to interspecies differences (as for example in the case of direct/pH-driven chemical action on tissue/cell membranes). In such cases, based on sound scientific reasoning, the 10-fold default factor might be reduced dependent on the mode of action.* [2]

- Toxicodynamic AF 2.5

  For oral and dermal local effects, a distinction has to be made between a) direct chemical reactivity which does not involve local metabolism, and b) other or unknown mechanisms. If it is known that the local effect is caused by direct chemical reactivity where metabolism has no role (e.g. simple membrane destruction by acids/bases), the factor 2.5 can also be omitted, leaving an interspecies AF of 1. This is because it is assumed that humans are not more sensitive than experimental animals to these direct local effects on the skin or the GI tract. On the other hand, if the mechanism is not known, or if local metabolism may have a role, then the factor 2.5 is applied. The possible influence of local metabolism is taken into account in this AF although it does not clearly form a part of either the toxicokinetic or the toxicodynamic component of the AF.

  For respiratory local effects, the toxicodynamic factor is applied because it is assumed that humans are more sensitive than animals to any effects on the respiratory tract, regardless of the mechanism. The AF 2.5 is therefore applied, but it is in reality an uncertainty factor rather than a toxicodynamic factor.

  > *"With regard to local effects on the respiratory tract, guidance is available e.g. from the EU project ACUTEX, which proposes to apply reduced interspecies AFs when extrapolating data obtained in rats to humans. Given that there could be significant quantitative differences in deposition, airflow patterns, clearance rates and protective mechanisms between humans and animals and when there is no data to inform on this uncertainty, it is prudent to assume that humans would be more sensitive than animals*

[2] TNsG on Annex I Inclusion; Chapter 4.1.3: Selection of Assessment Factors

to effects on the respiratory tract. In such a situation the default factor of 2.5 to address remaining uncertainties should be applied." [1]

Intraspecies AF

The intraspecies AF can be divided into components of 3.2 for toxicodynamic variability and 3.2 for toxicokinetic variability. It is considered that information on intraspecies variation for local effects is very scarce and it is therefore generally suggested not to refine these default factors. This is consistent with REACH guidance[3].

In some cases it may nevertheless be possible to reduce the intraspecies AF if there is sufficient information available on either human variation (human data), or on the mechanism of action of the local effect together with knowledge on human variation of the mechanistic process involved. This would be consistent with IPCS guidance[4]. For instance, intraspecies variability may be small for local effects exerted through an irritant effect. In such cases it may be appropriate to reduce the intraspecies AF.

If the mode of action does not involve local metabolism, the default toxicokinetic factor may be reduced to 1, as no kinetic or metabolic processes are involved.

However it should be acknowledged that dermal irritation is not an immunologic inert process but involves different cytokines and intercellular interactions leading to tumor, rubor, calor, dolor, functio laesa. Thus skin irritation is a complex process that may show intraspecies variability (Fluhr et al. 2008). It is important to note that the intraspecies AF of 10 and its subdivision into 3.16 for toxicokinetics and 3.16 for toxicodynamics (as proposed in the IPCS guidance) is so far only based on data for systemic but not local effects. In fact Basketter et al. 1996 reports substantial human intraspecies differences for acute local effects with SDS. Therefore a potential reduction of the AF to 1 for substances without critical local metabolism cannot be based on evidence for low human intraspecies variability. This reduction may eventually be based on the publications of Jirova et al. 2007 and Basketter et al. 2004 indicating that with regard to acute dermal irritation the animal test is very conservative. The available data indicate that the rabbit test data predict the 4h-human-patch-test data with a sensitivity of about 100% but with a specificity just of or below 50%. However in case there is scientific agreement on this false positive rate for acute local dermal classification, the results and NOAECs from repeated dose local dermal studies also may be considered as too uncertain to be used for a quantitative local RA at all.

A reduced intraspecies AF should always be justified.

For further reading on AF adjustment, please see [5].

