



*Scientific Principles for Soil Hazard
Assessment of Substances*

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EXECUTIVE SUMMARY

This report reviews the scientific principles that should underpin any future terrestrial (soil) hazard classification scheme for organic and inorganic substances. The terrestrial effects of a substance are determined by a combination of its intrinsic toxicity and bioavailability, and these two factors are considered to be the main drivers for soil hazard classification. Experimental effects data are always a combination of both factors, and need to be standardised and normalised before they are useful for classification purposes. Surrogate data, including read-across from aquatic effects data and equilibrium partitioning for organic substances, and 'transformation' data for substances of low solubility, may lead to both over- and under-estimates of terrestrial hazard, and should be applied with caution.

Modifying factors to be included in a soil classification scheme are related to elimination (the length of time a substance is likely to stay in the soil compartment) or possible long-term effects (long-term toxicity). Factors not recommended for inclusion in such a scheme are bioaccumulation and slope of the dose-response curve.

Certain main elements of a terrestrial classification scheme, including selection of the most appropriate terrestrial test guidelines, assessment of data quality and relevance, read-across methods from aquatic data and how to incorporate the modifying factors, merit further development.

The Task Force considered that the current EC risk phrases related to individual terrestrial organisms living in soil were not appropriate and would be better replaced by a classification based on two or three toxicity classes amended by a term for potential long-term effects. This is analogous to the existing classification scheme for the aquatic environment and would therefore encourage harmonisation, and improve transparency and ease of use.

1. INTRODUCTION

Whereas classification criteria for the aquatic compartment (R50 - R53) and the ozone layer (R59) have been established, as yet no criteria exist for terrestrial organisms (Table 1). Proposals for a detailed terrestrial classification scheme have been developed (KEMI, 1996; Tarazona *et al*, 1996; Tarazona and Fresno, 1997; Torstensson *et al*, 1997) and discussed in various EC and OECD working groups. However, in these approaches, four initial questions remain largely unanswered, namely:

- The purpose of such a terrestrial classification scheme;
- the added value of any terrestrial classification scheme over the existing aquatic scheme to meet environmental protection objectives;
- the priority to develop a terrestrial classification scheme;
- the underlying scientific principles.

The present report focuses on the last question, i.e. the scientific principles on which a terrestrial classification scheme should be based.

The general purpose and principles of classification and labelling of organic and inorganic substances are laid down in the so-called 'Dangerous Substances' Directive 67/548/EEC (EC, 1967) and its subsequent legal amendments and adaptations to technical progress. According to this directive, substances are ranked according to intrinsic hazardous properties covering physico-chemical data, human health and environmental impact. These potential hazards are described by 'R-phrases' and in some cases specific hazard symbols such as 'T' or 'N'. For labelling, the R-phrases are combined with safety advice (S-phrases).

The primary aim of any environmental labelling system is to inform users about the environmental properties/hazards of the substances (in the form of products) that they are handling, and provide guidance for risk management. Substances (products) are handled at several stages of the life cycle, from production and transport to consumer use and disposal.

Possible users of a classification system for substances exist in all three of these groups, but the quantities and form in which substances are handled may be quite different. Therefore it might be considered desirable to design a classification (labelling) system tailor-made for the needs of these three constituencies, providing specific information most relevant to the way in which the product is handled. For example, during transport, substances are normally handled in large quantities and the environmental hazard posed, in the case of an accident, is mostly acute or at least spatially limited. For the consumer/end-user, the quantity of product handled is likely to be much smaller, but its handling and disposal may be more continuous and widespread. Information on chronic hazards may be relevant for this type of user. Diversity in classification systems to suit the needs of the users would however not favour harmonisation. In the present report, the focus will therefore be on a single classification scheme that provides useful information for all three target groups.

It should be noted that the possible hazards as characterised by the R-phrases are different from the actual risk. Risk can be defined as the result of the combination of likely exposure and hazard, or the probability of adverse effects occurring as a result of exposure of an organism to hazardous concentrations of a substance. The exposure of organisms in soil, water or air is highly dependent on the use pattern, release and fate of the substance. Therefore, a compound that is, for instance, toxic to fish does not necessarily present a risk to the aquatic environment as long as its release to surface water is limited. For a comprehensive risk assessment on a substance the toxicity to organisms is compared with either the estimated or measured exposure of such an organism. Such a comparison is outside the scope of classification and labelling. If sufficient information on use scenarios and exposure patterns is available, substances should be evaluated by means of risk assessment procedures rather than by a hazard-based approach.

Many R-phrases prescribed in the Dangerous Substances Directive relate to toxicological properties with respect to humans. R-phrases concerning environmental properties of substances were enacted in later adaptations in Directives 92/32/EEC and 93/21/EEC (EC, 1992, 1993). Those environmental R-phrases are summarised in Table 1.

Table 1: Environmental risk phrases in the EU

| Number | Description |
|--------|--|
| R50 | Very toxic to aquatic organisms |
| R51 | Toxic to aquatic organisms |
| R52 | Harmful to aquatic organisms |
| R53 | May cause long-term adverse effects in the aquatic environment |
| R54 | Toxic to flora |
| R55 | Toxic to fauna |
| R56 | Toxic to soil organisms |
| R57 | Toxic to bees |
| R58 | May cause long-term adverse effects in the environment |
| R59 | Dangerous for the ozone layer |

Specific criteria for R-phrases related to aquatic organisms are laid down in Directive 93/21/EEC, as an adaptation to technical progress of Directive 67/548/EEC Annex VI (this report, Appendix A). A specific hazard symbol 'N' (dangerous for the environment) and a pictogram were also introduced. At present, substances are labelled as 'dangerous for the environment' in combination with the symbol 'N' if the phrases R50 or R50/R53 or R51/R53 apply.

In contrast to classifications for possible effects on human health, the use of R53 (for non-readily degradable or bioaccumulating substances) adds a parameter of potential long-term exposure and effects to the environmental classification process. For metals and metal compounds the R53 phrase is added only if there is evidence for long-term chronic effects (ECB, 1995). Additionally the R53 is applied as a safety-net procedure for insoluble metals and 'sparingly soluble metal compounds, if the corresponding soluble salt could give rise to classification'.

S-phrases giving safety advice relevant to the environment, have to be stated on the label in relation to the safe handling, transport and disposal of substances (Appendix A).

It should be noted that soil is an extremely heterogeneous compartment as compared to the aquatic environment. Soils vary in the mineral and organic matter content and sizes of particles, aggregates, pores and micropores. In addition they contain air and water. These create a wide spectrum of environmental conditions, which influence substances in a number of ways. Substances can be adsorbed to the organic matter or to mineral components of the soil, thus reducing their bioavailability. Soil pores and micropores may act as traps for desorbed matter. Substances may at the same time encounter aerobic and anaerobic conditions. Soil water may therefore be quite different in chemical reactivity from surface water. Thus, bioavailability, ageing, and other phenomena, influence and modify the effects of chemicals in soil.

2. AIMS

To develop further industry's understanding of the subject an ECETOC Task Force was established with the following Terms of Reference.

1. Identify and evaluate the parameters of fate, bioavailability and effects that determine the hazard of substances in soil, and in this respect:
 - Evaluate the applicability of existing test methods;
 - evaluate the advantages/disadvantages of extrapolating values derived from aquatic toxicity tests or quantitative structure-activity relationship (QSAR) based approaches.
2. Recommend the scientific principles to be taken into consideration for a future hazard-based classification scheme.

The above Terms of Reference limit the scope of the present report in three ways, namely:

1. The report deals with hazard assessment, including some elements of fate, but does not address risk directly.
2. The report is confined to the assessment of hazard for organisms living in the soil. The hazard that a substance may pose to organisms, such as bees, birds and upper parts of plants in the 'above-soil' compartment, or to groundwater, are not included in the scope of this report. The decision was made in parallel with the aquatic system on the grounds that the 'in-soil' is the sub-compartment in which exposure of terrestrial organisms is most likely to occur.
3. The Task Force activities were focused on the general scientific principles and parameters underlying a terrestrial (soil) classification scheme. Although the basis for a classification scheme has been proposed, the Terms of Reference did not require the proposal of a ready-to-use scheme (with criteria and risk phrases), nor discussion of the added value of such a scheme over the existing aquatic scheme in environmental classification. The Task Force considered both these activities to be important, and recommended that they be addressed elsewhere before a terrestrial (soil) classification scheme was applied.

The proposals made in the text below should be applicable to all organic and inorganic substances including metals, pharmaceuticals and plant protection products.

3. IDENTIFICATION OF MAIN DRIVERS

The classification of chemical substances is based on hazard assessment. Hazard is an intrinsic property of a substance and is unaffected by environmental variables. Indeed the distinction between hazard and risk is that risk relates environmental conditions, exposure scenarios and product properties to give a probabilistic determination that the hazard will be expressed. Basing classification on hazard is appropriate since it should be relevant for a range of environmental conditions and use scenarios.

Currently, for environmental classification and labelling, hazard is expressed in terms of intrinsic toxicity and the likelihood of long-term effects. In the aquatic environment, intrinsic toxicity is estimated by conducting tests in simple systems in which the bioavailability of the substance is maximised. To ensure this, the test vessels contain no sediment and, for volatile substances, are sealed. Reduced bioavailability is not specifically taken into account in aquatic classification and labelling because natural water bodies exist where the bioavailability may be close to 100%, e.g. in case of non-volatile, soluble substances in oligotrophic waters.

