Step 2: Gather published and unpublished literature for each category member.

18.   A literature search resulted in identifying data for most category members in the environmental fate, ecotoxicity and human health effect SIDS endpoints.

Step 3: Evaluate available data for adequacy.

19.   Again, as was discussed in the alpha-olefin example, evaluation of data adequacy is performed at the individual study level. [Guidance for Determining the Quality of Data for the SIDS Dossier (Interim SIDS Manual)]

Step 4: Construct a matrix of data availability.

20.   An analysis of available data resulted in a matrix as presented in Table B-2. Again, for simplicity not all data found or compiled are presented here. Note that three LAB formulations (Alkylate 215, Alkylate 225, and Alkylate 230) had data available in each of the major SIDS classes (environmental fate, ecotoxicity, and health effects), and they each represent one of the three subcategories presented in Table B-1.

Table B-2 STEP 4: Matrix of Available and Adequate Data on LAB CategoryMembers[1]								
LAB Formulation	Environ- mental Fate	Ecological Effects			Human Health Effects			
		Fish Acute	Daphnid Acute	Daphnid Chronic	Acute[4]	Repeated Dose[5]	Mutagen- icity[6]	Develop- mental[7]
Nalkylene 500					√			
Nalkylene 500L			-		-		-	
Alkylate 215	√	√	√	√	√	-	√	√
Nalkylene 550L			-		√		-	
Alkylate 225	√	√	√	-	√	√	√	-
Nalkylene 575L			-				-	
Nalkylene 600					√			
Nalkylene 600L			-		√		-	
Alkylate 230	√	√	√	√	√	√	√	√

[1] "√" denotes data are available and adequate. "-" denotes data are either not available, or are available and are judged inadequate. Shaded areas mark the three subcategories identified in Table B-1.

Step 5: Perform an internal assessment of the category.

21.   As with Table A-1 in the alpha-olefin example, the data in Table B-2 identifies where data gaps exist. Note that adequate data are available for most endpoints for the three LAB formulations mentioned above. Table B-3 is essentially the same table as Table B-2, except that the data values are placed in each cell so that they can be evaluated to determine the acceptability of the category approach for each endpoint.

22.   Table B-3 shows a consistent pattern of no discernible difference in aerobic degradation among the three LAB formulations tested (range of 56% - 61% of parent material evolved as carbon dioxide after a 35 day incubation period). Similarly, the acute fish toxicity, chronic daphnid toxicity, acute mammalian toxicity, reproductive/developmental toxicity, and mutagenicity data do not show differences across the tested formulations. However, the acute daphnid toxicity results, as well as the repeated dose toxicity tests in mammals suggest a pattern of increasing toxicity with an increase in the proportion of higher length carbon chains in the substituted alkyl group that appears to hold for each of these SIDS endpoints.

Step 6, 7 and 8: Prepare category test plan for review; Conduct necessary testing; and Perform an external assessment of the category.

23.   In this case, it was concluded that no further testing was necessary under the SIDS programme and that the existing data were sufficient for a screening level hazard assessment. Thus; it was not deemed necessary to test each LAB formulation given the results of testing in three separate formulations to represent the boundaries of the category.

24.   In this example, the test plan would include the rationale for "no testing" together with an evaluation of the existing data. Robust summaries for the individual supporting studies would also be available.

Table B-3
Evaluation of Matrix Data Patterns for LAB Category

LAB Formulation	Environmental Fate	Ecological Effects			Human Health Effects			
		Fish Acute	Daphnid Acute	Daphnid Chronic	Acute[4]	Repeated Dose[5]	Mutagenicity[6]	Developmental[7]
Nalkylene 500	Not tested	Not tested	Not tested	Not tested	>34 g/kg	Not tested	Not tested	
Nalkylene 500L	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	
Alkylate 215	56%[1]	>Water solubility	80 ppb[2]	7.5 to 15 ppb[3]	17 g/kg	100 mg/m^3	Negative	125 mg/kg
Nalkylene 550L	Not tested		Not tested		>5 g/kg		Not tested	
Alkylate 225	61%[1]	>Water solubility	9 ppb[2]	Not tested	28 g/kg	29 mg/m^3	Negative	Not tested
Nalkylene 575L	Not tested	Not tested	Not tested			Not tested	Not tested	
Nalkylene 600	Not tested	Not tested	Not tested		>35 g/kg	Not tested	Not tested	
Nalkylene 600L	Not tested	Not tested	Not tested		>5 g/kg	Not tested	Not tested	
Alkylate 230	56%[1]	>Water solubility	10 ppb[2]	13 to 23 ppb[3]	21 g/kg	<32 mg/m^3	Negative	125 mg/kg

[1] Percent of parent material evolved as carbon dioxide after 35 days in an aerobic biodegradation test.
[2] 48-hour LC50s.
[3] 21-Day No Observed Effect Concentration (NOEC).
[4] Oral LD50s in rodents.
[5] Four week inhalation studies in rats, values represent NOECs for the following effects: irritation of the eyes and nose and decreased body weight.
[6] Negative in vitro (bacteria - Ames; mammalian - Chinese hamster ovary cells) and in vivo (chromosomal aberration study in rats) tests.
[7] Developmental toxicity study (oral, rats, doses of 0, 125, 500, and 2000 mg/kg/d). Numbers in column represent no observed adverse effect level (NOAEL) for both maternal (weight gain) and developmental (ossification variations) endpoints.

