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Guidance for "What to Test" for the HPV Challenge

The issue of precisely what form of a chemical substance should be tested, or the "test substance," in the HPV Challenge's battery of health and environmental fate and effects testing is often a difficult, complex issue. This document is intended to serve as a starting point for discussions on the "what to test" issue by describing past experience and approaches used under the Organization for Economic Cooperation and Development's (OECD) Screening Information Data Set (SIDS) Program and TSCA §4 Test Rules. EPA recognizes that there can be many different manufacturers and different grades and purities of a chemical substance that can be described by the same CAS number and that there can be issues of whether these various products[1] are equivalent in toxicity.

Background

Companies that sponsor a chemical in the HPV Challenge Program should include in the Test Plan prepared for that chemical a detailed description of the test substance to be utilized (or what was tested in the case of existing studies) **and a rationale for the selection of the test substance to be used in new testing**. This description is a key component of the Test Plan. The description of the test substance will likely be carefully reviewed by EPA, and other members of the interested public, to ensure the technical needs of the HPV Challenge are being met. EPA recognizes that this guidance may not adequately address all "what to test" issues. Ultimately, it is the sponsor company's responsibility to use its best judgment in the selecting the test substance and be prepared to justify the selection.

The issue of what to test is especially important for multi-component chemical substances or Class 2 Substances because equivalency of chemical substances extends beyond the issue of purity of a single component and extends to compositional makeup. The Framework Document, which describes the design of the HPV Challenge, does not specifically discuss the "what to test" issue.

The SIDS Manual, which is available via an electronic link on the **epa.gov/chemrtk** web site, offers the following guidance on what to test:

> *"... it is recommended that tests for physical-chemical properties be conducted on the purified substance because they are basic information on a specific chemical. The other tests, however, should generally be carried out on the substance with any essential additives (e.g. stabilizers) and impurities it normally contains in order to know the effects of the marketed product. Ideally, the same batch of substance should be used. However,*

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[1]As noted, an Inventory-listed chemical (i.e. a CAS numbered substance on the TSCA Inventory) can be made commercially in different grades or forms by the same or multiple manufacturers. The term, "product", is used here to describe this concept of different grades or forms of a CAS-numbered Inventory entry made by one or more manufacturers.

gross amounts of water, mineral oil or other solvents that are sometimes present in the marketed product should be excluded."

The HPV List is made up of organic, non-polymer chemicals reported to the 1990 IUR that have a national aggregate production volume (including imports) greater than 1,000,000 lb/yr. Substances that are subject to IUR reporting are of two types: Class 1 Substances and Class 2 Substances.

Class 1 Substances are defined in the "Toxic Substances Control Act Chemical Substance Inventory, 1985 Edition" (EPA-560/7-85-002a) as chemical substances which are "single compounds composed of molecules with particular atoms arranged in a definite known structure." Examples are: acetone and benzene.

Class 2 Substances are defined as "chemical substances which may have variable compositions or be composed of a complex combination of different molecules. Class 2 Substances are further divided into three subgroups. The first subgroup includes substances which can be represented by definite Hill-ordered molecular formulae but have variable structural diagrams, e.g. xylene, in which location of substituent groups is variable. The second subgroup includes substances which can be represented by definite molecular formulae but have unknown structural diagrams, e.g. aluminum cerium nickel sulfide, Al Ce_3Ni S_7. The third group includes substances that have no definite molecular formulae representation and either partial structural diagrams or no structural diagrams. This kind of substance is typified by coke oven light oil (coal), which is defined on the Inventory as "the volatile organic liquid extracted from gas evolved in the high temperature (greater than 700^o C) destructive distillation of coal; composed primarily of benzene, toluene and xylenes; may contain other minor hydrocarbon constituents." Substances that fall within this group are often called UVCB substances, for "Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials." Note that UVCBs are defined by the commercial intent of the manufacturer because their components can not be characterized.

