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CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR REPEAT DOSE TOXICITY OF SUBSTITUTED DIPHENYLAMINES (SDPA)

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IOMC INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2016

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FOREWORD

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This case study was developed by Canada for illustrating practical use of IATA in a regulatory context and submitted to the 2015 review cycle of the IATA Case Studies project. This case study was reviewed by the project team and revised to consider the comments from reviewers. The document was endorsed at the 9th Task Force on Hazard Assessment meeting in June 2016.

The following three case studies were also reviewed in the project in 2015 and are published with this case study:

1. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR IN VITRO MUTAGENICITY OF 3,3' DIMETHOXYBENZIDINE (DMOB) BASED DIRECT DYES, ENV/JM/MONO(2016)49, Series on Testing & Assessment No. 251.

2. CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR HEPATOTOXICITY OF ALLYL ESTERS, ENV/JM/MONO(2016)51, Series on Testing & Assessment No. 253.

3. CASE STUDY ON THE USE OF AN INTEGRATED APPROACH FOR TESTING AND ASSESSMENT OF THE BIOACCUMULATION POTENTIAL OF DEGRADATION PRODUCTS OF 4,4'-BIS (CHLOROMETHYL)-1,1'-BIPHENYL, ENV/JM/MONO(2016)52, Series on Testing & Assessment No. 254.

In addition, a considerations document summarizing the learnings and lessons of the review experience of the case studies is published with the case studies:

REPORT ON CONSIDERATIONS FROM CASE STUDIES ON INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA) -First Review Cycle (2015): Case Studies on Grouping Methods as a Part of IATA- ENV/JM/MONO(2016)48, Series on Testing & Assessment No. 250.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

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INTRODUCTION

The Substituted Diphenylamines (SDPA) substances are part of the Groupings Initiative of the Government of Canada's Chemical Management Plan (CMP). The grouping consists of 14 substances that were identified as priorities for action as they met the categorization criteria under section 73 of CEPA 1999, and/or were considered as a priority based on ecological and/or human health concerns (Environment Canada and Health Canada 2013).

This case study aims to illustrate the application of read across to address data gaps within the SDPA grouping with respect to repeat dose toxicity via the oral route. The case study outlines available and relevant information considered by Health Canada for formation of subgroups and the application of read across between tested and non-tested substances.

The approach outlined in the case study was expanded to support further discussion and guidance development under the OECD Case Study Project. Accordingly, the analysis is subject to change based upon input received and may not reflect the final approach applied for the planned Screening Assessment Report currently under development by Health Canada and Environment Canada.

This document is not intended to provide complete characterization of health effects for the SDPAs. Also, it does not provide information regarding exposure of the general population of Canada to SDPAs. These elements, along with risk characterizations, will be presented in the subsequent screening assessments and related documents developed under Canada's Chemicals Management Plan.

1. PURPOSE

1.1 Purpose of use

A comprehensive risk assessment is not presented in this document. Rather, the case study focuses on applying a grouping and read across approach to address data gaps on untested substances for oral repeat dose toxicity. The purpose of the case study is to provide insights to further develop guidance related to grouping and IATA approaches. This case study is intentionally limited to repeat dose toxicity. Elements of the case study are intended to support regulatory decision making under Canada's Chemicals Management Plan (CMP).

1.2 Category definition

Subgroups of structurally related substituted diphenylamine (SDPA) compounds have been established in order to aid the oral repeat dose hazard characterization of these substances.

SDPAs are widely used lipophilic antioxidants. They are made up of a diphenylamine core and one to four alkyl or phenyl side chains. The common synthetic pathway for the production of SDPAs is through an electrophilic aromatic substitution reaction between an olefin and diphenylamine (DPA) through reductive alkylation. The starting material, DPA, is reacted with an olefin in the presence of hydrogen. The resulting reaction product is typically purified by distillation (RAPA 2003).

The general structure of SDPAs is presented in Figure 1-1. The amine group acts as an electron donating group and therefore the electrophilic aromatic substitution by alkenes of DPA will occur at the para (preferred) and/or ortho position to the amine. SDPAs in the subgroups have 1-4 substituents on the diphenylamine core.

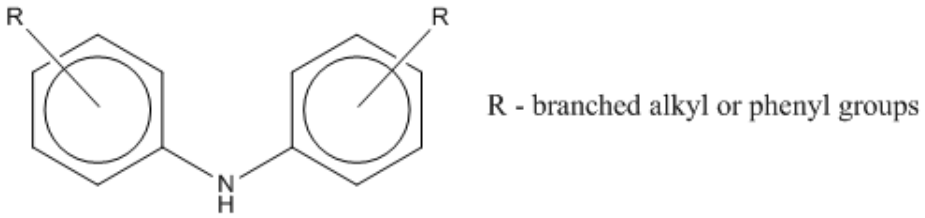


Figure 1-1 General structure common to SDPAs

The chemical structures of SDPAs vary according to the olefin used for synthesis, the manufacture process, and the number and position of substituents on the aromatic ring. Therefore most SDPAs are UVCB-type substances. However, in the grouping there are substances where the position and branching pattern of the side chain is specified in the chemical name (e.g Benzenamine, 4-(1,1,3,3-tetramethylbutyl)-N-[4-(1,1,3,3-tetramethylbutyl)phenyl]-).

The SDPA substances within this approach are further sub-grouped for applying read-across based on considerations of molecular structure including degree and type of substituent as well as composition for

UVCBs. Properties including molecular weight, logK_{ow}, and predicted oral bioavailability are also considered as the basis for forming subgroups. Differences in observed effects were also noted across the subgroups, although similarities were seen as well. The considerations for subgrouping are further discussed in Section 4.

The subgroups developed for the application of read-across are as follows:

- Subgroup 1 - Monoalkylated SDPAs
- Subgroup 2 - SDPAs with variable number of alkyl substitutions
- Subgroup 3 – Dialkylated SDPAs
- Subgroup 4 - SDPAs with variable number of phenyl substitutions

SDPAs not considered part of subgroups 1-4:

- SDPAs with variable number of alkyl *and* phenyl substitutions

1.3 Endpoint(s)

- Repeat dose toxicity via the oral route is the focus of the approach. The extent and nature of systemic effects following repeat dose oral exposures in rats vary depending on the SDPA (explained in section 4). However, depending on the subgroup, read across is conducted for the following effects:
    - Histopathology changes in the liver (e.g. centrilobular hepatocyte enlargement, lipid vacuolation and/or minimal hypertrophy) with accompanied alterations in clinical chemistry parameters associated with liver function.
    - Haematological effects related to coagulation postulated to be secondary to liver toxicity.
    - Histopathological changes in the kidney (degenerative changes) or thyroid (slight hypertrophy or hyperplasia) also postulated to be secondary to liver toxicity.
    - Pigment accumulation in the spleen (haemosiderin deposition), elevated haemoglobin and bilirubin attributed to erythrocyte destruction.

2. HYPOTHESIS

SDPAs share a common amine functional group within the diphenylamine core with one to four alkyl or phenyl side chains. The structural differences are related to the type and degree of substitution. It is hypothesised that subgroups of SDPAs can be formed based on structural similarity and as a result, the substances exhibit a similar trend in physicochemical properties, oral bioavailability, and observed toxicological effects. Read-across can be applied within subgroups for untested members and the associated uncertainty is acceptable to support a screening level risk assessment.

3. CATEGORY MEMBERS

3.1 Identification and selection of category members

The grouping consists of 14 substances that are on Canada's Domestic Substance List (DSL) identified as priorities for action as members met the categorization criteria under section 73 of the *Canadian Environmental Protection Act* (CEPA 1999) (Environment Canada and Health Canada 2013). The Canadian DSL inventory was searched using both automated and manual methods to derive the list of substances based on the criteria in Table 3-1.

Table 3-1 Selection criteria that reflect the read-across hypothesis were applied in order to identify suitable group members

Criteria for category members	Reasoning
1) Common functional group: - Must have the diphenylamine (DPA) substructure - No other functional groups are permitted.	The SDPA members share a common functional group based on the diphenylamine core, which is chemically reactive and considered to be relevant to the overall toxicity. The amine is considered responsible for the for the desired antioxidant effect for industrial use.
2) Side Chains: - Structural similarity criteria. Must have saturated alkyl chain and/or phenyl substitutions (number of substitutions can vary between 1 and 4). - The number of carbons on the alkyl side chains can vary between 4 and 9.	The SDPAs share one, two or more hydrophobic alkyl or phenyl side chains. It is expected that the degree of DPA substitution and number of carbons on the side chains will influence physicochemical properties and kinetics such as the oral bioavailability and metabolic pathway in mammals. On this basis the SDPAs can be further sub-grouped to account for these differences when applying read-across.

To facilitate a more robust group analysis and read-across for oral repeat dose toxicity, the following databases/tools were used to search for additional SDPAs meeting the criteria outlined in Table 3-1.

- OECD QSAR Toolbox version 3.3.0 (OECD 2014) was used to search for additional SDPAs with required endpoint data. A custom profiler was created specifically for SDPAs meeting the above criteria. All human health hazard databases included in this version were selected for the search.

- The Health Canada Existing Substances Risk Assessment Bureau (ESRAB) internal database of toxicity information (maintained in IUCLID 5.4) was also searched via the OECD Toolbox using the criteria set out in Table 3.1.

No additional SDPAs were identified beyond what was captured through searching the Canadian DSL. However, there was considerable overlap between the Canadian DSL and SDPA substances that are currently registered under the European Chemical Agency's REACH program and other international inventories (confirmed via OECD QSAR Toolbox).

3.2 List of category members

As previously noted, the chemical structures of SDPAs vary according to the olefin used for synthesis, and the production process. As a result, most substances covered in the approach are UVCB mixtures containing a number of different chemical structures in varying concentrations where the alkylation of diphenylamine by various olefins produces various substitution patterns and variable branching patterns. General chemical structures are provided in Tables 3-2 to 3-5. Where the branching pattern was known either through available information or by examining the substance name, it is used for the general structure. When the branching pattern for the olefin could not be determined, a general molecular formula was used to describe the side chain.

Table 3-2 Subgroup 1 - Monoalkylated SDPAs

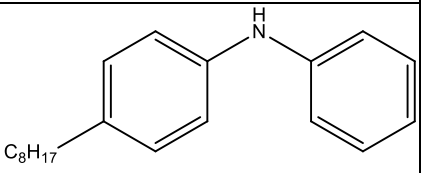
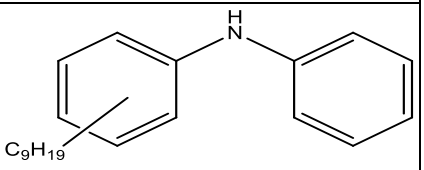
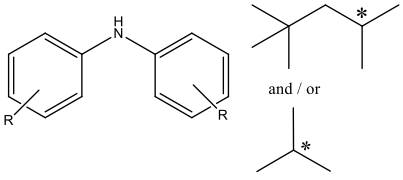
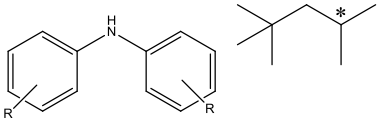
CAS RN	Chemical Name	Inventory Affiliation	General Chemical Structure
4175-37-5	Benzenamine, 4-octyl-N-phenyl-	Canadian DSL	
27177-41-9	Benzenamine, ar-nonyl-N-phenyl- (UVCB)	Canadian DSL	

Table 3-3 Subgroup 2 - SDPA mixtures with variable number of alkyl substitutions

CAS RN	Chemical Name	Inventory Affiliation	General Chemical Structure(s)
184378-08-3	Benzenamine, *N*-phenyl-, reaction products with isobutylene and 2,4,4-trimethylpentene (UVCB)	Canadian DSL; EPA HPV Challenge Program	R =  Attachment occurs at * carbon.
68411-46-1	Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (UVCB)	Canadian DSL; REACH Registered Substance; EPA HPV Challenge Program	R =  Attachment occurs at * carbon.