Example C: Brominated Diphenyl Ethers

Step 1: Identification of structure-based category and its members:

25.   The polybrominated diphenyl ether (PBDE) category theoretically contains a number of congeners (mono- through to decabromodiphenyl ether) but only three products are produced commercially: bis (pentabromophenyl) ether, also known as decabromodiphenyl ether (decaBDE); diphenyl ether, octabromo derivative (octaBDE); and diphenyl ether, pentabromo derivative (pentaBDE). The general chemical formula for this category is:

$$x + y = 5, 8, 10$$
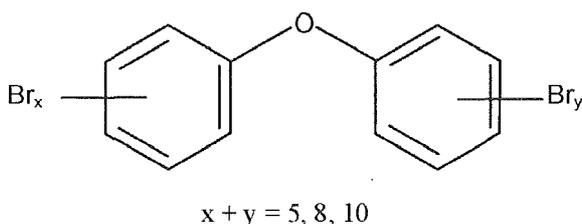
26.   This category is the "family of congeners" category type and is limited to three products produced commercially as most of the laboratory test data has been obtained with these products. DecaBDE is an essentially pure substance, but the other two are complex mixtures of related substances with varying degrees of bromination and substitution patterns. The actual compositions of the commercial products are summarised in table C-1. This particular example is limited to an analysis of ecotoxicity data.

Table C-1			
STEP 1: Typical compositions of commercially available PBDEs			
Congener	PentaBDE	OctaBDE	DecaBDE
tribromo-	0-1%		
tetrabromo-	24-38%		
pentabromo-	50-62%	1.4-12%[1]	
hexabromo-	4-12%		
heptabromo-	trace	43-58%	
octabromo-		26-35%	
nonabromo-		8-14%	≤3%
decabromo-		0-3%	≥97%
[1]This figure refers to the combined total of pentabromo- and hexabromo- congeners present.			

Step 2: Gather published and unpublished literature for each category member.

27.   A literature search identified some ecotoxicity data for all category members.

Step 3: Evaluate available data for adequacy.

28.   As with the other examples in this Appendix, evaluation of data adequacy is performed at the individual study level.

Step 4: Construct a matrix of data availability.

29.   Table C-2 presents a matrix for the available ecotoxicity data based on the literature search. SIDS data gaps exist for acute invertebrate testing (decaBDE) and for acute algal testing (octaBDE).

Table C-2 STEP 4: Available Acute Ecotoxicity Data on PBDEs[1]			
Test Organism	PentaBDE	OctaBDE	DecaBDE
Fish	√	√	√
Invertebrate	√	√	-
Algae	√	-	√
[1] "√" denotes data available and adequate; "-" denotes data not available, or available and not adequate.			

Step 5: Perform an internal assessment of the category.

30.   Table C-3 is essentially the same table as Table C-2, except that actual data replace the "√s".

31.   In evaluating these data, it was concluded that a decrease in aquatic toxicity could be expected with increasing bromine number. Since there were adequate aquatic toxicity data for the category member with the lowest number of bromine atoms (pentaBDE), it was not necessary to conduct additional acute toxicity tests on the remaining members with a higher number of bromine atoms. DecaBDE would not be more toxic to invertebrates than octaBDE, and the algal toxicity of octaBDE could be inferred from the data on penta- and decaBDE.

32.   In addition to the ecotoxicity data, available data on environmental monitoring, bioconcentration, and the physicochemical properties of the category members were evaluated. It was determined that there was a decreasing concern for bioaccumulation potential with an increase in bromine number; that all three compounds were not very water soluble; and that they all had high octanol-water partition coefficients (log K_{ow}). This suggested that the likely exposure scenario of concern would be to organisms exposed directly to sediment or soil.

Table C-3 Available Acute Ecotoxicity Data on PBDEs			
Test Organism	PentaBDE	OctaBDE	DecaBDE
Fish	Rainbow trout NOEC (96 hr) = 21 µg/L (>water solubility?)	Medaka LC_{50} (48 hr) = >water solubility	Medaka LC_{50} (48 hr) = >water Solubility
	Medaka LC_{50} (48 hr) = > water solubility		
Invertebrate	Daphnid EC_{50} (48 hr) = 14 µg/L NOEC (48 hr) = 4.9 µg/L (EC_{50} values close to water solubility)	Daphnid 21-day NOEC > 2 µg/L	No Data
Algae	*Selanastrum capricornutum* NOEC (96 hr) up to 26 µg/L (>water solubility?)	No Data	Three different species EC_{50} (72 hr) > water solubility
[1] Small freshwater fish (warm water species).			

Step 6: Prepare category test plan for review.

33.   Because of the concern for bioaccumulation and partitioning of thePBDEs to the sediment/soil environment, it was recommended that further testing (chronic aquatic toxicity, sediment toxicity, and soil toxicity) be conducted, begining with pentaBDE. Therefore, the final testing recommendation required "advanced" SIDS testing without filling the acute aquatic toxicity basic SIDS data gaps. The testing plan (Table C-4) was tiered, the results of the lower tiers determining the next set of tests.

