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# Environmental Risk Assessment Based on 6th Amendment Information

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# ENVIRONMENTAL RISK ASSESSMENT BASED ON 6TH AMENDMENT INFORMATION

(This is the first part of a report which will eventually include a consideration of toxicity to humans)

## A. INTRODUCTION

In 1979 the European Communities published a Council Directive amending for the sixth time Directive 67/548/EEC relating to the classification, packaging and labelling of dangerous substances, henceforth referred to as the "6th Amendment". This amendment has been incorporated into legislation by the member states. There are some differences in the text between the 6th Amendment and the national versions of it, and in this report the English text issued by the European Commission is used.

The 6th Amendment in Article 6.1. requires that a manufacturer or importer, before placing a new substance on the market, shall submit to the competent authority a notification including (to quote) :

"- a technical dossier supplying the information necessary for evaluating the foreseeable risks, whether immediate or delayed, which the substance may entail for man and the environment, and containing at least the information and results of the studies referred to in Annex VII, together with a detailed and full description of the studies conducted and of the methods used or a bibliographical reference to them;
- a declaration concerning the unfavourable effects of the substance in terms of the various uses envisaged;
- the proposed classification and labelling of the substance in accordance with this Directive;
- proposals for any recommended precautions relating to the safe use of the substance."

While information in the technical dossier serves to fulfil all of these requirements, this report is concerned only with the environmental

aspects i.e. with "evaluating the foreseeable risks, whether immediate or delayed, which the substance may entail for.....the environment", under normal conditions of use and disposal. Whereas (Art. 7.1 of 6th Amendment) the competent authority is "responsible for receiving the information provided for in Article 6 and examining its conformity with the requirements of the Directive, and in particular - the notifier's proposed findings on any foreseable risks which the substance may entail", it would be advisable for the manufacturer to submit his own risk assessment, and ECETOC recommends that he does so as an aid to the discussions with the authority. This report is thus meant to assist both notifiers and competent authorities.

There is no international agreement on the use and definition of the terms Hazard and Risk, and in current groups within the OECD and EEC the above "evaluation of foreseable risks" is generally called hazard assessment. ECETOC has defined these terms for consistency in its own internal use :

" Hazard is a function of toxicity and conditions of exposure which could result in an adverse biological effect".
" Risk is the probability that an adverse biological effect occurs, or may occur, in defined circumstances".

On this basis the term Risk Assessment seems more appropriate to the current exercise, and is used throughout the report.

The purpose of risk assessment as outlined in this document is to identify possible areas of risk to the environment, i.e. not to prove that a substance is "safe" but rather to indicate how potentially hazardous chemicals can be used, for the purposes notified, with minimum risk. It will enable "recommended precautions relating to the safe use of the substance" (6th Amendment, Art. 6.1) to be adopted, although this aspect is outside the scope of the present document.

The information required under the 6th Amendment concerns the fate and toxicity of a chemical and is developed at three Levels according to the expected tonnage to be marketed - see the attached Annexes VII and VIII of the 6th Amendment. Base-set information includes many parameters which are fundamental for risk assessment. The ecotoxicological tests in

Levels 1 and 2 comprise further studies on the ecotoxic effects of the substance and persistence/accumulation properties related to its environmental fate. An assessment of risk is made at each of the 3 Levels, for the tonnage and uses notified, from the information generated.

Base-set information (Annex VII, 6th Amendment) must be provided when the marketed volume of a new substance exceeds 1 tonne/year. Information necessary to enable a risk assessment to be made at Level I (Annex VIII) may be requested by the authorities after being informed that the tonnage has reached 10 t/y and must be requested by them at 100 t/y. The testing necessary at this Level should preferably be discussed between the manufacturer and the competent authority. When the marketed volume reaches 1000 t/y, a similar discussion of testing at Level 2 (Annex VIII) must take place.

Both annexes VII and VIII contain the sentence - "If it is not technically possible, or if it does not appear necessary to give information, the reasons shall be stated". This permits some flexibility in choosing logically which tests to carry out and in what sequence. The question therefore arises : what tests are necessary to provide data adequate for risk assessment at each Level, and at what point can the testing be terminated ? A fixed set of obligatory tests cannot serve for the assessment of risk for all substances. Justification has to be given if, exceptionally, it is decided to : carry out tests at an earlier or later Level than is indicated by tonnage; carry out tests not listed in Annex V of the Directive; omit certain tests. The overriding criteria for selecting studies are that the information developed is adequate and necessary for the assessment of the risks which may arise when the substance is used in practice.

It should be possible to harmonise the principles of risk assessment so that a common approach is used by notifiers and the authorities in various countries. It is not possible or desirable to harmonize the risk assessment in detail, because the toxicological and environmental characteristics, and their significance, will differ from chemical to chemical. It is strongly emphasised that the somewhat detailed guidance given in this document may not apply in all cases. The notification will normally be based on an expert interpretation of which sequence of tests (within

the limits of choice in the 6th Amendment) is optimum for the purpose, and how the risk assessment is to be made, for each individual substance. Water-insoluble and highly-volatile substances present problems in many of the required tests, and may need special treatment.