In the terrestrial environment, bioavailability will practically always be less than 100%. Some terrestrial test systems have been designed with the objective of maximising bioavailability; they are essentially water with a quartz sand substrate to represent soil. The use of such systems to generate toxicity data expands the taxonomic diversity of tested species beyond those considered under the existing aquatic classification and labelling scheme. However, the value of simply expanding the range of species is questionable. To add value to the existing aquatic scheme, terrestrial classification and labelling needs to take into account bioavailability. Thus, a terrestrial classification and labelling scheme should have two main drivers, toxicity and bioavailability.

Substances in the terrestrial environment can exist in one or more of five phases: sorbed to particles or solid phases, absorbed in particles, associated with colloidal materials (e.g. dissolved organic carbon), free in solution or air, or as independent solids. Physico-chemical properties of an ingredient and the properties of the different phases determine the extent to which a compound partitions among these phases.

Differences in the binding capacity of different soils are due to heterogeneous soil chemistry and physics, and correlate to variations in the proportions of the major components: mineral and organic matter, water and air. Relevant parameters comprise, among others, organic carbon, clay type and content, humidity, pH-value, cation exchange capacity (CEC) and temperature.

Association with solid phases is generally referred to as sorption; either adsorption onto a two-dimensional surface, or absorption into a three-dimensional matrix. The underlying processes of adsorption may be due to Van der Waals interactions, hydrophobic interactions, hydrogen bonding, charge transfer interactions, ligand exchange and ion bonding, direct and induced ion-dipole and dipole-dipole interactions or covalent binding. The underlying process of absorption may be due to diffusion into the pore structures of soil particles.

Integration of toxicity and bioavailability into a terrestrial classification system requires measurement or estimation of the effects of a substance in a standard soil with high, but sub-maximal, bioavailability. It is anticipated that the results of tests conducted with non-standard soils could be normalised to standard soil conditions, provided that the parameters defining bioavailability are known for the non-standard soil. The advantage of standardisation is that it permits comparison of the relative hazards of different substances. A number of parameters will be involved, depending on the nature of the contaminant. The precise description of the high bioavailability soil will be largely arbitrary, but it will almost certainly differ from the OECD artificial soil currently used in earthworm testing which, with a peat content of 10%, is atypical of most field soils.

As bioavailability is key to a discussion of terrestrial hazard classification, a review of the term is provided in Appendix B, exemplifying use of the term in different science fields. The available definitions of bioavailability that exist all have slightly different meanings. In the context of this report on terrestrial soil hazard assessment, bioavailability is defined as:

The fraction of the total quantity of a compound in the soil that interacts with an organism.

Importantly, the Task Force recognised that changes over time in the external environment of an organism could change the bioavailability of a chemical. Such changes may result from influences of the organism on its external environment. For instance, exudates of plants may affect soil pH, and thus change metal speciation and subsequently bioavailability. Another example is that extracellular enzymes excreted by micro-organisms may facilitate biodegradation outside the organism. Thirdly, gut-processing after ingestion of soil by an organism, which occurs in an enzymatically rich environment, may increase bioavailability as it will influence equilibrium partitioning conditions (Johnson and Jones, 1997).

4. MEASUREMENT OF TERRESTRIAL EFFECTS

Ideally for terrestrial classification purposes, and in alignment with the existing aquatic classification system, species tested should include representatives of different trophic levels such as plants, earthworms and potentially a third standard terrestrial test organism from a different taxonomic group. Any terrestrial testing will automatically incorporate bioavailability. Therefore, it is preferable to use data from tests that have been standardised in terms of soil composition and equilibration time after adding the test compounds, since these parameters influence bioavailability considerably. However, since the current terrestrial test guidelines allow the use of a range of soil types and qualities, normalisation of the test data may be needed (e.g. percentage organic carbon, clay content and pH) to correct for differences in bioavailability.

4.1 Available test methods for terrestrial effects

Internationally established test methods for effects on soil organisms are available for earthworms, biological soil functions (soil micro-organisms), higher plants and soil-dwelling beetles and spiders; another method exists for collembolans (Table 2).

With the exception of the soil micro-organism tests, the test organisms are transferred to the contaminated soil substrate, which is a characterised natural soil or a standardised artificial mixture. During the test period various endpoints such as mortality, growth, reproduction or metabolic activities are recorded and, where possible, dose-response curves are established. Since the standard test methods with soil micro-organisms, beetles and spiders are designed as limit tests, no dose-response relationship can be established in these studies. In general, analytical verification of test concentration in the soil substrate is not required according to the standard methodology; this is in contrast to aquatic testing methods. Effects should be expressed in terms of loading rates, because measurements of concentration include bioavailable and non-bioavailable fractions, and so do not reflect exposure. Further details on the individual test parameters are given in Table 2.

Table 2: Terrestrial ecotoxicity test guidelines

| Organism | Protocol | Substrate | Endpoint | Duration (d) | Dose-response | Reference |
|---|------------------------------|--|---|--------------|---------------|---------------------------|
| Earthworm (<i>Eisenia fetida</i>) | OECD 207 | Artificial (10% peat; pH 6) | Mortality (LC ₅₀), growth (NOEC) | 14 | Yes | OECD, 1984a |
| | BBA VI, 2-3 | Artificial (amended with 1% manure) | Reproduction, growth | 56 | No | BBA, 1994 |
| Soil micro organisms ¹ (metabolic activities) | BBA VI, 1-1 OECD Draft | Natural soils | Carbon and nitrogen turnover; recovery | 28 | No | BBA, 1990 |
| Rove beetle ¹ (<i>Aleochara XV/3 bilineata</i>) | IOBC/WPRS | Moist sand | Mortality, parasitisation rate | ? 80 | No | Moreth and Naton, 1992 |
| Carabid beetle ¹ (<i>Poecilus cupreus</i>) | IOBC/WRRS XV/3 | Moist sand | Mortality, feeding rate | 14 | No | Heimbach, 1992 |
| Lycosid spiders ¹ (<i>Pardosa sp.</i>) | BBA VI, 23- 2.1.9 | Quartz sand | Mortality, feeding rate | 21 | No | BBA, 1993 |
| Collembola (<i>Folsomia candida</i>) | ISO, 1999 | Artificial (10% peat, pH 6) | Reproduction (LOEC and NOEC) | 28 | Yes | ISO, 1999 |
| Terrestrial plants (various species) | OECD 208 | Potting soil (1.5% organic carbon, pH 5 - 7.5) | Emergence (LC ₅₀), growth (EC ₅₀) | 14 | Yes | OECD, 1984b |

¹ Developed for plant protection product registration in the EU

In addition to these international guidelines, other terrestrial test methods have been reported in the literature (Løkke and van Gestel, 1998). However, the suitability of these for use in hazard classification needs to be considered.

4.2 Need for standardisation

The guidelines summarised in Table 2 cover a wide range of soil-dwelling organisms and are considered satisfactory in this respect. However, they do not measure intrinsic toxicity to soil organisms of a substance, but rather a combination of intrinsic toxicity and bioavailability that is specific to the test system used. Therefore there is a need to consider further standardisation, particularly with respect to soil organic matter content and equilibration time after adding the test compound, since both parameters may have a major influence on bioavailability.

Evidence that soil organic matter content affects bioavailability was observed by plotting aquatic toxicity against soil toxicity; the correlation was very poor. However, if the soil toxicity data were normalised for organic carbon the correlation was improved (Torstensson *et al*, 1997).

Evidence that ageing of chemical residues in soil affects bioavailability is diverse. For example, Ma *et al* (1995) showed that worm bioaccumulation factors for fluoranthene rise and then fall with increasing exposure duration. This observation is presumed to reflect changes in the bioavailability of fluoranthene with time. Kelsey and Alexander (1997) showed that the amounts of atrazine, phenanthrene and naphthalene assimilated by *Eisenia fetida* were negatively correlated with time between mixing of the substance in soil and the start of exposure (aggressive extraction of the soil indicated that the substances had not degraded). Belfroid *et al* (1995a) examined the bioaccumulation of chlorobenzenes by earthworms from freshly contaminated OECD standard soil and from a field soil contaminated 20-30 years previously. After organic matter normalisation of the soil, there was still a difference in the soil-to-worm bioaccumulation factor of 2-30 times; this may be attributable to ageing of the chemical residues. A further comprehensive discussion on the influence of ageing on the hazard of substances in soil is included in Appendix C.

4.3 Need for quality control

The value of any classification exercise greatly depends on the quality of the data on which it is based. In addition to the need, described above, for the standardisation of terrestrial test methods and their results, there is also a need for the development of appropriate data quality parameters for such studies, e.g. positive controls and acceptable test responses, including data variability.

5. ESTIMATION OF TERRESTRIAL EFFECTS

Few terrestrial effects data have been generated for substances other than agrochemicals. Classification of such substances in the absence of terrestrial data would require an estimate to be made of their terrestrial effects by other means. Several potential approaches exist for different groups of compounds such as read-across from aquatic toxicity data, transformation testing for poorly soluble substances (e.g. metals) and use of Quantitative Structure Activity Relationships (QSARs). These approaches are discussed below in more detail.

