Determining the Adequacy of Existing Data

1.0 Introduction

The EPA's High Production Volume (HPV) Challenge Program ("Challenge Program") is an important and necessary step towards gathering basic hazard information on those chemicals manufactured at high volumes throughout the United States, and making this information publicly available. This program, described in detail on the EPA website *(http://www.epa.gov/chemrtk/volchall.htm),* follows the example of the Organization for Economic Cooperation and Development's (OECD) program known as the Screening Information Data Set (SIDS) program to initially characterize the hazard of HPV chemicals by means of a minimum data set.

The Challenge Program is similar in substance but distinct in process from the OECD SIDS program. However, the Challenge Program will benefit from the experience and judgment of the OECD SIDS process and can serve as a source for US contributions to the OECD effort.

This document provides guidance for determining whether existing published or unpublished data meet a minimum standard of acceptability for the purposes of the Challenge Program.

2.0 Purpose

There are approximately 2,800 chemicals[1] on the current EPA list of HPV chemicals (available at: *http://www.epa.gov/chemrtk/volchall.htm*). The Challenge Program is intended to develop a minimum set of hazard information on each of these chemicals.
This hazard information can either be existing data or data that need to be generated. An evaluation of the existing data on each chemical is necessary to determine whether additional testing is required.

The purpose of this document is to provide basic guidance for accepting or rejecting data used to describe the basic hazard of a chemical. The guidance is offered primarily for the entity submitting the data ("sponsor"), but will also be useful to EPA and other reviewers. Adherence to this guidance will facilitate international acceptance under the OECD SIDS program.

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[1] This figure represents the HPVs subject to the Challenge Program. The total number of HPVs, approximately 3,000 chemicals, has been pared down by eliminating polymers, inorganics, and chemicals already in the OECD SIDS program.

3.0 The SIDS as Screening Tool

Appendix A contains a list of the endpoints included in the SIDS. *The SIDS represents a minimum data set and thus should only be used for initial or screening level hazard assessments.* SIDS information may be used to make judgments on potential hazard and to set priorities for further work in order to refine those judgments.

For example, the physicochemical data provide information on the physical form and likely environmental medium in which the chemical will reside. This is important in order to determine the likely routes of exposure of humans and environmental species. The environmental fate and pathway data are important for trying to predict where a chemical may ultimately reside and how long it might persist. Ecotoxicity and health effects data are critical elements of hazard assessments and are important for guiding further testing to refine endpoints and dose-response relationships that might be useful in future assessment activities.

Each endpoint in Appendix A is an important piece of information on the potential hazard of a chemical. Ideally, tests to gather such information have been conducted under optimum conditions and follow good laboratory practice (GLP). However, for the purpose of satisfying a specific SIDS data element[2], it is important to consider existing information that might not have been generated under ideal conditions.

4.0 Tiered Approach to Evaluation of Sponsor Data Package

The Challenge Program will involve the gathering and evaluation of available data and submission of a proposed testing plan by an HPV chemical sponsor to fulfill the SIDS. Again, the purpose of the Challenge Program is to provide screening-level hazard information on all HPV chemicals manufactured or imported into the U.S. Therefore, it is not important whether this information comes from existing data or newly conducted studies - as long as the information is judged adequate and is available for public review.

EPA proposes a two-tiered system to evaluate existing data. In Tier I, criteria will be used to assess overall scientific integrity of the information. Any data or information which do not meet the Tier I criteria will be rejected from further consideration in the Challenge Program. In Tier II, a more rigorous evaluation of existing data that has passed Tier I will occur (existing data generated via OECD or equivalent guidelines can enter directly into Tier II evaluation).

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[2] If one can arrive at a conclusion about a specific endpoint from an "old" study, or by inference from other information (weight of the evidence analysis), it might not be necessary to do further testing for that specific endpoint.

Other methods are available for assessing "data adequacy", and one in particular has been recently proposed for use in Europe in developing the International Uniform Chemical Information Database (IUCLID). Klimisch et al. (1997) describe the method and propose that data evaluation be done systematically and that it include consideration of reliability, relevance, and adequacy. Klimisch et al. define adequacy as "the usefulness of data for risk assessment purposes", whereas in this document EPA uses the term to mean usefulness for hazard identification purposes.

The method described in Klimisch et al. (1997) is similar in principle to EPA's tiered approach in that both methods present specific criteria for evaluating existing data. In fact, the data reliability criteria presented by Klimisch and by EPA (in Tier I) are remarkably similar. The difference between the two approaches is in how the criteria are used.

Klimisch et al. use their criteria in the following scoring system for evaluating data reliability[3]: 1 = reliable without restrictions; 2 = reliable with restrictions; 3 = not reliable; and 4 = not assignable. The Klimisch ranking system does not conflict with the EPA approach. Assigning a numerical value to each study is both useful and comprehensive; however, EPA believes using the same criteria as a screen (Tier I as described below) results in the appropriate "weeding out" of data/studies not useful in describing an endpoint. For example, studies assigned Klimisch reliability codes "3" or "4" would not advance to Tier II in the EPA approach, except for those cases in which a weight-of-the-evidence analysis might be used (see below).