B. THE APPROACH TO RISK ASSESSMENT.

The environmental risk of a substance is assessed by comparing a no-effect concentration with the potential environmental concentration (PEC) of the chemical in the appropriate compartment after reasonable dispersion. This comparison is expressed as a ratio of the concentrations, allowing an estimate of the safety margin to be made, specific to the tonnage, use and disposal patterns, and the location. This assessment is based on all relevant data on effects, tonnage, use, disposal, receiving compartment and fate, available at the particular Level concerned.

Certain of the tests carried out under the 6th Amendment give information on effects for various species. Information from other tests (physical-chemical properties, stability, degradation, etc.) will usually make it possible to assess in which environmental compartment a chemical will mainly appear. It will often be possible to define scenarios taking into account the foreseen volume, use and disposal of the substance as the basis for estimating the PEC even from the data generated at the Base-set Level.

The concept of PEC in this document is very similar to that developed by the OECD (1982). It should not be confused with the OECD concept of PED in which generalised mathematical models are used to estimate the Potential Environmental Distribution. For risk assessment, in the sense of the term in this document, the PED is not adequate and the estimated PEC should be used.

Where broad scenarios for estimating PEC can be defined they should relate to the "fields of application" categories specified in section 2.1.2 of Annex VII, i.e.

i) Industries
ii) Farmers and skilled trades
iii) The public at large.

When the foreseeable uses in the above categories, and hence the corresponding environmental dispersions, are known, the most appropriate scenario or scenarios can be developed for estimating the PEC. After the substance has been placed on the market, measurements of environmental concentrations resulting from actual use may be available for estimating the PEC, and for the subsequent discussions of testing necessary, at Levels 1 and 2.

It is emphasised that if the comparison of data on toxic effects with estimated exposure at the Base-set Level indicates a need to refine the risk assessment, further information should be sought to improve the estimates of both the toxicological effects and the environmental fate.

There will undoubtedly be cases when scenarios adequate for the estimation of the PEC cannot be defined because the data on use and disposal are insufficient. In such cases it may be possible to estimate the PEC by analogy with existing chemicals for which information on environmental concentrations exists, and whose relevant characteristics are broadly similar to those of the new substances. For example, for a substance which goes mainly into surface waters a preliminary estimate of PEC can be adopted as follows. From data on the measured concentrations of industrial chemicals in surface waters it seems that after dispersion (i.e. excluding local discharge points) the concentrations rarely reach 10 µg/l even for products of which much more than 1,000 tonnes are produced per year - see Appendix *. If this is accepted as a useful generalisation, then a conservative estimate of the PEC would be 1 µg/l in the range 1 to 100 tonne/year, and 10 µg/l in the range 100-1000 tonne/year. At above 1000 tonnes/year (Level 2) it is more likely that an adequate scenario can be defined because more information is available, and a more direct estimate of PEC is possible.

Thus, either by calculation, by analogy with existing chemicals, or from actual field measurements, the PEC can be estimated for the relevant use, compartment and tonnage.

At each Level, for the various toxic effects which have been tested, the no-effect-level or $LC_{50}$ and the estimated PEC are compared to assess the risk in the relevant environmental compartment, and a judgement is then made whether the ratio of these indicates :

a) that the risk at this Level is not significant or can be adequately controlled by appropriate measures, and that no further testing or other action is necessary ;

or

b) that the risk is not adequately defined, and that further information is necessary to define it more accurately.

1. Some Useful Generalisations for Risk Assessment..

For making rational decisions on which tests are necessary to permit the assessment of risk at each Level, the following generalisations will prove to be useful.

1.1. Relationship of acute to sub-lethal and chronic effects in aquatic organisms. In the absence of information about chronic toxicity, a ratio of 100 between an $LC_{50}$ and the environmental concentration could be taken as guidance to indicate negligible risk for the following reason.

Sprague (1971) and Maki (1979) found that for the great majority of chemicals which they tested, sub-lethal and chronic effects on aquatic species are not likely to occur at concentrations below 1% of the acute $LC_{50}$ (Base-set tests on fish and Daphnia). Thus, when the PEC is below 1% of the $LC_{50}$ to both fish and Daphnia it can be considered as being below the threshold concentration for sub-lethal or chronic effects, and testing for such effects need not normally be performed. This may not apply if the curve of $LC_{50}$ vs time in the $LC_{50}$ test has not reached a plateau or if there is evidence that bioaccumulation may be significant.

1.2. Degradability. Evidence of biotic or abiotic degradability from the Base-set tests indicates routes by which a chemical can be removed from the environment. As a working hypothesis at the Base-set Level, it can be assumed that for a chemical which is readily biodegradable in any of the Base-set tests, 90% will disappear rapidly from the aqueous environment. Conversely, for a chemical which is not readily biodegradable, zero removal from water should be assumed unless there is evidence of degradation from tests at Levels 1 or 2, or of removal by other routes (eg. volatilisation or adsorption).

In estimating the PEC of a substance which reaches the soil it is useful to note that biodegradation in soil is usually at least as rapid as in surface waters because of the greater variety and density of micro-organisms in fertile soil.