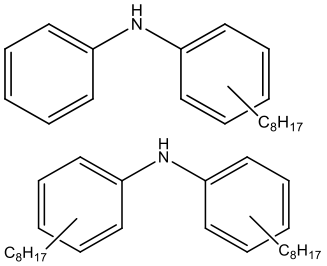
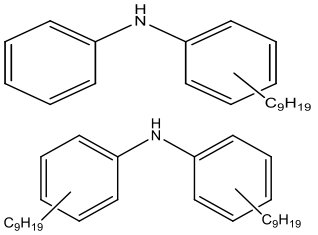
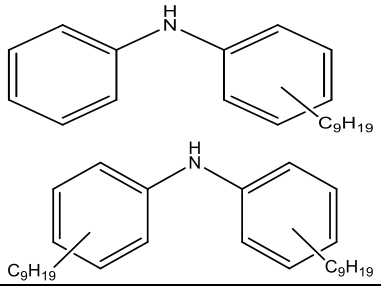
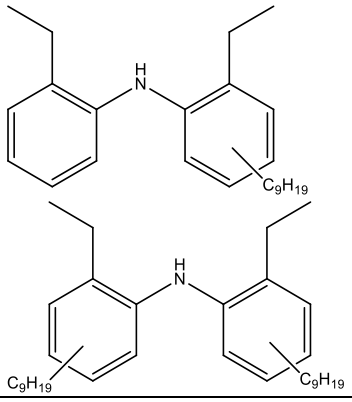
CAS RN	Chemical Name	Inventory Affiliation	General Chemical Structure(s)
26603-23-6	Benzenamine, ar-octyl-N-(octylphenyl)- (UVCB)	Canadian DSL	
36878-20-3	Benzenamine, ar-nonyl-N-(nonylphenyl)- (UVCB)	Canadian DSL; REACH Registered Substance; EPA HPV Challenge Program	
68608-79-7	Benzenamine, N-phenyl-, (tripropenyl) derivs. (UVCB)	Canadian DSL	
68608-77-5	Benzenamine, 2-ethyl-N-(2-ethylphenyl)-, (tripropenyl) derivs. (UVCB)	Canadian DSL; EPA HPV Challenge Program	

Table 3-4 Subgroup 3 – Dialkylated SDPAs

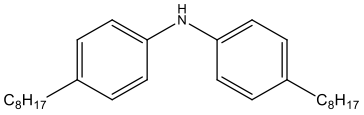
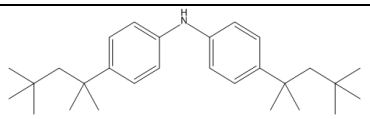
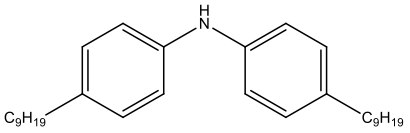
CAS RN	Chemical Name	Inventory Affiliation	General Chemical Structure(s)
101-67-7	Benzenamine, 4-octyl-N-(4-octylphenyl)-	Canadian DSL; EPA HPV Challenge Program	
15721-78-5	Benzenamine, 4-(1,1,3,3-tetramethylbutyl)-N-[4-(1,1,3,3-tetramethylbutyl)phenyl]-	Canadian DSL; REACH Registered Substance	
24925-59-5	Benzenamine, 4-nonyl-N-(4-nonylphenyl)-	Canadian DSL	

Table 3-5 Subgroup 4 – SDPAs with variable number of phenyl substitutions

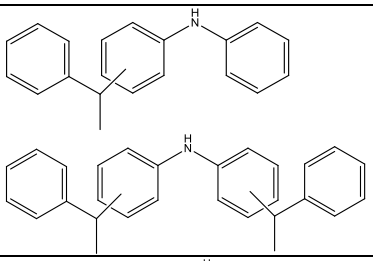
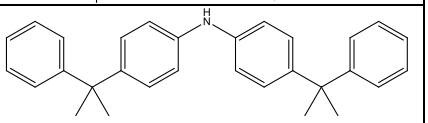
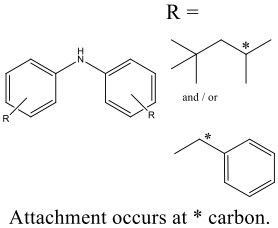
CAS RN	Chemical Name	Inventory Affiliation	General Chemical Structure(s)
68442-68-2	Benzenamine, N-phenyl-, styrenated (UVCB)	Canadian DSL	
10081-67-1	Benzenamine, 4-(1-methyl-1-phenylethyl)-N-[4-(1-methyl-1-phenylethyl)phenyl]-	Canadian DSL; REACH Registered Substance	

Table 3-6 SDPA mixture with variable number of alkyl and phenyl substitutions not considered part of a broader subgroup

CAS RN	Chemical Name[a]	Inventory Affiliation	Representative Chemical Structure(s)
68921-45-9	Benzenamine, N-phenyl-, reaction products with styrene and 2,4,4-trimethylpentene (UVCB)	Canadian DSL; REACH Registered Substance	

The general chemical structures in Table 3-2 to 3-6 are not amenable to modelling. For modelling, idealized representative structures for UVCB substance were selected based on synthetic chemistry principles and assumptions including:

- The aniline nitrogen will be ortho/para directing, however a preference is given to para-substitution due to steric effects of the N-phenyl group;
- For the olefins, the preferred reaction will proceed through the most stable carbocation intermediate ("Markovnikov type") to determine the point of attachment on the olefin(s);
- When the olefin is named in the CAS name of the substance, that olefin is used in deriving the representative structure / branching pattern;
- Where the branching pattern of the olefin was unknown, a branched isomer was selected for modelling. Branching of the alkyl chain does not have a significant effect on the calculated $\log K_{ow}$ or other toxicity-relevant phys-chem parameters (MW, pKa, or water solubility). The pivotal parameter is the number of carbons on the alkyl chain;
- The representative structures were also selected to span the anticipated range of oral bioavailability potential for the respective UVCB components.

Idealized representative structures are depicted in the accompanying data matrix file.

4. JUSTIFICATION OF DATA GAP FILLING

4.1 Data gathering

4.1.1 Empirical Data

For each SDPA, several available databases were searched with a focus on repeat dose toxicity via the oral route, physicochemical properties and toxicokinetics.

The available databases or tools used to conduct the data search included:

- OECD QSAR Toolbox datasets (publically available)
- ECHA REACH Registered Substances Database (publically available)
- EPA HPV Challenge Program
- Toxline (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE)
- ChemIDplus (http://chem.sis.nlm.nih.gov/chemidplus/)
- SciFinder (http://www.cas.org/products/scifinder)

- Literature searches using Scopus (using CAS RN, common name or chemical name)

Health Canada received study data submitted by stakeholders. In some cases, the data was requested confidential, particularly with respect to composition and as a result is not presented in this case study. The case study was limited to data available in the public domain or where permission to publically cite studies for CMP related assessment work has been granted.

4.1.2 Predicted Data

The models outlined in Table 4-1 were used to generate predictions or estimates to support the case study. For modelling, the representative structures outlined in the data matrix were used to derive the estimates. For UVCB substances, the representative structures of components were modelled individually.

Table 4-1 Models used to support the SDPA subgroup justification for read across

Model Name (version)	**Description of use**	**Available QMRF***	**Reference**
EPI Suite (v4.11) MPBPVP model KOWWIN model WSKOW model	Prediction of physical-chemical properties of category members.	No Similar Review	EPI Suite 2012
Lhasa, DEREK Nexus (v4.1)	Expert system used to screen category members for alerts related to systemic effects.	No	DEREK Nexus 2014
ACD Percepta (2012- v2076) PK Explorer	Estimates a number of parameters determining the pharmacokinetic profile by using a set of differential equations from a multi-compartment model describing the organism of an average statistical human: T_{max} and C_{max} AUC after oral admin Oral Bioavailability (%F)	No Information	ACD 2012
OASIS TIMES (v2.27.5) *In vivo* Rat Metabolism Simulator (v5.05)	The *in vivo* rodent metabolic simulator (transformation table) reproduces and predicts the metabolic pathways of xenobiotic chemicals *in vivo* in rodents	No Similar Appendix A	TIMES 2014

* (Q)SAR model reporting format (QMRF)

4.2 Data matrix

See data matrix file for data summary (Annex). Representative chemical structures are provided. Where observed, data on hepatotoxicity, clinical chemistry parameters, haematological effects, spleen, kidney and thyroid effects, physicochemical properties, and toxicokinetics parameters are all compiled in the data matrix. The overall NOAEL or LOAEL for systemic toxicity are defined. Individual LO(A)ELs for each sub-effect are also listed by sex in the data matrix (in parenthesis).

4.3 Justification

4.3.1 Structure

The SDPA members share a common functional group based on the diphenylamine core, which is chemically reactive and may relate to the toxicity of these substances. The amine is considered responsible for the desired antioxidant effect for industrial use. When screened for alerting groups with DEREK (Deductive Estimation of Risk from Existing Knowledge) Nexus software (DEREK Nexus 2014 (v4.1) from the LHASA Group, no alerts were found for any organ related effects. Although all substances share a common diphenylamine substructure, there are structural differences related to the degree of substitution and nature of the side chains. These differences correlate to observed differences in the physicochemical properties and toxicokinetics parameters. As a result, the SDPAs have been sub-grouped based on structural considerations of the side chains, namely the number of substitutions on DPA and type (alkyl vs phenyl) as well as composition for UVCBs. The members within each subgroup are considered structurally similar. The structurally related changes to properties including molecular weight, $logK_{ow}$, and predicted oral bioavailability are also considered as the basis for forming subgroups and outlined below.

4.3.2 Physicochemical properties and toxicokinetic parameters

Identified or modelled physicochemical properties for the substances covered under the proposed subgroups are presented in the data matrix file. Where empirical data was not available, EPI Suite was used to model $LogK_{ow,}$ water solubility, melting and boiling points, and vapour pressure. When comparing the empirical data for the non-mixture UVCB against the modelled data (e.g. Benzenamine, 4-(1-methyl-1-phenylethyl)-N-[4-(1-methyl-1-phenylethyl)phenyl]- CAS 10081-67-1) the models appear to provide a reasonable estimation in most cases for the parameters most likely to influence oral bioavailability (e.g. $logK_{ow}$). For UVCB mixture SDPAs, modelling the properties for the mixture was not possible and was conducted for the individual components representative of the larger portions in the UVCB.

