

# MANUAL FOR INVESTIGATION OF HPV CHEMICALS

## CHAPTER 4: INITIAL ASSESSMENT OF DATA

### 4.3 Guidance for the Initial Assessment of Health Effects<sup>1</sup>

#### 4.3.1 Introduction

This document provides guidance for the initial assessment of health effects of chemicals with a full SIDS. This document was first drafted based on relevant sections of the monographs of the International Programme on Chemical Safety (IPCS) (see list of references). These monographs can be consulted for information about making fuller assessments of chemical substances. The OECD Harmonised Integrated Hazard Classification System for Chemical Substances and Mixtures (OECD, 2001) and the Technical Guidance Document of the European Commission (2002) also provide detailed guidance on human health effects.

2. In the context of the refocused HPV Chemical Programme, the initial hazard assessment of a substance should be focused on hazard identification and dose (concentration) – response (effect) assessment:

- The aim of hazard identification is to identify the effects of concern.
- Dose (concentration) – response (effect) assessment is the estimation of the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect. At this step the no observed adverse effect level (NOAEL), or if this is not possible, the lowest observed adverse effect level (LOAEL), shall, where possible and appropriate, be determined for the observed effects. If appropriate, the shape of the dose-response curve should also be considered.

3. After adoption at SIAM, member countries can use this hazard assessment for hazard classification or for risk assessment purposes. For the dose (concentration) – response (effect) assessment, the focus should therefore be both on the derivation of NOAELs and LOAELs as well as on the description of the adverse effects and the magnitude of these effects as they are seen on exposure to a substance. Further information regarding the level of detailed description necessary for the purpose of classification can be found in OECD (2001).

4. It should be noted that this section deals with the assessment of all relevant available information. For requirements within the OECD HPV Chemicals Programme, chapter 2 of this manual should be consulted. It is assumed that for the initial assessment of human health effects, the SIDS requirements as described in chapter 2 are fulfilled. Nevertheless, clarifications regarding the requirements for genetic toxicity are provided in section 4.3.6.

5. Although the SIDS is the minimum requirement for making an initial assessment of High Production Volume (HPV) chemicals in the OECD Existing Chemicals Programme, for many chemicals currently under consideration there will be data already available in excess of SIDS; these should, of course, be assessed and taken into consideration when developing conclusions and recommendations. However, in making the initial assessment of health effects, the elements in the SIDS which are relevant in this respect are:

- Acute Toxicity;
- Repeated Dose Toxicity;

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<sup>1</sup> This document was prepared by the OECD Secretariat based on the agreements reached in the OECD Existing Chemicals Programme up to October 2003

- Genetic Toxicity; and
- Reproduction/Developmental Toxicity.

6. Furthermore, this document also gives guidance on the following non-SIDS endpoints, when test results are available:

- Irritation to skin and eyes;
- Sensitisation.

7. In the traditional assessment of repeated dose toxicity and reproduction/developmental toxicity, Uncertainty Factors (UFs) are used and the Estimated Level of Low Concern (EDLC) is calculated from the No-Observed-Adverse-Effect level (NOAEL) or, when not available, the Lowest Observed-Adverse-Effect level (LOAEL) derived from animal test results. In the context of the refocused OECD HPV Chemicals Programme, the derivation of EDLCs and hence the use of UFs are not necessary.

#### **4.3.2 Acute Toxicity**

8. In the assessment of the toxicity of a chemical, the determination of the acute toxicity is often the first step. Generally the objectives of investigating the acute toxicity are to find out (EC, 2002):

- in studies in animals an estimate of lethal dose levels and/or the dose levels that produce evident toxicity; and/or
- what toxic effects are induced following a single exposure to a substance by the oral, dermal or inhalation route, their time of onset, duration and severity (all to be related to dose); and
- when available, the slope of the dose-response curve; and
- when available, whether there are marked sex differences in response; and
- to obtain information necessary for the classification and labeling of the substance for acute toxicity.

9. In the initial assessment of SIDS chemicals, data on acute toxicity will usually not lead to recommending action for follow-up testing, although exceptional findings (high lethality, neurotoxicity seen at low doses, etc.) may warrant such action.

#### **4.3.3 Irritation**

10. No testing for skin or eye irritation is required under the OECD HPV Chemicals Programme. Available test results should nevertheless be described and assessed. The general objectives are to find out:

- whether the substance is, or is likely to be, corrosive;
- whether, in studies in animals or *in vitro*, there is evidence of significant skin, eye or respiratory irritation;
- whether there are indications from human experience with the substance of skin, eye mucous membrane or respiratory irritation following exposure to the substance;
- the time of onset and the extent and severity of the responses and information on reversibility.