It is emphasised that substances of low or zero degradability do not necessarily represent a hazard to the environment.

1.3. Bioaccumulation. Evidence that a substance may bioaccumulate, especially in a food-chain organism (such that organisms higher in the chain may be exposed to toxic levels), will influence the decisions involved in risk assessment.

It is now widely-accepted that deductions about the bioaccumulation of, in particular, non-ionised substances in aquatic species can be made from the partition coefficient of a chemical between n-octanol and water (Pow; Base-set test). If the Pow is below 1000 the risk of bioaccumulation in aquatic species is low (Bioconcentration Factor below 100) since the normal route of bioaccumulation is by migration from the external environment into lipids in the organism. If Pow is above 1000 it indicates that the possibility of significant bioaccumulation must be taken into account.

According to the OECD Test Guideline 305A, page 6, a bioaccumulation study is not justified for substances whose water-solubility exceeds 2g/l, irrespective of the results of any previous biodegradation test. Substances which are soluble in water to this extent are not considered likely to be sufficiently soluble in lipids to bioaccumulate.

1.4 Toxicity to higher plants. Information useful for deciding whether to test a chemical for effects on a higher plant has been provided by Kenaga(1981). He collected data on 131,596 varied chemicals regarding their lethality to 5 species of terrestrial plant seeds (pre-emergence or germination effects) or seedlings. Only 0.17% of these chemicals killed the seeds at a concentration of 1 ppm or lower. This strongly suggests that it is unnecessary to carry out the higher plant test if the concentration in soil in unlikely to exceed 1 ppm (1 mg/kg of soil).

## C. RATIONALE FOR TESTING AND RISK ASSESSMENT AT BASE-SET LEVEL.

The Base-set requirements are given in the attached Annex VII.

### 1. Physico-chemical Properties

The Directive requires that the tests be carried out on the substance as marketed. This should always be so for the biological tests. However, when impurities or additives (including formulation adjuvants) required for the purpose of placing an acceptable product on the market would so alter the result of a physical-chemical measurement as to make interpretation difficult, it may be preferable to test the purified compound. If it is not possible to isolate the purified compound, the test should be omitted. When a test is carried out on a material other than the substance as marketed, the notifier should state what material was tested and give the reasons for his choice and, where necessary, its implications.

Data on certain physical-chemical properties are used to identify the substance. Other physical-chemical properties, combined with information on use and disposal, are used to make an estimate of environmental fate and thus potential environmental concentration. Vapour pressure, solubility in water, and partition coefficient will (together with information on other factors, especially degradability) indicate the probable distribution of the substance between air, water, soil or sediments. The octanol-water partition coefficient is a guide to bioaccumulation potential, in particular for non-ionised compounds (see section B.1.3. above).

Most of the physical-chemical property determinations in solution depend on the availability of a sensitive analytical method. The sensitivity need not be greater than the level of accuracy needed for interpreting the results of each measurement. In view of the uncertainties in compartmentalisation estimates or models, high accuracy in measuring the physical-chemical properties related to such estimates is not necessary. Similarly, physical-chemical parameters required solely for assessing ecotoxicological effects need be measured only with the accuracy adequate for such assessments.

2. Degradation Tests

The main requirement in the Base-set is for a measure of ready biodegradibility, over 28 days, by any of the methods listed in Annex V of the 6th Amendment. If the compound is readily biodegradable in any of these stringent tests it can be assumed to degrade readily under aerobic environmental conditions. An even simpler test, the 5-day BOD, can give some indication of biodegradation, but only few chemicals would biodegrade substantially in the short time available. Even if the compound is not readily biodegradable in the above tests, it cannot automatically be considered as non-biodegradable in the environment, and, where appropriate, further testing by the methods in Levels 1 and 2 may establish its biodegradability.

Many compounds, because of their physical state and/or low solubility, will not readily degrade under the above conditions, while others will not be readily degraded for reasons of chemical structure. Although such resistance to breakdown must be taken as indicating potential persistence, it will not be automatically necessary to carry out more vigorous or prolonged testing at this Level. For example, materials which by their function are required to resist biodeterioration in service could be declared to be non-biodegradable without testing at any Level.

Information on abiotic degradation is required at this Level. Data on susceptibility to hydrolysis seem unnecessary for substances which have already proved to be biodegradable. The determination of photodegradability in the atmosphere is not justifiable at the very low tonnage involved because even for persistent substances the PEC, after dispersion of the product in air, will be negligible at up to 100 t/y.

3. Acute Toxicity to Aquatic Organisms

Data on $LC_{50}$ (the concentration which kills 50% of the test organisms, calculated on a statistical basis) for a fish species and Daphnia magna is required in the Base-set. These organisms were chosen to represent species in the aquatic environment. Daphnia stands between Algae and fish in the food chain.

In most cases the curve obtained by plotting toxic concentration to fish against time will reach a plateau value by 96 hours (the

specified test period is 48 hours, optionally extended to 96 hours). Failure to do so may indicate a need for further testing, normally at Level 1.

These $LC_{50}$ determinations need not be carried out when the chemical will not reach the aquatic environment, for example in the case of a very volatile, water-insoluble substance.