A summary of a guideline toxicokinetics study was available for a single category member as part of a dossier submission to the REACH program (ECHA 2014a). Di(dimethylstyrenated) DPA (CAS RN 10081-67-1) was studied in male Wistar rats at two single oral dose levels of 10 and 80 mg/kg. The dosing was selected based on a corresponding 28-day repeat dose study where the LOAEL for haematology and clinical chemistry parameters was found to be 80 mg/kg/day. Calculated toxicokinetics parameters as presented by study authors are summarized in the data matrix. Maximum plasmatic levels generally occurred ~7h after dosing, consistent with no high or rapid adsorption. The results from the distribution analysis do not suggest a potential for accumulation in any tested organs and tissues, apart from liver. The substance was predominantly excreted via the faeces (≥80% AA) and a small extent via the urine (0.3-0.4% AA), suggesting a significant non-renal pathway. The relative contributions of these routes were dose dependent. The excretion by faeces at low dose was higher and faster than at high dose (97 vs. 79% AA). In all cases, approximately 2.5-2.7% of the dose remained in the carcass of rats at 96h. Elimination via the bile ranged from less than 0.05% AA at both doses. The utility of this study to support the subgrouping of the SPDAs is limited as Di(dimethylstyrenated) DPA (CAS RN 10081-67-1) is in a subgroup with only one other member (styrenated DPA) which also has sufficient repeat dose toxicity data and read across is not required. Dimethylstyrenated DPA has similar relevant physicochemical properties (MW, $LogK_{ow}$) to some of the alkylated SDPAs (e.g. dioctyl DPAs and dinonyl DPA). Absorption may be similar between these substances but due to difference with respect to the side chain (alkyl vs. phenyl) metabolism and excretion may differ.

In order to compare kinetic parameters for the members of the subgroups, predictions were generated for representative structures using ACD Percepta PK Explorer (ACD 2012). The model estimates a number of parameters related to the toxicokinetic profile of a substance by using a set of differential

equations from a multi-compartment model describing the organism of an average statistical human. Calculated parameters include: T_{max} and C_{max}, AUC after oral administration and oral bioavailability (%F of parent compound that reaches systemic circulation). Each component SDPA was modelled using an oral dose of 350 mg (corresponding to the lowest NOAEL observed for all SDPA substances (5 mg/kg) using a body weight of 70kg for human dose). The PK Explorer model does not provide reliability or applicability domain information. The model training set contains 790 compounds that were compiled from reference pharmacokinetic tabulations and various articles from peer-reviewed scientific journals. A cursory examination of the training set revealed no SDPA substances. $LogK_{ow}$ observed for the training set may not cover some of the more lipophilic SDPAs. As a consequence, reliability in the quantitative values generated is considered low for the model results but the results are useful for a comparative analysis.

Subgroup 1 - Monoalkylated SDPAs

This group consists of mononononyl and monooctyl DPAs. The number of carbons on the side chain differs by one and as a consequence these two substances have very similar MW, $LogK_{ow}$, water solubility, melting and boiling points, and vapour pressures (see data matrix). The branching pattern for the monooctyl derivative is not known. However, the differences in branching are not expected to have a significant impact on the most relevant toxicity related phys-chem parameters such that the chemicals would no longer be considered similar with respect to properties. The exception to this is melting point as branching can significantly impact this parameter.

Taking the results from the short-term toxicity testing with the mononononylated DPA, the substance appears to be absorbed from the gastrointestinal tract as evident by the liver and spleen effects. Modelled toxicokinetics parameters are considered comparable between the two substances within this subgroup. Oral bioavailability of the parent compounds are not appreciably different (23.64 %F for monooctyl vs. 21.53 %F for mononononyl).

Subgroup 2 - SDPA UVCB mixtures with variable number of alkyl substitutions

Members within this group are complex mixtures where the number of DPA substitution varies within each mixture. The number of carbon atoms on the side chains also varies in the mixtures from 4 to 9 depending on the SDPA. As a consequence, the substances vary with respect to $LogK_{ow}$, water solubility, melting and boiling points, and vapour pressures (see data matrix) depending on the nature of the components in the mixture. For example, CAS 184378-08-3has components that have $logK_{ow}$ values that range from 5.2 (monobutyl DPA) to 10.8 (dioctyl DPA). There is a general trend for these parameters depending on the length of the side chain and number of substitutions. The substances are roughly ordered in the data matrix based on the presence of components with lower $LogK_{ow}$, molecular weight and water solubility which are thought to influence toxicity.

Likewise, there is variation with respect to predicted kinetic parameters based on the nature of the components in the mixtures. Monobutyl DPA has a predicted oral bioavailability (%F) of 39.35 vs. Dioctyl DPA with a much lower value of 0.43. Taking the results from the repeat dose toxicity testing across this subgroup for members with data, all substances appear to be absorbed from the gastrointestinal tract as evident by the observed effects. Since there is a variation across the subgroup with respect to relevant phys-chem properties and predicted oral bioavailability, we are proposing to apply read across between closest members of the subgroup by comparing structure/composition, relevant physicochemical properties and related change in oral bioavailability of the components.

Subgroup 3 – Dialkylated SDPAs

This group consists of dioctyl and dinonyl DPAs. In some cases the point of attachment of the side chain is specified in the chemical name. In other cases the substances is a mixture of isomers where attachment of the side chains onto DPA is variable. The number of substitutions does not vary and only the carbon numbers on the side chain vary by one within the subgroup. As a consequence these four substances have very similar, MW, $LogK_{ow}$, water solubility, melting and boiling points, and vapour pressures (see data matrix). The branching pattern varies across the group. However, the differences in branching are not expected to have a significant impact on the most relevant toxicity related phys-chem properties such that the chemicals would no longer be considered similar in this regard. The exception to this is melting point as branching can significantly impact this parameter.

Modelled toxicokinetics parameters are considered comparable between the four substances. Oral bioavailability of the parent compounds are considered low and not appreciably different (0.27 %F for dioctyl DPAs vs. 0.06 %F for dinonyl DPA).

Subgroup 4 – SDPAs with variable number of phenyl substitutions

This group consists of substances where the number of styrenated substitution on DPA varies. As a consequence, the substances vary with respect to $LogK_{ow}$, water solubility, melting and boiling points, and vapour pressures (see data matrix) depending on the nature of the components in the mixtures. There is a general trend for these parameters depending on the number of phenyl substitutions. The substances are roughly ordered in the data matrix based on the presence of components with lower $LogK_{ow}$, molecular weight and water solubility which are thought to influence toxicity.

Likewise, there is variation with respect to predicted kinetic parameters based on the nature of the components in the mixtures. Taking the results from the repeat dose toxicity testing across this subgroup, all substances appears to be absorbed from the gastrointestinal tract as evident by the observed effects.

Both substances have adequate repeat dose data and does not require read-across.

SDPAs not considered part of a broader subgroup:

CAS 68921-45-9

This is complex mixture where the number of DPA substitution varies within as well as the type (alkyl *and* phenyl substitutions). It has structural commonalties to both subgroups 3 and 4. However, it is considered appropriate to this separate from the other subgroups. This substance has adequate repeat dose data and does not require read-across.

4.3.3 Metabolism

A summary of a guideline toxicokinetics study (TG 417) was available for a single category member as part of a dossier submission to the REACH program (ECHA 2014a). Di(dimethylstyrenated) DPA (CAS RN 10081-67-1) was studied in male Wistar rats at two single oral dose levels of 10 and 80 mg/kg. The metabolite profile in the plasma was determined via LC/UV/MS. Parent compound was the main substance identified in plasma and feces (70-95% of radioactive dose), but one unidentified metabolite was observed in both plasma and feces (5% of radioactive dose in both media), and another unidentified metabolite was observed in feces (25% of the radioactive dose). These metabolites were thought to be

hydroxylated derivatives of CAS RN 10081-67-1. The results indicate that the substance is not extensively metabolized. Limited metabolism is also expected for other larger SDPAs that share similar phys-chem properties with CAS RN 10081-67-1.

Data pertaining to identification of metabolites for all other SDPAs was not available. A metabolic simulator (OASIS TIMES (v2.27.5) *in vivo* Rat Metabolism Simulator (v5.05)) was used to predict the metabolism of individual representative SDPA components (structures of predicted metabolites are in second tab in the data matrix excel file). The model predicts the metabolic pathways of xenobiotic chemicals *in vivo* in rodents based on a well characterized training set including 647 structurally different parent chemicals with 4382 observed metabolites. All SDPAs were considered within the property domain of the model but all were out of domain when atom centered fragments were considered. As a result, the confidence in the predictions is considered low. Both DPA ring-hydroxylation and side-chain hydroxylation are predicted. Oxidative metabolism at the secondary amine is not predicted for any of the substances. The mono alkylated SDPAs are predicted to undergo aromatic C-hydroxylation at the unoccupied para position to the amine. Subsequent quinone imine formation is predicted followed by imine hydrolysis to liberate a primary aromatic amine. The model indicates that both the probability and reliability of this occurrence is low due to other competing and more probable metabolic transformations. Coupling in phase II metabolism would result in more soluble metabolites that could be eliminated both via bile and kidney. Actual rate of absorption, metabolism and elimination across the SDPAs will depend on the degree and type of alkyl or styrene substitution as this affects solubility.

Subgroups were developed based on side chain substituents on the diphenylamine backbone and related differences on relevant phys-chem properties. It is reasonable to assume that substances that have similar structural features with respect to the side chains on one or both of the phenyl rings and phys-chem properties, would show similar metabolic profiles in mammalian organisms. This was also observed with the simulator.

While assessing similarity, when an analogue is structurally similar to the target based on common functional groups, the metabolism assessment focuses on the potential for the target and analogues to diverge in such a way that their toxicological properties become different (Wu et al. 2010). Within each subgroup, the metabolic profile for the analogues used for read across are not expected to substantial diverge in a way that their toxicological properties differ to their respective target chemicals.

4.3.4 Observed trends in endpoint data

Study summaries of available data for SDPA group members are presented below. Of the SDPAs tested, available data indicate that the substances have both similarities and differences with respect to repeat-dose toxicity depending on the subgroup.

Similarities in effects are observed with the target organ being mainly the liver with vacuolation, hepatocyte enlargement and/or minimal hypertrophy observed. These histopathological findings were accompanied by associated clinical chemistry parameters related to liver function, although with some exceptions treated as separate subgroups (see below). There were also haematopoietic effects related to blood clotting for some SDPAs but this has been attributed as secondary to liver toxicity by a few study authors and is not seen for all SDPAs. Thyroid also showed some effects for certain SDPAs but again has been attributed as secondary to liver toxicity and not always present. The spleen was a target organ for the subgroup containing exclusively monoalkylated SDPAs. More comparative discussion is provided for each subgroup below.