11. If results from *in vivo* animal studies are available, for adequate hazard identification, information on the local responses (erythema and/or oedema for skin; corneal opacity, iridal effects, conjunctival redness and/or swelling for the eye) following application of a single defined amount of the substance should be reported. The local responses are evaluated and graded for each exposed animal at specified intervals after application of the test substance. Information should also be reported on the time fully to establish reversibility (or on the lack of reversibility), on any other local effects (e.g. pain, ocular discharge, necrosis, irreversible coloration of eyes) or any other toxic effects. Appropriate details for interpretation can be found in OECD Test Guidelines 404 and 405.

#### 4.3.4 Sensitisation

12. No testing for sensitisation is required under the OECD HPV Chemicals Programme. Available test results should nevertheless be described and assessed.

13. When evaluating human data, attention should be paid to:

- the number of well-documented cases in relation to the size of the exposed population;
- the relevance of any described cases and the association between clinical symptoms and clinical test results and exposure;
- the type of exposure (including: adequate substance identification, frequency, duration and magnitude of exposure, the physical state of the substance and exposure to other structurally-related substances). Data from subjects where exposure was not to intact skin or from subjects with pre-existing asthma should be interpreted with caution;
- the quality of the epidemiological data.

14. Particular points to take into account when evaluating results from assays to predict skin sensitisation include:

- the choice of vehicle;
- whether skin irritation is observed at the induction phase of guinea pig tests;
- whether the maximal non-irritating concentration is used at the challenge phase of guinea pig tests
- whether there are signs of systemic toxicity
- dose response and statistical analysis in case a Local Lymph Node Assay (LLNA, OECD TG 429) was performed
- effects observed in a control group exposed to a known sensitiser.

15. Assessment of cutaneous reactions at the challenge phase of guinea pig tests should be conducted carefully to discriminate irritation from sensitisation. Further guidance can be found in EC (2002).

#### 4.3.5 Repeated Dose Toxicity

16. The primary objective of the assessment of the repeated dose toxicity is the identification and description of the adverse effects and their severity, including dose-response characteristics that may be associated with the chemical being reviewed. Furthermore another objective of any repeated dose study with a duration of administration of normally at least 28-days is to obtain a value for the No-Observed-Adverse-Effect level (NOAEL), or the Lowest Observed-Adverse-Effect level (LOAEL). The NOAEL is considered to be the highest daily dose or concentration of a substance at which there is no adverse alteration observed in the morphology, functional capacity, growth, development, etc. of the target. The LOAEL, on the other hand, is considered to be the lowest daily dose or concentration of a substance at which any of these adverse alterations is actually observed. In general, greater confidence for assessing the hazards of a substance is placed in a NOAEL than in a LOAEL; in a NOAEL obtained from a sub-chronic study rather than one from a sub-acute study; in a test which demonstrates a clear dose-response relationship; and in a test in which the manifestations of toxicity are well-defined. In principle, a NOAEL should be obtained in each repeated dose study and can be used to derive a standard considered to represent a level of exposure or dose at which it is believed there is little if any likelihood of adverse effects in humans. However, when a reliable dose-response relationship is obtained, and a NOAEL cannot be estimated, a LOAEL could be used if the fact that the LOAEL is being used is clearly stated and consideration is being given to the slope of the dose-response curve.

17. As an alternative to this "classical" NOAEL approach, where feasible the so-called "bench-mark dose" approach could also be adopted. However, as this latter system uses the lower confidence limit of the dose corresponding to the lowest increase judged to be toxicologically significant in the incidence of an effect, and calculated on the basis of at least two dose levels showing an effect, it is anticipated that the number of repeated dose studies where adequate quantal or continuous information is available will be limited. For more guidance on the "bench-mark dose", see US-EPA (1995) and Slob & Pieters (1998).

18. Crucial in the dose-response assessment, is the definition of "adverse effects". In repeated dose toxicity testing, the values of selected parameters are compared to the average values in untreated concurrent control animals. Adverse effects cannot be defined in purely statistical terms as significant changes relative to control values. A judgement regarding biological significance is necessary. What is considered to be an adverse effect is dependent on expert judgement. In those cases where an adverse effect is observed in, for example, a parameter which monitors an organ system, such as a clinical biochemical change in a measurement of liver function, more weight can be attributed to its significance if other observations for that organ system, such as necropsy findings and to a lesser extent organ weight difference, also indicate an adverse effect. In addition, the dose response of an adverse effect, i.e. the progression of a change in an organ system with the dose, is a factor which adds weight to the significance of the effect. It should be kept in mind that some of the tests approved for fulfilling SIDS elements [e.g. according to TG 421, 422] are screening tests. It may therefore be that only one higher dose provides data that suggests an adverse effect. Under such circumstances careful professional judgement is required to determine if such an effect is probable in the absence of dose-response or even statistical significance. In study designs where the data are sufficiently robust, other aspects to be considered include reversibility of the toxicity, severity of the effect, latency of the onset of the effect and the shape of the dose-response curve. Correlations observed between changes in several parameters, e.g. between clinical or biochemical and (histo)pathological effects, will be helpful in the evaluation of the adversity of effects.