Table C-4
OECD SIDS Proposed Ecotoxicity Testing Plan withPBDEs

Tier	Category Member	Ecotoxicity Test[1]	Result	Comment
I	PentaBDE	Fish early life stage test	Rainbow trout 60-day NOEC = 8.9 µg/l	Fish test to verify bioaccumulative potential.
		Daphnid reproduction test	Daphnid 21-day NOEC = 5.3 µg/l	Daphnid study to verify that acute effects were due to toxicity.
		Sediment (midge) toxicity test	*Chironomus riparius* 28-day NOEC = 16 mg/kg dry weight	To verify concerns identified in hazard/exposure assessment
		Sediment (oligochaete) toxicity test	*Lumbriculus variegatus* 28-day NOEC = 3.1 mg/kg dry weight	
		Sediment (amphipod) toxicity test	*Hyalella azteca* 28-day NOEC ~ 6.3 mg/kg dry weight	
		Soil (earthworm) toxicity test	*Eisenia fetida* 14-day NOEC >500 mg/kg dry weight	
		Soil (plant) toxicity test	Six plants – lowest 21-day EC_5 = 16 mg/kg dry weight	
		Soil (nitrification inhibition) toxicity test	Soil microorganisms 28-day NOEC ≥ 1 mg/kg dry weight	
II	OctaBDE	Sediment (oligochaete) toxicity tests using two sediment types	*Lumbriculus variegatus* 28-day NOEC ≥ 1,272 mg/kg dry weight	Tests were chosen based on the pentaBDE results – for example, sediment organism sensitivity is not expected to differ significantly and so only the most sensitive organism from the pentaBDE test series required testing.
		Soil (earthworm) toxicity test	*Eisenia fetida* 56-day NOEC ≥ 1,470 mg/kg dry weight	
		Soil (plant) toxicity test	Six plants – 21-day NOEC ≥ 1,190 mg/kg dry weight	
III	DecaBDE	Sediment (oligochaete) toxicity tests using two sediment types	*Lumbriculus variegatus* 28-day NOEC ≥ 3,841 mg/kg dry weight	As for octaBDE
		Soil (earthworm) toxicity test	*Eisenia fetida* 56-day NOEC ≥ 4,910 mg/kg dry weight	
		Soil (plant) toxicity test	Six plants – 21-day NOEC ≥ 5,349 mg/kg dry weight	

[1] All tests are beyond the basic SIDS requirements. The testing plan is presented to show how basic SIDS requirements were waived in order to proceed to a more meaningful testing scheme.

Step 7. Conduct necessary testing.

34.   The results of the testing plan are summarised in Table C-4.

Step 8. Perform an external assessment of the category.

35.   The Tier I testing was carried out using pentaBDE alone and consisted of long-term toxicity tests with exposure via water (tests with daphnids and rainbow trout), sediment (tests with midges, oligochaetes and amphipods) and soil (tests with earthworms, plants and soil microorganisms). The results of these tests verified concerns over the toxicity of the substance to fish and daphnids over long-term exposures, and confirmed that the substance could also elicit toxic effects on sediment- and soil-dwelling organisms. The Tier II (octaBDE) and Tier III (decaBDE) testing was actually performed in parallel, based on the most sensitive soil and sediment organisms identified in the Tier I testing.

36.   In addition to the prescribed test plan, new information also became available on the accumulation potential of one member of the category, namely decaBDE. This showed that decaBDE was present in certain predatory birds and their eggs, as well as certain terrestrial mammals. Before these data were available it was assumed that decaBDE had a very limited potential for uptake and accumulation in organisms because of its large molecular size and low fish bioconcentration factor. In addition, new data also became available that appeared to show that the substance may cause neurotoxic effects in young laboratory mice at doses much lower than those causing effects for other toxicity endpoints (although further testing is currently underway to confirm this effect and establish a NOAEL). These new data required the assumptions over the low accumulation and hazard potential of decaBDE to be re-evaluated.

Step 9: Fill the data gaps

37   A major problem encountered with this category was that two members (octa- and decaBDE) showed effectively no toxicity in any of the aquatic (water exposure), sediment or soil toxicity tests undertaken. For aquatic toxicity it was possible to read-across between the various members of the category to fill the data gaps once the further testing for pentaBDE was completed, as follows. All three category members showed essentially no toxicity to fish from acute exposures. Penta- and decaBDE showed essentially no toxicity to algae, and so it was possible to infer that octaBDE would also show essentially no toxicity to algae. The long-term toxicity data for pentaBDE showed that invertebrates were the most sensitive trophic level. As octaBDE had no effect on daphnids following long-term exposure at concentrations up to its solubility limit, it was also possible to infer that decaBDE would show no effects with daphnids. Furthermore, both octa- and decaBDE were also considered unlikely to show any long-term effects in fish tests at concentrations up to their respective solubility limits (since both have a lower fish bioconcentration factor than pentaBDE).

38.   In retrospect, it would have been possible to read-across from the octaBDE data to predict that decaBDE would also show little or no toxicity to soil and sediment organisms. However, at the time it was not possible to carry out this read-across with any certainty; regulatory experience with these types of tests is less extensive than for aquatic tests (for industrial chemicals), and the possible exposure in sediment and soil is not necessarily limited by the water solubility of the substances (oral exposure could be important). There was also a policy need to complete the testing as quickly as possible, and so the testing on both octaBDE and decaBDE was performed at the same time.

39.   It should be noted that the main driver for the further testing requirements of octa- and decaBDE was consideration of the main compartments through which organisms may be exposed (i.e. sediment and soil) rather than to complete the SIDS endpoints (mainly related to exposure through water).

40.   Overall, read-across of data was compromised by the fact that two members out of three essentially showed no toxicity in standard tests. The number of members in the category is an important consideration because it is very difficult to reliably identify trends from two data points (for example, it would generally not be possible to predict the behaviour of pentaBDE from that of octa- or decaBDE). The patterns and trends in the data for this category only really became evident once a substantial amount of testing had been carried out. However, it was useful to identify the most sensitive species in tests with the most toxic member of the category, which could then be used to target the testing for the other members of the category.

41.   Another problem encountered with this category was that, with the exception of decaBDE, the commercially supplied substances contain a mixture of congeners. Much of the available ecotoxicity information was obtained with these commercial products and these data did not allow the actual toxicity of the individual congeners to be ascertained, except for the broad trends. This contrasts with the situation regarding the data on the environmental exposure of this group of substances, where a large database of monitoring data relating to specific isomers is available.