5.1 *Read-across from aquatic toxicity*

The toxicity of a substance to terrestrial biota may be estimated from aquatic toxicity data on the same substance. This approach is based on the general paradigm that terrestrial organisms are primarily exposed through the soil porewater, and that porewater concentrations can be estimated from basic physico-chemical properties of the substance in combination with a simple characterisation of a standard soil environment. At present, porewater concentrations are derived from bulk soil concentrations on the assumption that the partitioning between soil solids and porewater is determined by the soil/water partition coefficient (K_{oc}) of the substance (Appendix C.1.5).

This approach raises the following questions:

- Are there data that can be used to validate the use of aquatic toxicity data as surrogates for terrestrial effects, through the use of equilibrium partitioning;
- if data do not exist, are there other lines of evidence to support the acceptability, or otherwise, of the equilibrium partitioning approach to estimating terrestrial toxicity.

The results of a literature search to address these questions (Appendix C) highlight two major problems in evaluating the use of equilibrium partitioning for determining terrestrial toxicity. Firstly, there are insufficient data to evaluate the theory, although the existing data are broadly supportive. Secondly, the current approach may simplify the exposure of terrestrial organisms leading to an over- or under-estimate of terrestrial toxicity, when compared to aquatic systems (Table 3).

Table 3: Impact of parameters on terrestrial toxicity

| Parameter | Impact on terrestrial toxicity (over/under-estimate) | Remark |
|---|--|---|
| Oral exposure | Under | For substances with $\log K_{ow} > 5$ terrestrial toxicity may be underestimated by a factor up to 2.5 |
| Lack of exposure to porewater | Over | May be important for isopods or other soil dwelling organisms with a less permeable body wall |
| Sorption to soil components other than organic matter | Over | Clay may reduce the bioavailability of other substances in soil with an organic carbon content $< 0.1\%$ |
| Nature of the organic matter | Over or under | - |
| Ionisation of the chemical | Over | Compensation for ionisation can be made provided the pKa of the chemical and pH of the test medium is known |
| Behaviour of the test organism | Over or under | - |
| Ageing of chemical residue | Over | Bioavailability may decrease over time |
| Porewater not at equilibrium | Over or under | Directly after spiking concentration in the porewater may be higher than those at equilibrium leading to overestimation of toxicity. Depletion of porewater by bio-accumulation may lead to underestimation of toxicity |
| Dissolved but complexed and not bioavailable | Over | |

In an evaluation of the equilibrium partitioning approach to bioaccumulation, Belfroid *et al* (1996) concluded that the factors above may lead to an error in either direction (overestimation or underestimation) of an order of magnitude or more. It may be concluded that a similar magnitude of error may apply to toxicity estimation through the use of equilibrium partitioning.

5.2 Transformation testing for poorly soluble substances

The lack of terrestrial toxicity data on poorly soluble substances such as metals and metal compounds may be offset by a further development of equilibrium partitioning theory, using the transformation protocol developed for metals and metal compounds in the aquatic compartment (ECB, 1995) (Appendix D).

In a similar manner to the aquatic system, classification can be effected through comparison of the data obtained from transformation tests and standard ecotoxicity tests on a readily soluble compound of the metal. An outline of such a transformation test, compared to a standard soil test, is given in Table 4 below. By measuring the metal concentration in the porewater after standardised equilibration, the transformation test can estimate the amount of metal available in the soil and as such its 'bioavailability'.

Table 4: Standard and transformation testing of metals and sparingly soluble metal compounds

A. Standard terrestrial ecotoxicity test on readily soluble and neutral metal compounds

1. Load (by mixing a given mass of) substance to standard (wet) soil
2. Equilibrate for a given period ¹
3. Perform standard ecotoxicity test(s)
4. Determine corresponding (critical: EC₅₀) porewater metal concentration, e.g. by centrifugation of soil sample through 0.45 mm filter

B. Transformation test on metal or sparingly soluble metal compound

1. Load (by mixing a given mass of) substance to standard (wet) soil
2. Equilibrate for fixed period ¹
3. Determine porewater metal concentration after centrifugation through 0.45 mm filter
4. Compare porewater concentrations obtained with the different mass/surface loadings with the critical porewater concentration, determined on the soluble metal compound

¹ Equilibration time needed to reach an asymptotic metal concentration in the supernatant of a centrifuged soil sample

A few practical problems remain to be solved before this methodology can be applied. These are related to the mode of application of the substance to soil (i.e. in solution, as a slurry or as a dry powder), soil moisture content at time of application (i.e. should soils be soaked to ensure proper dissolution of the soluble fraction of the substance, or mimic specific environmental conditions) and determination of the appropriate equilibration time.

5.3 Quantitative Structure Activity Relationships

QSARs are usually linear regression equations that relate some structural descriptor or descriptors of a group of substances to a specific activity of those substances. The specific activity may be a biological endpoint, such as the EC₅₀ for an aquatic organism, or a physico-chemical property such as solubility or volatility. QSARs used for the assessment of the environmental fate and effects of substances are of two types. The first is based on a mechanistic understanding of the relationship between chemical structure and endpoint. Although it seems intuitive that this is the best approach, recognition of the actual mechanistic relationship (e.g. the mode of action), requires considerable expertise and may, therefore, be contentious. The second type is based only on a mathematical or empirical relationship, with no understanding of why the relationship

exists. The structural range of substances (the chemical domain) in a mechanistic QSAR tends to be more easily defined as are the limitations of the relationship. The various QSARs used in ecotoxicology have been reviewed by ECETOC (1998).

Most environmental QSARs have been developed to predict the aquatic toxicity of substances from a hydrophobicity descriptor. This descriptor is usually closely related to the octanol/water partition coefficient K_{ow} , which is considered to be a good indicator of the partitioning of a chemical into aquatic organisms that are exposed to the dissolved form.

Compared to the aquatic environment there has been little development of QSARs that could predict the toxicity of substances to terrestrial organisms. Van Gestel and Ma (1990) determined 2-week LC_{50} values of 7 organic chemicals (5 chlorinated phenols, 1,2,3-trichlorobenzene and 2,4-dichloroaniline) in 2 species of earthworms using 4 different soils. The LC_{50} values were related to porewater concentrations, which were calculated from soil adsorption data. On this basis the authors proposed linear regression equations that related terrestrial toxicity to $\log K_{ow}$. The correlations were reasonable, although the domain of the relationship was not specified.

For the earthworm *Lumbriculus rubellus*:

$$\log LC_{50} (? \text{ mol/l}) = -0.52 \log K_{ow} + 3.57 \quad r = -0.804 \quad \dots\dots\dots(\text{Eq. 1})$$

For the earthworm *Eisenia andrei*:

$$\log LC_{50} (? \text{ mol/l}) = -0.76 \log K_{ow} + 4.2 \quad r = -0.942 \quad \dots\dots\dots(\text{Eq. 2})$$

These findings suggest that QSARs may be developed for predicting the toxicity of substances to terrestrial organisms, provided the toxicity data are expressed on the basis of the exposure concentrations actually experienced by the organisms, i.e. primarily the porewater concentrations. To relate such an estimated value to soil bulk density levels it must be combined with some estimate of bioavailability.

6. IDENTIFICATION OF MODIFYING FACTORS

In Section 3, intrinsic toxicity and bioavailability were identified as the main drivers for a terrestrial classification scheme. In order to pose a hazard to soil organisms, a substance needs to be both intrinsically toxic and bioavailable. In addition to these main drivers, a number of other parameters/properties influence the hazard that a substance may pose to organisms in the soil. These include elements of environmental fate, such as (bio-)degradation and mobility (leaching and volatility). Both these parameters provide information on the length of time a substance is likely to remain in the soil compartment following contamination. Substances with equal intrinsic toxicity and bioavailability will not pose the same hazard to soil organisms if the period that they remain in the soil is different. As in the existing aquatic classification scheme, factors influencing the potential for long-term effects of a substance could be included as modifying factors in a terrestrial (soil) classification scheme.

6.1 *Parameters included as modifying factors*

6.1.1 Long-term effects

Effects of substances on organisms in the environment may occur both after short- and long-term exposure. The toxicity data used to derive classification criteria for the aquatic environment (R50, R51 and R52) are mainly drawn from short-term experiments (acute 48-hour toxicity to daphnia, acute 96-hour toxicity to fish and 72/96-hour algal growth inhibition) because data from long-term studies are generally not available. Thus, the log K_{ow} and lack of ready biodegradability are used as surrogate indicators of a substance's potential to cause long-term effects (R53).

In the case of terrestrial classification, log K_{ow} is not considered to be a valuable indicator of long-term effects in the terrestrial environment (Section 6.2.2). Due to the importance of this type of effect, a classification scheme should ideally be able to take into consideration data for long-term effects when such data are available. However, there are few standardised test guidelines to assess the effects of long-term exposure in the terrestrial environment (Table 2).

6.1.2 Biodegradation

Biodegradability is often the most important factor controlling the persistence of a chemical in water and soil. Therefore biodegradability data provide useful information on the potential to cause long-term effects.