4. Risk Assessment

The Base-set information can already permit a preliminary assessment of the environmental compartment(s) in which the substance will appear. A comparison of the PEC relating to the appropriate use-scenario with the information on degradability, and toxicity to fish and Daphnia will often enable a first risk assessment to be made for the substance in surface waters. It may also be possible to exclude the likelihood of risk in other compartments if it is clear that the substance is not likely to reach them in significant amounts.

For some substances the risk assessment at the Base-set Level may prove to be adequate for the higher Levels also. When this is not the case, risk assessment at the Base-set Level assists in selecting tests which it is logical and necessary to perform when Levels 1 and 2 are reached, so that the assessment can be improved.

## D. RATIONALE FOR TESTING AND RISK ASSESSMENT AT LEVELS 1 AND 2

The tests in Levels 1 and 2 include further studies on the potential ecotoxic effects of the substance, and on its persistence/accumulation properties. They enable the data on toxicity and fate generated in the Base-set to be refined so that the risk assessment can be correspondingly improved. The tests on Algae, Daphnia, fish, higher plant, and earthworms yield data on toxicity, while those on biodegradation, accumulation and mobility give information relevant to the estimation of the PEC.

1. Level 1

1.1. Algae test. This test involves measuring the effect of a substance on the growth of a unicellular Algae species, selected because it is common and convenient to use in the laboratory and is

a primary food source for certain aquatic organisms. The results, expressed as an $EC_{50}$ (the concentration of the chemical causing a 50% inhibition of growth) and as a highest-tested no-effect level, can be compared with the PEC for risk assessment.

When the $LC_{50}$ for Daphnia and fish are more than 100 times the PEC, there may be no need to perform the test on Algae. Kenaga and Moolenaar (1979) compared the acute toxicity of many thousands of chemicals, of various structures, towards a number of fish species, Daphnia magna and Alga chlorella. The fish and Daphnia proved, in most cases, to be at least as sensitive as Algae chlorella in indicating toxic effects. The results showed that only rarely would a substance with an adequate safety margin for fish and Daphnia be acutely toxic to Algae chlorella.

On the contrary, if a chemical seems likely to be stable in water and has an acute toxicity to fish or Daphnia high enough to raise concern when compared with the PEC at 10 t/y, it may be of value to carry out the Algae test at this stage in Level 1, so as to gain further information on its toxicity to aquatic species.

1.2. Prolonged (21-day) toxicity to Daphnia magna

Daphnia magna is a convenient aquatic organism for studying the potential effect of chemicals on reproduction, a chronic effect. Because (see B.1.1) the chronic no-effect concentration for aquatic organisms is generally above 1% of the acute $LC_{50}$, it is not necessary to carry out the prolonged Daphnia test on chemicals for which the PEC is below one-hundredth of the $LC_{50}$ to Daphnia.

Should the Base-set results show that a chemical is likely to be stable in water, and comparison of its acute toxicity to Daphnia with the PEC causes concern (particularly if the substance has the potential for significant bioaccumulation), it may be of value to get information on chronic toxicity by carrying out the prolonged Daphnia test at 10 tonnes/year in Level 1, instead of at the 100 tonnes/year stage.

1.3. Higher plant test

This test gives information on the effect of the chemical, expressed as an $EC_{50}$, on the germination and growth of a higher plant. It is not normally necessary at Levels 1 or 2 for chemicals shown to be readily biodegradable in water (see comment on biodegradation in soil, B.1.2) or not likely to reach the soil in concentrations above 1mg/kg (see B.1.4).

1.4. Earthworm Test

This test gives information on the effect of a chemical on earthworms, a particularly important soil macro-organism. The result is expressed as an $LC_{50}$. An earthworm test is not normally necessary for chemicals which are not likely to reach the soil in significant concentrations, eg. via application of sewage sludge or by other direct means.

1.5. Prolonged (14-day) fish toxicity test.

When, in the $LC_{50}$ (Base-set) test on fish, no plateau has been reached in the curve of concentration against time, this prolonged study should be carried out (see C.3). It is not normally necessary to perform it if a plateau has been reached in the Base-set test and the PEC is below one-hundredth of the $LC_{50}$ for fish.

If the Base-set results indicate that the substance is likely to be stable in water, and a comparison of its acute toxicity to fish with the PEC causes concern (particularly if the substance has the potential for significant bioaccumulation) it may be valuable to obtain information on chronic toxicity by carrying out the prolonged test at 10 t/y instead of 100 t/y at Level 1.

1.6. Test for species accumulation

This test indicates the potential of the substance to concentrate in higher organisms and, hence, to expose higher trophic levels. It provides a measure of the bioconcentration factor (BCF) of a chemical, which is the ratio of the concentration in, eg. a fish (the preferred species) to the concentration in water, at the steady state. As explained in section B.1.3., significant bio-concentration (BCF above 100) is not likely for non-ionised substances if the Partition Coefficient octanol-water (Pow) is below 1000 or if its water-solubility is above 2g./l. Thus a species accumulation test would usually be critical for the risk assessment only if the substance is not readily degradable, its Pow is greater than 1000, and its water-solubility is below 2g./l. If the Pow is high enough to suggest that bioaccumulation is likely, the test may be considered at the 10t/y level.