Subgroup 1 - Monoalkylated SDPAs

CAS 27177-41-9

One member within this subgroup was tested in a short-term 28 day repeat dose gavage study. The test substance identified as monononylated (branched) DPA (CAS 27177-41-9), was administered by gavage to rats at dose levels of 0, 15, 150 or 500 mg/kg bw/day for 28 days. Two recovery groups (5/sex/group) were treated with the high dose (500 mg/kg bw/day) or the vehicle alone for 28 consecutive days and then maintained without treatment for a further 14 days. The NOAEL was determined to be 15 mg/kg bw/day based on clinical signs of toxicity (increased salivation), increased relative liver weights, histopathological effects in the liver and spleen, as well as changes in haematological and clinical chemistry parameters in both sexes at the next highest dose of 150 mg/kg bw/day.

Histopathology findings in the liver included lipid vacuolation and centrilobular hepatocyte enlargement for either sex treated with 500 or 150 mg/kg bw/day. Recovery 500 mg/kg bw/day animals showed some regression in the hepatic conditions. The changes at 500 mg/kg bw/day were accompanied by changes in clinical chemistry parameters including reductions in total plasma protein and albumin concentration together with increases in plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) concentration when compared with controls. Males from this treatment group also showed a reduction in plasma creatinine concentration whilst females additionally showing an increase in plasma triglyceride level in comparison with controls. Effects extended to the 150 mg/kg bw/day treatment group with animals of either sex showing an increase in plasma ALP concentration when compared with controls. Females from this treatment group also showed a reduction in total plasma protein and albumin together with an increase in plasma triglyceride concentration in comparison with controls. Taken together, the histopathological findings in the liver with associated changes in clinical chemistry parameters are considered of toxicological relevance.

Animals of either sex treated with 500 mg/kg bw/day showed increase in prothrombin time (PT) when compared with controls. Males from this treatment group also showed an increase in activated partial thromboplastin time (APTT). These effects extended to the 150 mg/kg bw/day treatment group with males showing an increase in APTT and PT.

Histopathology findings in the spleen included pigment accumulation at 500 or 150 mg/kg bw/day for females. There was also an indication among males of a delayed splenic effect. The findings in the spleen at 500mg/kg bw/day were accompanied by reductions in haemoglobin and haematocrit for both sexes. Females additionally showed a reduction in mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in comparison with controls. Effects extended to the 150 mg/kg bw/day treatment group with females showing a reduction in MCH and MCHC when compared with controls. The changes are indicative of anaemia postulated to be haemolytic in nature. Associated with haemolysis are the elevated levels of bilirubin detected for both sexes indicating an increased degradation of haemoglobin attributable to erythrocyte destruction (Safepharm Laboratories 1999)

Lipid vacuolation and centrilobular hepatocyte enlargement are noted for SDPAs in other subgroups along with changes in clinical chemistry parameters associated with the liver. Changes associated with blood clotting are also found with other SDPAs. Effect levels are difficult to compare due to dose spacing. The observed spleen histopathology and reductions in haemoglobin and haematocrit are unique to this test substance (CAS 27177-41-9).

Subgroup 2 - SDPA UVCB mixtures with variable number of alkyl substitutions

CAS RN 184378-08-3

CAS 184378-08-3 is a UVCB mixture with components that span a range of oral bioavailability. Monobutyl DPA is on the high end while Dioctyl DPA is on the low end of bioavailability for components in this mixture. A combined repeat-dose/reproductive/development toxicity study was available using rats administered CAS RN 184378-08-3 via gavage for 43 days (males) or 54 days (females, PND4), consecutively, at dose levels of 0, 5, 25 or 125 mg/kg bw/day. A parental NOAEL of 5 mg/kg bw/day for systemic effects was determined based on liver hepatocyte enlargement in females at the next highest dose of 25 mg/kg bw/day (observed in males at 125 mg/kg bw/day), with associated clinical chemistry effect in both sexes and decreased white blood cell counts in males. At 125 mg/kg-bw/day, males had elevated absolute and relative liver weight. Skeletal muscle effects were also observed in females at 125 mg/kg bw/day. Haematological evaluation indicated increased levels of activated partial thromboplastin time (APTT) at 125 mg/kg bw/day in both sexes. There was also a decreased platelet count in both sexes prior to mating (SafePharm Laboratories 2006b). The clinical chemistry parameter changes (decreased total plasma protein, albumin and the albumin/globulin ratio levels, with elevated aspartate aminotransferase (AST) and alkaline phosphatase levels (ALP)) were indicative of liver toxicity, which were consistent with the observed centrilobular hepatocytes enlargement in females at ≥ 25 mg/kg bw/day and in males at 125 mg/kg bw/day.

CAS RN 68411-46-1

CAS 68411-46-1 is a UVCB mixture with components that span a range of oral bioavailability. Monooctyl DPA is on the high end while Dioctyl DPA is on the low end of bioavailability for components in this mixture. In one combined repeat-dose/reproductive/development toxicity study rats were administered CAS RN 68411-46-1 via gavage for 28 days (males) or 53 days (females), consecutively, at dose levels of 0, 25, 75 or 225 mg/kg bw/day. A parental NOAEL of 25 mg/kg bw/day for systemic effects was determined based on minimal hepatocyte hypertrophy and vacuolation at the next highest dose of 75 mg/kg bw/day with associated clinical chemistry effect in both sexes (decrease in plasma protein (females); decrease in albumin, increase in total bilirubin and increase in ALP (both sexes)). Similar effects were also noted for animals of both sexes at 225 mg/kg bw/day with increased severity. The study authors suggest that at 75 mg/kg bw/day, morphologic liver findings were regarded to be an adaptive, non-adverse response based on the minimal severity of the hypertrophy (correlated to pale discoloration) and low incidence and minimal degree of vacuolation in the females. However, when taken together with similar changes seen in clinical biochemistry parameters as high dose animals, and an approximate relative liver weight increase of 24% and 17% for males and females, respectively, the findings were also considered toxicologically relevant. Follicular cell hypertrophy of the thyroid gland was noted at an increased incidence in males treated at all dose levels. The study authors state that, the increased incidence of hypertrophy of the follicular epithelium was considered to be non-adverse based on the absence of a clear dose response and/or relation in severity of hypertrophy of the follicular epithelium of the thyroid gland, presence of hypertrophy of the liver, decrease in total T4 and increase in TSH (hypertrophy of the follicular epithelium of the thyroid glands may reflect an increase in thyroxin production in response to feedback mechanisms as a result of increased turnover of thyroxin). Additionally, the recorded severities/incidences of the hypertrophy of the follicular epithelium of thyroid were within background levels noted in male rats of this strain and age (ECHA 2014d).

In a range finding study conducted over a period of 28 days in rats, CAS 68411-46-1 was administered by gavage at 0, 125 and 300 mg/kg bw/day. At 125 mg/kg bw/day minimal to slight centrilobular hepatocellular hypertrophy, minimal single cell necrosis and increased severity of lymphoid cell infiltrates were noted in the liver. This was accompanied by an increase in ALP (ECHA 2015b).

CAS RN 36878-20-3

CAS 36878-20-3 is a UVCB mixture with components that span a range of oral bioavailability. Monononyl DPA is on the high end while dinonyl DPA is on the low end of bioavailability of components in this mixture. A subchronic toxicity study was available for CAS RN 36878-20-3. The test substance was administered by gavage to rats for 92-93 days at dose levels of 0, 100, 300 or 1000 mg/kg bw/day. The NOAEL could not be established as adverse effects were seen at the lowest tested dose (LOAEL). Males and females of all treatment groups showed liver cell hypertrophy and fatty change. Males showed in addition a minimal increase in single cell necrosis, when compared to control animals at the low-dose. The liver weight increase in males of all treated test groups and females mid and high dose were regarded to be consequence of the liver cell changes observed. Associated clinical chemistry parameters were also changed. There was a decrease in albumin in both sexes at the high dose, increase in ALP activities (starting at the low dose for females and mid-dose males), increase in triglyceride in females at mid-dose and decrease in bile acids for both sexes at the low dose. Taking the observations regarding the liver with the findings in clinical chemistry parameters, the liver cell hypertrophy was regarded to be treatment related and adverse at all doses groups in males and females.

Hypertrophy/hyperplasia and altered colloid of follicular cell in the thyroid gland of males and females were observed in all treated test groups. This was regarded by the study authors to be a secondary event to the liver cell changes and most likely induced by liver enzyme induction. Therefore it was regarded to be treatment-related but not adverse.

Prolonged prothrombin time (PTT) in males was observed in the mid and high dose group. The study authors postulated that this was also related to liver effects based on a decreased synthesis of coagulation factors.

Subgroup 3 – Dialkylated SDPAs

CAS RN 101-67-7

In a combined repeated dose reproductive/developmental toxicity study in rats, 4,4'-dioctyldipehnylamine (CAS 101-67-7) was administered via gavage at 0, 25, 75, and 250 mg/kg bw/day for 42 days for both sexes. A parental lowest observed adverse effect level (LOAEL) of 75 mg/kg bw/day (NOAEL of 25mg/kg bw/day) was determined based on a dose-related increase in the haematological measure, activated partial thromboplastin (APTT) time in male rats, with prothrombin time (PT) increased in males at 250 mg/kg bw/day. At the end of a 14-day recovery period, although, PT and APTT showed no abnormalities, decreased RBC, and increased MCHC and reticulotytes were noted in females in the 250 mg/kg recovery group. No effects were observed on detailed clinical observation, reflexes/reactions, body weight, food consumption, clinical chemistry, necropsy or histopathology examination (Japan CERI 2007).

The absence of histopathological findings in the liver or changes in clinical chemistry parameters up to 250 mg/kg bw/day distinguishes this SDPA from other alkylated DPAs previously described. An important difference between this substance and other alkylated DPAs with similar testing is that this substance does not contain any monoalkylated DPAs, which are perceived to have more favourable absorption and oral bioavailability due to more moderate values of $LogK_{ow}$. The test substance is described as a linear dioctylated DPA where attachment on DPA is known (4,4' positions). It is postulated that the absence of the smaller mono alkylated DPAs in the test substance could be related to the absence of liver related histopathology at the dose range tested. The diakylation with octyl chains could also be increasing steric hindrance around the amine functional group. This may also influence toxicity but without a mode of action for the liver related effects for other SDPAs, it is speculative. Parameters, related to blood clotting were altered with this test substance which could be secondary to a liver effect noted by

study authors for other SDPAs. However, in the absence of histopathological changes in the liver or other clinical chemistry parameters related to the liver, this is difficult to assume. According to the REACH registration dossier for CAS 15721-78-5 (Benzenamine, 4-(1,1,3,3-tetramethylbutyl)-N-[4-(1,1,3,3-tetramethylbutyl)phenyl]-) there is a planned repeat-dose 90-day guideline study (ECHA 2014b). This substance is also a discrete dioctylated DPA but with branched side chains. The results of this test would be beneficial to confirm the absence of liver effects for mono-constituent dioctylated DPAs.