19. The decision as to whether or not a local effect should be considered as a substance-related adverse effect or caused by treatment procedures (e.g. adverse effects in the upper gastro-intestinal tract, mediastinum and lungs following bolus application in oral gavage studies), should be based on expert judgement. If local effects are clearly identified after repeated dosing, a NOAEL or LOAEL should be established for these effects in addition to N(L)OAELs for systemic effects.

20. More guidance on the analysis and evaluation of repeat-dose toxicity studies can be found in OECD (2000).

#### **4.3.6 Genetic Toxicity**

21. Testing for genetic toxicity is conducted so that chemicals may be assessed for their potential to cause transmissible damage to the genetic material of somatic cells (with potential carcinogenic or other consequences) and germ cells (which may result in heritable damage to the offspring).

22. It is essential to differentiate between the *in vitro* tests which are primarily used to investigate intrinsic potential of chemicals to cause genetic damage and the *in vivo* tests which investigate if these intrinsic properties are expressed in whole animals.

23. For the initial assessment, results of at least two tests for genetic toxicity will generally be provided in the SIDS. These are expected to include results of an *in vitro* point mutation test and a test for structural chromosomal damage (either *in vitro* or *in vivo*). A wealth of genotoxicity data may nevertheless be available from studies conducted *in vitro* and/or *in vivo* but many of the tests may have been conducted using methods different from the standard OECD Test Guidelines. The validity and usefulness of each of the data sets to the overall assessment of genotoxicity should be individually assessed, taking account of

protocol design (including route of administration) and current expert views on the value of the test systems.

24. Evaluation of genotoxicity test data should be made with care, taking into account all available information. Particular points to take into account when evaluating "negative" test results include:

- the doses or concentrations of test substance used (were they high enough?);
- the volatility of the test substance (were concentrations maintained in tests conducted *in vitro*?);
- for studies *in vitro*, the possibility of metabolism not active in the system including those in extrahepatic organs;
- the bioavailability of the substance to the target organ;
- the reactivity of the substance (e.g. rate of hydrolysis, electrophilicity, presence or absence of structural alerts and other available indications related to potential mutagenic activity of the chemical structure);
- the response of the positive and negative controls (important to both *in vitro* and *in vivo* assays).

25. Contradictory results between different test systems should be evaluated with respect to their individual significance. Examples of points to be considered are as follows.

- Conflicting results obtained in non-mammalian systems and in mammalian cell tests may be addressed by considering possible differences in metabolism or in the organisation of genetic material. Additional information may be needed to resolve contradictions.
- Positive results in the *in vitro* SCE assay should be viewed with caution, as this assay is associated with a relatively high incidence of false positive results. Thus, a positive result in this assay would not be considered to be evidence of a significant clastogenic potential *in vitro* if negative results were available in an *in vitro* chromosome aberration assay.
- Similarly, interpretation of results from DNA binding assays should be viewed with caution as these assays are only considered to be indicators of DNA damage. Consequently, the observance of *in vivo* DNA adducts alone in the absence of positive findings from *in vitro* assays is generally not considered sufficient evidence of a significant genotoxic potential *in vivo*."
- If contradictory findings are obtained *in vitro* and *in vivo*, in general, the results of *in vivo* tests indicate a higher degree of reliability. However, for evaluation of "negative" results *in vivo*, it should be considered whether there is adequate evidence of target tissue exposure.

26. The consequences of "positive" findings only at highly toxic/cytotoxic concentrations, and the presence or absence of a dose-response relationship should be considered. The default assumption for genotoxic chemicals, in the absence of mechanistic evidence to the contrary, is that they have a linear dose-response relationship. However, both direct and indirect mechanisms of genotoxicity can be non-linear or threshold, and sometimes this default assumption may be inappropriate. When interpreting positive results, considerations of the dose-response relationship and of possible mechanisms of action are important components of a hazard assessment. Examples of mechanisms of genotoxicity that may be demonstrated to lead to non-linear or threshold dose-response relationships include extremes of pH, ionic strength and osmolarity, inhibition of DNA synthesis, alterations in DNA repair, overloading of defence mechanisms (anti-oxidants or metal homeostasis), interaction with microtubule assembly leading to aneuploidy, topoisomerase inhibition, high cytotoxicity, metabolic overload and physiological perturbations (e.g. induction of erythropoiesis).