For organic compounds that ionise under physiological conditions (pH 3 - 9), the Pow may be less useful for predicting bioaccumulation, especially if the chemical is likely to bind to cell constituents. If such ionic substances are sparingly soluble in water and are not biodegradable, the bioaccumulation test with fish may be necessary. For metallo-organic chemicals, the bioaccumulation potential cannot be established unequivocally by the Pow, and if they are stable in water the bioaccumulation test should be carried out.

Static, semi-static and continuous-flow techniques are available for this test . At Level 1 a static test is probably sufficient in the above cases. The OECD Test Guideline 305A (Bioaccumulation : Sequential Static Fish Test) gives the following criteria for applicability :

"This test procedure is applicable as long as the test chemical can be reliably analysed in the test organisms and the test medium, and as long as it can be demonstrated that sorption isotherms have been measured (i.e. the bioconcentration factor (BCF) given is based on steady-state measurements). If these prerequisites can be observed (generally with chemicals having a Pow $< 10^5$), the Test Guideline

can be used at both the screening and the confirmatory testing level."

1.7. Prolonged biodegradation study.

This study should be reserved for chemicals found not to be readily biodegradable and for which the Base set data indicate that biodegradability is important for assessing environmental risk. If a chemical is highly toxic to fish or Daphnia, or has a high Pow in the Base-set tests and is not readily biodegradable, it may be of interest to carry out this "inherent biodegradability" test at the 10 t/y level. Failure to biodegrade in this test can usually be taken to indicate that the substance will not biodegrade in the environment, but does not exclude the possibility that a study under conditions more closely simulating the environment (see Level 2) may show that it will in fact biodegrade.

2. Level 2

The details of the study programme at Level 2 will be agreed in discussions between the notifier and the competent authority. These discussions should, in particular, identify the need for further environmental research, taking into consideration the information already available from the Base-set and Level 1. The over-riding criteria for further testing are that the tests are adequate and necessary for refining the knowledge of environmental concentrations and effects consequent to the notified use, such that a risk assessment can be made at this Level. The tests should be appropriate to the chemical properties, uses and release pattern of the substance, and should be relevant to the environmental conditions.

At Level 2 the nature of the necessary studies will vary so much from substance to substance that only very general comments and guidelines can be given.

2.1. Additional tests for accumulation, and degradation

a) Accumulation. If the n-octanol/water partition coefficient (Base-set) or the initial Level 1 test on bioconcentration have shown that there is no potential for bioaccumulation there is no justification to run a further test for this. If on the basis of previous data there is a reason for concern, this test will enable

the bioconcentration factor to fish, and possibly the kinetics of uptake and depuration of the chemical, to be more precisely assessed.

b) Degradation. At Level 2, a biodegradation study under conditions simulating the removal of the substance under sewage treatment conditions, or a study of the rate of biodegradation in selected environmental situations, is envisaged where appropriate.

For substances not readily biodegradable in a Base-set test but which have subsequently shown a clear potential for biodegradation at Level 1, the Level 2 study may be necessary in order to characterise the biodegradation more precisely so that the estimate of PEC can be improved.

It may also be relevant to study photodegradation at this stage, under experimental conditions corresponding to the likely distribution of the chemical.

2.2. Prolonged toxicity study with fish (including reproduction)

If earlier acute toxicity tests with fish indicate that potential environmental concentrations are likely to exceed 0.01 x the $LC_{50}$ , a chronic test which also gives information on reproduction is required. In this case, a risk may exist and it is necessary to define more accurately the threshold concentration to fish, determined in the laboratory as the highest No-Observed-Effect-Concentration in a chronic exposure test.

At present there is no agreed test method for a reproduction study on fish. In the meantime the "early life-stage" type of test (see for example OECD draft Test Guideline ET-82.1), in which is measured the toxicity of the substance to fish eggs, embryo and fry, can be considered.

2.3. Additional toxicity study with birds

This test should normally be considered only if the substance is not degradable, has a whole-body bioconcentration factor in fish of at least 1000 (i.e. could be significant for fish-eating birds), or can

actually reach birds directly in amounts which a consideration of other toxicity data indicates may be harmful.

2.4. Additional toxicity study with other organisms

When the predicted environmental concentration of a chemical is likely, even after dispersion, to approach undesirable levels in specific parts of the environment, additional toxicity tests with other organisms more relevant to these compartments should be considered. (e.g. benthic organisms, marine organisms, higher plants etc.).

2.5. Adsorption/desorption and mobility studies

If a substance is not biodegradable, a major influence on its fate may be adsorption onto, or desorption from, solids such as soil, sediments, sewage solids, and suspended solids. In such a case the study of adsorption/desorption characteristics will provide information useful for assessing its mobility and environmental concentration.

A first estimate of the adsorption of un-ionised organic chemicals onto soil, and thus their mobility in soil, can be obtained from their water-solubility or Pow. A number of authors have shown that there is a good correlation between Koc (the soil adsorption coefficient normalised to 1% organic carbon) and water-solubility or Pow (Karickhoff, 1981; Means et al., 1980; Schwarzenbach and Westall, 1981; Means et al., 1982).