42.   Perhaps most importantly for this category, it was not possible to identify or infer the particular accumulative properties of decaBDE using a read-across approach. For example, the available laboratory studies show that pentaBDE has a much higher accumulation potential than decaBDE and this is also demonstrated by the majority of the available environmental monitoring data (particularly for the aquatic environment). However, it has recently become apparent that decaBDE can be widely found in certain predatory birds and their eggs and some predatory mammals, possibly linked to the terrestrial food chain. This finding means that it is necessary to re-visit the category assumptions when new information on a category member is made available.

Example D: Butene Isomers and their Mixtures

Step 1: Define the category

43.   An example of a category of isomeric substances and their mixtures is the butenes. This category includes four isomers (two structural isomers, butene-1 and isobutene), two geometric isomers, *cis*-butene-2 and *trans*-butene-2 and two mixtures (see table D-1) Substance structures are shown in Figures D-1 to D-4.
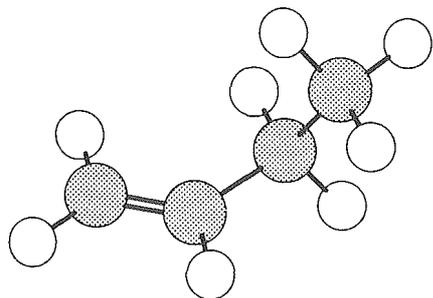
Figure D-1.   Molecular Structure of Butene-1 (C4H8)

Figure D-2. Molecular Structure of Isobutene (2-methyl propene) (C4H8)
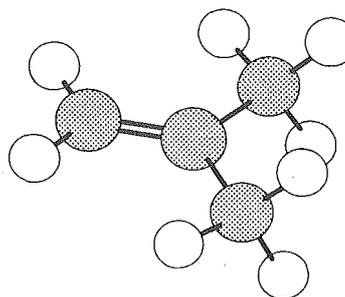
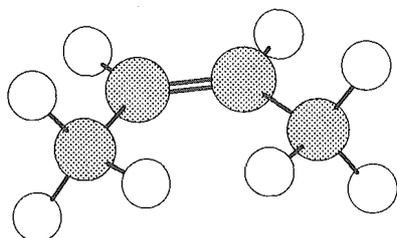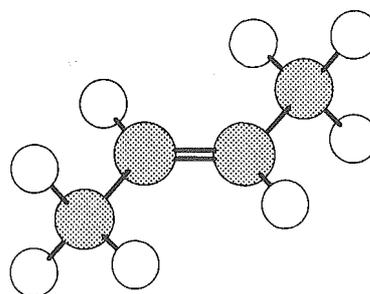Figure D-3.   Molecular Structure of *cis*-Butene-2 (C4H8)
Butene-2 (C4H8)

Figure D-4.   Molecular Structure of *trans*-Butene-2 (C4H8)

44.   Each butene isomer contains four carbon and eight hydrogen atoms, with one double bond between carbon number one and two or carbon number two and three. These are simple hydrocarbons in a class referred to as alkenes. At standard temperature and pressure (STP), they are gaseous.

45.   Butenes are a good example of an isomer category because their physicochemical and biological properties are closely aligned. Based on existing data for toxicological endpoints within the HPV Chemicals Programme and the overall toxicological knowledge of hydrocarbons, the Butenes Category members that do not have toxicological data would be expected to exhibit similar if not identical effects as category members with data (the toxicological endpoints under consideration include mammalian and environmental as specified under the Programme). Additionally, because of their similarity with respect to physicochemical and biological parameters, data on the individual substances can be used to estimate values for the mixtures.

46.   It should be noted that the Butenes Category example is relatively simple with a limited number of isomers. As the number of carbon atoms and functional moieties increase, the number of isomers increases illustrating the importance of appropriately defining isomer categories when optimising the use of existing data.

Step 2: Gather published and unpublished data for each category member

47.   A comprehensive literature search identified information on all SIDS endpoints for the category members. The majority of information was found on the single isomers rather than on the mixed isomers. Participating companies in the consortium were asked to review their internal files for appropriate information.

Step 3: Evaluate available data for adequacy

48.   Each study identified by the literature search was reviewed and assessed for quality and reliability (section 3.1). Data were entered into IUCLID 4.01 under the specific CAS number for each the isomers or as mixture as part of a "Butenes Isomers and their mixtures", category record. Those studies that were considered appropriate were classified as "Critical study for SIDS Endpoint". For several health related endpoints, more than one "critical study" was identified.

Step 4: Construct a matrix of data availability

49.   The IUCLID category matrix report was used to automatically generate tables containing substance identity, end point, data availability and their values. The table is not reproduced here to save space, but a summary is given in Table D-1.

TABLE D-1

Matrix of available and adequate data on butenes: isomers and their mixtures						
Test	*1-Butene* 106-98-9	*Butene, mixed -1 and -2- isomers* 107-01-7	*2-Methylprop ene* 115-11-7	*Butene, mixed isomers* 25167-67-3	*cis-2-Butene* 590-18-1	*trans-2-Butene* 624-64-6
Physicochemical Properties						
Melting Point	√	–	√	–	√	√
Vapour Pressure	√	–	√	–	√	√
Partition Coeff.	√	–	√	–	√	√
Water Solubility	√	–	√	–	√	√
Environmental Fate						
Biodegradation	–			–	–	–
Ecotoxicity						
Acute Fish	√	–	√	–	√	√
Acute Daphnid	√	–	√	–	√	√
Alga	√	–	–	–	√	√
Terrestrial	√	–	√	–	√	√

Human Health Effects						
Acute Oral	_	_	_	_	_	_
Acute Inhalation	_	√	√	_	_	_
Acute Dermal	_	_	_	_	_	_
Repeated Dose	√	√	√	_	_	_
Genotoxicity (*in vitro*)	√	√	√	_	_	_
Genotoxicity (*in vitro* - non-bacterial)	_	√	√	_	_	_
Genotoxicity (*in vivo*)	√	_	√	_	_	_
Repro/Developmental	√	√	_	_	_	_
(√) = Data available and considered adequate; (-) = No data available, or available data considered inadequate.						