5.0 Tier I

Tier I is a screening process designed to allow only potentially useful information to move to Tier II in the assessment of existing data for the purposes of the Challenge Program. Tier I assesses "experimental fitness"; thus, existing data rejected in Tier I for a particular endpoint may be identified as a data gap that needs to be filled by testing.

The 1997 SIDS Manual *(http://www.epa.gov/chemrtk/volchall.htm)* provides some guidance on how to assess existing data to determine whether additional testing is needed to fulfill one or more of the SIDS endpoints (Sections 3.4 and 3.5 of SIDS Manual, attached as Appendix B). Based on this information, along with EPA's experience in receiving and evaluating many different types of studies, the following general criteria have been developed for determining whether existing information is worthy of further scrutiny in Tier II:

[3] It should be noted that the Klimisch et al. scoring system is proposed for use with ecotoxicology and health effect studies and is not applicable to physicochemical and environmental fate studies.

Minimum scientific/experimental/reporting requirements. End products of the Challenge Program include a test plan[4] and a "robust summary [4]". For each endpoint for which testing is not proposed, robust summary(ies) must be prepared which include an objective, discussion of methods, and results and conclusions. The following minimum requirements need to be met by presenting the information in a robust summary, and if one or more are not met, those data will not advance to Tier II and that endpoint will be identified as a data gap that needs to be filled with testing under the Challenge Program :

5.1 Required for all SIDS endpoints:

A. Test Substance Identification. Adequate description of test substance, including chemical purity and identification/quantification of impurities to the extent available.

5.2 Required for Physicochemical endpoints:

A. Temperature (for vapor pressure, octanol/water partition coefficient, and water solubility values).

5.3 Required for Environmental Fate Endpoints:

A. Temperature. Must be recorded and reported for all endpoints/estimations.

B. Controls. Appropriate controls must be used and reported.

5.4 Required for Ecotoxicity and Human Health Effect Endpoints:

A. Number of Organisms. The number of organisms in each dose/concentration group must be reported. If appropriate (i.e., for mammalian studies but not ecotoxicity studies), the number per sex must be recorded.

B. Dose/Concentration Levels. The number and amount of each dose/concentration used in the experiment must be reported.

C. Route/Type of Exposure. The route/type of exposure (e.g., oral, inhalation, etc. for mammalian studies) or test system (static, flow-through, etc. for ecotoxicity) used must be reported.

D. Duration of Exposure. Duration of exposure must be reported. The time will change by endpoint/study type (see Appendix A).

E. Species. The species (and strain if appropriate) must be reported.

[4] Guidance on the content and format of both a test plan and a robust summary is presented in Appendix C.

F. Controls. All studies must have negative controls, and some studies (e.g., biodegradation, *Salmonella*/Ames assay) must also have positive controls. If a vehicle is used in administration of test agent, vehicle controls should be established and reported. Exceptions may be allowed for acute mammalian toxicity studies.

G. Statistical Analysis. Statistical analyses must be described and appropriate results presented with all tests, with some exceptions (e.g., the *Salmonella*/Ames assays).

The purpose of Tier I is to identify adequate studies early in the HPV Challenge Process so they may advance to Tier II. However, some studies that do not advance to Tier II may still be useful in a weight-of -the-evidence analysis. Whether used or not, it is prudent to make publicly available all the studies reviewed[5], possibly in the form of a bibliography.

6.0 Tier II

A review of the adequacy of data that have passed through Tier I requires a more rigorous analysis. Tier II analysis determines the pertinence of the data in describing the endpoint being measured or estimated. The SIDS endpoints listed in Appendix A can be grouped under four categories (physicochemical properties, environmental fate and pathways, ecotoxicity tests, and health effects tests). Each of the studies included in the SIDS is unique in what it assesses and in terms of cost, time, and sophistication; therefore, determination of data adequacy will be done at the individual study level on a case-by-case basis, although the presence of multiple studies on a given endpoint may be used in the overall judgment of hazard (as discussed later in this section).

Guidance is provided below for each of the SIDS study types/estimation methods on information that is necessary to give credence to a study. For the most part, the guidance presents criteria for accepting data generated under old or not widely used protocols, and is based on experience in the OECD SIDS program. For reference purposes, the applicable OECD guidelines are listed. The full text of these guidelines is available online by clicking on the "SIDS Test Guidelines" button at http://www.epa.gov/chemrtk/volchall. (NOTE: The reader is referred to each OECD guideline for additional, study-specific information on minimum requirements.)

The use of sound scientific judgment is the most important principle in determining whether data are adequate for a given requirement. For example, there may be several repeated dose studies available on a particular chemical, none of which would be acceptable due to some deficiency (i.e., low number of test animals/dose group, only one dose group in addition to

[5] See the EPA guidance document on literature search strategies for more information on this issue (under development).

control group, change in dose amount or frequency during the course of the study, etc.). Collectively, however, the different studies show effects in the same target organ at approximately the same dose and time. This could satisfy the repeated dose toxicity data element.