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*ANNEX VII*

## INFORMATION REQUIRED FOR THE TECHNICAL DOSSIER ('BASE SET') REFERRED TO IN ARTICLE 6 (1)

When giving notification the manufacturer or any other person placing a substance on the market shall provide the information set out below.

If it is not technically possible or if it does not appear necessary to give information, the reasons shall be stated.

Tests must be conducted according to methods recognized and recommended by the competent international bodies where such recommendations exist.

The bodies carrying out the tests shall comply with the principles of good current laboratory practice.

When complete studies and the results obtained are submitted, it shall be stated that the tests were conducted using the substance to be marketed. The composition of the sample shall be indicated.

In addition, the description of the methods used or the reference to standardized or internationally recognized methods shall also be mentioned in the technical dossier, together with the name of the body or bodies responsible for carrying out the studies.

### 1. IDENTITY OF THE SUBSTANCE

**1.1 Name**

1.1.1. Names in the IUPAC nomenclature

1.1.2. Other names (usual name, trade name, abbreviation)

1.1.3. CAS number (if available)

**1.2. Empirical and structural formula**

**1.3 Composition of the substance**

1.3.1. Degree of purity (%)

1.3.2. Nature of impurities, including isomers and by-products

1.3.3. Percentage of (significant) main impurities

1.3.4. If the substance contains a stabilizing agent or an inhibitor or other additives, specify: nature, order of magnitude: ... ppm; ...%

1.3.5. Spectral data (UV, IR, NMR)

**1.4. Methods of detection and determination**

A full description of the methods used or the appropriate bibliographical references

### 2. INFORMATION ON THE SUBSTANCE

**2.1. Proposed uses**

2.1.1. Types of use

Describe: the function of the substance .....

the desired effects .....

2.1.2. Fields or application with approximate breakdown

(a) closed system

— industries .....

— farmers and skilled trades .....

— use by the public at large .....

(b) open system

— industries .....

— farmers and skilled trades .....

— use by the public at large .....

2.2. **Estimated production and/or imports for each of the anticipated uses or fields of application**

2.2.1. Overall production and/or imports in order of tonnes per year 1; 10; 50; 100; 500; 1 000 and 5 000

— first 12 months .....tonnes/year

— thereafter .....tonnes/year

2.2.2. Production and/or imports, broken down in accordance with 2.1.1 and 2.1.2, expressed as a percentage

— first 12 months .....

— thereafter .....

2.3. **Recommended methods and precautions concerning:**

2.3.1. handling .....

2.3.2. storage .....

2.3.3. transport .....

2.3.4. fire (nature of combustion gases or pyrolysis, where proposed uses justify this)

2.3.5. other dangers, particularly chemical reaction with water

2.4. **Emergency measures in the case of accidental spillage**

2.5. **Emergency measures in the case of injury to persons**
(e.g. poisoning)

3. PHYSICO-CHEMICAL PROPERTIES OF THE SUBSTANCE

3.1. **Melting point**

.....°C

3.2. **Boiling point**

..... °C ..... Pa

3.3. **Relative density**

..... ($D_4^{20}$)

3.4. **Vapour pressure**

..... Pa at ..... °C

..... Pa at ..... °C

3.5. **Surface tension**

..... N/m (..... °C)

3.6. Water solubility

..... mg/litre (..... °C)

3.7. Fat solubility

Solvent — oil (to be specified)

..... mg/100 g solvent (..... °C)

3.8. Partition coefficient

n-octanol/water

3.9. Flash point

..... °C ☐ open cup ☐ closed cup

3.10. Flammability (within the meaning of the definition given in Article 2 (2) (c), (d) and (e))

3.11. Explosive properties (within the meaning of the definition given in Article 2 (2) (a))

3.12. Auto-flammability

..... °C

3.13. Oxidizing properties (within the meaning of the definition given in Article 2 (2) (b))

4. TOXICOLOGICAL STUDIES

4.1. Acute toxicity

4.1.1. Administered orally

$LD_{50}$..... mg/kg

Effects observed, including in the organs .....

4.1.2. Administered by inhalation

$LC_{50}$..... (ppm) Duration of exposure .....hours

Effects observed, including in the organs .....

4.1.3. Administered cutaneously (percutaneous absorption)

$LD_{50}$..... mg/kg

Effects observed, including in the organs .....

4.1.4. Substances other than gases shall be administered via two routes at least, one of which should be the oral route. The other route will depend on the intended use and on the physical properties of the substance.

Gases and volatile liquids should be administered by inhalation (a minimum period of administration of four hours).

In all cases, observation of the animals should be carried out for at least 14 days.

Unless there are contra-indications, the rat is the preferred species for oral and inhalation experiments.

The experiments in 4.1.1, 4.1.2 and 4.1.3 shall be carried out on both male and female subjects.

4.1.5. Skin irritation

The substance should be applied to the shaved skin of an animal, preferably an albino rabbit.

Duration of exposure ..... hours

4.1.6. Eye irritation

The rabbit is the preferred animal.