50.   As butenes are gaseous at STP the data for environmental toxicity are calculated values, using the ECOSAR program. Human health data are available for all endpoints, with a minimum of two category members having reliable data.

Step 5: Perform an internal assessment of the category

51.   The information in Table D-1 was assessed for trends. For environmental toxicity data, values were calculated for each endpoint. These values were similar for each member, and are therefore considered to be applicable to the mixed isomer category members, for which it is not possible to calculate values.

52.   For the health endpoints, where there were data, they were similar for all category members within the considerations of study variability. Table D-2 shows selected data from the IUCLID category report.

TABLE D-2

Endpoint	Substances					
	1-Butene	*Butene, mixed -1 and -2- isomers*	*2-Methylpropene*	*Butene, mixed isomers*	*cis-2-Butene*	*trans-2-Butene*
	106-98-9	107-01-7	115-11-7	25167-67-3	590-18-1	624-64-6
Acute Inhalation Toxicity		LC50: male/female rat > 10057 ppm for 4 hour(s)	* LC50: rat = 620 mg/l (270000ppm) for 4 hour(s)			
Repeated Dose Toxicity	Male/female rat; other: vapour inhalation; 28 days; 6 hours/day, 7 days/week; Doses: 0, 500, 2000 and 8000 ppm; Method: OECD	Male/female rat; inhalation; 2 weeks NOAEL: = 2500 ppm	Male/female rat; inhalation; 13 weeks NOAEL: 8000 ppm *			

	combined study TG422; NOAEL: = 8000 ppm					
Genetic Toxicity *in vitro*	* Salmonella negative	* Salmonella negative	* Mouse lymphoma L5178Y TK+TK- negative			
Genetic Toxicity *in vivo*	Mammalian Bone Marrow Erythrocyte Micronucleus Test Negative		Micronucleus assay mouse negative			
Toxicity to Reproduction	OECD Guide-line 422 parental NOAEL = 8000 ppm	OECD 422 NOAEL Maternal toxicity: = 2500 ppm; NOAEL Teratogenicity: 5000 ppm				
Developmental Toxicity / Teratogenicity	OECD Guide-line 422 NOAEL parental: = 2500 ppm; F1: >= 5000 ppm		OECD 414 NOAEL Maternal toxicity: > 8000 ppm; NOAEL Teratogenicity: > 8000 ppm			

* Endpoint where there was more than one study identified as a "critical" study. For the sake of this illustration only one study is shown. Multiple Endpoint data were not conflicting with data shown.

Step 6: Prepare category test plan

53.   No testing was proposed.

Step 7: Conduct the necessary testing

54.   No testing was proposed.

Step 8: Perform an external assessment of the category

55.   No new information was generated so this step was not performed.

Step 9: Fill data gaps by read-across, extrapolation, interpolation etc

56.   Read-across techniques can be applied to pure isomers within a category. For example, the log octanol-water partition coefficient (log P_{ow}) of *cis*-butene-2 is 2.3. If log P_{ow} data were not available for trans-butene-2, based on the structural similarity, the data for *cis*-butene-2 could be used to estimate the log P_{ow} of trans-butene-2, e.g. log P_{ow} =2.3. Selected properties of the pure isomers can also be used to

characterise a mixture containing the isomers. Using butene isomer log P_{ow} data as an example, if only butene-1 and isobutene data were available, 2.4 and 2.3, respectively, those data could be used to characterise the log P_{ow} of a substance containing all the butene isomers. The log P_{ow} value for such a substance could be represented as 2.3 to 2.4.

57.   Although the previous discussion focused on a physicochemical endpoint, extrapolation of environmental and mammalian toxicity data are also possible from category members with data to those without data. It was possible to calculate select acute aquatic toxicity endpoints, including those within the Programme. However, if that had not been possible, the acute fish toxicity data for cis-butene-2 could have been used to estimate the toxicity of trans-butene-2. The appropriateness of this read-across would be justified based on the knowledge that the mode of toxic action for hydrocarbons is non-polar narcosis and that the toxic mechanism is disruption of biological membrane function. Therefore, because these substances would exert a similar biological effect and they would be expected to do so over a relatively narrow range. Based on their similar log P_{ow} values, an effect value (i.e., 96-hour fish LC50) for one butene isomer could be used to estimate the toxicity of another (the calculated fish toxicity values for the butene isomers range from 17 to 21 mg/L). Equally, if acute fish toxicity data were available for cis-butene-2, those data could be used to characterise the toxicity of a substance that contained cis-butene-2 and trans-butene-2.

58.   An example of read-across as it can be applied to human health endpoints includes the application of repeated dose toxicity studies for three of the substances in this category (butene-1, isobutene, and butene mixed -1 and -2 isomers) to characterise the three substances without data (cis-butene-2, trans-butene-2, and butene mixed isomers). The three substances with data clearly indicate that they have a low order of subchronic toxicity. Adaptive and reversible changes in the liver indicate butenes were widely distributed within the body and metabolised. No Observed Adverse Effect Levels (NOAEL) from studies for tested members over 28-days to 2 years range from 2,000 ppm to 8,000 ppm. By read-across, the untested butene substances would also be expected to demonstrate a low order of subchronic toxicity over the extent and within the range to which the tested butenes were subjected, or to the lowest concentration of the range at a minimum.

