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

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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