The examination of biodegradability of chemicals is organised in a logical series of tests of increasing complexity from ready biodegradability (OECD 301) and inherent biodegradability (OECD 302) to simulation tests such as degradation in wastewater treatment plant models or in soil (OECD 303). With the exception of soil and water degradation studies used to study the environmental fate of crop protection products, information about degradation products other than CO_2 is not normally obtained in these studies (Appendix E).

Ready biodegradability test methods are stringent in that they are low biomass systems and provide limited opportunity for biodegradation and acclimatisation to occur. Most existing methodology is concerned with biodegradation in aquatic media. There is a standard terrestrial biodegradation method (OECD 304) but studies according to this method are performed on a regular basis only for plant protection chemicals. Due to the lack of test results for the majority of chemicals, there is a need to examine the possibility of estimating biodegradation from aquatic data or QSARs.

The QSAR work on biodegradation, in common with that on environmental toxicity, has been centred on the aquatic environment. This work has been reviewed by ECETOC (1998). A number of the QSARs do not give quantitative predictions but, instead, indicate whether a substance is likely to be readily or ultimately biodegradable (SRC, 1994; Langenberg *et al*, 1995). These are better defined as SARs than QSARs. Some of the SARs are based on the presence or absence of specific structural fragments in the substance as an indication of good or poor biodegradability. Other SARs are expert systems based on the combined judgement of experts in biodegradation assessment. It is possible that some of the approaches used to predict biodegradation in aqueous systems could be applied to soil. However, at present there are no SARs available to do this.

The Task Force concluded that data from long-term toxicity studies, in combination with data on degradation and fate when available, should be used to modify the terrestrial classification of a substance.

6.1.3 Abiotic degradation

In addition to bio-degradation, substances may undergo other (abiotic) processes in soil, which may lead to their ultimate disappearance from soil. Abiotic degradation tests in soil are not commonly carried out, but for some groups, such as plant protection products, information on hydrolysis and photolysis in and on soil is routinely generated. If data from such studies exist and provide evidence for rapid degradation in soil, a classification system should ideally take this into account. This is especially true for those substances for which abiotic degradation is the principal degradation pathway and for which biodegradation is slow or does not occur.

6.1.4 Volatilisation

In case of spills or intentional application onto soil, it is useful to know how long a substance will persist in the soil, and volatilisation is one of the factors on which persistence depends. The rate at which a chemical volatilises from soil is affected by many factors, and these may be divided into four groups:

- Intrinsic properties of the chemical: solubility in water, K_{ow} , K_{oc} and vapour pressure;
- soil properties: diffusion characteristics, density, CEC, water and organic matter content;
- chemical concentration;
- local environmental conditions: airflow rate over the soil surface, humidity, temperature;
- ground cover, terrain, weather, soil pH, method of incorporation into the soil.

All these factors can influence the distribution of a compound between soil, soil water, soil air, and atmosphere and should be considered if volatilisation is to be used as a modifying factor in terrestrial hazard classification. A comprehensive model of the volatilisation process would have to consider all the factors mentioned above. ECETOC (1994) discussed the relevance of this for ammonia.

The studies of Burkhard and Guth (1981) have shown an excellent correlation between calculated and determined volatilisation rates for crop protection products. With knowledge of the water solubility, vapour pressure, and soil/water adsorption coefficient, the volatility of substances from a moist soil can be calculated.

The relationships between volatility and vapour pressure, water solubility and soil adsorption coefficient were established for 9 chemicals applied to the soil surface (Dow Chemical Company, personal communication). The half-life ($t_{1/2}$) for depletion of the chemical from the soil surface was described by:

$$t_{1/2} = 1.58 \times 10^{-8} \times (K_{oc} \times S / P_{vp}) \text{ [days]} \dots\dots\dots(\text{Eq. 3})$$

where K_{oc} = soil adsorption coefficient
 P_{vp} = vapour pressure
 S = water solubility

Thus, the rate constant for volatilisation (k_v) could be expressed as:

$$k_v = 0.693 / t_{1/2} \dots\dots\dots(\text{Eq. 4})$$

Although the effect of environmental conditions (such as soil moisture, soil type, temperature and wind) are not incorporated, this simple model may be used for classification purposes if no measurements are available. The use of this model may enable a distinction to be made between substances that are of low, moderate or high volatility from soil.

6.1.5 Leaching

Substances can move out of the upper soil layer by downward transport (leaching). Substances with a high leaching potential will not persist in the soil compartment. In the context of a classification scheme specifically targeted at assessing hazard to soil organisms (excluding consideration of groundwater quality) leaching should be considered as a possible pathway for disappearance of the substance from the relevant compartment. Leaching studies are not commonly carried out for chemical substances, and so few data are available which could be directly used as modifying factors in a terrestrial (soil) classification scheme. Gustafson (1989) described a model to estimate the leaching behaviour of a substance in soil using simple properties:

$$\text{GUS index} = \log DT_{50} \times (4 - \log K_{oc}) \dots\dots\dots(\text{Eq. 5})$$

where DT_{50} = disappearance time for 50% of the substance
 K_{oc} = soil/water partition coefficient

The GUS index can be used to classify substances as having a high, low or moderate potential for leaching. The GUS index is thus a simple tool that may be useful as a modifying factor of persistence in soil.

6.2 Factors not included as modifiers

6.2.1 Bioaccumulation

In the aquatic classification scheme, bioaccumulation is considered an important modifying factor because extensive literature data are available that demonstrate that organisms can accumulate substances within their tissues at levels far in excess of the surrounding medium (water). For the soil compartment, much less research has been conducted (see below), but the weight of scientific evidence from the available studies indicates that bioaccumulation by soil organisms from bulk-soil is of minor importance, as compared with the aquatic environment. The steady-state bioaccumulation factor (BCF), i.e. the ratio of concentration in organism to concentration in soil at equilibrium, is below 1 for most substances. Even for highly lipophilic substances, for which an aquatic BCF in excess of 10,000 has been determined, the soil BCF based on soil bulk-density was below 10 (Opperhuizen and Sijm, 1990). Belfroid *et al* (1995b) examined the uptake of large (> 0.95 nm cross section), highly lipophilic molecules in earthworms to evaluate the relative contribution of ingestion and found that BCFs were low, although there was no evidence of steric hindrance. Therefore, the Task Force concluded that bioaccumulation should not be included as a modifying factor. This is further supported by consideration of the use of QSARs for assessing bioaccumulation in terrestrial organisms.

There are a number of QSARs that have been developed to predict bioaccumulation in fish. Most of these are based on $\log K_{ow}$ as the chemical descriptor. There are very few QSARs that can predict the bioaccumulation of a substance in terrestrial organisms. The terrestrial bioaccumulation QSAR referred to most widely is that of Connell and Markwell (1990) who developed a relationship for the soil porewater concentration of lipophilic substances and their bioaccumulation in earthworms:

$$\log BCF = 1.0 \log K_{ow} - 0.6 \quad (n = 100, r^2 = 0.91) \dots\dots\dots(\text{Eq. 6})$$

The QSAR is based on literature data on 32 compounds (mostly plant protection products) that are considered to be relatively persistent in soil. Although 100 data points were used to generate the QSAR, some of the data were for the same chemical. The domain of the QSAR is restricted to organic chemicals with a $\log K_{ow}$ between 1 and 6. Ionic substances and organometallic chemicals are excluded.

The QSAR indicates uptake of substances from soil-porewater to be highly dependent on the lipophilicity (K_{ow}) of the substance. However, when related to soil bulk-density the BCF is strongly correlated with worm (lipid content) and soil parameters (fraction of organic carbon in soil) but only very weakly dependent on the lipophilicity (K_{ow}) of the substance.

$$BCF = (Y_l/x F_{oc}) K_{ow}^{b-a} \dots\dots\dots(Eq. 7)$$

In which: Y_l = lipid content of earthworms
 F_{oc} = organic carbon fraction in soil
 x = constant calculated to be equal to 0.66 for earthworms
 b = constant
 a = constant

The authors state that “since b and a are often both close to unity, the dependence of BCF on K_{ow} is generally limited or zero”. Thus, bioaccumulation from bulk soil is not considered to be a useful modifying factor for classification of substances.

6.2.2 Slope of dose-response curve

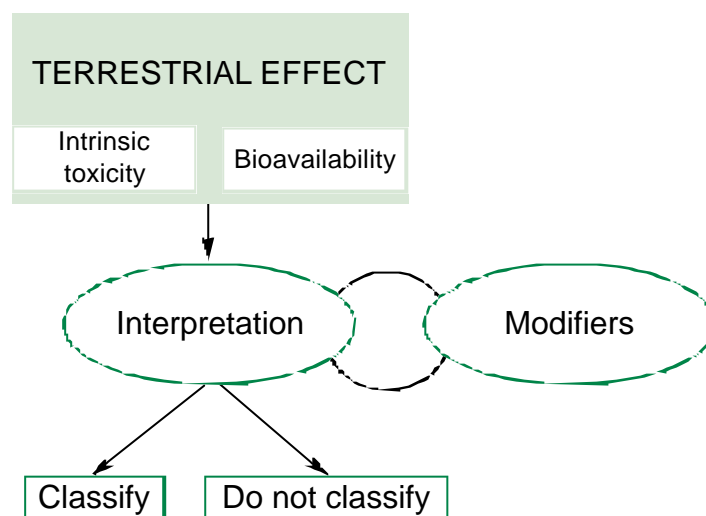
The intrinsic toxicity of a substance can be characterised by its EC_{50} or EC_x and the slope of the concentration-response relationship, observed for a given endpoint. It is clear that a substance that causes an adverse effect only after application of a large dose is less toxic than one that causes the effect at lower dose.