*Further work in the OECD HPV Chemicals Programme*

NOTE: While considering whether further work is necessary, it is assumed that all relevant available information, including information on analogues and/or structure-activity relationships have been assessed and taken into account. It should also be kept in mind that the guidance below is specific

to the OECD HPV Chemicals Programme, and that further work might be applicable in national/regional review programmes.

27. In general, substances for which only the SIDS elements are available and for which both the point mutation test and the chromosomal aberration test are negative can be considered as non-genotoxic.

28. Substances, for which positive *in vivo* test results are available, are usually considered to be of concern. Proposals for post-SIDS work should only be considered where there is a real and demonstrable need, as the *in vivo* test would normally be considered to be sufficient to regard a substance as genotoxic.

29. If only *in vitro* test results are available and one of the two *in vitro* tests is positive, further work is usually necessary within the SIDS context:

- When the mammalian cell test *in vitro* is negative, it will be necessary to decide whether further work is needed at this stage on a case-by-case basis. Further testing could be either *in vitro* or *in vivo*. Suspicion that a positive response observed in the bacterial test was due to a specific bacterial metabolism of the test substance could be explored further by investigation *in vitro*. Alternatively, an *in vivo* test may be required.
- Following a positive result in an *in vitro* mammalian cell mutagenicity test, adequately conducted *in vivo* testing, such as the micronucleus test or bone marrow chromosomal aberration test is usually required to ascertain if this potential can be expressed *in vivo*. In exceptional cases, where it can be sufficiently deduced that a positive *in vitro* finding is not relevant for *in vivo* situations, *in vivo* testing will not be necessary.

For chemicals which are *in vitro* mutagens, and are handled and used as if they were *in vivo* mutagens (i.e. it can be shown that the exposure is reduced to the lowest level which is technically feasible), any further *in vivo* tests may be considered for post-SIDS assessment.

30. Before undertaking any *in vivo* testing, a review of the *in vitro* test results and all available information on the toxicokinetic and toxicodynamic profile of the test substance is needed, as well as consideration of available information about structure-activity relationships. A particular *in vivo* test should be conducted only when it can be reasonably expected from all the properties of the test substance and the proposed test protocol (using the most appropriate route of administration) that the specific target tissue will be adequately exposed to the test substance and/or its metabolites. If necessary, an investigation of toxicokinetics could be conducted before progressing to *in vivo* testing. If the *in vivo* test is negative, the need for further work could still be considered (such as testing in a second tissue to supplement a negative *in vivo* assay when positive results have been seen in an *in vitro* point mutation assay). In this regard, attention should be paid to the quality and relevance of all the available data, the adequacy of target tissue exposure and the potential for human exposure.

#### **4.3.7 Reproduction/Developmental Toxicity**

31. Reproduction toxicity represents any effect on fertility and reproduction that can adversely affect the continuation of the species. Developmental toxicity is any adverse effect induced during the developmental period, i.e. from conception through puberty. The major manifestations of developmental toxicity include death of the developing organism, structural abnormalities, altered growth and functional deficiencies. Developmental toxicity can be considered a component of reproductive toxicity, and sometimes it is difficult to distinguish between effects mediated through the parents versus direct interaction with developmental processes.

32. The organisation of the information for an assessment of reproduction and developmental toxicity is described in a number of OECD Test Guidelines related to these endpoints (TG 414, 415, 416) and the guidelines for the Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity

Screening Test (TG 422) and the Reproduction/Developmental Toxicity Screening Test (TG 421). For example, toxic response data should be considered by sex and dose and, when possible, be sub-divided into reproductive and developmental effects. Reproduction effects would include, *inter alia*, altered fertility indices for males and females, effects on mating performance or other factors affecting reproductive function and, when related to the nursing capacity of the females, postnatal viability indices for the offspring or other postnatal signs of toxicity. Developmental effects, either as a consequence of maternal toxicity or as a direct effect on the developing organism, would include, *inter alia*, decreased numbers and percentages of live offspring per litter, and increased numbers and percentage of affected offspring (male, female or combined) per litter. Data on maternal toxicity and on certain metabolic or kinetic observations need to be considered when determining the nature, severity and relevance of developmental toxicity.

33. Reproductive and developmental effects exhibit dose-response relationships, and where these effects are not genotoxic (e.g. heritable) thresholds are generally assumed to exist. It is thus possible to estimate exposure levels unlikely to produce effects in humans on the basis of a NOAEL obtained in an animal experiment, in a similar manner to that for repeated dose toxicity.

34. The occurrence of a dose level producing well defined toxicity is considered of crucial importance in reproductive toxicity studies. This is called for in the OECD Test Guidelines for both screening tests, 421 and 422. Tests in which toxicity is not observed should, therefore, not be considered as adequate tests unless the limit concentration of 1000 mg/kg bw/d or a higher dose level (when relevant) has been included.

35. In addition, useful information can be derived from the repeated dose toxicity study, e.g. pathology in the reproductive organs, if specific histological examination has been carried out and a comparison of dose-response curves for such an effect between males and females could be made both in the repeated dose toxicity and the reproduction toxicity study.

36. To satisfy the SIDS requirements for reproductive toxicity, information (e.g. test data from studies in animals) is required which addresses both reproductive parameters (including fertility) and developmental toxicity. Examples of acceptable information are provided below:

- Requirements are met if existing data on the chemical include a developmental toxicity study and a 90-day (or longer) repeated dose study that sufficiently documents that reproductive organs were examined histologically and indicate no effects. If results from a developmental toxicity study are not available then such a study is required (e.g. OECD Test Guideline 414).
- When either a  $\geq 90$ -day (with no evaluation of reproductive organs) or a 28-day repeated dose study is the only repeated dose study available, it is recommended that at least a reproduction/developmental toxicity screening test (e.g. OECD Test Guideline 421) be carried out, in order to satisfy the requirements for the reproductive/ developmental toxicity endpoint.
- When a repeated dose toxicity test of 28-days or longer is not available, then a combined repeated dose toxicity test with a reproductive/developmental screening test (e.g. OECD Test Guideline 422) can be carried out to satisfy the requirements for repeated dose and reproductive/developmental toxicity. (This option uses the lowest number of test animals to satisfy both the repeated dose and the reproduction toxicity requirements.)
- If reliable tests results from well performed tests according to OECD Test Guidelines 415 or 416 (one or two generation reproductive toxicity) are available, the SIDS requirements for reproductive/developmental toxicity are met.

37. Data from animal studies ideally should provide clear evidence of specific reproductive toxicity in the absence of other, systemic, toxic effects. However, if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of the generalised adverse effects should be assessed to the fullest extent possible. The preferred approach is to consider adverse effects in the embryo/foetus first, and then evaluate maternal toxicity, along with any other factors which are likely to have influenced these effects, as part of the weight of evidence. In general, developmental effects that are observed at maternal toxic doses should not be automatically discounted. Discounting developmental effects that are observed at maternal toxic doses can only be done on a case-by-case basis.

38. If appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. Generally, the presence of maternal toxicity can not be used to negate findings of embryo/foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects. This is especially the case when the effects in the offspring are significant, e.g. irreversible effects such as structural malformations. In some situations it is reasonable to assume that reproductive toxicity is due to a secondary consequence of maternal toxicity and discount the effects, for example if the chemical is so toxic that dams fail to thrive and there is severe inanition; they are incapable of nursing pups; or they are prostrate or dying. Further guidance on the interpretation of developmental vs. maternal toxicity is described in OECD (2001).

#### **4.3.8 Other human health endpoints**

##### **Carcinogenicity**

39. No detailed guidance on the assessment of carcinogenicity is provided in this document. Carcinogenicity is not a SIDS element, and information is rarely available for substances assessed under the OECD HPV Chemicals Programme. If information on the potential carcinogenicity is available for a substance, it should be described and assessed in the SIDS Documents in the same way as information on a SIDS element. Guidance on the analysis and evaluation of carcinogenicity studies can be found in OECD (2002). A conceptual framework for evaluating mode of action has recently been developed by the Harmonisation Group within IPCS and was recently published (Sonich-Mullin et al., 2001). These harmonised approaches should be taken into account when assessing existing tests results on carcinogenicity in the SIAR. If internationally agreed assessments are available (e.g. by IARC), the conclusions of those assessments should be reflected in the SIAR.

##### **Neurotoxicity**

40. No detailed guidance on the assessment of neurotoxicity is provided in this document. Neurotoxicity is not a SIDS element, and information is rarely available for substances assessed under the OECD HPV Chemicals Programme. If information on the potential neurotoxicity is available for a substance, it should be described and assessed in the SIDS Documents in the same way as information on a SIDS element. Guidance on the assessment of neurotoxicity studies can be found in IPCS (2001a).

## References

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