Prior Applied Experience with Regard to the "What to Test" Issue

Class 1 Substances

- The SIDS program guidance recommends testing a chemical substance in the form in which it is marketed as cited above. Consequently, SIDS Dossiers, which describe the results of toxicological and other testing, have characterized the test substance with purity specifications.

  An example of this approach is mesityl oxide. The test substance had a purity greater than 95% with a major impurity of 4-methyl-4-penten-2-one noted.

  Similarly, acetacetanilde was tested in SIDS at a purity of greater than 99% by weight.

- Test rules promulgated under section 4 of TSCA have generally required the test substance to have a purity of 99%. On occasion there have been chemical substances that can not be isolated at a purity of 99% and EPA has adjusted its purity requirement on the test substance to as low as 95%. Sponsors can find examples of purity requirements in a number of Final Test Rules available at 40 CFR 799.

Class 2 Substances

- Only a limited number of Class 2 Substances have been tested within the TSCA Test Rule Program. Commercial hexane is one example. In this case an ASTM Standard, which specifies the compositional makeup of commercial hexane, was used for defining the test substance.

- C9 Aromatics are another example. The Test Rule specified the volume per cent of the major components in the test substance.

- Determining test substance in the Aryl Phosphates Proposed Test Rule and subsequent ongoing ECA negotiations has been a difficult issue because of the complexity of the equivalence issue for these substances amongst different manufacturers. The original Proposed Rule would require testing for 11 aryl phosphate base stocks identified by CAS numbers, most of which are Class 2 Substances. ECA discussions refined this list to 8 CAS numbers. The ECA discussions used detailed compositional analyses to ascertain that certain substances with the same CAS number, manufactured by several manufacturers, contained roughly the same components and were likely to be toxicologically equivalent. This approach lead to agreement on the equivalence for six CAS-numbered substances. A similar conclusion on equivalence could not be made for the remaining CAS-numbered substances and an approach to defining the test substance has not been finalized although it may involve product-specific testing by each of the manufacturers.

- Determining the test substance for a category of Class 2 substances is potentially even more challenging. There has been limited experience in testing categories of Class 2 Substances in SIDS. Nonylphenol ethoxylates are currently just entering the SIDS process and the proposed strategy for identifying the test substances for this category is instructive. This category of substances encompasses a large number of compounds which may have varying degrees of ethoxylation and different isomeric configurations. A single CAS number and description may include nonylphenol ethoxylates having from one to more than 20 ethoxylate units. In addition, due to the common use of a variety of nomenclature systems, a single molecular configuration may be represented by multiple CAS numbers. To address the issue of what to test, the sponsors have proposed to split nonylphenol ethoxylates into three ethoxylation subcategories based on the average ethoxylate units (EOs). One subcategory contains substances with 1 - 5 EOs, one has 5 - 20 EOs, and the last has greater than 20 EOs. The nonylphenol ethoxylates in each

subcategory are expected to have similar physical, chemical and toxicological properties. Existing testing for each subcategory will be assessed for adequacy and after data gaps are identified, Test Plans specifying additional testing developed. The Test Plan developed for each subcategory will also specify test substances for each subcategory.

- Another Class 2 example from the SIDS experience is the linear alkyl benzene (LAB) category. The LAB category was determined to be comprised of nine different commercial formulations. Each formulation contains various proportions of individual LABs. For data adequacy analysis the LAB category was further subdivided into three subcategories based on the percentage of alkyl substituents with a low (C10-C11), mid (C11-C13), and high (C13-C14) proportion of carbon chain lengths. In the SIDS process the existing data for all of the subcategories were judged to be sufficient for screening level hazard assessment and additional testing was not required.

- When deciding what to test for a Class 2 Substance, the TSCA regulatory history suggests sponsors consider the following:

  Does the proposed test substance conform to the CAS narrative definition of the sponsored substance?

  Is there a consensus standard, such as an ASTM standard, that specifies the chemical substance? Is some other recognized specification or industry standard for the chemical substance available?