However, if classification is based on EC_{50} values it is less clear whether a dose-response curve with a steep slope indicates a more hazardous substance than one with a shallow slope. Consider two dose-response relationships with a similar concentration-response curve but with different slopes; at concentrations greater than the EC_{50} , the steeper curve causes a greater effect than the more shallow curve, but at concentrations less than the EC_{50} the reverse will be true. Clearly the threshold concentration causing effects is sharper for the steeper curve whereas the shallower curve indicates some effect over a wider range of concentrations. The Task Force was unable to relate unequivocally the steepness of a dose-response curve to the intrinsic hazard of a substance. The slope of the dose-response curve should, therefore, not be used in a classification scheme.

7. SUMMARY AND CONCLUSIONS

The scientific principles that should underlie any future terrestrial (soil) classification scheme have been identified; they are applicable to all organic and inorganic substances. It is recommended that, if a soil classification scheme is required, the general structure should allow both terrestrial effects data and the factors that can modify such data to be taken into account. This is schematically presented in Figure 1.

Figure 1: General classification strategy



Terrestrial effects are determined by a combination of intrinsic toxicity and bioavailability, and these two factors should be considered as the main drivers for soil classification. Experimental effects data are always a combination of both factors. Therefore, terrestrial effects data need to be standardised and normalised before they are useful for classification.

There is a general lack of experimental terrestrial (soil) toxicity data, and therefore the availability and usefulness of surrogate data should be considered, including read-across from aquatic effect data and equilibrium partitioning for organic substances, and 'transformation' data for substances of low solubility. In considering such sources of surrogate data it was concluded that these were prone to many sources of uncertainty, potentially leading to both over- and under-estimates of hazard. Read-across should therefore be applied with caution.

Modifying factors that were considered and recommended to be included in a soil classification scheme are given in Table 5. The factors were selected either because they either present a relevant indicator as to how long a substance is likely to stay in the soil compartment (elimination) or provide information on possible long-term effects (long-term toxicity).

Table 5: Modifying factors

| Modifying factor | Parameter | Test method |
|---|------------------|---|
| Elimination | | |
| Biotic degradation, abiotic degradation | DT ₅₀ | OECD guideline 304A or read-across from OECD guideline 301 |
| Volatilisation | DT ₅₀ | Vapour pressure, OECD guideline 104 |
| Leaching | GUS index | Half-life and K _{oc} |
| Long-term toxicity | Chronic NOEC | Long-term terrestrial toxicity test data, chronic transformation data for insoluble (in)organic compounds |

Modifying factors that were also considered, but that could not be recommended for inclusion were bioaccumulation (due to its relatively low importance as compared to the aquatic environment) and slope of the dose-response curve (because the Task Force could not see how this parameter could be used unequivocally to either identify increases or decreases in hazard).

Certain key elements of a terrestrial classification scheme would merit further development. These include selection of the most appropriate terrestrial test guidelines, assessment of data quality and relevance, read-across methods from aquatic data, and how to incorporate the modifying factors.

Based on the above discussion of main drivers and modifying factors, the Task Force believed that the current R-phrases related to individual terrestrial organisms living in soil are not appropriate and would be better replaced by a classification based on two or three toxicity classes amended by a term for potential for long-term effects. This is analogous to the existing classification scheme for the aquatic environment and would therefore increase harmonisation, transparency and ease of use.

If sufficient information on use scenarios and exposure patterns is available, substances should be evaluated by means of risk assessment procedures rather than a hazard-based approach.

APPENDIX A. ENVIRONMENTAL RISK AND SAFETY PHRASES

Further to Table 1, specific criteria for R-phrases related to aquatic organisms were laid down in Directive 93/21/EEC, as an adaptation to technical progress of Directive 67/548/EEC Annex VI (Table A.1).

Table A.1: Criteria for environmental R-phrases (EC, 1993)

| Number | Criteria |
|--------|---|
| R50 | LC/EC ₅₀ ? 1 mg/l for fish, daphnia or algae |
| R51 | 1 < LC/EC ₅₀ < 10 mg/l for fish, daphnia or algae |
| R52 | 10 < LC/EC ₅₀ < 100 mg/l for fish, daphnia or algae or evidence of danger to structure and/or function of aquatic ecosystems |
| R53 | Not readily degradable in aquatic systems or log Pow ? 3.0, unless BCF ? 100 (special considerations for metals) |
| R54 | None |
| R55 | None |
| R56 | None |
| R57 | None |
| R58 | None |
| R59 | Substances covered by Council Regulation 594/91/EEC ¹ |

¹ As amended by Council Regulation 3952/92/EEC, lists substances that deplete the ozone layer

S-phrases with safety advice relevant to the environment that have to be stated on the label concern the safe handling, transport and disposal of substances (Table A.2).

Table A.2: Environmental S-phrases (EC, 1993)

| Number | Phrase | Criteria |
|--------|---|--|
| S35 | This material and its container must be disposed of in a safe way | Dangerous for the environment and used by the public |
| S56 | Dispose of this material and its container to hazardous or special waste collection point | Dangerous for the environment, symbol 'N' and used by the public |
| S57 | Use appropriate containment to avoid environmental contamination | Symbol 'N' and not used by the public |
| S59 | Refer to manufacturer / supplier for information on recovery/recycling | Dangerous for the environment, dangerous for the ozone layer, symbol 'N' |
| S60 | This material and its container must be disposed as hazardous waste | Dangerous for the environment, symbol 'N', not used by the public |
| S61 | Avoid release to the environment. Refer to special instructions/safety data sheet | Dangerous for the environment, symbol 'N', all substances classified dangerous for the environment not covered above |

APPENDIX B. REVIEW OF BIOAVAILABILITY

As bioavailability is key to a discussion of terrestrial hazard classification, a review of the term is provided here, exemplifying use of the term in different science fields.

B.1 In pharmacokinetics and toxicokinetics

The extent of systemic absorption of a xenobiotic can be determined experimentally by comparing the plasma AUC (Area Under the Curve) after intravenous vs. oral dosing. The resulting index is called bioavailability (Gallo and Doull, 1991). Casarett and Doull (1975) argued that bioavailability is an exceedingly important concept as the most critical factor in exerting toxicity is not necessarily the dose but the concentration of a toxicant at the site of action. Toxicants are delivered to most organs by the systemic circulation. Therefore, the fraction of a chemical reaching the systemic circulation is of critical importance. Three factors can greatly alter this systemic availability, namely limited absorption after oral dosing, intestinal or hepatic first-pass effect and mode of formulation affecting, for example, dissolution rate or incorporation into micelles.

B.2 In aquatic ecotoxicity assessment

Van Leeuwen and Hermens (1995), in the glossary, define bioavailability as:

“The ability of a substance to interact with the biosystem of an organism.”

Systemic bioavailability will depend on the chemical and physical reactivity of the substance and its ability to be absorbed through the gastrointestinal tract, respiratory tract, or skin. It may be locally available at all these sites.

Another definition of bioavailability is given by Spacie and Hamelink (1995):

“The portion of the total quantity or concentration of a chemical in the environment or a portion of it that is potentially available for biological action, such as uptake by an aquatic organism.”

More specifically, bioavailability is that portion of a chemical that an organism encounters that it actually absorbs.

Bioavailability is a basic requirement for uptake of a chemical substance from water, with uptake normally viewed in terms of absorption from true water solutions (Manahan, 1992). Hence, bioavailability is a function of the concentration of the ‘free’ toxicant in water, which depends on the binding affinities that the various components of materials in contact with the water have for the toxicant and the concentration of each of these components in the water phase (Lores *et al*, 1993). Schrap and Opperhuizen (1990) suggested that a reduction of the uptake of substances is caused by a reduced availability of the compound due to the sorption on particles. The components of primary importance in free toxicant concentration are organic and inorganic materials such as dissolved organic carbon (DOC) and sediment (Lores *et al*, 1993). In addition, exposure systems affect the direct and indirect bioavailability of the test substances, as they may affect the behaviour of the exposed test species and the behaviour of the test species may affect the bioavailability of the chemical (Van Leeuwen and Hermens, 1995).

B.3 In terrestrial effects assessment

An international workshop was convened on the subject of bioavailability as a key property in terrestrial ecotoxicity assessment and evaluation (Herrchen *et al*, 1997). The workshop recognised that a prerequisite to a comprehensive assessment system is that exposure should be correctly quantified, since considerable differences between contamination (as assessed by destruction procedures) and exposure are often found in soil and sediment toxicity evaluations. The consequence is that analytically detectable concentrations of pollutants in environmental samples do not enable a sure prediction of a harmful biological or ecological effect. Exposure is to be understood as the time- and space-dependent fate of the substance in soil. Exposure is dependent on the potential availability under realistic abiotic conditions (chemical availability) as well as the biological counterpart, which includes species-specific uptake routes. The abiotic and biotic processes are complementary. The workshop defined in this context bioavailability as follows:

“The amount/percentage of a compound that is actually taken up by an organism as the outcome of a dynamic equilibration of organism-bound uptake processes, and soil particle related exchange processes, all in relation to a dynamic set of environmental conditions.”