59.   Members of the Butenes Category that have been tested do not produce mutagenic responses either in in vitro or in vivo test systems. Butene-1, butene 1 and 2 mixed isomers, and isobutylene did not induce gene mutations in reverse mutation assays conducted in S. typhimurium and/or E. coli either in the presence or absence of metabolic activation. Butene-2 was not clastogenic to rat lymphocytes in vitro. Isobutylene tested negative in an in vitro cell transformation assay using a mouse embryo fibroblast derived cell line and in a mouse lymphoma assay both in the presence or absence of metabolic activation. In addition, neither 1-butene nor isobutylene induced micronuclei formation in mouse bone marrow cells. By read-across, these data support characterising the untested members of the Butenes Category as also having a low potential for carcinogenicity.

60.   Inhalation reproductive/developmental toxicity studies conducted with butene-1, butene-2 mixed isomers, and isobutylene resulted in a NOAEL for each study that was the highest exposure concentration tested (5,000 to 8,000 ppm). Based on the weight of the experimental evidence and the consistent absence of observed significant toxic findings, the untested members of the Butenes Category would be expected to have a low potential for chronic, reproductive, and/or developmental toxicity and cancer.

61.   Supportive evidence to consider the butenes as a category for mammalian toxicity endpoints is provided by an understanding of metabolism for selected butenes and their mode of action. Additionally, physicochemical data add to an overall understanding of the potential for distribution and data confirm that isomer form does not change toxicity. Metabolism of isobutylene via cytochrome P450 to an epoxide,

2-methyl-1,2-epoxypropane (MEP), has been demonstrated, and MEP has been identified as the primary metabolite in liver tissue of various species, including man. Epoxidation of *cis-* and *trans*-butene-2 has also been demonstrated. It is known that inhalation of these substances, only at very high concentrations, can produce central nervous system depression, anaesthesia and/or asphyxiation. For example, isobutylene is predicted to produce narcosis in man at concentrations exceeding the lower explosion limit of 18,000 ppm. Other butene substances would therefore also be expected to exhibit a similar low order of acute toxicity. Both the log P_{ow} and water solubility favour absorption via the lung and their small molecular weight and log P_{ow} suggest that butenes are likely to be widely distributed within the body. From data on tested butenes, it can be concluded that branching does not affect the toxicity of butenes. Evaluation of this information provides further support beyond the base toxicity data that butene substances would behave similarly in mammalian systems and exert similar degrees or lack of effects.

Example E : HYDROCARBON SOLVENTS

[Note: The categories for Hydrocarbon Solvents have not been assessed by SIAM yet. The description below is based on the preparatory work of the sponsor organisations (International Hydrocarbon Solvents Consortium and US-EPA), as well as the discussions held at the OECD workshop on the development and use of chemical categories held in January 2004. The descriptions below might have to be revised once the categories have undergone assessment at SIAM.]

Background

62.   Hydrocarbon solvents assessed under the OECD HPV Chemicals Programme include aliphatic hydrocarbon solvents in the C5-C20 range and aromatic hydrocarbon solvents in the C9-C12 range. Production of hydrocarbon solvents is differentiated from other refinery substances such as gasoline and diesel fuel by additional processing steps leading to finished substances with narrow distillation range, a defined aromatic content, removal of benzene, polyaromatic hydrocarbons (PAHs), sulfur- and nitrogen-containing compounds, and low color. These additional refining steps are necessary in order to make substances with qualities suitable for consumer applications. The specific isomer content of most hydrocarbon solvents is not specified as these products are complex substances, not preparations or intentional mixtures, and may contain dozens or even hundreds of individual isomers. However, these substances are well characterized for their general chemical composition in terms of their paraffin, cycloparaffin, and aromatic content. In addition, the composition of specific constituents of concern (e.g., n-hexane, naphthalene) is well defined.

Aliphatic Hydrocarbon Solvents

63.   Aliphatic hydrocarbons consist of carbon and hydrogen molecules arranged as straight chain (n-paraffins or n-alkanes), branched chain (isoparaffins), or cyclic hydrocarbons (naphthenes). They contain no reactive functional groups (e.g., alkenes, sulfides, alcohols, etc.). Commercial aliphatic hydrocarbon solvents can be individual hydrocarbons (e.g., n-pentane) or multi-constituent combinations of aliphatic hydrocarbons. These multi-constituent hydrocarbon solvents include products that are composed of one type of hydrocarbon chemistry (e.g., isoparaffins) or multiple types (e.g., white spirits, Varnish Makers and Painters (VM&P) Naphtha). Multi-constituent hydrocarbon solvents are primarily defined by distillation range and flash point. In addition, aliphatic hydrocarbon solvents are also be defined by the amount of aromatic compounds they contain. The petroleum feedstocks used to make many aliphatic hydrocarbon solvents contains some aromatic hydrocarbons (normally less than 23%). For some aliphatic hydrocarbon solvents, such as regular mineral spirits or white spirits, these aromatic hydrocarbons are purposely retained in the final product to achieve or enhance certain solvency properties. The term dearomatized refers to those products that have undergone an additional step to remove or hydrogenate the aromatic compounds contained in the feedstock (e.g., dearomatized mineral spirits), resulting in an aliphatic hydrocarbon solvent with little or no aromatic content (≤2%). Some aliphatic hydrocarbon solvents, such as isoparaffins or n-paraffins, are produced through a polymerization process that involves the hydrogenation of an olefin (e.g. ethylene, propylene) oligimerization product. Petrochemicals produced in this manner are commonly referred to as synthetics because they are synthesized from smaller molecules rather than being derived from petroleum streams.