Subgroup 4 – SDPAs with variable number of phenyl substitutions

CAS RN 68442-68-2

In a combined repeated-dose/reproductive/developmental toxicity screening test, rats were administered CAS 68442-68-2 via gavage at 0, 50, 250 and 600 mg/kg-bw/day for 43 (males) and 54 (females) days. Absolute and relative liver and adrenal weights were increased in both sexes at the 600 mg/kg-bw/day. Histopathological examination of the liver revealed centrilobular hepatocyte enlargement in all treated females and in males treated with 250 and 600 mg/kgbw/day. Reduced cholesterol levels were reported in males at 250 and 600 mg/kg-bw/day, and an increased activity for alkaline phosphatase (ALP) was noted in males at 600 mg/kg-bw/day. The authors suggest that the changes seen in liver are adaptive in nature. No haematological effects were observed. Follicular cell hypertrophy was observed for both sexes treated with 600 and 250 mg/kg/day. No such effects were noted for animals of either sex treated with 50 mg/kg/day. The authors suggest that thyroid follicular cell hypertrophy is commonly associated with liver changes due to the induction of thyroxine metabolising enzymes and resulting changes in thyroid follicular cells in rats are often seen at a higher incidence in males than females (SafePharm Laboratories 2006a [cited in ACC 2006; US EPA 2009]).

The histopathological findings in the liver, coupled with the decrease cholesterol and follicular cell hypertrophy of the thyroid are indicative of changes in liver function at 250 mg/kg bw/day in males, although possibly adaptive in nature, are considered of toxicological relevance. As such the NOAEL is interpreted to be 50 mg/kg bw/day by Health Canada.

CAS RN 10081-67-1

In a short-term 28-day repeat dose study, CAS RN 10081-67-1 was administered via gavage at 0, 10, 40, and 80 mg/kg bw/day to rats. Twelve animals (per sex) were used at each dose level. In addition, a satellite group of 8 animals (per sex) of control and 8 animals (per sex) of the highest dose group were used for information about reversibility, persistence or delayed occurrence of any toxic effects after 14 day recovery period. A significant increase of the relative weights of liver was observed in high and medium dose group of males in comparison to control group. The relative weight of left kidney was significantly increased in low dose group of females. A significant increase of the relative weight of right adrenal was found in females from medium dose group in comparison to control group. The test article caused increase of ALP in males and females in the high dose group. There was some indication that this effect was reversible; satellite animals see trend of return to normal physiological state. A decrease in creatinine and an increase of total bilirubin in males of the high dose group was observed. The changes were reversible. Finally, a reversible increase of triacyglycerols in males of the high dose group and decreased of total cholesterol in satellite males of this dose group was observed. These changes were observed in the absence of any histopathological findings in the liver at any dose for both sexes compared to control. The authors state that decrease of total cholesterol might be considered as retarded effect and of possible toxicological significance. Histopathological findings in the kidney included moderate degenerative changes nephrosis in proximal tubules of female's kidneys of all dose groups. This injury of kidneys was stated in the report

to be probably reversible and the degenerative changes were not dose dependent. Also, a toxicokinetics study of this test substance indicates that the test material is not absorbed by the kidneys. The NOAEL for the study was determined to be 40 mg/kg bw/day based on effects seen at the next highest dose of 80 mg/kg bw/day based on relative liver weight increase coupled with an increase in ALP activity, increased bilirubin, increase in triacylglycerols and the retarded decrease of total cholesterol at this dose in males. The registrant plans to submit a 90-day repeat-dose toxicity study to further investigate the delayed cholesterol effect (ECHA 2014a).

The recent release of the EPA ToxCast/Tox21™ data pipeline (MySQL database (introdb_v1) and R package (tcpl_1.0)) was used to search for category members with high throughput *in vitro* data (US EPA 2014a). Only this substance (CAS RN 10081-67-1) has been tested in 169 assays spanning a wide range of biological activity. The substance was considered "active" in six of the assays (~3.5% of assays). The details of how the EPA establishes the "active" calls are described elsewhere (US EPA 2014b). The "active" calls are very weak with significant activity only observed at the highest concentration tested which barely exceeded the noise threshold. A list of active assays is presented in Table 4-3 along with the corresponding 50% activity concentration (AC50) values. CAS RN 10081-67-1 was not active in any of the cytotoxicity assays in the high throughput test battery across the dose ranges tested. Therefore, the activity seen in these active assays is not likely confounded by cytotoxicity.

Table 4-3 List of active assays in the ToxCast/Tox21™ High Throughput Screening Program

List of Assays with Hit Call (N=6)	Activity (AC50 µM)	Species / Cell Line	Description
ATG_TGFb_CIS_up	65	Human / HepG2 (hepatocyte)	Multiplex reporter gene assay for cell growth and differentiation; TGFb pathway.
ATG_PXRE_CIS_up	2.01	Human / HepG2 (hepatocyte)	Multiplex reporter gene assay for the PXR nuclear receptor.
Tox21_ERa_BLA_Antagonist_ratio	35	Human / HEK293T (kidney)	Reporter gene assay for estrogen receptor antagonist pathway.
Tox21_HSE_BLA_agonist_ratio	36	Human / HeLa (cervix)	Reporter gene assay for heat shock response element (HSE). Detection of activators of the heat shock / unfolded protein response.
Tox21_ESRE_BLA_ratio	38.5	Human / HeLa (cervix)	Reporter gene assay for endoplasmic reticulum stress response element (ESRE). Detection of agonists/antagonists of the ER stress signalling pathway.
NVS_NR_mERa	17.5	Mouse / cell free	Mouse estrogen receptor binding assay.

CAS RN 10081-67-1 appears to activate the estrogen receptor (ER) pathway according to active calls from two ER pathway related assays. However, this activity was cross-verified with 16 other ER related

assays available in the ToxCast data pipeline where the compound was not considered active. Furthermore, EPA recently published an ER pathway integrated network model ("ER AUC model") that uses all 18 ER related assays (US EPA 2014c) to classify ER pathway activity in the ToxCast assays and results are available online[1]. CAS RN 10081-67-1 is not considered an ER agonist or antagonist in this model. The activity seen in these two ER related assays are likely false positives for this pathway.

CAS RN 10081-67-1 appears to activate assays related to detoxification (PXR) and cell growth and differentiation (TGFb) in the liver. The AC50 values for these two assays were 2.01 and 65 µM respectively. Human toxicokinetic data (*in vivo* or *in vitro*) for this substance such as clearance or serum binding do not exist. Therefore *in vitro* to *in vivo* extrapolation (IVIVE) for this activity to a human oral equivalent dose (as described in Wetmore et al. 2012) is not possible. However, there is a toxicokinetics study in rats for this compound (see Section 4.3.3). At a single dose of 80 mg/kg (the daily repeated dose over a 28 day period where relative liver weight increase coupled with an increase in ALP activity, increased bilirubin, increase in triacylglycerols and the retarded decrease of total cholesterol in male rats are seen *in vivo)* the $AUC_{0\text{-}inf}$ of the substance was found to be 122.13 µg.h/mL. The toxicokinetics study only used a single dose and as such steady state blood concentration at a dose of 80 mg/kg/day was not determined. Average plasma concentration at steady state (C_{ss}) at this dose can be estimated from the single dose study according to equation (1).

$$(1) \qquad C_{ss} = AUC_{0\text{-}inf} / \tau$$

Using an $AUC_{0\text{-}inf}$ of 122.13 µg.h/mL from the single dose toxicokinetics study at a dose of 80 mg/kg over a dosing interval (τ) of 24 hours gives an average C_{ss} of approximately 12.54 µM which is in the concentration range of activity (AC50) seen in the high throughput *in vitro* testing.

High throughput *in vitro* data is not available for other category members; therefore a comparative analysis of activity across category members to support the subgrouping and read-across is not possible. However, it appears from the above analysis that perturbation in biological activity of liver cells *in vitro* occurs within expected plasma concentrations from dosing related to *in vivo* effects in rats. An analysis of the perturbed biological activity of the hepatocyte as a cellular event related to the observed *in vivo* effects is outside the scope of this case study.

SDPAs not considered part of other subgroups:

CAS RN 68921-45-9

Chronic repeat-dose toxicity data were available for CAS RN 68921-45-9. In this study, rats were administered the test substance in the diet for 64 weeks at dose levels of 0, 125, 250 or 500 mg/kg bw/day. A LOAEL of 125 mg/kg bw/day, the lowest dose tested, was determined based on decreased body weight gain in all females, hepatomegaly in both sexes, and diffuse hepatic degeneration in all animals. It is reported that, diffuse hepatic degeneration was observed in all test animals. The degenerative changes in the liver were described as diffuse cloudy swellings and fatty metamorphosis of the cytoplasm of the hepatocytes (cited in ACC 2006; US EPA 2009; ECHA 2014c). No data on haematology or clinical chemistry parameters were reported.

4.3.5 Investigations on Mode of Action

There is limited data with respect to mode of action or adverse outcome pathway interpretations for the effects seen across the SDPAs.

[1] EPA EDSP Dashboard: actor.epa.gov/edsp21

A metabolome study was available that looked at the plasma taken from fasted rats after a 28-day treatment with CAS 68411-46-1 (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene) at doses of 0, 125, and 300 mg/kg bw/day. This was also compared with the metabolome of CAS 36878-20-3 (Benzenamine, ar-nonyl-N-(nonylphenyl)-) after similar testing. The analysis included 225 endogenous plasma components. At 300 mg/kg bw/day, 24 metabolites were increased and 35 were decreased compared to the control group. Increased were many complex lipids, fatty acids and derivatives such as nervonic acid, ceramides, lysophophphatidylcholines and phosphatidylcholines as well as ornithine and phosphates, testosterone and androstenedione. A similar, but less pronounced pattern was observed at the lower dose group of 125 mg/kg bw/day. Creatinine, creatine, phosphcreatine, urea, many amino acids and homovanillic acid were decreased. According to the study authors, dysregulations in the liver cell metabolism of rats can be assumed because of lower urea and amino acid levels, indicating a decreased protein metabolism. In addition, creatine, creatinine, phosphocreatine and urea were significantly decreased whereas citrulline, uric acid, ornithine and phosphate (inorganic and from organic phosphates) were significantly increased indicating a slight functional effect on the kidneys or an alteration on the urea cycle. Changes in testosterone and androstenedione levels in the 300 mg/kg dose group are regarded to be incidental. The changes were compared to those obtained after 28-day treatment with CAS 36878-20-3. It was found that a majority of metabolome changes was similar for both compounds in terms of significance and direction of change (either increased or decreased.) Applying a Pearson-based statistical correlation of the whole plasma metabolome, CAS 36878-20-3 and CAS 68411-46-1 were the most similar compounds in terms of metabolome changes out of a database consisting of more than 750 substances. These two substances are in the same subgroup under this approach although both have sufficient toxicity data.

A correlation analysis of the whole plasma metabolite profile of CAS 68411-46-1 against the metabolite profiles of the reference compounds for patterns in a database of 750 substances showed no matches which would give a clear indication for a certain toxicological mode of action.