B.4 For microbial degradation in soil

Bioavailability of a substance for microbial degradation in soil is the extent to which that substance is available as a substrate to micro-organisms. It is believed that the primary factor limiting bioavailability to micro-organisms in the soil subsurface is the association of the substance with solid surfaces. Therefore, factors influencing bioavailability are focused primarily on physical, chemical or electrostatic sorption of the substance. Bioavailability can also be limited by reduced transport to the microbial cell surface, e.g. slow diffusion of the substance from within a soil aggregate to a microbial cell on the outside of the aggregate, or by a strong binding reaction such as chemisorption that renders the substance unavailable for transport to the cell. Furthermore, the micro-organism may lack a metabolic pathway for the utilisation of the substance.

The complex set of factors that influence bioavailability to micro-organisms can be grouped into three types: cell-surface interactions, cell-contaminant interactions, and contaminant-surface interactions.

Since (xenobiotic) substances are often localised near solid surfaces (aquifer solids), the ability of a cell to colonise that surface and utilise sorbed substrates can influence bioavailability (cell-surface interactions). An organism must be able to reach the substance and degrade it via some metabolic pathway, so specific enzyme availability and transport mechanisms which facilitate the delivery of the substance to the cell, and scavenging mechanisms such as bio-surfactant production, may all influence bioavailability (cell-contaminant interactions). Finally, the nature in which a chemical is associated with the surface will impact its degree of bioavailability. It may be present as a soluble

substance within intra-aggregate or intra-particle pores, inaccessible to cells, it may be associated with a charged particle by electrostatic effects, or it may be chemically bound to surface functional groups. Each different type of surface association of the substance will influence its physical availability to the cell, and consequently, its bioavailability (contaminant-surface interactions).

B.5 Evaluation

Existing definitions of bioavailability all have slightly different meanings. The definition used in terrestrial effects assessment (Section B.3) includes chemical and biological availability, as well as the possibility for interactions. When compared with the microbiological field, it restricts itself on the biological part to actual uptake of material. Though this may be appropriate for terrestrial effects assessments for higher organisms, it seems too restrictive for application in the field of biodegradation and persistence. Hence, in the context of hazard classification, the following definition is proposed for terrestrial bioavailability:

“The fraction of the total quantity of a compound in soil that can interact with an organism.”

APPENDIX C. ESTIMATION OF TERRESTRIAL EFFECTS

C.1 Read-across from aquatic toxicity

A literature search was performed to find:

- Datasets that examine the use of aquatic toxicity data as surrogates for terrestrial species;
- circumstantial evidence to evaluate the equilibrium partitioning approach to estimating terrestrial toxicity.

This evidence addressed the following assumptions:

- The toxicity of substances to soil organisms is correlated to the concentration in the soil porewater;
- the distribution of the substance between soil and porewater is at equilibrium;
- the sensitivity of terrestrial species and aquatic species is similar;
- the porewater concentration for organic substances is determined by the K_{oc} of the product.

C.1.1 Datasets on the use of aquatic toxicity data as surrogates for terrestrial species

The published literature contains few direct comparisons of aquatic and terrestrial effects data. The work of Van Gestel and Ma (1988, 1990) figures strongly in such studies. Comparison of data for earthworms and fish showed that the toxicity to earthworms expressed in terms of porewater concentrations was similar to the toxicity to fish.

Van Gestel *et al* (1996) examined the toxicity of chlorophenols and chloroanilines to lettuce (*Lactuca sativa*). They conducted tests in two soils, measured the porewater concentrations and compared the results to those from lettuce grown in nutrient solution. The results were compared with published data on the toxicity of these products to the alga *Selenastrum capricornutum*. The chlorophenol porewater concentrations were not modified to take account of pH. The data for chlorophenols showed a reasonable correlation between the toxicity to lettuce (expressed in terms of porewater concentration) and toxicity to *S. capricornutum*, but the dataset is fairly small.

Hulzebos *et al* (1993) investigated the toxicity of 76 substances to lettuce and reviewed the literature for data on algal toxicity for the products they included in their programme. The authors did not regress algal EC_{50} values against lettuce EC_{50} values, expressed in terms of porewater, nor did they list the calculated porewater concentrations in the lettuce soil tests. Therefore, it was impossible to determine the correlation or the relative sensitivity (intrinsic toxicity) of algal and lettuce porewater EC_{50} values.

This is the extent of published comparisons of aquatic and terrestrial toxicity data. The existing data support the use of equilibrium partitioning to estimate terrestrial toxicity in the absence of terrestrial test data. However, given the paucity of data, the basic assumptions involved in extrapolating from aquatic to terrestrial data using equilibrium partitioning have been examined.

C.1.2 Circumstantial evidence to evaluate the equilibrium partitioning approach

C.1.2.1 Porewater exposure

The use of the soil/water partition coefficient K_{oc} and aquatic effects data to estimate terrestrial effects is based on the assumption that exposure is primarily via the porewater. Originally, Di Toro *et al* (1991) pointed out that similarity between porewater toxicity and aquatic toxicity did not indicate that the porewater was the only route of exposure. Rather, since soil solids, porewater and biota may all be in equilibrium, the route of exposure cannot be definitively deduced. Nevertheless, in the Technical Guidance Document for EU Risk Assessment, the correlation of porewater toxicity and aquatic toxicity has been taken to mean that porewater is the primary route of exposure (EC, 1996).

Ronday *et al* (1997) studied at the toxicity of carbofuran and parathion to the collembolan *Folsomia candida* and found that for collembola exposed to soil, expressing toxicity in terms of porewater concentration provided the best correlation with the toxicity to collembola exposed to water only or isolated porewater.

Hulzebos *et al* (1993) compared EC_{50} values for lettuce (*Lactuca sativa*) shoot weight in plants grown in nutrient solution and in soil. They calculated the soil EC_{50} values on the basis of estimated porewater concentrations and regressed these data against the nutrient solution EC_{50} values. The results are shown in Table C.1.

Table C.1: Regression of EC_{50} for lettuce shoot weight (Hulzebos *et al*, 1993)

| Compound class | $\log EC_{50}^1, \text{soil}$ | r | n |
|-----------------------|--|------|----|
| Phenols | $1.08 \times \log EC_{50, \text{nutrient}} + 0.58$ | 0.95 | 17 |
| Chloroanilines | $1.16 \times \log EC_{50, \text{nutrient}} + 0.15$ | 0.9 | 12 |
| Chloro(nitro)benzenes | $1.48 \times \log EC_{50, \text{nutrient}} - 0.21$ | 0.86 | 13 |
| Miscellaneous | $1.3 \times \log EC_{50, \text{nutrient}} + 0.27$ | 0.87 | 19 |

¹ Expressed in $\mu\text{mol/l}$

r = correlation coefficient

n = number of data points on which correlation is based

Hulzebos *et al* (1993) suggest the differences between $EC_{50, \text{soil}}$ and $EC_{50, \text{nutrient}}$ may be due to differences in experimental design. For values of $EC_{50, \text{nutrient}}$ in the range 0.1-10 $\mu\text{mol/l}$, values of $EC_{50, \text{soil}}$ are within a factor of 5 of values of $EC_{50, \text{nutrient}}$. Outside this range, the porewater EC_{50} correlates less well with the nutrient EC_{50} . These data suggest that the porewater concentration is a reasonable basis for predicting effects on plants.

Connell and Markwell (1990) stated that if soil organisms were only exposed through the soil porewater, bioaccumulation from bulk soil to worms should be dependent only weakly on the octanol-water partition coefficient K_{ow} . This would arise since bioaccumulation would be a product of two partitioning processes: soil to water and water to organism. $\log K_{ow}$ is largely cancelled out in the two equilibria. The authors showed weak dependence of bioaccumulation on $\log K_{ow}$ for 32 agrochemicals for which soil to worm bioaccumulation factors had been reported, suggesting that porewater was the relevant route of exposure. However, this result could also be achieved if oral uptake only occurred.

It has been stated that for very lipophilic substances or for species that are exposed primarily through food, equilibrium partitioning may underestimate exposure. The EC Technical Guidance Document for risk assessment (EC, 1996) recommends that for substances with a $\log K_{ow} > 5$, these other processes may be important and compensation should be made by increasing the PEC_{soil} by a factor of 10. Belfroid *et al* (1995c) calculated the relative contribution of uptake from porewater to the total uptake in earthworms for organic hydrophobic substances with $\log K_{ow}$ between 2 and 7 in three different soils with 20, 8 and 3% organic matter. They calculated that in the most extreme combination of a soil with 20% organic matter and a chemical with $\log K_{ow}$ of 7, the porewater would contribute > 40% of the uptake, i.e. the correction factor should be < 2.5. For a soil with a more commonly found organic matter content (< 3%), the porewater would contribute over 90% of the earthworms' uptake. Belfroid *et al* (1996) admitted that their 1995 modelling of the relative importance of uptake from the porewater left many uncertainties unresolved, but nevertheless it seemed that porewater exposure would be the primary route of exposure for most substances in most soils.