Aromatic Hydrocarbon Solvents

64.   Aromatic hydrocarbons in the C9-C12 range are either one aromatic-ring structures with alkyl side chains (alkylbenzenes) or two aromatic-ring structures with alkyl side chains (alkylnaphthalenes). C9 aromatic hydrocarbon solvents contain isomers of trimethylbenzene, isomers of ethyltoluene, cumene (isopropylbenzene), n-propylbenzene and small amounts C8 and C10 aromatic hydrocarbons. Aliphatic

hydrocarbons of similar molecular weight may also be present in very small amounts, generally <1%. These products contain only traces of benzene (<10 ppm) and toluene (<100 ppm). Aromatic hydrocarbon solvents in the C10-C12 range contain isomers of alkylbenzenes and alkylnaphthalenes; some contain up to 10% naphthalene.

Step 1: Identify structure-based category and its members.

Hydrocarbon Solvent Categories

65.   Nine categories were identified to address the different types of commercial hydrocarbon solvents in the C5-C20 range. These categories are listed below and each category is addressed in separate test plans.

> C5 Aliphatic Hydrocarbon Solvents
> C6 Aliphatic Hydrocarbon Solvents
> C7-C9 Aliphatic Hydrocarbon Solvents
> C9-C13 Aliphatic [≤2% Aromatics] Hydrocarbon Solvents
> C9-C13 Aliphatic [2-23% Aromatics] Hydrocarbon Solvents
> C14-C20 Aliphatic [≤2% Aromatics] Hydrocarbon Solvents
> C14-C20 Aliphatic [2-35% Aromatics] Hydrocarbon Solvents
> C9 Aromatics Hydrocarbon Solvents
> C10-C12 Aromatics Hydrocarbon Solvents

66.   There are many different hydrocarbon solvent substances within the C5-C20 range being addressed. While there may be several potential ways to categorize and review these substances, the above categories appear to be the best way of organizing the substances based on physical-chemical properties (e.g. vapor pressure), commercial applications, uses, and toxicity. In developing these categories, it was intended that similar commercial products, with similar applications, be in the same category. Substances with similar hydrocarbon structures (e.g., aromatic) would be placed in the same category. Finally, special consideration was given to previously reviewed substances. For example, a separate category was developed for C9 Aromatic Hydrocarbon Solvents because the of U.S. EPA Toxic Substances Control Act (TSCA) C9 Test Rule on C9 aromatics (ethyltoluene and trimethylbenzene isomers).

Underlying Hypothesis for the Formation of the Categories

67.   There are essentially two key underlying hypotheses for the formation of the hydrocarbon solvent categories. The first is that hydrocarbon solvents can be grouped around major product types (e.g., hexanes, VM&P Naphtha, Mineral Spirits/White Spirits, etc.). Because there are so many different commercial and trade names used for hydrocarbon solvents, it was decided that generic names (e.g., C7-C9 Aliphatic Hydrocarbon Solvents) would be the most appropriate for the OECD HPV Chemicals Programme. While commercial hydrocarbon solvent products are frequently defined by distillation range, it was felt that grouping hydrocarbon solvents by predominant carbon number range would be easier and more practical than grouping the hydrocarbon solvents by boiling range because of simpler nomenclature, easier identification, and selection of representative constituents for modelling. The second hypothesis for the formation of the categories is that aliphatic isomers within a particular carbon range, regardless of them being straight-chained, branched, or cyclic, and aromatic hydrocarbons within a particular carbon range have similar physicochemical, environmental fate, and toxicological properties. The two defining toxicological characteristics of hydrocarbon solvents are central nervous system depression and upper respiratory tract irritation observed at high airborne concentrations. The hypothesis that these two toxicity endpoints are related to and can be predicted by carbon chain length and aromatic content is

supported by reviews appearing in various standard toxicology texts, including Patty's Industrial Hygiene and Toxicology, Browning's Toxicology of Industrial Solvents, and Caserett and Doulls Toxicology. The single well-known exception is n-hexane, which produces a specific type of axonapathy resulting from metabolism to the gamma diketone 2,5-hexanedione. The specific structural requirement for the axononapthy induced by gamma diketones is also described in standard toxicology texts.

Technical and Practical Issues in Forming the Categories

68.   The grouping of hydrocarbon solvents was proposed around hydrocarbon chemistry (e.g. isoparaffins, n-paraffins, alkylbenzenes) and major product types (C9-C13 Multi-constituent Hydrocarbon Solvents). There are a number of important technical issues when evaluating complex substances. One is that environmental fate and physicochemical data cannot be modelled for complex substances. To address this issue, representative constituents were identified for each category to cover the carbon range and hydrocarbon structures of the category. These constituents were then modelled for the SIDS environmental fate and physicochemical endpoints. Measured data on the commercial products is available for most of the physicochemical endpoints, so this is also provided. Generally the modelled constituent data and measured whole product showed good correlation, further supporting the categories. Major usage categories were also a practical consideration in forming the categories.

As an example, Table E.1 presents the proposed category for C7-C9 Aliphatic Hydrocarbon Solvents.

Table E.1: Substances in the C7-C9 Aliphatic Hydrocarbon Solvents Category

Class/Dossier	CASRN	Chemical Name
Normal Paraffins	142-82-5	Heptane
	111-65-9	Octane
	111-84-2	Nonane
Isoparaffins	70024-92-9	Alkanes, C7-8-iso-
	90622-56-3	Alkanes, C7-10-iso-
Multi-constituent	8032-32-4	Ligroine
C7-C9 Aliphatics	64741-63-5	Naphtha, (petroleum), light catalytic reformed
	64741-84-0	Naphtha, (petroleum), solvent-refined light
	64742-48-9	Naphtha, (petroleum), hydrotreated heavy
	64742-49-0	Naphtha, (petroleum), hydrotreated light
	64742-89-8	Solvent naphtha, (petroleum), light aliph.
	92045-53-9	Naphtha (petroleum), hydrodesulf. light, dearoma.
	426260-76-6	Heptane, branched, cyclic and linear

Step 2: Gather published and unpublished data for each category member.
Step 3: Evaluate available data for adequacy
Step 4: Construct a matrix of data availability (SIDS endpoints vs. category members) and indicate in the cells of the matrix whether existing data are available.