4.3.6 Observed trends in for other endpoints

An extensive review of available data for other endpoints including genotoxicity, acute toxicity and skin sensitisation is not provided in this case study. However, other reviews for the SDPAs have been provided under the US EPA High Production Volume Program (ACC 2006; US EPA 2009)). Data from bacterial mutation assays, *in vitro* chromosome aberration assays, as well as *in vivo* micronucleus assays, were reviewed; the findings indicated a low concern for SDPAs based on lack of positive response.

Overall, based on the empirical data available for the substances, SPDAs have low acute toxicity via the oral and dermal routes. Where data exists, the overall weight of evidence across the group indicates that the SDPAs are not skin sensitizers. Based on available information for reproductive and developmental toxicity in this case study, three substances show similarity in effects including decreased viability indices. These observations always occurred in the presence of maternal toxicity. The diaklyated SPDA did not show reproductive or developmental effects, nor liver effects in maternal animals.

5. STRATEGY FOR AND INTEGRATED CONCLUSION OF DATA GAP FILLING

5.1 Integrated conclusion

As justified above, subgroups of SDPAs can be formed on the basis of structure/composition of the sides chains, physical chemical properties as well as predicted toxicokinetics parameters. The extent and nature of systemic effects following repeat dose exposures in rats vary depending on the SDPA. Although

there are similarities in observed systemic effects across the various subgroups there are observed differences which were also considered when forming the subgroups.

For oral repeat dose toxicity the read-across approach for untested members involves applying the effect level from the most similar substance (nearest neighbour) within each subgroup considering the structure/composition of the sides chains, physicochemical properties as well as toxicokinetic parameters (mainly predicted oral bioavailability) (Table 5-1 to 5-5). Read-across of effect levels from the nearest neighbour is one method that can be used to compare against an exposure estimate within a screening level risk assessment in order to establish margin of exposures to assess risk for data poor chemicals.

Table 5-1 Oral Repeat Dose Toxicity Effect Levels and Application of Read-Across for Subgroup 1: Monoalkylated SDPAs

Substance Identity CAS RN (DSL Name)	**Representative SDPA Components**	**Repeat Dose Toxicity (Oral)**
4175-37-5 (Benzenamine, 4-octyl-N-phenyl-)	Monooctyl DPA	**Read-across** Nearest neighbour based on structure/composition, properties (MW, LogP, WS) and predicted oral bioavailability (CAS 27177-41-9) NOAEL = 15 mg/kg/day LOAEL = 150 mg/kg/day Liver, spleen, haematological effects / clincal biochem para. 28 day gavage study (Rat)
27177-41-9 (Benzenamine, ar-nonyl-N-phenyl-) isomer mixture	Monononyl DPA	NOAEL = 15 mg/kg/day LOAEL = 150 mg/kg/day Liver - Centriolobular hepatocyte enlg. / Lipid vacuolation (150mf) Haematological - ↑APTT (150m/500f) ↑PT (150m/500f)↓haemoglobin and haematocrit (500mf) ↓ MCH/MCHC (150f) Clincal biochem para - ↓ plasma protein ↓ALB (500m/150f); ↑AST (500mf)↑ALT (500mf) ↑ALP (150m/150f) ↓creatinine (500m) ↑phosphorus and triglyceride (150f) ↓Ca (150m/500f) ↑total bilirubin (500mf) Spleen - ↑pigment accumulation (probably haemosiderin) (150f) 28 day gavage study (Rat) (Japan MHW Guideline 1986 GLP) (SafePharm Laboratories 1999)

Table 5-2 Oral Repeat Dose Toxicity Effect Levels and Application of Read-Across for Subgroup 2: SDPA UVCB mixtures with variable number of alkyl substitutions

Substance Identity CAS RN (DSL Name)	**Representative SDPA Components**	**Repeat Dose Toxicity (Oral)**
184378-08-3 (Benzenamine, N-phenyl-, reaction products with isobutylene and 2,4,4-trimethylpentene) UVCB	Monobutyl DPA Dibutyl DPA Monooctyl DPA Monobutyl Monooctyl DPA Dioctyl DPA	NOAEL = 5 mg/kg/day LOAEL = 25 mg/kg/day Liver - Centriolobular hepatocyte enlg. (125m/25f) Haematological - ↑PTT ↓ platelet count (125mf) Clincal biochem para - ↓ plasma protein (125m/25f); ↓ALB ↓ALB/G ↑AST ↑ALP (125m/25f) 43-54 days gavage repro/devo study (Rat) (OECD TG 422) (SafePharm Laboratories 2006b [also in US EPA 2009])
68411-46-1 (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene) UVCB	Monooctyl DPA Dioctyl DPA	NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day Liver - Minimal hypertrophy and vacuolation (75mf) Haematological - ↑ APTT (25f); ↓ lymphocytes and red blood cell counts (25m) Clincal biochem para - ↓ plasma protein (75f); ↓ALB ↑total bilirubin ↑ALP (75mf) 28 (males) - 53 (female) day gavage study (Rat) OECD TG 422 (ECHA 2014d)
26603-23-6 (Benzenamine, ar-octyl-N-(octylphenyl)-) UVCB	Monooctyl DPA Dioctyl DPA	Nearest neighbour based on structure/composition, properties (MW, LogP, WS) and predicted oral bioavailability (CAS 36878-20-3) LOAEL = 100 mg/kg/day* Liver, haematology, thyroid effects / clinical biochem para. 90 day gavage study (Rat) OECD TG 408 (ECHA 2015a) *lowest dose tested
36878-20-3 (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene) UVCB	Monononyl DPA Dinonyl DPA	LOAEL = 100 mg/kg/day* Liver - Centrilobular hypertrophy (grade 1) (100mf), fatty change (100m / 300f), single cell nercrosis (300m) Haematology - ↑PTT (300m) Clinical biochem para - ALB (1000mf) ↑ALP (100f / 300m) ↑Triglyceride (300f), ↑ Glucose (1000f) ↓Bile acid (100mf) Thyroid - hypertrophy / hyperplasia (grade 1) (100mf) 90 day gavage study (Rat) OECD TG 408 (ECHA 2015a) *lowest dose tested
68608-79-7 (Benzenamine, N-phenyl-, (tripropenyl) derivs.) UVCB	Monononyl DPA Dinonyl DPA	Read Across Nearest neighbour based on structure/composition, properties (MW, LogP, WS) and predicted oral bioavailability (CAS 36878-20-3) LOAEL = 100 mg/kg/day* Liver, haematology, thyroid effects / clinical biochem para. 90 day gavage study (Rat) OECD TG 408 (ECHA 2015a) *lowest dose tested

68608-77-5 (Benzenamine, 2-ethyl-N-(2-ethylphenyl)-, (tripropenyl) derivs.) UVCB	Diethyl monononyl DPA Diethyl dinonyl DPA	Read Across Nearest neighbour based on structure/composition, properties (MW, LogP, WS) and predicted oral bioavailability (CAS 36878-20-3) LOAEL = 100 mg/kg/day* Liver, haematology, thyroid effects / clinical biochem para. 90 day gavage study (Rat) OECD TG 408 (ECHA 2015a) *lowest dose tested

Table 5-3 Oral Repeat Dose Toxicity Effect Levels and Application of Read-Across for Subgroup 3: Dialkylated SDPAs

Substance Identity CAS RN (DSL Name)	Representative SDPA Components	Repeat Dose Toxicity (Oral)
101-67-7 (Benzenamine, 4-octyl-N-(4-octylphenyl)-)	Dioctyl DPA	NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day Liver - no histopath findings (250mf) Haematological - ↑PTT (75m) ↑APTT (75m) Clinical biochem para - no changes (250mf) 42 day gavage comb. rep dose/repro/devo study OECD TG422 (Rat) (Japan NITE 2008)
15721-78-5 (Benzenamine, 4-(1,1,3,3-tetramethylbutyl)-N-[4-(1,1,3,3-tetramethylbutyl)phenyl]-)	Dioctyl DPA	Planned OECD TG408 90 - day gavage (ECHA 2014b) **Read-across** Nearest neighbour based on structure/compostion, properties (MW, LogP, WS) and oral bioavailability (CAS 101-67-7) NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day haematological effects 42-53 day gavage comb. rep dose/repro/devo study OECD TG422 (Rat)
24925-59-5 Benzenamine, 4-nonyl-N-(4-nonylphenyl)-	Dinonyl DPA	**Read-across** Nearest neighbour based on structure/compostion, properties (MW, LogP, WS) and oral bioavailability (CAS 101-67-7) NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day haematological effects 42-53 day gavage comb. rep dose/repro/devo study OECD TG422 (Rat)

Table 5-4 Oral Repeat Dose Toxicity Effect Levels and Application of Read-Across for Subgroup 4: SDPAs with variable number of phenyl substitutions

Substance Identity CAS RN (DSL Name)	Representative SDPA Components	Repeat Dose Toxicity (Oral)
68442-68-2 (Benzenamine, N-phenyl-, styrenated) UVCB	Monostyrenated DPA Distyrenated DPA	NOAEL = 50 mg/kg/day LOAEL = 250 mg/kg/day* Liver - centrilobular hepatocyte enlargement (250m / 50f) Haematology - no effects Clinical biochem para - ↓ cholesterol (250m); ↑ALP (600m) Thyroid - Follicular cell hypertrophy (250m) Repro - pre-implantation loss (600f) 43-54 days gavage repro/devo study OECD TG 422 (Rat) (SafePharm Laboratories 2006a [also in ACC 2006]; US EPA 2009) *LOAEL - as interpreted by Health Canada
10081-67-1 (Benzenamine, 4-(1-methyl-1-phenylethyl)-N-[4-(1-methyl-1-phenylethyl)phenyl]-)	Dimethylstyrenated DPA	NOEL = 40 mg/kg/day LOEL = 80 mg/kg/day Liver - no significant histopath findings at high dose (80mf) Clinical biochem para -↑Bilirubin (80m) ↓ cholesterol (80m) ↑ALP (80mf) ↑Triacylglycerols (80m) ↓Creatine (80m) Kidney - degenerative nephrosis in proximal tubles (10f) 28 day gavage rep. dose OECD TG407 (Rat) (ECHA 2014a)

Table 5-5 SDPAs not considered part of other subgroups

Substance Identity CAS RN (DSL Name)	Representative SDPA Components	Repeat Dose Toxicity (Oral)
68921-45-9 (Benzenamine, N-phenyl-, reaction products with styrene and 2,4,4-trimethylpentene) UVCB	Monooctyl DPA; Monostyrentated DPA; Distyrenated DPA; Dioctyl DPA; Monooctyl monostyrenated DPA	LOAEL = 125 mg/kg/day (2500 ppm in diet)* Liver - degenerative changes (fatty change, diffuse cloudy swellings) Haemetological - no data Clinical Biochem para - no data 64 week diet study (non-GLP) (Rat) (1957 report cited in ECHA 2014c; cited in US EPA 2009) *lowest dose tested

5.2 Uncertainty

The potential for the chemical grouping and read across approach to introduce uncertainty into the hazard assessment of chemicals is well acknowledged by regulatory agencies including Health Canada and Environment Canada. There are various areas that contribute to the overall uncertainty when applying the approach. There is uncertainty associated with the data, assumptions, and predictions used to justify similarity and analogue suitability between the group members. There is also toxicological uncertainty

with the prediction of hazard that can be derived when using quantitative read-across (evaluated based on the number and suitability of analogues contributing data, source study quality, severity of the critical effect, likelihood of effect and potency concordance between target and source chemical).