This is not an endorsement for initiating studies using other than currently approved OECD or equivalent test guidelines for submission under either the U.S. HPV Challenge Program or the OECD SIDS program. Rather, it is an attempt to use all available data for the purpose of hazard screening. All newly conducted testing shall be done using current OECD or equivalent test guidelines to ensure Mutual Acceptance of Data.

6.1 Physical/Chemical Property Tests[6]

Information under this category is important to describe the physical state and basic chemical properties of an HPV chemical. For all the physicochemical data discussed below, numerical values will usually be acceptable if taken from standard references (e.g., CRC Handbook of Chemistry).

Melting point (OECD Guideline 102). Melting points of less than 0°C do not need to be specified ("< 0°C" is sufficient).

Boiling point (OECD Guideline 103). Boiling points above 300°C do not need to be specified, but may be estimated from vapor pressure or boiling point under reduced pressure. (While this would be acceptable under the Challenge Program, in other programs EPA prefers that testing be done if estimated boiling points < 400°C.)

Vapor pressure (OECD Guideline 104). Calculations showing a value $<1 \times 10^{-5}$ KPa at 25^0C may be acceptable in lieu of measuring vapor pressure. (While this would be acceptable under the Challenge Program, in other programs EPA prefers that testing be done if estimated vapor pressures $> 1 \times 10^{-7}$ KPa.)

Octanol/water partition coefficient (OECD Guidelines 107 and 117). Calculated or estimated values are encouraged; however, if the estimated log K_{ow} is greater than 6, it may not be considered reliable.

Water solubility (OECD Guideline 105). Quantitative values are needed, however, no

[6] Additional information is available in Appendix B (Sections 3.4 and 3.5 of the OECD SIDS Manual).

testing is needed if water solubility values are ≤ 1 ug/L (1 ppb); qualitative descriptions (e.g., very soluble, insoluble) are not acceptable. For acids and bases, dissociation constants (pKa) and the conditions under which they were measured should be reported *(OECD Guideline 112)*.

6.2 Environmental Fate and Pathway Tests

After a substance is released to the environment its fate or behavior is determined by its intrinsic (physical/chemical) properties, reactivity (i.e., rates of transformation), and characteristics of the environment. Basic properties were addressed in the previous section. Data requirements in this section consist of rates or half-lives for the most important transformation (degradation) processes, which vary by compartment for air, water, soil and sediment; and the overall assessment of environmental behavior as a complex function of all these inputs. Multimedia models are most often used for the latter purpose.

Photodegradation (OECD Guideline 113). Estimation methods may be acceptable. (See Section 3.4 in Appendix B).

Stability in water (OECD Guideline 111). Estimation methods may be acceptable. (See Section 3.4 in Appendix B).

Biodegradation (OECD Guidelines 301a-f for ready biodegradability, 302a-c for inherent biodegradability). The following information would describe the results of an adequate biodegradability test:

- source and concentration of the microbial inoculum;
- pre-acclimation of the test chemical;
- initial concentration of test chemical;
- temperature of incubation;
- analytical method used to measure biodegradation;
- time required for 10% degradation; and
- total degradation at the end of the test.

Transport and distribution (See Section 3.4, Appendix B). Given the data collected on photodegradation, stability in water, and biodegradation, sponsors are encouraged to evaluate this information to assess environmental distribution and fate (e.g., half-life determination). The OECD program advocates the use of computer modeling techniques such as FUGMOD (a fugacity-based model) to estimate the partitioning and distribution of a chemical in the environment. EPA aggrees with this approach but suggests that a more recent model be used - the EQC model. The EQC model is described and reviewed in Mackay et al. (1996), and the authors have made the model available on the internet at

http://www.trentu.ca/envmodel.

While the OECD -- and by extension the HPV Challenge Program -- accepts Level I[7] fugacity modeling to estimate transport/distribution values, EPA believes that values based on a Level III fugacity model are more realistic and useful for estimating a chemical's fate in the environment on a regional basis and recommends their use.

6.3 Ecotoxicity Tests

Traditionally, SIDS ecotoxicity evaluations have focused on characterizing hazards in the aquatic environment. Toxicity to terrestrial organisms, while not part of the SIDS, may be required based on the physicochemical properties and environmental fate and pathway tests/estimations, and the potential use of the HPV chemical of concern (see Appendix B).