Duration of exposure ..... hours

4.1.7. Skin sensitization

To be determined by a recognized method using a guinea-pig.

4.2. **Sub-acute toxicity**

4.2.1. Sub-acute toxicity (28 days)

Effects observed on the animal and organs according to the concentrations used, including clinical and laboratory investigations .....

Dose for which no toxic effect is observed .....

4.2.2. A period of daily administration (five to seven days per week) for at least four weeks should be chosen. The route of administration should be the most appropriate having regard to the intended use, the acute toxicity and the physical and chemical properties of the substance.

Unless there are contra-indications, the rat is the preferred species for oral and inhalation experiments.

4.3. **Other effects**

4.3.1. Mutagenicity (including carcinogenic pre-screening test)

4.3.2. The substance should be examined during a series of two tests, one of which should be bacteriological, with and without metabolic activation, and one non-bacteriological.

5. ECOTOXICOLOGICAL STUDIES

5.1. **Effects on organisms**

5.1.1. Acute toxicity for fish

$LC_{50}$..... (ppm) Duration of exposure determined in accordance with Annex V (C)

Species selected (one or more) .....

5.1.2. Acute toxicity for daphnia

$LC_{50}$..... (ppm) Duration of exposure determined in accordance with Annex V (C)

5.2. **Degradation**

— biotic

— abiotic

The BOD and the BOD/COD ratio should be determined as a minimum

6. POSSIBILITY OF RENDERING THE SUBSTANCE HARMLESS

6.1. **For industry/skilled trades**

6.1.1. Possibility of recovery .....

6.1.2. Possibility of neutralization .....

6.1.3. Possibility of destruction:

— controlled discharge .....

— incineration .....

— water purification station .....

— others .....

6.2. **For the public at large**

6.2.1. Possibility of recovery .....

6.2.2. Possibility of neutralization .....

6.2.3. Possibility of destruction:

— controlled discharge .....

— incineration .....

— water purification station .....

— others .....

*ANNEX VIII*

ADDITIONAL INFORMATION AND TESTS REQUIRED UNDER ARTICLE 6 (5)

Any person who has notified a substance to a competent authority in accordance with the requirements of Article 6 of this Directive shall provide at the request of the authority further information and carry out additional tests as provided for in this Annex.

If it is not technically possible or if it does not appear necessary to give information, the reasons shall be stated.

Tests shall be conducted according to methods recognized and recommended by the competent international bodies where such recommendations exist.

The bodies carrying out the tests shall comply with the principles of good current laboratory practice.

When complete studies and the results obtained are submitted, it shall be stated that the tests were conducted using the substance marketed. The composition of the sample shall be indicated.

In addition the description of the methods used or the reference to standardized or internationally recognized methods shall also be mentioned in the technical dossier, together with the name of the body or bodies responsible for carrying out the studies.

LEVEL 1

Taking into account:

— current knowledge of the substance,

— known and planned uses,

— the results of the tests carried out in the context of the base set,

the competent authority may require the following additional studies where the quantity of a substance placed on the market by a notifier reaches a level of 10 tonnes per year or a total of 50 tonnes and if the conditions specified after each of the tests are fulfilled in the case of that substance.

Toxicological studies

— Fertility study (one species, one generation, male and female, most appropriate route of administration)

  If there are equivocal findings in the first generation, study of a second generation is required.

  It is also possible in this study to obtain evidence on teratogenicity.

  If there are indications of teratogenicity, full evaluation of teratogenic potential may require a study in a second species.

— Teratology study (one species, most appropriate route of administration)

  This study is required if teratogenicity has not been examined or evaluated in the preceding fertility study.

— Sub-chronic and/or chronic toxicity study, including special studies (one species, male and female, most appropriate route of administration)

  If the results of the sub-acute study in Annex VII or other relevant information demonstrate the need for further investigation, this may take the form of a more detailed examination of certain effects, or more prolonged exposure, e.g. 90 days or longer (even up to two years).

The effects which would indicate the need for such a study could include for example:

(a) serious or irreversible lesions;

(b) a very low or absence of a 'no effect' level;

(c) a clear relationship in chemical structure between the substance being studied and other substances which have been proved dangerous.

— Additional mutagenesis studies (including screening for carcinogenesis)

A. If results of the mutagenesis tests are negative, a test to verify mutagenesis and a test to verify carcinogenesis screening are obligatory.

If the results of the mutagenesis verification test are also negative, further mutagenesis tests are not necessary at this level; if the results are positive, further mutagenesis tests are to be carried out (see B).

If the results of the carcinogenesis screening verification test are also negative, further carcinogenesis screening verification tests are not necessary at this level; if the results are positive further carcinogenesis screening verification tests are to be carried out (see B).

B. If the results of the mutagenesis tests are positive (a single positive test means positive), at least two verification tests are necessary at this level. Both mutagenesis tests and carcinogenesis screening tests should be considered here. A positive result of a carcinogenesis screening test should lead to a carcinogenesis study at this level.

Ecotoxicology studies

— An algal test: one species, growth inhibition test.

— Prolonged toxicity study with Daphina magna (21 days, thus study should also include determination of the 'no-effect level' for reproduction and the 'no-effect level' for lethality).

The conditions under which this test is carried out shall be determined in accordance with the procedure described in Article 21 in the light of the methods laid down in Annex V (C) for acute toxicity tests with Daphnia.

— Test on a higher plant.

— Test on an earthworm.

— Prolonged toxicity study with fish (e.g. Oryzias, Jordanella, etc.; at least a period of 14 days; thus study should also include determination of the 'threshold level').

The conditions under which this test is carried out shall be determined in accordance with the procedure described in Article 21 in the light of the methods adopted under Annex V (C) for acute toxicity tests with fish.

— Tests for species accumulation; one species, preferably fish (e.g. Poecilla reticulata).

— Prolonged biodegradation study, if sufficient (bio)degradation has not been proved by the studies laid down in Annex VII, another test (dynamic) shall be chosen with lower concentrations and with a different inoculum (e.g. flow-through system).

In any case, the notifier shall inform the competent authority if the quantity of a substance placed on the market reaches a level of 100 tonnes per year or a total of 500 tonnes.

On receipt of such notification and if the requisite conditions are fulfilled, the competent authority, within a time limit it will determine, shall require the above tests to be carried out unless in any particular case an alternative scientific study would be preferable.

LEVEL 2

If the quantity of a substance placed on the market by a notifier reaches 1 000 tonnes per year or a total of 5 000 tonnes, the notifier shall inform the competent authority. The latter shall then draw up a programme of tests to be carried out by the notifier in order to enable the competent authority to evaluate the risks of the substance for man and the environment.

The test programme shall cover the following aspects unless there are strong reasons to the contrary, supported by evidence, that it should not be followed:

— chronic toxicity study,

— carcinogenicity study,

— fertility study e.g. three-generation study); only if an effect on fertility has been established at level 1,

— teratology study (non-rodent species) study to verify teratology study at level 1 and experiment additional to the level 1 study, if effects on embryos/foetuses have been established,

— acute and sub-acute toxicity study on second species: only if results of level 1 studies indicate a need for this. Also results of biotransformation studies and studies on pharmacokinetics may lead to such studies,

— additional toxicokinetic studies.

Ecotoxicology

— Additional tests for accumulation, degradation and mobility.

The purpose of this study should be to determine any accumulation in the food chain.

For further bioaccumulation studies special attention should be paid to the solubility of the substance in water and to its n-octanol/water partition coefficient.

The results of the level 1 accumulation study and the physiocochemical properties may lead to a large-scale flow-through test.

— Prolonged toxicity study with fish (including reproduction).

— Additional toxicity study (acute and sub-acute) with birds (e.g. quails): if accumulation factor is greater than 100.

— Additional toxicity study with other organisms (if this proves necessary).

— Absorption — desorption study where the substance is not particularly degradable.

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*ANNEX IX*

A. PROVISIONS RELATING TO CHILD-RESISTANT FASTENINGS: for the record

B. PROVISIONS RELATING TO TACTILE WARNINGS OF DANGER: for the record

G. APPENDIX

SOME MEASURED CONCENTRATIONS OF LARGE-TONNAGE INDUSTRIAL CHEMICALS IN SURFACE WATERS

| Product | Average Concn. for Year µg/l | Site | Year | Source of Information* |
|---|---|---|---|---|
| Chloroform | 22 | Rhein | 1978 | LWA/NRW |
| | 4,5 | Rhein | 1980 | LWA/NRW |
| | 0,8 | Rhein | 1981 | ARW |
| | 1,6 - 26 | Main | 1980 | Hess.Ministerium für Umwelt |
| Methylene chloride | 15 | Rhein | 1978 | LWA/NRW |
| | not detectable | Rhein | 1980 | LWA/NRW |
| Carbon tetrachloride | <0,1 | Rhein | 1980 | Holländische Rheinkommission |
| | 0,45 | Rhein | 1981 | ARW |
| | 0,01 - 5,5 | Lippe | 1980 | LWA/NRW |
| Trichloroethylene | 0,32 | Rhein | 1978 | LWA/NRW |
| | 0,3 | Rhein | 1980 | LWA/NRW |
| | 0,18 | Rhein | 1981 | ARW |
| | 0,7 - 8,8 | Main | 1980 | Hess.Minesterium für Umwelt |
| Perchloroethylene | 0,6 | Starnberger See | | Wacker Chemie GmbH |
| | 1,7 | Rhein | 1973 | Diet et al. (Ruhrverband) |
| | 0,5 - 4 | Main | 1980 | Hes.Ministerium für Umwelt |
| Chlorbenzene | 2 | Rhein | | VCI |
| | 1,2 | Rhein | 1975 | LWA/NRW |
| Dichlorobenzne o- | 0,16 | Rhein | 1977 | LWA/NRW |
| | 5,2 | Main | 1980 | Hess.Ministerium für Umwelt |
| p- | 0,1 | Rhein | 1980 | LWA/NRW |
| | | Main | 1980 | Hess.Ministerium für Umwelt |
| m- | not detectable | Rhein | 1980 | LWA/NRW |
| Nitrobenzene | 0,12 | Rhein | 1978 | LWA/NRW |