There is some evidence that bioconcentration of substances by worms may be affected by worm behaviour. Ma *et al* (1995) found that polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene and fluoranthene bioaccumulate up to 8x more in starved worms than in well-fed worms, a difference that could not be explained by differences in worm fat content. Ma *et al* (1995) hypothesised that earthworms increase their oral intake of soil particles when driven by hunger stress and consequently take up more PAH via the gastro-intestinal tract.

Belfroid *et al* (1996) reviewed the literature available on the influence of soil particle size on the bioaccumulation of substances. Since small particles tended to have a higher organic matter content than larger particles and since organic substances sorb to organic carbon, the exposure of an organism that selectively feeds on smaller particles might be expected to be greater than that of an organism that feeds less selectively. There was some evidence indicating that sediment-dwelling organisms feed on smaller particles, but little evidence from the terrestrial environment. Van Brummelen *et al* (1996) showed that the chemical body burden of a variety of soil organisms was more closely correlated with the concentration of the substances in humus and fragmentation material than with the concentration in litter and mineral soil. This is consistent with the feeding behaviour of these organisms. Thus, food selection may be a determinant of exposure for substances that are not homogeneously distributed in the soil.

In conclusion, there is some circumstantial evidence that the porewater concentration is a major determinant of the exposure of soil organisms. Other factors may be of subsidiary importance for soil animals. These include oral ingestion and the behaviour of the soil organisms.

C.1.3 Is soil/porewater partitioning at equilibrium?

The use of equilibrium partitioning to estimate effects on terrestrial biota depends on the assumption of equilibrium between the concentration in the porewater, that adsorbed to the soil matrix and that within soil organisms (Belfroid *et al*, 1996). In this context, ageing phenomena and effects of soil organism behaviour on exposure are of relevance (Section 4.2).

The relationship between ageing and time cannot be derived, nor can the influence of $\log K_{ow}$ be discerned (Belfroid *et al*, 1996), but since bioaccumulation may decline over time by a factor of 2 - 30, failure to take account of ageing may overestimate exposure and hence the toxicity of a substance to soil-dwelling organisms. Alternatively, if tests are conducted over timescales during which equilibrium between soil solids, the porewater and the test organism is not reached or during which degradation occurs, toxicity may be underestimated.

Van Brummelen *et al* (1996) raised the issue that for substances or taxa for which ingestion is an important route of exposure, equilibrium across the gut wall will also be important. The authors speculated that inter-species differences in bioaccumulation might be attributable to different gut residence times.

C.1.4 Sensitivity of terrestrial species and aquatic species

By using read-across from aquatic toxicity, the assumption is made that there are no differences in general in the sensitivity to substances between terrestrial and aquatic organisms. In testing such an assumption, the problem occurs that toxicity tests measure effects rather than true intrinsic toxicity. As explained in Section 3 of this document, effects occurring in terrestrial (soil) tests are the result of both bioavailability and intrinsic toxicity (sensitivity). Therefore, differences in apparent toxicity (effects) between aquatic and terrestrial organisms could be attributed either to differences in bioavailability or to true differences in species sensitivity.

Hendriks (1995) suggested that when the toxicity of substances without a specific mode of action is measured in terms of body burden, fish might be more sensitive than aquatic invertebrates and plants. These data need to be extrapolated to terrestrial invertebrates and plants, especially in the light of the evidence from studies with chlorinated compounds that show little difference between fish and earthworm toxicity measured in terms of concentrations in the water surrounding the test organisms (Van Gestel and Ma, 1988, 1990).

Most terrestrial effects data relate to earthworms, and there is some evidence that earthworm exposure is through the porewater and the body wall (Lord *et al*, 1980; Van Gestel and Ma, 1988, 1990). For soil-dwelling animals such as isopods, the exoskeleton is less permeable (Warburg, 1987) and hence routes of exposure and time to equilibration may differ. Van Brummelen *et al* (1996) observed a negative relationship between bioaccumulation of PAHs and $\log K_{ow}$ in some but not all of the isopod species they examined. They suggest several plausible explanations including that the isopods' exoskeleton limits exposure to the porewater. Consequently, while there is some evidence justifying the use of read-across from aquatic species to earthworms, additional information is required before read-across can be applied to other soil dwelling organisms.

C.1.5 Porewater concentration is determined by the K_{oc} of the substance

Equilibrium partitioning theory assumes that for uncharged organic substances, K_{oc} largely determines the porewater concentration. However for inorganic substances such as metals and polar organic substances, the concentration of dissolved (bioavailable) material is in equilibrium with the amount of substance sorbed to clay, organic matter (humic compounds), hydroxides of Fe, Mn and Al, and dissolved chelates. The equilibrium is dependent on physico-chemical conditions in the soil including salinity, pH, dissolved oxygen and particle composition. Simple equilibrium partitioning will therefore be inappropriate.

Basing the calculation of porewater concentration on the $\log K_{oc}$ of a chemical assumes that all organic matter in soil is alike. Belfroid *et al* (1996) reviewed the evidence underlying this assumption and concluded that the polarity, aromaticity, 3-dimensional structure and humification of the organic matter may affect the sorption of a chemical by a factor of up to an order of magnitude. This was not taken into account in any of the models used to calculate porewater concentration. The authors added that when the organic carbon content is very low (< 0.1%), sorption to clay may be an important determinant of sediment porewater concentrations. It was concluded that organic carbon content is the most important factor determining the bioavailability of substances in soil, with ageing and the composition of the organic matter being of next importance. Models have been developed to determine soil-water partitioning for ionised substances (Bintein and Devillers, 1994), but the application of such models to determine toxicity to terrestrial organisms has not been evaluated extensively.

For ionising substances it may be appropriate to determine the concentration of the non-ionised form. However, Van Gestel *et al* (1996) (Section C.1.1), found that such a modification did not consistently improve the correlation between porewater and nutrient solution toxicity; the similarity between porewater and nutrient solution EC_{50} , soil and EC_{50} , nutrient was better for pentachlorophenol, reduced for 2,4,5-trichlorophenol and little affected for 3-chlorophenol and 3,4-dichlorophenol. The data suggested that for uncharged organic substances, lettuce EC_{50} values expressed as porewater concentrations correlated with EC_{50} values determined in nutrient solutions. For charged substances factors other than, or in addition to, pH appear to determine the bioavailability and hence the toxicity.

C.2 QSARS for K_{OC}

The sorption of non-polar substances can be regarded as a distribution process between the polar phase (the porewater) and the organic phase of the soil. The equilibrium constant of this partitioning between the solid and solution phases constitutes the adsorption coefficient for soil. The sorption coefficient (K_d) at steady-state is defined as follows:

$$K_d = C_s / C_w \dots\dots\dots (Eq. C.1)$$

where C_s = concentration of chemical sorbed to soil or sediment (mol/kg)
 C_w = concentration of chemical in the porewater (mol/l).

K_d -values for the same compound may extend over several orders of magnitude. Normalisation to fraction of organic carbon (OC), the principal interaction site for non-ionic (neutral) organic substances, is used to reduce the variance of sorption coefficients measured in different soils. The carbon normalised partition coefficient is defined as:

$$K_{oc} = K_d \times (100 / \% \text{ OC}) \dots\dots\dots (Eq. C.2)$$

Several methods exist to determine K_{oc} experimentally. However, if no K_{oc} is available, QSARs may be used to estimate K_{oc} .

Reviews of existing QSAR models can be found in Lyman (1982), Güsten and Sabljic (1995a,b), Watts *et al* (1995) and ECETOC (1998).

Many of the QSARs relate $\log K_{oc}$ directly to $\log K_{ow}$ (octanol-water partition coefficient) and have the general form:

$$\log K_{oc} = a \times \log K_{ow} + b \dots\dots\dots (Eq. C.3)$$

where a = slope of the linear regression line
 b = intercept with the Y-axis

The simplest and perhaps most widely used QSAR at the moment is based on Karickhoff (1981):

$$\log K_{oc} = 1.00 \times \log K_{ow} - 0.21 \dots\dots\dots (Eq. C.4)$$

A somewhat more accurate estimate of $\log K_{oc}$ is obtained by using a QSAR based on a set of substances from the substance's own compound class.

Approaches based on structural sub-units (Meylan *et al*, 1992a) or other molecular descriptors (Sabljic, 1987) have also been developed. A group contribution method described by Meylan *et al* (1992b) incorporates molecular connectivity indices (MCIs). A regression for the non-polar substances against first-order MCIs was obtained and this was then corrected by the addition of group contributions from a polar set of substances. This model is available in computerised form: PCK_{oc}Win (SRC, 1994).

APPENDIX D. USE OF TRANSFORMATION IN THE CLASSIFICATION OF METALS AND SPARINGLY SOLUBLE INORGANIC COMPOUNDS

D.1 Bioavailability of metals and sparingly soluble metal compounds in aqueous solution

In aquatic ecotoxicity testing, the test organism is not directly exposed to a given dose of the test substance, but to the concentration of this substance, dissolved in water. Although this concentration may reflect exposure conditions in reality, it implies that the bioavailability of the substance is of crucial importance for its potential ecotoxicological effects. This issue is particularly relevant for metals and sparingly soluble compounds, which will become dissolved as ionic and/or complexed species that are potentially bioavailable to a rather limited extent only after reaction with the components of the water.