Acute toxicity to fish (OECD Guideline 203); Acute toxicity to aquatic invertebrates (OECD Guideline 202); and Acute toxicity to algae (OECD Guideline 201). The SIDS manual provides guidance on data adequacy for ecotoxicity tests (see Section 3.5 of the SIDS Manual in Appendix B). Included in this guidance are some additional details provided by both the EPA and the Netherlands. Since that time, EPA has published a more complete list of details that describe an adequate ecotoxicity study (Smrchek and Zeeman, 1998). Only those items not in the SIDS manual are presented below[8]:

- description of deviations from OECD guidelines;
- test substance (describe analytical procedures, any impurities, and water solubility);
- test procedures and conditions (standard/recognized procedures, acceptable test species, appropriate acclimation procedures followed, certain conditions noted (test temperature, dissolved oxygen levels, pH, lighting), and placement of test units to avoid position effects);
- test medium and dilution water (correctly made, specified hardness and salinity range, all contaminants reported, acceptable levels of particulates, total organic carbon, chemical oxygen demand, un-ionized ammonia, residual chlorine, pesticides, heavy metals, and PCBs);

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[7] The Levels correspond to the following conditions/assumptions: I = steady-state, equilibrium, closed system, and no degradation; II = steady-state, equilibrium, with degradation and advection; and III = steady-state, non-equilibrium, degradation and advection, and intermedia transfer (Mackay et al., 1996).

[8] Only those parameters relevant to acute toxicity tests are listed. The full list in Smrchek and Zeeman (1998) includes necessary details for subchronic and chronic studies that may be appropriate for some situations in the Challenge Program.

- test concentrations/dose levels (measured concentrations preferred over nominal concentrations, replication adequate, concentrations maintained during test);
- controls (number adequate, upper limit on mortality not exceeded, response acceptable);
- test endpoints and reported data (specified endpoints adequate, dose-response or concentration-response evident, data quality assured and good laboratory practices followed);
- statistical analyses (correct tests or procedures used (e.g., parametric or non-parametric) to determine the difference between treated and control groups; discuss normality of data, any variances, the minimal detected difference between treatments and controls, level of significance and power, and the precision and accuracy of the test data); and
- **S** results and conclusions (address any indirect effects (e.g., oxygen depletion or disease outbreak), results should be compatible with other data on same substance).

6.4 Health Effects Tests

The series of studies discussed below represent a variety of endpoints (general systemic toxicity under acute and repeated dose exposure scenarios, genetic toxicity, reproductive toxicity, and developmental toxicity) that are a starting point to identify the potential health hazards of a chemical.

In general, the following details should be used to describe an adequate health effects study: descriptions of test species, test concentrations and dose levels, test endpoints evaluated and reportable data/procedures. Some of these requirements are not relevant for some assays, e.g., genetic toxicology studies using cell cultures, in which case other information, such as culture conditions, should be included.

Acute toxicity. (OECD Guidelines 401-403,420, 423, and 425). The following endpoints are of interest in acute toxicity studies: lethality, tolerance, and qualitative information on potential target organs. Key parameters are the number of animals/dose (usually 5), the number of dose levels or concentrations (usually >2), and the number of days of observation (14) following the single dose (or multiple doses over a 24-hour period). If no mortality is observed at dose levels of 2000 mg/kg (oral and dermal) or 5 mg/L, or the maximum attainable or safe air concentration given the physicochemical properties (e.g., $\leq$ 50% of the lower flammability limit) of the test agent (inhalation), then no testing is required and the LD_{50}/LC_{50} is reported as greater than the dose level used.

In order to minimize the use of animals in lethality studies, estimation of lethality (an LD_{50} or an LC_{50}) from less than perfect existing data may be acceptable. For example, the use of 2-3 animals (same sex)/dose, or 7-14 days of observation may be acceptable assuming there are no residual health effects for several consecutive days prior to study termination. This information would be enhanced by supportive repeated dose toxicity data. On the other hand, if no data exist, the EPA recommends the recently adopted OECD Guideline 425 (oral toxicity, Up and Down Method), to minimize the number of animals needed to estimate lethality.

Genetic Toxicity (in vitro and in vivo). Genetic toxicity testing provides data useful in assessing the potential of chemicals to cause mutations, which are implicated in the induction of carcinogenicity, heritable mutagenicity, cellular aging, etc. The two major endpoints in genetic toxicity that must be addressed in the Challenge Program are gene mutation and chromosome aberration. This is achieved by testing under *in vitro* or *in vivo* conditions.

The OECD SIDS program recognizes a total of 11 different studies to assess these two major endpoints: three *in vitro* and three *in vivo* assays to assess mutations at the gene level and two *in vitro* and five *in vivo* assays to assess chromosomal aberrations[9] (two assays have the potential to detect both types of mutations). EPA's policy has been to require *in vivo* testing for chromosomal aberrations when there are no data available[10].

Key parameters for all genetic toxicology studies include the selection of dose levels, the number of cultures/dose or number of animals/dose (the latter usually 5), the number of doses (usually $\geq$ 3), the number and timing of harvest/sacrifice times, the presence of activation systems, and the route of administration.

Genetic toxicity (in vitro). (OECD Guidelines 471-473, 476, 479-482). Bacterial (*Salmonella typhimurium*, *Escherichia coli*) or non-bacterial (*Saccharomyces cerevisiae*, various mammalian cell lines, etc.) gene mutation tests, and mammalian cell chromosomal studies (many different cell lines). The following details would describe an adequate *in vitro* study[11]:

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[9] The OECD SIDS includes four more assays (three *in vitro* and one *in vivo*) that assess DNA effects.

[10] EPA places greater weight on results from *in vivo* tests than from *in vitro* tests in order to account for whole animal metabolism, DNA repair capabilities, and target organ specificities. Also, positive *in vitro* results without an indication of mutagenic activity *in vivo* may not provide a sufficient weight-of-the-evidence to justify additional testing (e.g., heritable mutagenicity or a cancer bioassay). The OECD also places greater weight on *in vivo* testing than on *in vitro* testing (OECD, 1998a). More details on this rationale have been published (USEPA, 1986; Dearfield et al. 1991; and Auletta et al. 1993).

[11] Some information taken from Klimisch et al. (1997).

- **S** the dose/concentration relative to the toxicity of the test material in the test system used;
- **S** information on volatility (if applicable);
- **S** information on confounding factors that need to be considered to interpret results (solubility, impurities, pH shifts, influence on osmolarity, etc.);
- **S** listing of appropriate negative and positive controls; and
- **S** conduct of the study both with and without mammalian metabolic activation.

Genetic toxicity (in vivo). (OECD Guidelines 474-475, 477-478, 483-86). A variety of acceptable test organisms for assessing gene mutations (fruit fly, mouse) or chromosomal aberrations (mouse, rat, hamster) exist. For these studies, adequate descriptions should contain all the general information discussed above for health effects tests, plus documentation of the use of positive/negative controls, and of the specific protocol followed for the listed endpoint (e.g., the number of cells counted in a mouse micronucleus test).

Repeated dose toxicity (OECD Guidelines 407, 410, 412, 422). The purpose of repeated dose studies is to provide information on general toxicity, including potential target organs, severity of effect, tolerance, and possible reversibility of effects. Although the OECD guidelines (Guidelines 407, 410, and 412) are for 28-day studies, EPA recognizes that adequately run 90-day studies would also be acceptable to meet the repeated dose SIDS endpoint. The following details would describe adequate repeated dose studies:

- **S** Body weight/body weight changes;
- **S** Food and water consumption;
- **S** General toxic response by sex and dose;
- **S** Nature, severity and duration of clinical observations (whether reversible or not);
- **S** Clinical biochemistry and hematological tests, both with relevant base-line values;
- **S** Time of death during the study or whether animals survived to termination;
- **S** Body weight and organ weights at sacrifice;
- **S** Necropsy findings; and
- **S** Detailed description of histopathological analysis.

Reproductive toxicity (OECD Guidelines 415, 416, 421, 422). Reproductive toxicity studies are designed to assess potential adverse effects on reproductive organs and function. The following details would describe adequate reproductive toxicity studies:

- **S** Effects on reproduction, fertility, gestation;

- Number of live births and post implantation losses; and
- Number of implantations, corpora lutea, litter size, and litter weights at time of recording.

The OECD SIDS program accepts an existing, adequate 90-day repeat dose study that "demonstrates no effects on reproductive organs, in particular the testes, then a developmental study (e.g., OECD Test Guideline 414) can be considered as an adequate test for information on reproduction/developmental effect" (SIDS Manual, Section 3.3, paragraph 13).

Developmental toxicity (OECD Guideline 414, 421, 422). Potential adverse effects on the developing fetus is the focus of developmental toxicity tests. The following details would describe adequate developmental toxicity studies:

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- Effects on offspring, postnatal growth (depending on protocol);
- Number of pups with grossly visible abnormalities;
- Number of implantations, corpora lutea (recommended), litter size, and litter weights at time of recording.

Integrating Repeat Dose, Reproductive, and Developmental Toxicity Endpoints. If a sponsor finds no adequate data available for their HPV chemical(s) under the SIDS endpoints assessing reproductive or developmental toxicity, then the current OECD options for meeting these endpoints should be considered. Table 2 lists the various study design scenarios acceptable to the OECD.

Extensive data beyond SIDS endpoints. Some HPV chemicals have been tested in a variety of studies that are more sophisticated (e.g., neurotoxicity, carcinogenicity, fish chronic toxicity test, etc.) Than the SIDS. In such cases, if a rationale can be presented to show that such non-SIDS tests adequately describe the SIDS endpoint of concern, a new test for that particular endpoint may not be necessary.

Table 2: Possible Study Designs to Meet Selected Mammalian Toxicity Endpoints			
Study Designs	General Toxicity	Reproductive Toxicity	Developmental Toxicity
28-day (such as OECD Guidelines 407, 410, or 412) or 90-day repeated dose studies	✔		
90-day repeated dose study with adequate histopathology and assessment of reproductive organs	✔	✔	
Combined repeated dose study and a developmental/ reproductive toxicity screen (OECD Guideline 422)	✔	✔	✔
Reproductive toxicity test (OECD Guideline 415 or 416)		✔	
Developmental toxicity test (OECD Guideline 414) (EPA prefers OPPTS Guideline Number 870.3700, available at http://www.epa.gov/docs /OPPTS_Harmonized)			✔
Reproductive/developmental toxicity screen test (OECD Guideline 421)		✔	✔

7.0 Additional Guidance on Difficult to Test Substances

EPA understands there may be some HPV chemicals on the 1990 IUR list that, for a number of reasons described below, are difficult to test for some, or all, SIDS endpoints. Nonetheless, basic hazard information on these chemicals needs to be made available through the Challenge Program. In such cases, EPA encourages sponsors to follow appropriate SIDS guidance, where available, and develop a rational test plan with the necessary alternative test battery. Some examples include:

Unstable chemicals. HPV chemicals shown to be unstable in abiotic or biotic systems may not be easily tested; however, known degradation products (if potentially present) should be appropriately characterized.