[3] Guidance for the implementation of REACH (2008), Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose [concentration]-response for human health, point 4.3.1
[4] WHO/IPCS (2005), Chemical-specific adjustment factors for interspecies differences and human variability: Guidance document for use of data in dose/concentration-response assessment. WHO Press, Switzerland
[5] JMPR 2008, general consideration 4; JMPR 2008 summary report, pp 24-28, http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2008AnnexIFinal.pdf

Appendix 2: Literature references

This Appendix is provided by AT. It provides and briefly summarises some primary literature references that may be useful in judging the uncertainty of RC for local effects, in the selection and discussion of AF, and when considering qualitative *vs.* quantitative RC and data requirements.

7. Kalberlah F, Föst U, Schneider K. 2002. Time extrapolation and interspecies extrapolation for locally acting substances in case of limited toxicological data. Ann Occup Hyg. 46(2):175-85.

   The tabled review indicates the variation between rat and human N/LOAELs over 33 ATSDR reports (e.g. particle exposure: factor >100 for 32% and factor 5-100 for 21% of reports; gas exposure: factor >100 for 6% and factor 5-100 for 42% of reports). The author comments that the comparability of the human observations and the endpoints analyzed within the animal studies is problematic and limits his interpretation of the data to supporting that humans on average are marginally more sensitive than rats.

8. Jirova *et al*. 2007. Comparison of human skin irritation and photo-irritation patch test data with cellular in vitro assays and animal in vivo data. AATEX 14, Special Issue, 359-365.

9. Basketter *et al*. 2004. Determination of skin irritation potential in the human 4-h patch test. Contact Dermatitis, 51:1-4.

   With regard to skin irritation, Jirova and Basketter (publications 2 and 3) indicate that acute dermal irritation studies in rabbits show a sensitivity of about 100% but specificity of or below 50% for the prediction of 4h-human-patch-test data. The new *in vitro* human skin method EU-B46 (full replacement of *in vivo* method) seems to perform superior. However no discussion is available of the implications of these data for interspecies uncertainty estimates for local dermal repeated dose NOAECs.

10. Basketter *et al*. 1997. The classification of skin irritants by human patch test. Food Chem Toxicol. 35(8):845-52

11. York *et al*. 1996. Evaluation of a human patch test for the identification and classification of skin irritation potential. Contact Dermatitis 34(3): 204-12

12. Robinson *et al*. 2001. Validity and ethics of the human 4-h patch test as an alternative method to assess acute skin irritation potential. Contact Dermatitis 45(1): 1-12)

    Basketter, York and Robinson (publications 4, 5, 6) inform on the protocol for the 4h-HPT: 30 human volunteers are exposed to the substance with 0.2g/25mm plain Hill chamber for up to 4 hours. As soon as weak but unequivocal erythema is observed exposure is stopped in the respective individual and counted as positive response. The substance is considered as skin irritant (R38), when the incidence of positive irritation reactions to the undiluted test substance is statistically significantly ≥ the level of reaction in the same panel of volunteers to 20% SDS

13. Basketter *et al*. 1996. Individual, ethnic and seasonal variability in irritant susceptibility of skin: the implications for a predictive human patch test. Contact Dermatitis 35, 208-213

    With regard to skin irritation Basketter *et al*. reports substantial human intraspecies differences for acute local effects with SDS: while up to 76% of humans showed irritation with up to 20% of SDS still up to 9% of humans showed irritation with 0.25%. Also seasonal effects are reported.

14. Fluhr *et al*. 2008. Skin Irritation and Sensitization: Mechanisms and New Approaches for Risk Assessment. Skin Pharmacology and Physiology 2008, 21: 124-135

Fluhr *et al*. reviews that dermal irritation is not an immunologic inert process but involves different cytokines and intercellular interactions, however he provides just qualitative information on individual and environment related variables

15. Falk-Filipsson *et al* 2007. Assessment factors-applications in health risk assessment of chemicals. Environ. Res. 104, 108-127