The 'dissolved' fraction is in this respect experimentally defined as the fraction passing through a 0.45 mm filter, and this is considered as an estimate of the bioavailable fraction (ECB, 1995).

D.2 Transformation as a surrogate for aquatic ecotoxicity testing

Rather than having to comply with a multitude of ecotoxicity tests, complicated by the bioavailability issue, the metals industry, together with the competent authorities in the EU and OECD, has developed a 'transformation' test strategy, as a surrogate for ecotoxicity testing. Transformation is defined in this context as the totality of all physico-chemical reactions, resulting in a given concentration of 'dissolved' metal species under a given set of reaction conditions (ECB, 1995).

The objective of a transformation test is to determine experimentally the rate and the extent of dissolved metal species formation in test water, starting from a metal or a sparingly soluble metal compound. The test is carried out according to a standardised protocol, in which the most important parameters influencing transformation and ecotoxicity in water are set. Transformation is measured as a function of time until an apparent equilibrium state is reached between the substance and the test solution. Subsequent classification follows from the comparison of the dissolved metal concentration measured at apparent equilibrium or at a standardised point in time, with the lowest reliable ecotoxicity value obtained on a readily soluble compound of the given metal under the same test conditions.

APPENDIX E. EXISTING GUIDELINES FOR BIODEGRADATION TESTING

In general, the biodegradation of a substance is measured by the determination of parameters such as DOC removal, CO₂ production and O₂ uptake. Measurements are made at sufficiently frequent intervals to allow the identification of the onset and end of biodegradation.

E.1 Methods

Six internationally standardised methods are available that permit the screening of substances for ready biodegradability in an aerobic aqueous medium. Depending on the physical characteristics of the substance to be tested, a particular method may be preferred (Table E.1).

Table E.1 : Methods for ready biodegradability screening (OECD, 1998)

| Guideline | Name of test |
|-----------|--|
| OECD 301A | DOC die-away |
| OECD 301B | CO ₂ evolution (Modified Sturm) |
| OECD 301C | MITI ¹ (I) |
| OECD 301D | Closed-bottle |
| OECD 301E | Modified OECD screening |
| OECD 301F | Manometric respirometry |

¹ Ministry of International Trade and Industry, Japan

The minimum criteria for ready biodegradability are 70% removal of DOC and 60% Total Oxygen Demand (TOD) or total CO₂ production for respirometric methods. The stringent nature of these tests does not necessarily mean that failure of a substance to meet the criteria will lead to an absence of biodegradation in the environment. A proposal is currently under discussion within OECD to lower the pass level for biodegradation to 35 % in 28 days.

Inherent biodegradability tests (OECD 302 series) are tests in which a substance is exposed over a prolonged period of time to degradation by micro-organisms. The extended exposure period allows for adaptation of the micro-organism community. The tests are to be conducted under environmentally relevant conditions with a compound/biomass ratio that favours bio-degradation.

Simulation tests are designed to simulate the rate of biodegradation under certain environmentally relevant conditions, including an (aerobic or anaerobic) biological wastewater treatment plant, a river, lake, estuary or sea, and soil.

If a substance is judged not to be readily biodegraded, the first test applied may be one for inherent biodegradability. Other tests that can be demanded for any chemical are those for anaerobic biodegradation, degradation in soil, and photo-degradation. Nevertheless even 'ready' data are unavailable for many substances and biodegradability must then be estimated.

In order to measure the biodegradation kinetics of an organic substance in soil, one must be aware of the factors that may affect the chemical's fate in soils. These factors are not only dependent on the chemical itself, but equally on the biological and physical nature of the soil. Interactions of the substances with the soil matrix generally result in the chemical becoming less bioavailable to the microbial community for immediate mineralisation. In extreme cases, a substance may be no longer bioavailable and therefore not biodegradable. Mineralisation models suitable for classification should ideally incorporate a parameter that allows for losses due to, or changes in, bioavailability.

Despite major efforts, up to now generally applicable QSARs could not be formulated for the aerobic biodegradation in the compartments surface water, soil and the aeration tank of a communal wastewater treatment plant.

Due to their design, present screening tests in water allow for adaptation as a result of proliferation of specific micro-organisms that can grow using the chemical present as the sole carbon and energy source. To achieve a similar situation for soil, tests need to be carried out at relatively high concentrations of the substance in the soil substrate. When assuming that degradation in soil takes place mainly in the porewater, it follows that the degradation rate in soil is highly influenced by the solubility of the substance in (pore) water. In this case the degradation rate in soil can be described accurately using solubility data and results of aquatic ready biodegradation tests. Providing the above assumption holds true, it is reasonable to assume that, for classification purposes, aerobic biodegradation is generally about as rapid in the upper layers of soil as in fresh water.

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- No. 83 The Use of T25 Estimates and Alternative Methods in the Regulatory Risk Assessment of Non-threshold Carcinogens in the European Union
- No. 84 Scientific Principles for Soil Hazard Assessment of Substances

Joint Assessment of Commodity Chemicals (JACC) Reports

| No. | Title |
|--------|---|
| No. 1 | Melamine |
| No. 2 | 1,4-Dioxane |
| No. 3 | Methyl Ethyl Ketone |
| No. 4 | Methylene Chloride |
| No. 5 | Vinylidene Chloride |
| No. 6 | Xylenes |
| No. 7 | Ethylbenzene |
| No. 8 | Methyl Isobutyl Ketone |
| No. 9 | Chlorodifluoromethane |
| No. 10 | Isophorone |
| No. 11 | 1,2-Dichloro-1,1-Difluoroethane (HFA-132b) |
| No. 12 | 1-Chloro-1,2,2,2-Tetrafluoroethane (HFA-124) |
| No. 13 | 1,1-Dichloro-2,2,2-Trifluoroethane (HFA-123) |
| No. 14 | 1-Chloro-2,2,2-Trifluoromethane (HFA-133a) |
| No. 15 | 1-Fluoro 1,1-Dichloroethane (HFA-141B) |
| No. 16 | Dichlorofluoromethane (HCFC-21) |
| No. 17 | 1-Chloro-1,1-Difluoroethane (HFA-142b) |
| No. 18 | Vinyl Acetate |
| No. 19 | Dicyclopentadiene (CAS: 77-73-6) |
| No. 20 | Tris-/Bis-/Mono-(2 ethylhexyl) Phosphate |
| No. 21 | Tris-(2-Butoxyethyl)-Phosphate (CAS:78-51-3) |
| No. 22 | Hydrogen Peroxide (CAS: 7722-84-1) |
| No. 23 | Polycarboxylate Polymers as Used in Detergents |
| No. 24 | Pentafluoroethane (HFC-125) (CAS: 354-33-6) |
| No. 25 | 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0) |
| No. 26 | Linear Polydimethylsiloxanes (CAS No. 63148-62-9) |
| No. 27 | n-Butyl Acrylate (CAS No. 141-32-2) |
| No. 28 | Ethyl Acrylate (CAS No. 140-88-5) |
| No. 29 | 1,1-Dichloro-1-Fluoroethane (HCFC-141b) (CAS No. 1717-00-6) |
| No. 30 | Methyl Methacrylate (CAS No. 80-62-6) |
| No. 31 | 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2) |
| No. 32 | Difluoromethane (HFC-32) (CAS No. 75-10-5) |
| No. 33 | 1,1-Dichloro-2,2,2-Trifluoroethane (HCFC-123) (CAS No. 306-83-2) |
| No. 34 | Acrylic Acid (CAS No. 79-10-7) |
| No. 35 | Methacrylic Acid (CAS No. 79-41-4) |
| No. 36 | n-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9) |
| No. 37 | Methyl Acrylate (CAS No. 96-33-3) |
| No. 38 | Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3) |
| No. 39 | Tetrachloroethylene (CAS No. 127-18-4) |
| No. 40 | Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions |

Special Reports

| No. | Title |
|--------|---|
| No. 8 | HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances |
| No. 9 | Styrene Criteria Document |
| No. 10 | Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1) |
| No. 11 | Ecotoxicology of some Inorganic Borates |
| No. 12 | 1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0) |
| No. 13 | Occupational Exposure Limits for Hydrocarbon Solvents |
| No. 14 | n-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document |
| No. 15 | Examination of a Proposed Skin Notation Strategy |
| No. 16 | GREAT-ER User Manual |

Documents

| No. | Title |
|--------|--|
| No. 32 | Environmental Oestrogens: Male Reproduction and Reproductive Development |
| No. 33 | Environmental Oestrogens: A Compendium of Test Methods |
| No. 34 | The Challenge Posed by Endocrine-disrupting Chemicals |
| No. 35 | Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances |
| No. 36 | Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals |
| No. 37 | EC Classification of Eye Irritancy |
| No. 38 | Wildlife and Endocrine Disrupters: Requirements for Hazard Identification |
| No. 39 | Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach |
| No. 40 | Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene |
| No. 41 | Persistent Organic Pollutants (POPs) Response to UNEP/INC/CEG-I Annex 1 |
| No. 42 | Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction |