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The EEC Sixth Amendment: A Guide to Risk Evaluation for Effects on Human Health

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TECHNICAL REPORT

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THE EEC SIXTH AMENDMENT :
A GUIDE TO RISK EVALUATION
FOR EFFECTS ON HUMAN HEALTH

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A. SUMMARY

The European Communities' Directive for the notification of new chemicals (Council Directive amending for the sixth time Directive 67/548/EEC, henceforward referred to as the 6th Amendment) requires a manufacturer or importer of a new substance to submit "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...". This report is concerned with the foreseeable risks to man. These are evaluated by first performing toxicological studies chosen from a series specified in the 6th Amendment and then evaluating the risk from the results plus all other relevant information available.

The studies and the risk evaluations are carried out at three Levels (Base set, Level 1 and Level 2) according to the tonnage marketed. The evaluation of risk at each Level influences the decisions about testing at the same, or a later, Level. A decision is required, on a case by case basis, as to which studies are necessary to provide data adequate for evaluating the risks at each Level and for deciding at which point further studies are unnecessary. These questions are addressed in this report in which a rationale is given, a) for the logical choice of studies to be carried out, or in some cases omitted, and b) for the evaluation of risk to humans, at each Level. The over-riding criteria for selecting studies is that the information developed is adequate and necessary for the evaluation of risks which may arise when the substance is used in practice.

Harmonisation of the principles of risk evaluation should be sought, but it is not possible to harmonise the details because the toxicological and exposure characteristics, and their significance, will differ from chemical to chemical.

B. 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 as 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 fulfill all of these requirements, this report is concerned only with "evaluating the foreseeable risks, whether immediate or delayed, which the substance may entail for man", under normal conditions of use and disposal. According to Art. 7.1 of the 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 foreseeable risks which the substance may entail". Information is required at three Levels (Base set, Level 1 and Level 2) depending on the tonnage marketed, and at each Level the notifier has to evaluate the risk as a guide to the further toxicological

studies required at the next Level, or to a decision that further studies are unnecessary.

The purpose of risk evaluation as outlined in this report is to identify possible areas of risk to human health, 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 made, although this aspect is outside the scope of the present document.

The technical dossier to be supplied under the 6th Amendment includes data on the toxicity of a chemical, and also information which indicates the probable human exposure. The data and information are developed at three Levels according to the expected tonnage to be marketed - see Annexes VII and VIII of the 6th Amendment, reproduced as Appendix 1 in this document. Base-set information includes many parameters which are fundamental for risk evaluation, and the toxicological studies in Levels 1 and 2 give further information on the effects of the substance. As information accumulates from studies in the sequence Base-set, Level 1, Level 2, it contributes to the decision on which tests are appropriate to enable the risk evaluation to be made at each Level according to the uses and tonnage notified.

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 evaluation 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 studies necessary at Level 1 should preferably be discussed between the manufacturer and the competent authority. When the marketed volume reaches 1000 t/y, a similar discussion of studies 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 qualification allows some flexibility in choosing logically which tests to carry out and in what sequence. Thus the question arises : what studies are necessary to provide data adequate for

risk evaluation at each Level, and at what point can they be terminated ? A fixed set of obligatory studies cannot serve for the evaluation of risk for all substances. Justification has to be given when it is decided to : require a study at an earlier Level, or postpone a study to a later Level, than is indicated by tonnage; carry out studies not listed in Annex V of the Directive; omit certain studies. The overriding criteria for selecting studies are that the information developed is adequate and necessary for evaluating the risks which may arise when the substance is used in practice. If only for ethical reasons, the study scheme should be aimed at obtaining the maximum of data with the minimum use of animals.

Harmonisation of the principles of risk evaluation should be sought so that a common approach is used by notifiers and the authorities in the various countries. Harmonisation of the details of risk evaluation is not possible because the toxicological and exposure 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 studies (within the limits of choice in the 6th Amendment) is optimum for the purpose, and how the risk evaluation is to be made, for each individual substance.

C. RATIONALE FOR TOXICOLOGICAL STUDIES IN THE BASE-SET

The Base-set requirements are given in the attached Annex VII of the 6th Amendment. Sections 1 (Identity of Substance), 2 (Information on the Substance) and 3 (Physico-chemical Properties) provide information and data relevant to the exposure of humans to the substance. Section 4 (Toxicological Studies) sets out the health effects studies to be performed.

In the majority of cases it will be appropriate to carry out all or most of the Base-set studies, although Annex VII states that "If it is not technically possible or if it does not appear necessary to give information, the reasons shall be stated". Whether a study is technically possible has to be judged against the protocols prescribed in Annex V of the 6th Amendment. A decision whether "it does not appear necessary to give information" will depend on whether the data from a study is relevant

to the evaluation of risk. The relevance of a study, at any Level, depends markedly on the exposure profile, and due consideration should be given to :

i) the route(s) of exposure;
ii) the duration of exposure, and whether it is continuous or intermittent;
iii) the number of people exposed;
iv) the operating conditions, eg. whether they comprise an open or closed system;
v) the population exposed.

One or more of the above considerations may indicate that a particular study should be omitted. On the other hand, even at the notification level of 1t/y exceptional circumstances may make it advantageous to perform additional studies, eg. if a substance to which women may be exposed is structurally closely-related to a known teratogen. Guidance on the choice and sequence of studies in the Base-set is given below.

Ethical considerations may lead to a decision not to perform certain studies at any Level : eg. animal studies would not be appropriate if the physical-chemical properties of a substance indicate that it is corrosive, strongly acidic or strongly basic, or possesses extremely hazardous physical properties (eg. ignites on contact with air) under the conditions of the test.

1. Acute Oral Toxicity (4.1.1. in Annex VII).

Before deciding to carry out the acute oral (LD_{50}) study, performance of the "Limit Test" at a dose level of at least 2000 mg/kgbw should be considered. In protocol B.1, Annex V of the 6th Amendment, a dose level of 5000 mg/kgbw is prescribed for this test, but the highest level related to classification and labelling (see Annex VI A, 6th Amendment) is 2000 mg/kgbw and it is therefore more relevant to use the latter. If this Limit Test produces substance-related mortality then the full acute oral toxicity study is necessary.

This study is inappropriate for a substance which is a gas at room temperature and pressure (acute oral exposure of humans to a gas is, in any case, improbable, but if it is likely then special techniques may be used). It is similarly inappropriate for substances which cannot be dissolved or suspended in a suitable medium. For substances known to be

unstable in the formulation to be administered, careful judgement is required as to the relevance of the data generated and in some circumstances it may not be possible to design an adequate study.

Determination of the acute oral toxicity will be possible for most substances , and indeed seems to be obligatory according to section 4.1.4 of Annex VII.

2. Acute Inhalation Toxicity (4.1.2., Annex VII).
Before deciding to carry out this study, performance of the Inhalation Hazard Test (Annex to OECD Test Guideline 403) should be considered if the substance is a vapour or is volatile under the conditions of human exposure. In this Hazard Test the maximum concentration attainable under the study conditions is used, and if there is no substance-related mortality the lethal concentration may be quoted as greater than the concentration used and no further acute inhalation testing is necessary. If substance-related mortality occurs it may be necessary to perform the full acute inhalation toxicity study, although prior to this the "Limit Test" (protocol B.2, Annex V, 6th Amendment) may be performed at an exposure concentration of 20 mg/l for a gas, or 5 mg/l for an aerosol or particulate (the practical feasibility of working with such a high concentration of an aerosol or particulate is very questionable). Substance-related mortality in the Limit Test indicates that the full study should be performed.

The acute inhalation toxicity study is technically impossible, and is not necessary, for solids which do not sublime or produce respirable particles, and for non-volatile liquids which do not produce aerosols under the notified conditions of use.

3. Acute Dermal Toxicity (percutaneous absorption) (4.1.3. Annex VII).
Before deciding to carry out this (LD_{50}) study, performance of the Limit Test (protocol B.3, Annex V, 6th Amendment) at 2,000 mg/kgbw should be considered. If substance-related mortality occurs in this Limit Test, the full study should be performed. It is not possible, by the prescribed methods, to perform the study on substances which are gaseous at room temperature and pressure.

For some classes of chemical (eg. certain polymers of high molecular weight) the skin is known to be an effective barrier to percutaneous absorption, and for substances in such classes this study may be unnecessary.

4. Choice of Route of Exposure in Acute Studies

Section 4.1.4 of Annex VII requires that "Substances other than gases shall be administered via two routes at least, one of which should be the oral route". Thus for liquids and solids a decision has to be made whether to perform either the acute inhalation or acute dermal study, or both. This will depend on the likely human exposure and the factors set out in sections 2 and 3 above. It should be noted that a whole-body acute inhalation study inevitably involves dermal contact with, and oral intake (via preening) of, the test substance. The most common technique, however, is to use "nose-only" exposure which avoids such dermal and oral intake, but includes oral ingestion by swallowing.

5. Skin Irritation (4.1.5, Annex VII).

Preliminary indications that a substance has potential to irritate the skin might be obtained from the above inhalation and dermal studies. The skin irritation test should normally be carried out unless the substance is gaseous at room temperature and pressure, when the prescribed method is not practicable. If the structure of the substance indicates that it may be severely irritant or corrosive it could be reported as such, in which case the test could be omitted.

6. Eye Irritation (4.1.6, Annex VII)

The same remarks apply as in the preceding paragraph.

7. Skin Sensitisation (4.1.7, Annex VII)

This study should normally be performed except for substances which are :

- severely irritant or corrosive at the concentration at which they are used, or for which protective measures related to a high dermal toxicity have to be taken;
- gaseous at room temperature and pressure (prescribed method is inapplicable);

If the structure of the substance is closely-related to that of a known skin sensitiser, or an impurity known to be a skin sensitiser is present, the substance may be notified as a sensitiser without testing. Alternatively, the notifier may choose to carry out the study to assess whether, indeed, the substance has sensitisation potential.

8. Sub-acute Toxicity. (4.2.1, Annex VII)
Because people may be repeatedly exposed to a substance, it is required to assess the effect of such exposure via the sub-acute study which gives data on cumulative toxicity and the no-adverse-effect level. Ideally, the route of administration should be that which is most relevant to human exposure and to the information on acute toxicity as generated above. In practice, the oral route may be preferable because, i) in the case of large particulates, inhalation results in substantial oral intake by swallowing, and ii) it may be impossible to determine the toxicity profile of a substance by the dermal route because absorption through the skin is low.

If an oral or dermal sub-acute study by one of these routes is indicated but the oral or dermal acute toxicity is low, then a Limit Test (Annex V, protocols B.7 oral, B.9 dermal) should be carried out at a concentration of 1000 mg/kgbw, or at a higher concentration if it is suspected that humans could be so exposed. If no toxic effects are observed in the oral or dermal Limit Test, the full sub-acute study by these routes is normally not necessary and the no-adverse-effect level is taken to be at least 1000 mg/kgbw.

Omission of the sub-acute study might be considered if all of the following conditions are met :

- the acute toxicity is low,
- the acute toxicity tests reveal no delayed effects,
- the bioavailability is low,
- the potential for bioaccumulation is low.

If the oral, dermal or inhalational acute toxicity is high enough to indicate that there may be a risk to human health at the expected exposure levels, a sub-acute study is normally necessary. For specific reasons, and on a case by case basis, the notifier may perform only a

limited number of laboratory or histopathological observations, but such limitation must be justified.

If a notifier believes that the quantity of a substance marketed will move rapidly to a Level beyond that of the Base-set, it may be more cost-effective to carry out the (Level 1) sub-chronic study at the Base-set level.

9. Mutagenicity (4.3.1, Annex VII)

A bacterial and non-bacterial test should normally be carried out on all substances. When the physical-chemical properties of a substance do not permit one or both types of study to be carried out according to the methods prescribed in Annex V, there may be alternative test systems which it is feasible and relevant to use.

If one of the above tests gives a positive result it may be necessary to perform additional, complementary short-term tests for mutagenicity as in Level 1 (see section D.3.).

D. RATIONALE FOR TOXICOLOGICAL STUDIES AT LEVEL I

At Level 1 there is some freedom of choice in the sequence of studies and the study conditions because :

i) Base-set toxicological information is to hand;
ii) within the prescribed test methods there is scope for a rational choice of the most appropriate conditions to be used.

In this chapter a rationale for choosing which studies to perform at Level 1 is given. All the information available, plus any guidance arising from a knowledge of the toxicity of structurally-related chemicals, should be taken into account on a case-by-case basis.

The need to perform the fertility and teratology studies should be questioned if :

i) from the sub-acute or sub-chronic studies there is no evidence of toxic effects,

or ii) there are good reasons, or experimental evidence, indicating that the bioavailability is low,

or iii) it is evident from the use-pattern that human exposure will be restricted and well-controlled.

1. Fertility Study

A one-generation, single-species study by the route of administration most appropriate to the expected human exposure may be required at Level 1, normally at the 100 t/y level. If there are equivocal findings in the reproductive performance of the parents (Fo), or effects in the first (F1a) litter, a further litter (F1b) may be examined before the second (F2) generation is studied (see Level 2). This examination of the F1b litter, although not specified in the 6th Amendment, may be necessary to resolve the equivocal results obtained with the F1a litter and is, in fact, an option in the OECD Test Guideline no. 415. If clear effects on reproductive performance and/or effects on the progeny are thus established at Level 1, there may be no need to carry out the 2-generation study at Level 2. (Note that the Level 2 study is referred to as a "3-generation" study in Annex VIII of the 6th Amendment. Because it involves the parents plus F1 and F2 generations, in the present document it is called a "2-generation" study).

If in the sub-acute or sub-chronic studies the toxicity of the substance appears to be low, a Limit Test for fertility at a single dose level of 1000 mg/kgbw in the diet might be performed. In the absence of adverse effects on reproduction, no further fertility testing is necessary and the no-adverse-effect level can be taken to be at least 1000 mg/kgbw.

The need to perform the fertility study at the 10 t/y stage in Level 1 should be considered if in the sub-acute or sub-chronic studies there are :

i) significant changes in the weight of the testes or ovaries; macroscopic findings in the reproductive organs; or significant histopathological changes in the male or female sex organs;

and ii) positive results from mutagenicity tests on mammalian systems which are relevant to adverse effects on reproduction;

and iii) concern about the expected level of human exposure.

Structure-activity relationships are very rarely of value for predicting potential toxic effects on reproduction, but may provide some supporting evidence concerning the need for studies.

If a substance is likely to pass rapidly from Level 1 to Level 2, then it may be more economical to carry out the Level 2 (2-generation) study directly at Level 1, omitting the 1-generation study. In this case the sub-chronic study should precede the 2-generation fertility study so that the dose-range for the latter can be set. Embryotoxic and teratogenic effects may be revealed in an additional part of this study, if included.

2. Teratology Study

This study, in one species and by the most appropriate route of administration, is normally required at Level 1 (100 t/y) if teratogenicity has not been evaluated as an additional part of the above fertility study. Oral administration to the rat is commonly used in these studies, particularly since the results can be more easily assessed in relation to the Base-set and Level 1 toxicity studies on the same species and by the same route. The use of other routes of administration is not excluded if extrapolation of the oral results to the human situation is not possible. If in the sub-acute and sub-chronic studies the toxicity of the substance appears to be low, a "Limit Test" at 1000 mg/kgbw (OECD Test Guideline 414) should be carried out. In the absence of teratogenic effects no further teratogenicity study is necessary.

In exceptional circumstances the need to perform the teratogenicity study at the 10 t/y stage in Level 1 should be considered, i.e.

i) if positive results have been obtained in mutagenicity tests with mammalian systems relevant to embryotoxicity, such as systems which reveal chromosome aberrations and gene mutations. (Such results do not prove unequivocally the existence of teratogenic potential, but indicate that it is possible);

and ii) if the expected human exposure causes particular concern;

or iii) if toxic effects have been found in rapidly-replicating tissue, such as bone marrow, ovaries or testes.

While structure-activity relationships give very little guidance about embryotoxic and teratogenic potential, there may occasionally be cases in which their consideration assists in the above decision.

If in the additional part of the one-generation fertility study (see section D.1) no teratogenic effects have been revealed, no further teratogenicity study is necessary at any Level. If teratogenic effects are observed and a comparison of the no-adverse-effect level with the expected level of human exposure indicates that there is an adequate safety margin, no further study is necessary at Level 1. If the safety margin is inadequate, or if the results are equivocal, the performance of a teratogenicity study on a second species should be considered at Level 1, 100 t/y. The results are more relevant if abnormal findings in the foetus occur for both species. If the sensitivity (no-adverse-effect level) differs widely between the species, additional studies may be advisable to clarify the findings.

3. Sub-chronic, Chronic and Additional Mutagenicity Studies.

If the prerequisite conditions in Annex VIII (Level 1, Toxicological studies) are fulfilled, a sub-chronic and/or chronic (beyond 90 days) study, which might include "special studies", may be required prior to the 100t/y stage in Level 1. In considering the need to carry out a sub-chronic study at the 10t/y stage the most important evidence is the toxicity profile as revealed by the acute and sub-acute studies. Information will normally be available on the target organ(s), cumulative effects, dose-response and no-adverse-effect level. The following considerations could justify a sub-chronic study at 10t/y :

i) the finding of adverse effects at dose levels which when compared to the expected level of human exposure do not lead to an adequate safety margin;

ii) marked evidence of cumulative toxicity;

iii) the failure to establish a no-adverse-effect level in the Base-set sub-acute study.

The finding of potentially-irreversible lesions (eg. kidney necrosis, liver lesions, anaemia, paralysis, CNS effects) at doses relevant to those which would result from the expected human exposure also justifies consideration of the sub-chronic study at 10 t/y. On the other hand, if the no-adverse-effect level in the sub-acute study is

high compared with the expected level of human exposure, and there is no evidence of cumulation, the sub-chronic study could be deferred until Level 2.

Special studies (eg. acute and sub-acute studies on a second species, metabolism, pharmacokinetics and toxicokinetics) are required at Level 2. It is a matter of expert judgement whether in exceptional cases particular studies (often incorporated in the sub-chronic study) should be performed at Level 1. This judgement has to be made by a case-by-case consideration of all the available evidence, and no general guidance can be given.

The need for a carcinogenicity study at Level 1 is evaluated by a careful assessment of all toxicological data. Although indications of carcinogenic potential may occasionally be obtained from the sub-chronic study, this is normally of too short a duration to reveal such potential. The most important evidence is that from the short-term tests for mutagenicity in the Base-set (on a bacterial and a non-bacterial system) and Level 1 (verification test or tests, chosen from a range of possible systems). For interpreting the evidence from such short-term tests the guidance in two ECETOC monographs (ECETOC 1980,1982) should be followed. In conjunction with the other toxicological evidence available at this stage, the results of short-term tests are used to assess whether a substance has carcinogenic potential. Consideration of the combined results leads to a decision that the overall result is a "confirmed negative", "confirmed positive" or "unconfirmed" (See Appendix 2). While a "confirmed negative" result in the two Base set tests does not constitute proof of non-carcinogenicity, it indicates that a carcinogenicity study is unnecessary at Level 1. Unless consideration of the structure of the substance suggests otherwise, a confirmed negative result in two tests with unrelated end-points also means that no further mutagenicity testing is necessary.

A "confirmed positive" result, which leads to the substance being considered as a "questionable human chemical carcinogen", together with the finding of proliferative lesions in the sub-chronic study suggests that further studies to determine the relevance of these results may be necessary. This may lead to consideration of the carcinogenicity study

at Level 1 unless the probable human exposure is insignificant in relation to the assumed carcinogenic potential of the substance. This latter concept is in line with recent opinions expressed by the US Food and Drug Administration who made it clear (FDA, 1982) that for direct food additives their concern is minimal if the level of exposure is very low - they quote an intake of 0.00031 mg/kg/day or less. Failure to confirm the first result indicates that it would be useful to study the comparative pharmacokinetics and metabolism, and then to re-assess the risk prior to a decision whether a carcinogenicity study is required.

When an "unconfirmed result" is obtained, complementary short-term tests should be considered in an attempt to confirm the result as "positive" or "negative".

E. RATIONALE FOR TOXICOLOGICAL STUDIES AT 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 toxicological studies, taking into consideration the information already available from the Base-set and Level 1. The over-riding criteria for further studies are that they are adequate and necessary for refining the knowledge of toxicological effects consequent to the notified use, such that a risk evaluation can be made at this Level. At Level 2 the nature of the necessary studies will vary so much from substance to substance that only very general comments and guidance can be given.

If on the basis of the evidence relevant to carcinogenicity it is accepted that the substance is handled as a carcinogen, there may be no need to carry out the fertility study, or the teratology and acute/sub-acute studies on a second species suggested at Level 2, because exposure will already be stringently controlled.

1. Chronic Toxicity and Carcinogenicity Studies.

At Level 2, the results of the short-term tests for mutagenicity, and other aspects of the toxicological profile which might be available such as data on bioavailability, metabolism, cellular or functional targets (eg. hormone balance and immunological status), and species differences, may be useful in deciding whether to carry out the carcinogenicity study.

In the absence of other relevant toxicological data, a confirmed negative result from short-term tests is a good indication of non-carcinogenicity. However, other considerations, e.g. the size of the exposed population and the likely dose absorbed, may make it prudent to carry out additional testing in whole-animal systems. When consistently positive indications of carcinogenic potential are obtained in this phase, consideration should be given to carrying out the long-term animal study. Alternatively, it may be accepted that the substance has carcinogenic potential and should be handled accordingly, without further studies.

If the substance has shown low acute toxicity, high no-adverse-effect levels in sub-chronic studies with only negligible effects, no evidence of teratogenicity, fertility and mutagenicity, lack of bio-availability and biotransformation, and if human exposure is expected to be low, then there seems no reason to perform a chronic study.

If the substance has consistently failed to show any adverse effects in the Base-set and Level 1 studies at the highest doses tested, and if there is a large safety margin between these doses and the expected level of human exposure, then the need for a study of the bioavailability of the substance (and hence its capacity to cause chronic effects) is questionable. In this case it may be more valuable to carry out the biotransformation and toxicokinetic studies since if they confirmed that the substance is not bioavailable there would normally be no need to perform the long-term studies.

2. Fertility Study

In the 6th Amendment it is recommended that a 2-generation study is performed at Level 2 only if an effect on fertility has been established in the Level 1, one-generation study. It is ECETOC's view,

however, that if clear effects on reproductive performance and and/or effects on the progeny have been established in the 1-generation study, there is no scientific justification for a 2-generation study.

3. Teratology Study
   This study, in a non-rodent species, should be performed to verify the results at Level 1.

4. Acute and/or Sub-acute Study on Second Species
   These studies are necessary only if the Level 1 results are equivocal and would be elucidated by such studies.

5. Additional Toxicokinetic Studies
   These studies are necessary only when clear adverse effects have been established but there is a need to clarify their relevance to man for risk evaluation.

F. RISK EVALUATION BASED ON INFORMATION GENERATED UNDER THE 6TH AMENDMENT

In evaluating the risk to human health, all data available from the toxicity studies are taken into account. The minimum dose inducing toxic effects and the no-adverse-effect level are compared with the expected level of human exposure, and the likelihood that the toxic effect will occur is evaluated. The dose-effect relationship should also be taken into account.

While any deviations from the observations made on the actual and historical controls in a toxicological study have been accepted by some authorities as adverse effects, this is not necessarily justified. Whether such deviations indicate a toxic action depends on their nature. Professional experience and judgement are required to distinguish between those observed effects which are of no toxicological significance and those which indicate toxic potential. Thus, certain effects may result merely from adaptation to exposure. Furthermore, deviations from an effect in the current control animals may lie within the normal range of the findings in compatible controls of the same strain and may therefore be judged to have no toxicological significance.

When interpreting data from inhalation studies or studies on dermal toxicity it should be recognised that simultaneous oral ingestion is possible unless stringent precautions are taken.

When the toxicological properties of the notified substance have been established in the animal studies, the relevance of the observed effects for man has to be established. This is a complex process requiring expert judgement in integrating all of the factors mentioned above and relating them to the knowledge of human exposure. Some of the information generated under the 6th Amendment is quantitative (eg. that from the LD_{50}, LC_{50}, fertility and teratogenicity studies) and enables effect-levels and no-adverse-effect levels to be compared with estimated levels of human exposure in evaluating risk. Other information (eg. on sensitisation, mutagenicity and carcinogenicity) is less firmly quantitative and needs additional consideration in using it for risk evaluation.

Information relating to the expected human exposure to the substance is requested mainly at the Base-set Level. Consideration of the various uses and modes of handling of the substance provides key parameters for this, i.e. the number and type of people exposed (see the 3 categories in section 2.1.2. of Annex VII, 6th Amendment), the main exposure route(s), the expected level of exposure, and its frequency and duration. These depend, in part, on certain physical-chemical properties of the substance, e.g. its physical form, vapour pressure, reactivity to water, etc.

Information necessary for "evaluating the foreseeable risks" is required at each Level in the 6th Amendment. There is overlap in the type of information generated at each Level (eg. mutagenicity testing is required in the Base-set and at Level 1) and therefore the guidance to risk evaluation given here is not sub-divided to correspond to each Level but is treated according to the type of toxicological information generated. A case-by-case approach is recommended and has the advantage of enabling special or unusual effects to be dealt with.

At the Base-set Level the results of evaluating risk are expressed mainly in the recommendations for classification, labelling and safety precautions, but they also contribute to the risk evaluation, and decisions about studies to be carried out, at Levels 1 and 2.

1. Acute Toxicity Including Median Lethal Dose/Concentration (LD_{50} and LC_{50})

These studies indicate the lethality of a chemical to the test species, and give details of its toxic effects after acute exposure. The results can be used to indicate levels at which a hazard to humans may exist, but it is unwise to use them to define levels at which the risk to humans is low (Royal Society, 1983). The acute toxicity data are especially useful in identifying hazards arising from exposure following an accident.

2. Sub-acute Toxicity.

This study gives a no-adverse-effect level, which is important for risk evaluation. Other information useful for evaluating risk is also generated, e.g. the dose-effect curve, the target organ(s) and systems, and an indication of cumulative toxicity (the adverse effects of repeated doses resulting from the prolonged action of the substance on susceptible tissues, or from an increased concentration of the substance or its metabolites in them).

2.1. Oral and percutaneous exposure. For evaluating risk to humans the no-adverse-effect levels (NAELs) resulting from sub-acute studies with oral or percutaneous exposure should be directly compared with the expected level of human exposure. When the latter is considerably below the animal NAEL, the risk is evaluated as being negligible. If, however, the expected human exposure level is comparable to the NAEL, the slope of the dose-effect curve and the nature and severity of the lesions at doses above the animal NAEL will help in evaluating the risk. Data from sub-chronic and chronic studies, and on reversibility, toxicokinetics and metabolism which might be available at the later Levels, may help to refine the risk evaluation.

In studies with repeated exposure, the sensivity of animal skin to irritants may preclude the use of a dose which is above the expected level of human exposure. In this case the animal model cannot give results of value for risk evaluation.

2.2 Inhalation exposure. When the expected level of human exposure is

considerably below the animal NAEL, the risk is evaluated as being negligible. If, however, it is comparable to the NAEL an assessment of the slope of the dose-effect curve, and the nature and severity of the lesions at doses above the animal NAEL, will help in evaluating the risk. Data from sub-chronic and chronic studies and on reversibility, toxicokinetics and metabolism, which may be available at the later Levels, could help to refine the risk evaluation.

2.3 Limit test. If a sub-acute Limit Test (oral or percutaneous) is performed and there are no observable toxic effects, the risk to man can be evaluated as negligible at the dose used.

3. Skin and Eye Irritation/Corrosivity

The studies on skin and eye irritation yield semi-quantitative results expressed as scores derived from arbitrary scales related to the severity of the response. The primary use of the results is for classification and labelling, and for indicating safety precautions.

In considering the risk to humans posed by a substance when it is used as notified, the following are taken into account :

i) the severity of the response in the animal study;
ii) the exposure conditions in the study (use of occlusive patch or semi-occlusive dressing; whether rinsing was carried out, etc.) compared with the likely conditions of human exposure;
iii) the concentration of the substance and duration of contact, especially for consumer uses;
iv) the efficacy of good industrial hygiene practices for avoiding contact in an industrial situation.

4. Skin Sensitisation

Extrapolation to man of the results of a skin sensitisation study on animals is valid to a limited degree. One valid generalisation is that substances which are strong sensitisers in guinea pigs frequently cause sensitisation reactions in man, whereas weak sensitisers in guinea pigs are less likely to cause such reactions. If exposure of the skin has to be limited by taking suitable protective measures because of other effects, eg. strong irritation and/or corrosivity, the risk of skin sensitisation is automatically minimised.

5. Fertility and Teratology

When a chemical has shown no adverse effects on reproduction in the animal model(s), and the study or studies were conducted with a route and level of exposure relevant to that expected for humans, it can be assumed that the risk to humans is negligible.

If adverse effects on reproduction are observed, the relevance to man should be evaluated by taking into account the circumstances under which they occurred, the frequency and nature of the anomalies (target organs or systems), the NAEL and dose-effect relationship, and the number of species in which they were observed. When the expected level of human exposure is considerably below the animal NAEL, the risk is evaluated as being negligible. If, however, it is comparable to the NAEL, the slope of the dose-response curve and the nature and severity of the effects at doses above the NAEL will help to evaluate the risk. Evidence about the biological mechanism responsible for the development of the reproductive abnormalities may also be available, from special studies carried out at Levels 1 or 2, and will aid the evaluation.

6. Mutagenicity

Information from the mutagenicity studies should enable the presence or absence of mutagenic potential to be established. Chemically-induced mutations in in vivo or in vitro short-term tests, or tests which indicate interference with DNA, do not necessarily indicate a mutagenic hazard to humans. It is not possible to use information from mutagenic studies to make a quantitative evaluation of risk to humans at our present state of knowledge.

7. Carcinogenicity

For a detailed account of evaluating the risk from carcinogens see the ECETOC Monographs (ECETOC, 1980, 1982). In summary, the carcinogen is evaluated as being of high, medium or low potency, and by relating this to the exposure conditions the risk is evaluated in as quantitative a manner as possible. This process is limited by the fact that "in the case of carcinogens the expression of both dose and intensity, or incidence, is so complex as to preclude the calculation of a simple numerical index" (for potency) (ECETOC, 1982, p. 14).

Some indication of the presence or absence of carcinogenic potential is derived from short-term tests on mutagenicity in the Base-set and Level 1. Firmer evidence is provided by the Level 2 carcinogenicity study. These results should be considered, together with all other relevant toxicological information available to aid in interpreting them, prior to evaluating the risk.

7.1 Indications from short-term tests (see Appendix 2).

The two tests in the Base-set may prove to be adequate for defining the overall result as a "confirmed negative". When such a result is obtained and there is, in addition, evidence which leads to a decision to omit the carcinogenicity study (Chapter E, section 1), the risk of a carcinogenic effect is assessed as negligible.

Positive findings in at least two standardised, short-term mutagenicity tests which have been verified as useful in screening for carcinogenic potential and have unrelated end-points, indicate that the substance is a "questionable human chemical carcinogen". For such chemicals more evidence, eg. from the carcinogenicity study, will become available and may allow their re-classification either as a "putative human chemical carcinogen" for which risk evaluation is possible, or as a "human chemical non-carcinogen" for which the risk of a carcinogenic effect is taken to be negligible.

The "test to verify carcinogenesis screening" which is obligatory at Level 1 provides an opportunity to complement the Base-set studies. If an appropriate system is chosen, an "unconfirmed result" in the Base set may become a "confirmed positive" or "confirmed negative", and, in addition, the presence or absence of genotoxic action may be established or confirmed.

If the overall result remains unconfirmed then expert judgement regarding its significance is required when it is used, with all the other factors available, as one element in the risk evaluation.

7.2. Evidence from a carcinogenicity study.

Positive evidence of carcinogenic potential from the carcinogenicity study will indicate that a substance is a "Putative human chemical carcinogen" provided that the study was carried out with "adequate

animal experimentation under exposure conditions which correspond with those in man, on where the relevance of the exposure conditions can be deduced" (Appendix 2). In this case it is necessary to quantify as far as is possible the carcinogenic potency of the substance. By a consideration of all available data, carcinogens can be categorised with reasonable confidence according to potency in the species tested, although in the present state of knowledge they can be put only into broad classes of high, medium and low potency.

The following are some of the factors from the data in the animal study which would lead experts to judge that a putative human chemical carcinogen was of high carcinogenic potency :

i) a large increase in the incidence of malignant neoplasms at low exposure levels;
ii) a large number of malignant neoplasms per individual;
iii) a short latency period;
iv) development of malignant neoplasms after a single dose or few doses;
v) induction of malignant neoplasms in a variety of organs;
vi) induction of malignant neoplasms in organs with a low natural incidence of neoplasms;
vii) induction of a high incidence of malignant neoplasms in a number of strains and species;
viii) ancillary information on mode of action, metabolism and tissue dose, when available;

Conversely, the following are some of the factors which may lead to categorising a putative human chemical carcinogen as one of low carcinogenic potency :

i) a small increase in the incidence of malignant neoplasms;
ii) a long latency period;
iii) the induction of malignant neoplasms only of a type with high and variable natural incidence;
iv) the induction of malignant neoplasms only at excessive exposure levels;
v) the absence of carcinogenic activity in a number of species;
vi) ancillary information on mode of action, metabolism and tissue dose, when available.

The mode of action of a carcinogen is an important consideration for risk evaluation. "Certain chemical carcinogens have the ability to induce self-replication errors in the genetic material and it is assumed that this is a mechanism by which they induce cancer. Such chemicals are said to have genotoxic activity. There is now a large body of evidence which supports the view that malignant neoplasms may be produced by chemicals which do not induce self-replicating errors in the genetic material. Such chemicals are said to have non-genotoxic activity" (ECETOC, 1982, p.17). For chemicals acting by non-genotoxic mechanisms it is often possible to identify convincing reasons why there should be no appreciable risk under conditions of low exposure.

7.3. Final evaluation of risk.

The factors related to exposure are analysed so that the significant sources of exposure are identified and it can be estimated quantitatively. The following need to be considered :

- the intrinsic properties of the substance;
- factors related to its production, use and disposal;
- factors related to personnel.

In the case of occupational exposure these factors have been considered in more detail by ECETOC (1982, pp.22-23 and Appendix 2, reproduced as Appendix 3 in this document). They can be readily adapted to cover user and disposal situations.

When the likely potency of the carcinogen to man and the expected exposure of humans have been assessed, a judgement is made as to whether there is likely to be a risk of excess cancers in the exposed population under the conditions of exposure. This judgement can be made only by experts.

G. BIBLIOGRAPHY

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H. APPENDICES

Appendix 1: Annexes VII and VIII, 6th Amendment.

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.

— 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 Daphnia magna (21 days, this 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; this 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. Poecilia reticulata).

— Prolonged biodegradation study, if sufficient (biodegradation 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 physicochemical 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 greater than 100.

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

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

Appendix 2

CONCLUSIONS BASED ON SHORT-TERM TESTS FOR CARCINOGENICITY WHEN PART OF A REGULATORY BASE SET

Most toxicological information is quantitative, and the majority of the information in, for example, the EEC proposed toxicological base-set is no exception. Thus, for example, the reporting of acute oral or dermal toxicity is given as an LD_{50} expressed as a numerical value of dose per unit body weight. One notable exception to this rule is short-term testing for mutagenicity (see Appendix A) or carcinogenicity. There are in fact very good scientific reasons, e.g. quantitative and qualitative differences in metabolism, for not using data from short-term tests for a numerical quantification of mutagenic and/or carcinogenic risk in man. Such data are relevant to assessing carcinogenic potential.

The consequence is that the qualitative nature of the result (either positive or negative) puts particular emphasis on its accuracy. It is not possible to calculate the probability of error of a result expressed only as «positive» or «negative». This emphasises the need for a high degree of certainty that the qualitative result is correct, so that subsequent decisions are made on a sound footing.

In considering what decisions should be taken on the basis of a positive or a negative short-term test, the first and most important step is to establish that the result is «confirmed». There are several conditions which must be met before a result can be considered confirmed, and one of the most important is that the result from a second short-term test should be in agreement (see below). Carcinogenic or non-carcinogenic activity is suggested only when this and the other conditions are met.

Special problems, which are not yet resolved, are presented by mixtures and thus results from the testing of mixtures should be regarded with caution.

2.1. Requirements for a «confirmed» result

For the result of testing a chemical in short-term tests to be considered «confirmed», it must meet the following criteria :

2.1.1. The result should be derived from a test carried out to a protocol meeting minimum criteria (i.e. the protocol should be supported by a formal validation study) or the «chemical class control pairs» should have performed as expected in the same experiment. (A «chemical class control pair» is defined as a pair of chemicals, both structurally-related to the chemical under test, one of which is carcinogenic and the other non-carcinogenic).

2.1.2. The result should be the same in two test systems with unrelated end-points.

2.1.3. The result should be consistent with the experimental design, e.g. there should be a clear dose-response relationship ; where no increase in colonies is seen in a bacterial mutation test, it should be established that this is not due to high toxicity ; in a bacterial mutation test, an increase in colony counts should be confirmed by replicate plating

2.1.4. The positive controls used to check the reliability of the test should be used in parallel with each experiment.

2.1.5. The result should be reproducible if performed at different times in separate laboratories.

2.1.6. An assessment should be made of the likely contribution of impurities to the test result. Impurities which are strongly positive in the test system or highly toxic to the test organism can lead to incorrect results.

2.2. An unconfirmed result

If any one of the above criteria is not met, the result becomes an unconfirmed result.

2.3. Consequences of a confirmed positive result

Since there are sufficient controls and checks in the experiments leading to a confirmed result, it has certain characteristics, namely it is reproducible ; it is consistent with the known response of the tests to that chemical class ; each test is shown to have been performing accurately at the time of the experiment ; the chemical itself is responsible for the result ; and the result is the same in two tests. These controls and checks provide a result which is the best possible indication of carcinogenicity short of actually carrying out an animal carcinogenicity study, although it falls short of proof of carcinogenicity. If any one of the criteria for regarding a result as confirmed is not met, it should be considered an unconfirmed result.

2.4. Consequences of a confirmed negative result

In the absence of other relevant toxicological data, a confirmed negative result from short-term tests is a good indication of non-carcinogenicity. However, other considerations, eg. the size of the exposed population and the likely dose absorbed, may make it prudent to carry out additional testing in whole animal systems.

2.5. Consequences of an unconfirmed result

There are many occasions when the result is unconfirmed eg. if it is positive in only one test, or if the results are not fully reproducible. An unconfirmed result is a temporary situation and generally needs further studies in order to confirm the positive result or to obtain a confirmed negative result. If the result remains unconfirmed, then expert judgement regarding the significance of the unconfirmed result is required when it is used, with all the other factors available, as one element in the process of the risk assessment. Whatever decision is taken, it should be reviewed when new information becomes available and the result becomes confirmed.

The data produced by short-term tests can be considered as part of the process of identifying carcinogenic potential. Other points (including chemical and toxicological properties and exposure conditions), have to be taken into account for risk assessment and, thus, the decisions taken must be based on a consideration of all the data for each chemical and situation.

Appendix 3

DETAILS OF FACTORS INFLUENCING EXPOSURE

1. Factors Related to Intrinsic Properties of the Material

1.1. Physical and chemical properties and physical form :

- — gas
- — liquid (boiling-point, vapour pressure)
- — solid (wet or dry, particle size)
- — solubility
- — reactivity (possibility of neutralisation, etc)
- — ease of removal (in case of spill, escape)

1.2. Toxicological properties which themselves lead to a limitation of exposure, for example irritancy, corrosivity, etc.

1.3. Detectability

- — odour
- — availability of analytical methods for determining exposure level
- — availability of analytical methods for detection in effluents, exhaust air, reaction mixtures, residues, etc
- — availability of monitoring methods

2. Factors Related to the Process

2.1. Status of material under consideration in the process :

- — starting material (reactant)
- — major intermediate (isolated / not isolated)
- — minor or unstable intermediate
- — desired product
- — impurity
- — solvent or other auxiliary substance

2.2. Type of process :

- — batch
- — continuous

2.3. Size of process :

- — gross material turnover per year
- — batch size
- — number of batches per year

2.4. Processing system :

- — open / closed equipment
- — open-air or enclosed plant

2. 5. Sources of workplace contamination (exposure) in the processing system :

- — charging of materials
- — ventilation (filters, washers, cyclones, etc)
- — effluents
- — leaks (especially gases and liquids)
- — spillage (especially dusts, and splashing of liquids)
- — maintenance and repair operations
- — going on- and off-stream

2. 6. Sources of workplace contamination (exposure) in ancillary systems :

2. 6. 1. Transport

- — bulk material (especially liquids and gases)
- — packed material
- — loss of containment due to road or rail accidents
- — loss of containment due to breakage or perforation of individual containers
- — effect of small leaks (especially from punctured bags or leaking flanges)
- — means of decontamination and cleaning of vehicles and spilling areas

2. 6. 2. Storage

- — above, at or below atmospheric pressure
- — warehouse or open-air storage
- — suitability of storage area (ventilation, size, height, possibility of cross-contamination, etc)
- — fire and explosion hazards
- — handling during storage (loading, unloading, repacking, etc)

2. 6. 3. Disposal

- — residues from technological processes to be considered with respect to quantity, frequency and physical form (liquids, solids, tars, etc)
- — other materials to be disposed of e.g. packaging materials, cleaning materials, disposable protective equipment, equipment to be scrapped
- — way of disposal, e.g. burning (type, suitability and location of incinerator) ; depositing (controlled / uncontrolled, bulk / packed, above-ground / underground, geological and atmospheric conditions, etc) ; others (regeneration, recycling, shredding, etc).

3. Factors Related to Personnel

3.1. Personal protective equipment (to be assessed for suitability, duration of use, cleaning methods, etc) :

- protective clothing (full or partial protection)
- goggles / face shields
- respirators / gas masks
- separate air supply

3.2. Hygiene measures :

- availability of working overalls and underwear (number ; disposable or to be washed)
- cleaning of clothes (who, where)
- frequency and obligation of changes
- availability and use showers / baths
- type and suitability of cloak-rooms
- eating, drinking, smoking in working-area
- ease, frequency and methods of cleaning working area
- possibilities of spreading contaminating material outside working area (by persons)
- protection of outsiders coming into working area (restricted areas)

3.3. Training, instruction :

- extent, adequacy
- effect, identification
- adherence

I. AUTHORS OF REPORT (TASK FORCE : RISK EVALUATION - TOXICITY)

Dr. H.K. Leist (chairman)	HOECHST (Frankfurt)
Dr. G. Calvin	PROCTER AND GAMBLE (Brussels)
Dr. R. Millischer	ATOCHEM (Paris)
Dr. R.J. Staab	ESSOCHEM (Brussels)
Dr. C. Rhodes	ICI (Alderley Park)

J. ECETOC SCIENTIFIC COMMITTEE

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Responsible editor : L. Turner, ECETOC
Avenue Louise 250, Bte 63
B-1050 Brussels, Belgium.

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