Explosive/flammability hazards. HPV chemicals with known explosive/flammable properties at ambient (or near ambient) conditions (temperature and pressure) may not need to be tested for certain SIDS endpoints because of the potential hazard to laboratory personnel. The EPA has a precedent of not requiring inhalation toxicity testing for chemicals at air concentrations $\geq$ 50% of the lower limit of explosivity or flammability.

EPA has issued additional HPV Challenge guidance documents on a variety of issues: (1) *Development of Chemical Categories for the HPV Challenge Program*; (2) *Guidance for "What to Test" in the HPV Challenge Program*; and (3) *Guidance for Testing Closed-System Intermediates for the HPV Challenge Program* (USEPA, 1999a-c).

In building the SIDS, the OECD has developed test guidelines for each SIDS endpoint. Many of these guidelines have been updated or changed over the years, and will continue to evolve as the state of the science in each field progresses. Other organizations have developed comprehensive experimental protocols for a variety of studies and for a variety of chemicals. EPA recognizes this and encourages HPV Challenge sponsors to use appropriate test guidelines for HPV chemicals presenting special circumstances. To help ensure that testing conducted using non-OECD guidelines will likely be acceptable for "Mutual Acceptance of Data" purposes, the sponsor is encouraged to contact EPA before commencing testing. Some examples and references include:

- American Society of Testing and Materials (ASTM) methods to measure water solubility and octanol/water partition coefficients for petroleum substances (http://www.astm.org);
- ISO/DIS Method 14593 to measure biodegradation of petroleum substances (http://www.iso.ch:8080ISOWeb.html); and
- possible use of proposed methods by (1) a recently formed ECETOC Task Force (Ruffli et al., 1998) and (2) a draft OECD guidance document (OECD 1998b), which both attempt to address the aquatic toxicity of difficult-to-test substances, such as sparingly soluble, volatile, and unstable substances.

10.0 References

Auletta, AE, KL Dearfield and MC Cimino. 1993. *Mutagenicity test schemes and guidelines: US EPA Office of Pollution Prevention and Toxics and Office of Pesticide Programs.* Environmental and Molecular Mutagenesis (21):38-45.

Dearfield, KL, AE Auletta, MC Cimino and MM Moore. 1991. *Considerations in the U.S. Environmental Protection Agency's testing approach for mutagenicity.* Mutation Research (258):259-283.

Klimisch, HJ, Andreae E, and Tillmann, U. 1997. *A systematic approach for evaluating the quality of experimental and ectoxicological data.* Regulatory Toxicology and Pharmacology 25:1-5.

Mackay, D, A DiGuardo, S Paterson and C Cowan. 1996. *Evaluating the environmental fate of a variety of types of chemicals using the EQC model.* Env. Tox. and Chem. (15)(9):1627-1637.

OECD. 1998a. Introduction to the OECD Guidelines on genetic toxicology testing and guidance on the selection and application of assays, *In,* **OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.**

OECD. 1998b. Guidance Document on Aquatic Toxicity Testing of Difficult Substances. Draft dated June, 1998.

Ruffli H, PR Fisk, AE Girling, JMH King, R Lange, X Lejeune, N Stelter, C Stevens, P Suteau, J Tapp, J thus, DJ Versteeg, and HJ Niessen. 1998. *Aquatic toxicity testing of sparingly soluble, volatile, and unstable substances and interpretation and use of data.* Ecoto. Env. Safety (39):72-77.

Smrchek, JC and MG Zeeman. 1998. Assessing risks to ecological systems from chemicals, Chapter 3 *In* Handbook of Environmental Risk Assessment and Management, edited by P. Calow. Blackwell Science,Ltd., Oxford, UK. Pages 24-90.

USEPA. 1986. Guidelines for mutagenicity risk assessment. Federal Register 51: 34006-34012.