69.   These three steps were essentially done almost in parallel once the categories were formed. Available data from company proprietary files, the peer-reviewed literature, and modelled data for environmental fate and physicochemical endpoints were collected, placed in a matrix and evaluated. Data were evaluated for study reliability in accordance with the OECD guidance. Only studies which met the reliability criteria of "1" (valid without restriction) or "2" (valid with restrictions) were included in this review. In a few cases, additional data for substances with the same CAS RN exist in the ECB (European Chemicals Bureau) IUCLID (International Uniform Chemical Information Dataset). These data were evaluated and included in the review only if it was determined that the substances were compositionally relevant and met the reliability criteria of "1" or "2." After these data evaluations were complete, the any necessary testing to fill data gaps was proposed. Preliminary recommended changes from EPA on the test plans included the proposed addition of chronic aquatic toxicity data for categories with a log Kow above 4.2. Table E.2 and E.3 provide the matrices for the C7-C9 Aliphatic Hydrocarbon Solvents Category.

Table E.2 C7-C9 Aliphatic Hydrocarbon Solvents Category – Toxicity Endpoints Matrix

Endpoints	Multi-Constituent Substances (i-,n-,cy-)	Isoparaffins	Normal Paraffins
Acute	1[1] - Low	- Low	- Low
Assigned Value[2]	**Low**	**Low**	**Low**
Repeat-Dose	- No Target Organ	- No Target Organ	- CNS/No Target Organ
Assigned Value	**No target organ effects; CNS at high conc.**	**No target organ effects; CNS at high conc.**	**No target organ effects; CNS at high conc.**
Genetic – *in vitro*	-Negative (bac.) -Negative (mam.)	-Negative (bac.) -Negative (mam.)	-Negative (bac.) Read-Across (mam.)
Assigned Value	**Negative**	**Negative**	**Negative**
Genetic – *in vivo*	-Negative (new testing)	-Negative	Read-Across
Assigned Value	**Negative**	**Negative**	**Negative**
Reproductive	- No Repro Effects	Read-Across	Read-Across
Assigned Value	**No Repro Effects**	**No Repro Effects**	**? – Under Discussion**
Developmental	- No Develop Effects	- No Develop Effects	Read-Across
Assigned Value	**No Develop Effects**	**No Develop Effects**	**No Develop Effects (?)**

[1] - Study Available.
[2] Assigned Value – Given available data, information, and construct of category, the value assigned to cells for which there are no data.

Table E.3 C7-C9 Aliphatic Hydrocarbon Solvents Category – Aquatic Endpoints Matrix

Endpoints	Multi-Constituent Substances (i-, n-, cy-)	Isoparaffins	Normal Paraffins
Acute Fish	D[1] – Moderate	Read-across	D - Moderate
Assigned Value[2]	Moderate	Moderate	Moderate
Acute Invertebrate	D - Moderate	Read-across	D - Moderate
Assigned Value	Moderate	Moderate	Moderate
Algae Toxicity	D - Moderate	Read-across	Read-across
Assigned Value	Moderate	Moderate	Moderate
Chronic Invertebrate	D (Test Underway)	Read-across	Read-across
Assigned Value			

[1] D - Study available
[2] Assigned Value – Given available data, information, and construct of category, the value assigned to cells for which there are no data.

Use of Read-Across Data

70.   The categories were initially developed around commercial hydrocarbon solvents. The available data were then reviewed and the categories were evaluated based on this data; in some case the categories were reorganized.   As these product categories are in sequence and data are available at a number of points across the spectrum of hydrocarbon solvents, use of read-across data was considered in some cases. Use of read across is limited to chemicals of very similar structure. For example, in the C7-C9 Aliphatic Hydrocarbon Solvents category data for C7-C9 normal paraffins, C7-C9 isoparaffins, and complex C7-C9 multi-constituent aliphatic substances (products containing normal, iso-, and cycloparaffins) were used to read across (either quantitatively or qualitatively) to cover all the substances in the category. Where data were not available and read-across could not be justified, additional testing was proposed.

Step 5: Evaluate the category approach.

71.   Since most of the SIDS endpoint data for hydrocarbon solvents were already available, the verification of the two key category hypotheses generally occurred simultaneously to the assembly and evaluation of the data for each category.   Generally the IHSC found that good correlation of the data suggesting that the proposed categories are appropriate. In one case, the initial assembly and evaluation of the data caused the reorganization of the categories. In this case, originally one category was considered for the C9-C13 Aliphatic Hydrocarbon Solvents. The toxicology endpoint data showed good correlation across this category; however, the products with higher aromatic content (up to 23%) in this category showed somewhat greater respiratory irritation and more water solubility due to the greater solubility of the aromatic fraction, resulting in correspondingly greater potential to cause aquatic toxicity at similar loading rates. To address this difference in irritation, water solubility and aquatic toxicity, it was decided to divide the category into two categories – one with essentially no aromatic content and one with products up to 23% aromatic content. Once the testing is complete, where planned, these additional data will be evaluated to determine whether the categories are valid or need to be reorganized.

Step 6: Prepare category test plan

72.   Category test plans have been developed and were submitted to the Sponsor Country (U.S.) for evaluation and consideration prior to submission to OECD.

Step 7: Conduct necessary testing

73.   Underway for those categories that have been evaluated by EPA.

Step 8: Evaluate new and existing data for the category and make robust study summaries for new data

74.   To be completed upon completion of testing.