Presented here is a preliminary effort to document the various areas of uncertainty associated with the data and methods used for the similarity justification (Section 5.2.1). Some consideration of the uncertainty with prediction of hazard when applying read across for repeat dose toxicity is also conducted in a separate step (Section 5.2.2). Limited guidance is available in the open literature for assessing uncertainty with respect to the read across approach. Two recent and related published frameworks were consulted when assessing the read across uncertainty within our case study (Wu et al. 2010, Blackburn and Stuard 2014).

5.2.1 Analogue Suitability Rating for Each Case of Read Across

The process used in this case study to justify similarity within subgroups of SDPAs follows closely the framework proposed by Wu et al. 2010. The overall flow chart from this publication was used to assign a 'suitability rating'. For each use of read-across, Table 5-6 to 5-8 outlines the applicable questions from this flow chart and the associated areas of uncertainty based on the data, assumptions, and predictions used for the determination. The 'suitability rating' is then used in a subsequent step to assign an uncertainty category to the overall read across.

Table 5-6 Analogue suitability rating for read-across within subgroup 1: monoalkylated SDPAs

Chemical ID		
Target Chemical(s) CAS: 4175-37-5		*Source Chemical (analogue)for read across* CAS: 27177-41-9
Similarity Justification		
Evaluation Criteria	**Decision tree question(s) in Wu et al. 2010**	**Uncertainty**
Structure and reactivity	Do the target & analogue have similar structural features & chemical reactivity? **YES** Do the target & analogue contain different or potentially different alert functional groups? **NO**	The branching pattern on the side chain for the monooctylated substance (target) is unknown. The analogue is a mixture of branched monononylated DPA isomers whereas the source is a discrete monooctylated DPA with attachment at the 4-position on DPA. The impact of these structural differences on toxicity is unknown. The target and source chemicals were screened for alerts related to systemic toxicity using DEREK (Lhasa 2014). No differences were found.
Metabolism	Do the target & analogue have similar metabolic pathways? **YES**	Empirical data on metabolism is lacking for both the target and source chemicals. Similarity in metabolism was inferred based on known xenobiotic transformations employed within a predictive tool. There was domain of applicability concerns when applying the metabolic model which increases uncertainty. The rate and extent of metabolism between the two substances is not known. However due to minor differences in structure and phys-chem properties the substances likely follow a similar

		metabolic pathway.
Physicochemical Properties and Toxicokinetics Parameters	Do the target & analog have similar phys-chem properties? **YES** Could these phys-chem differences fundamentally alter toxicological profile? **N/A.**	Empirical data on phys-chem properties is lacking for both the target and source chemicals. Well documented models (EPI Suite) were used to provide estimations. Empirical data on toxicokinetics parameters is lacking for both target and source chemicals. Predicted models were used to generated estimations for oral bioavailability, AUC, Cmax, and Tmax for comparative purposes. No major difference in oral bioavailability or other predicted kinetic parameters. Although the training set for the model was broad, there was some concern with applicability domain with respect to SDPA chemicals.
Overall "suitability rating" based on Wu et al. 2010 decision tree: **Suitable** Uncertainty in the similarity justification would be reduced with empirical data on metabolism, physicochemical properties, and mode of action.		

Table 5-7 Analogue suitability rating for read-across within subgroup 2: SDPA UVCB mixtures with variable number of alkyl substitutions

Chemical ID		
Target Chemical(s) CAS: 68608-79-7; 68608-77-5; 26603-23-6		*Source Chemical (analogue)for read across* CAS : 36878-20-3
Similarity Justification		
Evaluation Criteria	**Decision tree question(s) in Wu et al. 2010**	**Uncertainty**
Structure and reactivity	Do the target & analogue have similar structural features & chemical reactivity? **YES** Do the target & analogue contain different or potentially different alert functional groups? **NO**	All four substances are complex UVCB mixtures. The composition and branching pattern of source and target chemicals is not well documented leading to uncertainty. However, there is confidence that the representative structures cover the larger portions of the mixtures (based on knowledge of synthetic chemistry and chemical name). The target and source chemicals were screened for alerts related to systemic toxicity using DEREK (Lhasa 2014). No differences were found.
Metabolism	Do the target & analogue have similar metabolic pathways? **YES**	Empirical data on metabolism is lacking for both the target and source chemicals. Similarity in metabolism was inferred based on known xenobiotic transformations employed within a predictive tool. There was domain of applicability concerns when applying the metabolic model which increases uncertainty. The rate and extent of metabolism between the two substances is not known. However, due to minor differences in structure and phys-chem properties the substances likely follow a similar metabolic pathway.
Physicochemical Properties and Toxicokinetics Parameters	Do the target & analogue have similar phys-chem properties? - YES **(CAS 68608-79-7)** - Minor differences **(CAS 68608-77-5; 26603-23-6)**	Empirical data on key phys-chem properties (e.g. $LogK_{ow}$) is lacking for both the target and source chemicals. Well documented models (EPI Suite) were used to provide estimations.

	Could these phys-chem differences fundamentally alter toxicological profile? - **NO.** Lower or similar predicted oral bioavailability for target (CAS 68608-77-5; 26603-23-6) compared to analogue.	Empirical data on toxicokinetics parameters is lacking for both target and source chemicals. Predicted models were used to generate estimations for oral bioavailability, AUC, Cmax, and Tmax for comparative purposes across the representative components. Although the training set for the model was broad, there was some concern with applicability domain with respect to SDPA chemicals.
Overall "suitability rating" based on Wu et al. 2010 decision tree: **Suitable** (CAS 68608-79-7; 26603-23-6); Suitable with interpretation (CAS 68608-77-5) Uncertainty in the similarity justification would be reduced with empirical data on metabolism, physicochemical properties, and mode of action.		

Table 5-8 Analogue suitability rating for read-across within subgroup 3: Dialkylated SDPAs

Chemical ID		
Target Chemical(s) CAS: 15721-78-5; 24925-59-5		*Source Chemical (analogue)for read across* CAS: 101-67-7
Similarity Justification		
Evaluation Criteria	**Decision tree question(s) in Wu et al. 2010**	**Uncertainty**
Structure and reactivity	Do the target & analogue have similar structural features& chemical reactivity? YES Do the target & analogue contain different or potentially different alert functional groups? NO	The branching pattern on the side chain for 101-67-7 and 24925-59-5 are not well known. The analogue is likely linear dioctyl DPA (from description in study report). The impact of these structural differences on toxicity is unknown. The target and source chemicals were screened for alerts related to systemic toxicity using DEREK (Lhasa 2014). No differences were found.
Metabolism	Do the target & analogue have similar metabolic pathways? YES	Empirical data on metabolism is lacking for both the target and source chemicals. Similarity in metabolism was inferred based on known xenobiotic transformations employed within a predictive tool. There was domain of applicability concerns when applying the metabolic model which increases uncertainty. The rate and extent of metabolism between the two substances is not known. However due to minor differences in structure and phys-chem properties the substances likely follow a similar metabolic pathway.
Physicochemical Properties and Toxicokinetics Parameters	Do the target & analog have similar phys-chem properties? YES Could these phys-chem differences fundamentally alter toxicological profile? N/A.	Empirical data on key phys-chem properties (e.g. $LogK_{ow}$) is lacking for both the target and source chemicals. Well documented models (EPI Suite) were used to provide estimations. Empirical data on toxicokinetics parameters is lacking for both target and source chemicals. Predicted models were used to generated estimations for oral bioavailability, AUC, Cmax, and Tmax for comparative purposes across the representative components. Although the training set for the model was broad, there

		was some concern with applicability domain with respect to SDPA chemicals.
Overall "suitability rating" based on Wu et al. 2010 decision tree: **Suitable** Uncertainty in the similarity justification would be reduced with empirical data on metabolism, physicochemical properties, and mode of action. Additional repeat dose data for CAS 15721-78-5 is proposed in the REACH dossier. This would decrease uncertainty within the category by ruling out liver related effects. For dioctylated DPAs at the dose range tested.		

5.2.2 Assigning an Uncertainty Category for Read-Across

A systematic framework to describe potential areas of additional uncertainty that may arise in read across (evaluated based on the number and suitability of analogues contributing data, severity of the critical effect, and effects and potency concordance) has been recently published (Blackburn and Stuard 2014). The framework was developed for consistent application through the use of a questionnaire for evaluating and documenting consideration of these potential additional sources of uncertainty by risk assessors. For each instance of read across within this case study, the questionnaire was completed (Appendix B). The answers from the questionnaires were compared against the developed uncertainty categories from this publication. The authors propose additional uncertainty factors to apply based on the different uncertainty categories for read-across. For this case study, only the qualitative descriptions of uncertainty are used. For each case of read across presented, a summary of the uncertainty category is provided in Table 5-9 below.

Table 5-9 Uncertainty categories for each use of read across in the case study

Target Chemical(s) CAS RN	**Source Chemical (analogue) CAS RN**	**Uncertainty Category and Reasoning (Blackburn and Stuard 2014)**
4175-37-5	27177-41-9	**Low** - Read across data (of sufficient quality for risk assessment) is contributed by at least 1 'suitable' analogue for the target.
68608-79-7; 68608-77-5; 26603-23-6	36878-20-3	**Low** - For CAS 68608-79-7 and CAS 26603-23-6 read across data (of sufficient quality for risk assessment) is contributed by at least 1 'suitable' analogue for the target. Highly concordant toxicity effects and potency (indicating low/moderate hazard) in data set across subgroup. The analogue has structural features that collectively bracket the structural features of the target. **Low** - For CAS 68608-77-5 read across data (of sufficient quality for risk assessment) is thought to be from a 'worst-case' analogue based on higher oral bioavailability of the comparative components. Highly concordant toxicity effects and potency (indicating low/moderate hazard) in data set across subgroup.
15721-78-5; 24925-59-5	101-67-7	**Low** - For all targets read across data (of sufficient quality for risk assessment) is contributed by at least 1 'suitable' analogue for the target.

Under Canada's Chemicals Management Plan where the purpose of the read across is to establish critical effect level(s) to generate margin of exposures (MOEs) for screening level risk assessments, we consider the proposed read across within this case study as acceptable. Although there is some degree of uncertainty associated with the justification of similarity between the analogues and target chemicals, it is not expected to invalidate the read across for the intended screening level purpose. It is Health Canada's

current practice for screening based assessments to examine all areas of uncertainty related to the risk assessment and compare against the magnitude of the established MOE. The tolerance for the level of uncertainty within the screening based assessments can be examined against the derived MOE. This determination will be made within the screening assessment itself and is outside the scope of this document.

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APPENDIX A – DESCRIPTION OF OASIS TIMES *IN VIVO* RAT METABOLIC SIMULATOR

Endpoint

The *in vivo* rodent metabolic simulator (transformation table) reproduces and predicts the metabolic pathways of xenobiotic chemicals *in vivo* in rodents (mostly rats).