USEPA 1999a. *Development of Chemical Categories in the HPV Challenge Program* (click on appropriate button at http://www.epa.gov/chemrtk/volchall)

USEPA 1999b. *Guidance for "What to Test" in the HPV Challenge Program* (under development)

USEPA 1999c. *Guidance for Testing Closed System Intermediates in the HPV Challenge Program* (under development)

APPENDIX A

SCREENING INFORMATION DATA SET (SIDS)

SIDS Endpoints		
SIDS Category	Test/Estimation Endpoint	OECD Guideline (or equivalent[1])
Chemical and Physical Properties	Melting point	OECD 102
	Boiling point	OECD 103
	Vapor pressure	OECD 104
	Partition coefficient (log K_{ow})	OECD 107, 117
	Water solubility	OECD 105, 112
Environmental Fate and Pathways	Photodegradation	OECD 113
	Stability in Water	OECD 111
	Biodegradation	OECD 301, 302
	Transport/Distribution	EQC Model[3]
Ecotoxicity Tests	Acute toxicity to fish	OECD 203
	Acute toxicity to aquatic invertebrates	OECD 202[2]
	Toxicity to aquatic plants	OECD 201[2]
	Chronic aquatic invertebrate test (When appropriate)	OECD 211[2]
	Terrestrial toxicity test (When appropriate)	OECD 207, 208[2]
Human Health Effects	Acute Toxicity	OECD 401-403,420, 423, 425
	General Toxicity (repeated dose)	OECD 407-413, 422
	Genetic Toxicity (effects on the gene and chromosome)	OECD 471-486
	Reproductive Toxicity	OECD 415, 416, 421, 422
	Developmental Toxicity	OECD 414, 421, 422

[1] EPA recognizes that alternate, equivalent test guidelines exist for some of the listed endpoints: for example, guidelines listed by EPA, ASTM, etc. The OECD Guidelines are presented here for both illustration purposes and because the Challenge Program is based on the OECD SIDS program.
[2] The OECD is in the process of updating this Guideline.
[3] See text at Section 6.2 of this document.

APPENDIX B

Sections 3.4 and 3.5 of the OECD SIDS MANUAL

APPENDIX C

The HPV Challenge Program Test Plan and Robust Summary

TEST PLAN AND ROBUST SUMMARIES

When companies volunteer to participate in the HPV Challenge Program, the first step is to identify, compile, and evaluate existing information for each HPV chemical they intend to sponsor. Evaluation of these data requires an assessment of data adequacy, which is the subject of this guidance document. Once the evaluation is complete, however, the company (sponsor) must make the information available to the EPA, and it will be made publicly available through the appropriate mechanisms. EPA believes the most appropriate method for doing this is through the submission of a *test plan*, as identified and used by the OECD (see SIDS manual at http://www.epa.gov/opptintr/sids/sidsman.htm) A test plan is a listing of all SIDS endpoints and whether adequate data exist for that endpoint, or whether the specific test needs to be done as part of the Challenge Program. If adequate data do exist, the test plan would reflect this, but the information would be submitted separately as a *robust summary*. Full study reports would be made available to EPA upon request. Some details, guidance, and examples of test plans and robust summaries are provided below.

The HPV Challenge Test Plan

A test plan is of a summary of the available data and data gaps for each SIDS endpoint identified in Appendix A. It is submitted as a summary table in the OECD SIDS program. Below is an example of a test plan that could be used in the Challenge Program. Note that the table has two parts. Part A includes chemical identity information and an overall summary. It includes non-hazard information, such as production volume and use pattern, which, while optional under Challenge Program, helps place the hazard data in the proper context. A concise summary of any proposed testing, if necessary, is also presented.

In Part B, details of the data availability and adequacy for each SIDS endpoint are listed. Some of the studies/information listed in Part B are not part of the basic SIDS. Again, like the non-hazard information in Part A, submission of such information, to the extent available, is important for placing all other information in the proper context for future assessment activities. Examples include: density, environmental monitoring, human experience (exposure data), and "other" information pertinent to the chemical but not covered by any of the listed information categories.

Example HPV Test Plan: Part A	
1.01A	***CAS No.***
1.01C	***Chemical Name***
1.01D	***CAS Descriptor***
1.01G	***Structural Formula***
	Other Chemical Identity Information
1.5	***Quantity Produced Per Year[1]***
1.7	***Use Pattern[1]***
1.9	***Sources and Levels of Exposure[1]***
Test Plan Justification ***(Discuss data adequacy, no testing, SAR, etc. Robust summaries of individual tests should be submitted separately via the electronic Health and Safety Data form.***	

[1] **Not requried under the HPV Challenge Program.**

Example HPV Test Plan: Part B							
CAS No:	Info Avail?	GLP	OECD Study	Other Study	Estim. Meth.	Accept-able?	SIDS Testing Required?
	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
Physicochemical							
Melting Point Boiling Point Density[1] Vapor Pressure Oct:water part.coef Water solubility pKa		--	--	--	--	--	--
Other		--	--	--	--	--	--
Environmental Fate and Pathway							
Photodeg Stability in water Monit. Data[1] Transp/Dist Biodeg		--	--	--	--	--	--
Other		--	--	--	--	--	--
Ecotoxicology							
Acute Fish Acute Daph. Acute Algae Chron. Daph[2] Terr. Tox.[2]							
Other		--	--	--	--	--	--
Toxicology							
Acute Rep. Dose Genetic Repro Devel/Terat Human Experience[2]		--	--	--	--	--	--
Other		--	--	--	--	--	--

[1] Not required for SIDS Base Set
[2] Conditional SIDS studies

The HPV Challenge Robust Summary

If available and adequate data exist on an HPV chemical for a given SIDS endpoint, then the pertinent information needs to be made publicly available under the HPV Challenge Program. This is accomplished in the form of a robust summary of the study as opposed to providing the full technical report. A *robust summary* should provide enough information on a study to allow the technically qualified reader to understand and evaluate: (1) the study's objective(s); (2) the method used; (3) the results (outcome); and (4) the conclusions.