  Can a single substance or mixture be selected which would be representative, for toxicological, environmental effects and environmental fate purposes, of the substances to which persons and the environment are exposed?

  Is there a particular substance or mixture which is representative of most of the commercial production of the chemical substance?

  Should a commercial (marketed) substance be selected for testing instead of a synthetic mixture?

Alternatives/Options

The goal of the HPV Challenge Program (and the related OECD SIDS Program) is to develop a screening level of understanding of the hazards presented by the chemical and make summary information available to the public. Given the ultimate use of the test data will be for screening level assessment, as opposed to a detailed risk assessment, it may be reasonable not to require definitive toxicological equivalence and hence the issue of test substance selection may be somewhat diminished. However, the test substance selected must at some level be sufficiently representative to minimize incorrect hazard screening judgements.

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It should be recognized that when a test substance is selected under the HPV Challenge Program, the specific material to be tested will likely be selected from among the product(s) made by the sponsor(s). Consequently the product(s) made by non-sponsors will likely not be tested. Ideally, sponsors will take into account the various products produced by all the possible manufacturers, including non-sponsors when selecting the test substance and be prepared to justify their selection.

A number of alternative approaches to addressing the "what to test" issue have been identified and are described below.

EPA Recommendations for Class 1 Substances:

Given (1) the compositional variability (based largely on purity differences) which is likely to exist across Class 1 Substances made by the same or different manufacturers (e.g., production and sale of different grades) and (2) EPA's interest in avoiding complex transactions to determine an appropriate "as marketed" version of the chemical, EPA here presents a clarification of the OECD preference for testing of a chemical "as marketed." EPA recommends that the "pure" HPV chemical (including non-separable solvents and needed stabilizers) be used to perform physical-chemical tests included in the test battery. This approach is consistent with that recommended by the SIDS manual. EPA recommends that the highest commercially available purity be used as the test substance in the remaining SIDS tests as determined by or among the sponsor(s) for a given chemical. This is a departure from the SIDS manual approach but avoids complications associated with selecting one "as marketed" form as the test substance. To avoid any possibility of confusion, purities should be described using percentages by weight. The Test Plan should provide the basis (including, e.g., approximate number of products, range of purities, difficulties in preparing "pure" chemical, etc.) for the selections made.

EPA recognizes that there may be instances when sponsor companies believe that some other procedure to select a test substance is preferred to the above recommendation. For example, there may be situations where it is possible to identify a most representative commercial sample and use it as the test substance. In these circumstances sponsors should explain and justify their selection in the Test Plan.

Class 2 Substances:

Class 2 Substances include multi-component chemical substances, such as reaction products and other substances of undefined or variable composition. EPA recognizes the problems in testing only one of what could be a myriad of different Class 2 products all having the same CAS number. Ideally, the test substance should be representative of the hazard that might be associated with a particular CAS number and the selection should be a science-based decision. EPA proposes the following options for consideration and requests comments and the identification of additional approaches:

- use as the test substance any product that meets a consensus specification (e.g.ASTM) or similar industry accepted specification for the CAS number of the sponsored chemical substance.

- use as the test substance the product which is produced at the highest volume by the sponsor(s).

- randomly select one product for the test substance from among all the different forms which are made by the sponsor(s).

- alternatively, or in addition, if there are unresolvable issues regarding toxicological equivalence, and if there are large number of different products made by the sponsor(s), randomly select 1 out of every 3, 5, or 10 products as the test substance.

- identify the 3, 5, or 10 highest volume products made by the sponsors and randomly select 1 (or more?) as the test substance(s).

EPA recognizes that it may not be possible to identify a single approach for handling Class 2 substances and asks for comment on whether the options might be structured in a hierarchy (which could involve grouping of options) or whether a series of equivalent options might be offered. In all cases the Test Plan would need to describe the situation and provide the basis for the selection(s) of the test substance.