Information Required

All the information listed under Tier I and some Tier II information in the Data Adequacy Document is anticipated to cover the objectives, methods, and some results of all studies. The conclusions should be a narrative describing the observed effects and objective interpretation. The robust summary should be presented electronically using a consistent format. Two formats have been suggested and are being evaluated for this purpose: the EPA HaSD (Health and Safety Data) format and the IUCLID (International Uniform Chemical Information Database) format. While a final decision on the format has not been made by EPA, until such time sponsors should use the EPA HaSD form (available at http://cyber22.dcoirm.epa.gov/oppt/tsca.nsf /HaSDForm /openform).

Examples of "Robust Summaries"

In order to provide initial guidance on developing robust summaries, EPA has taken several existing summaries from: (1) IUCLID (International Uniform Chemical Information Database); (2) OECD SIDS Dossiers; or (3) contract lab/study report abstracts. On the following pages are illustrative examples of summaries that provide a level of detail which is acceptable versus ones that EPA does not consider acceptable. The format of the information varies, however, the goal is to illustrate the qualities and level of detail found in an acceptable robust summary. Thus, these examples are not intended to convey formatting recommendations. Additional guidance on formatting will be provided when EPA has completed its evaluation of the available options and alternatives (e.g., HaSD versus enhanced IUCLID).

EXAMPLE A
Acute Toxicity Study in Rats
(Unacceptable)

Study type: Acute oral toxicity
Species: Rat, Carworth-Wistar
Sex: Males
Number of animals: 5
Vehicle: ???
Method: Other
Year: 1962
GLP: No
Test Substance: XX
CAS No.: XXX
Method (conditions): 5 non-fasted Carworth-Wistar male rats per group, 4-5 weeks old. 14-day observation period.

Results: LD_{50} = 700 mg/kg.

Reference: *Full citation is listed.*

Unacceptable because:
- lack of information relative to GLPs
- no discussion of method
- number and amount of doses not provided
- no details on observed deaths/symptoms, etc.

EXAMPLE B
Acute Toxicity to Rats
(Acceptable)

The test substance was suspended in 0.25% aqueous methylcellulose and administered to fasted male rats at dosages of 1000, 3000, or 5000 mg/kg and to fasted female rats at a dosage of 5000 mg/kg. The rats were observed for clinical signs of toxicity approximately 1 hour after dosing and over a 14-day observation period. The observation period for 1 male rat treated at 5000 mg/kg was extended to test day 21. All rats that were found dead or sacrificed by design at the end of the observation period were given a gross pathological examination.

Deaths occurred in 0/5, 0/5, and 4/5 male rats treated at 1000, 3000, and 5000 mg/kg, respectively. No deaths occurred in female rats treated at 5000 mg/kg. The oral $LD_{50}$for male rats was calculated to be 4550 mg/kg and the LD_{50} for the female rats was greater than 5000 mg/kg.

No clinical signs of toxicity were observed in male rats treated at 1000 or 3000 mg/kg. The appearance of clinical signs in the other treatment groups was delayed during the study. Clinical signs of toxicity observed in male rats treated at 5000 mg/kg included ruffled fur, lethargy, hunched posture..... Females treated at 5000 mg/kg exhibited.....

EXAMPLE C
Acute Toxicity in Daphnids
(Unacceptable)

The acute toxicity of the test material was determined in D. magna in a static (without renewal) 48-hr. test. Each treatment group consisted of 20 animals (4 replicates of 5 animals each) exposed to individually prepared WAFs at treatment levels of 2 mg/L, 1 mg/L, 0.5 mg/L, 0.25 mg/L and 0.13 mg/L. At the 2 day observation point, total mortality (100%) was observed in all test groups. All control group animals survived to study termination. A 50% lethal loading level (LL_{50}) could not be determined.

Unacceptable because:
- test method not given
- no details on method (test substance, dilution water, nominal vs. measured conc.)
- explanation of test results not sufficient

EXAMPLE D
Acute Toxicity in Daphnids
(Acceptable)

Protocol Title	Acute Toxicity to Water fleas (*Daphnia magna)* under static conditions, following OECD Guideline 202.....
Test Substance	A light beige liquid with a purity of 37%.....
Species	*Daphnia magna*, age < 24 hours, source provided
Dilution water	Fortified well water (pH, conductivity, total hardness and alkalinity provided)
Test Temperature	20ºC
Nominal Test Conc.	Eight conc. ranging from 0.037 mg a.i./L to 10 mg a.i./L
Mean Meas. Conc.	0.027 mg a.i./L to 7 mg a.i./L
Results:	The 48 hour EC_{50} was calculated by probit analysis to be 0.32 mg a.i./L (95% C.I. of 0.23 - 0.43). The NOEC was determined to be 0.061 mg a.i./L.