Data

The metabolism training set contains experimentally observed (documented) *in vivo* metabolic pathways for 647 structurally different parent chemicals, and 4382 observed metabolites compiled into a searchable electronic database. Published data on the *in vivo* metabolism of these chemicals in rodents, collected mainly from research publications in scientific journals and, also, from some websites were extracted and introduced into an electronic database [1].

Model

The simulation of metabolism is focused on the correct reproduction of experimentally observed metabolites [2]. The current *in vivo* rat metabolic simulator (transformation table) represents electronically designed set of 622 structurally generalized, hierarchically arranged biotransformation reactions. These molecular transformations are characteristic for the *in vivo* metabolism in rats. Each transformation in the simulator consists of source and product structural fragments, and inhibiting "masks". A probability of occurrence is ascribed to each transformation, which determines its hierarchy in the transformation list. Thus the modeling is based on the set of principal molecular transformations, and the in vivo "logic" of the commonly observed xenobiotics metabolism in living rats.

The following types of molecular transformations are included into *in vivo* simulator:

- 26 abiotic (non-enzymatic) reactions. The highest priority (probability of occurrence) is assigned to these reactions. This subset of reactions includes also transformations of highly-reactive functional groups and intermediates, such as tautomerizations, arene epoxide rearrangements to phenols, etc. which occur spontaneously;
- 479 enzymatic phase I transformations such as aliphatic C-oxidation, aromatic C- hydroxylation, oxidative N- and O-dealkylation, epoxidation, ester and amide hydrolysis, carbonyl group reduction, nitro and azo group reduction, N- hydroxylation, oxidative deamination, beta-oxidation, ring cleavage, hydrolytic cleavage, aromatization, decarboxylation, dehalogenation, etc.
- 104 enzymatic phase II transformations, such as glucuronidation, sulfation, glutathione and mercapturic acid conjugation, N-acetylation, etc., which, unlike the *in vitro* systems, are believed to occur with high priority *in vivo*.

Domain

The stepwise approach [3] was used to define the applicability domain of the model. It consists of the following sub-domain levels:

- General parametric requirements – includes ranges of variation *log K*OW and *MW*
- Structural domain – based on atom-centered fragments (ACFs)

Statistics (Simulator Performance)

Average Sensitivity:

S = [X/(X + Y)].100 [%]

Average Predictability:

P = [X/(X + Z)].100 [%]

X: number of correctly generated (predicted) metabolites, observed also experimentally ("true positives");
Y: number of non-generated (non-predicted) but experimentally observed metabolites ("false negatives");
Z: number of generated but not experimentally observed metabolites ("false positives").

Simulator performance parameters:

Average sensitivity: S = 77.2 %

Average predictability: P = 45.2 %

References:

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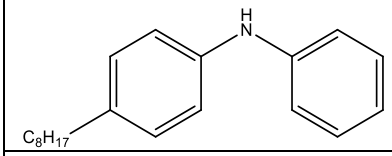
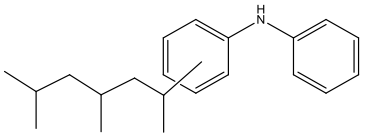
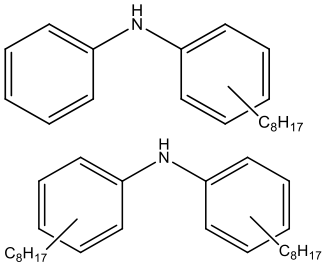
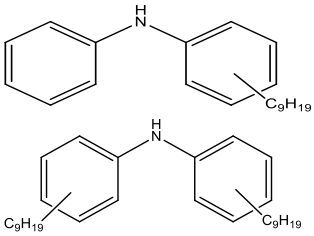
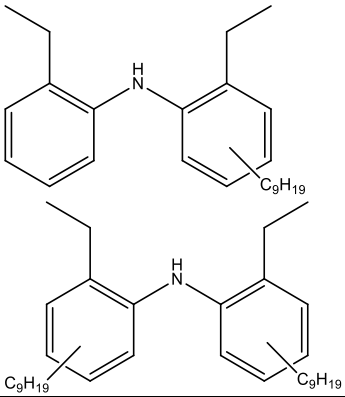
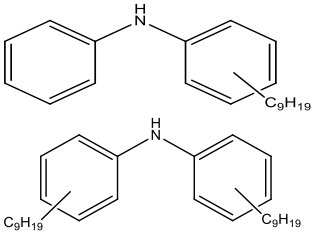
APPENDIX B - READ-ACROSS UNCERTAINTY EVALUATION QUESTIONNAIRES (MODIFIED FROM BLACKBURN AND STUARD 2014)

Target Chemical(s) CAS: 4175-67-5  	**Source Chemical (Analogue)** CAS: 27177-41-9 
Questions	**Responses by Endpoint**
Section I. Think about the chemical similarity between target and source chemicals:	
What is the "similarity rating" of the analogue(s) or category members?	_X_ Suitable __ Suitable with precondition ___ Suitable with interpretation ___ Not Suitable ** Stop questionnaire
Are there any differences in functional groups and associated reactivity expected to be in the conservative direction (i.e. analogues would be expected to be more reactive than target?)	___ Yes _X_ No ___ Unknown Explanation/Notes (if any):
Are there any differences in predicted metabolic pathways/rate expected to be in the conservative direction (e.g. where target and analogues are predicted to undergo activating metabolism and analogue metabolism to active/toxic species is more rapid than the target OR where the target and analogues are predicted to undergo deactivating metabolism and analogue metabolism to detoxification products proceeds more slowly than the target)? *Note: some knowledge of MOA will be necessary to answer.*	___ Yes ___ No _X_ Unknown ___ No Differences Explanation/Notes (if any): The metabolic pathway is expected to be similar. Differences in rates are unknown.
Are there any differences in predicted metabolites expected to be in the conservative direction (i.e. data on analogue metabolites is more toxic than the predicted metabolites of the target)?	___ Yes _X__ No ___ Unknown ___ No Differences Explanation/Notes (if any): Both are expected to yield hydroxylated derivatives of the parent structure. Differences in toxicity between metabolites are not expected.
Are there any differences in physical-chemical properties expected to be in the conservative direction (i.e. bioavailability of the analogue would be expected to be greater than or equal to the target)?	___ Yes ___ No ___ Unknown _X__ No Differences Explanation/Notes (if any): Predicted oral bioavailability was comparable.

Are there structural characteristics that would be expected to decrease toxicity of target relative to analogues (e.g. structural hindrances for metabolism or receptor binding)?	___ Yes ___ No ___ Unknown _X_ No Differences Explanation/Notes (if any):
Section II. Think about the quality and consistency of the data being contributed by different analogues to the endpoint read across:	
Did the endpoint data set include more than one study on the same analogue?	___ Yes _X__ No Was the single study of sufficient quality? _X__Yes ___ No (stop questionnaire) Explanation/Notes: Data for read across originates for a guideline GLP study considered of sufficient quality for risk assessment.
Were data from multiple analogues considered in the read across assessment for the endpoint?	_X_ Yes ___ No Explanation/Notes (if any): The read-across in this subgroup is one to one. However, a broader group of SDPA substances was also looked at in parallel and similarities in liver effects were seen across multiple SDPAs.
Were most of the studies generally of sufficient quality to serve as a basis for risk assessment?	_X_ Yes ___ No Explanation/Notes (if any): Data for read-across originates from a guideline GLP study. Most of the studies across the broader group of SDPAs were GLP guideline studies.
Was there <u>any</u> evidence in the entire analogue data set of a specific toxic effect(s) for the endpoint (i.e. some target organ toxicity; some reproductive toxicity; or some developmental toxicity)? For example, answer no if there was only general toxicity such as reduced body weight and no specific target for the repeat dose toxicity endpoint.	_X_ Yes (continue questionnaire) ___ No (STOP questionnaire) Explanation/Notes (if any): The liver was the target organ for most SDPAs.
If multiple analogues contributed to the endpoint data set, was there concordance in endpoint-specific adverse effects across analogues, suggesting they have same targets of toxicity? Examples: Repeat Dose Toxicity: liver toxicity, renal effects, heme changes etc. Reproductive Toxicity: repro organ weight change, estrous cycle change, % resorptions, etc. Developmental Toxicity: pup body weights, litter sizes, malformations, etc.	_X__ Yes ___ No Explanation/Notes (if any): Histopathological findings included hepatocyte enlargement, vacuolation or minimal hypertrophy with associated changes in clinical biochemical parameters related to the liver across multiple SDPAs. Other common effects were also noted including effects on blood clotting, thyroid and kidney. These were considered to be secondary to liver toxicity in most cases. Histopathological findings in the spleen are unique to this subgroup of monoalkylated SDPAs but were found to have commonalities with the starting material DPA.
If multiple analogues contributed to the endpoint data set, was there	_X__ Yes ___ No

concordance in endpoint-specific adverse effects and/or PODs (at least where dose spacing was comparable) across analogues, suggesting they have similar potency for the critical effects in the endpoint?	Explanation/Notes (if any): Potency comparison based on LOAEL was difficult to assess due to dose spacing differences across multiple studies. However, NOAELs are considered comparable across multiple SDPAs for the effect. Alkylated SPDAs had a NOEAL between 5 and 25 mg/kg bw/day for the liver effects.
Severity of effect. Does the endpoint-specific analogue data set demonstrate very low/no toxicity (e.g. very high dose point of departure or free-standing NOAELs or no selective repro/devo effects seen)?	___ Yes _X__ No Explanation/Notes (if any): The effects related to the liver and changes in clinical chemistry parameters are considered of toxicological significance. However, the effect is considered adaptive in many toxicity studies and there is some evidence that the hepatic effects are reversible once dosing is ceased. The severity of this effect is considered low. However, for this subgroup, the pigmentation in the spleen is indicative of haemolysis. This effect is considered more severe in nature.
Is the most conservative point of departure in the endpoint-specific analogue data set being used as the read-across for that endpoint? If not, is there a robust rationale to support why (i.e. clear rationale for selecting nearest neighbor approach to account for differences?	___ Yes _X__ No Explanation/Notes (if any): Sub-groups the SDPAs were formed based on differences in side chains, phys-chem properties, predicted oral bioavailability and differences in observed effects outside of the liver toxicity. Based on these differences it is more defensible to select the closest neighbour within each subgroup to apply read-across.
Section III. Think about consistency between analogue and COI data across all endpoints:	
For the analogues used in the endpoint data set, do these analogues have data on another endpoint (anchor data) for which there are also direct data on the target, are the data concordant?	___ Yes _X_ No
Section IV: For Read Across based on metabolites and/or 'suitable with precondition' analogues requiring a metabolic precondition to be met: N/A	
<u>Conclusions from Read-Across Uncertainty Evaluations</u> Use questionnaire responses above and weight of evidence of available data to select the appropriate uncertainty category for each endpoint (see uncertainty descriptions in Appendix D)	
Based on the analogue data set considered, read across is:	_X_ Actionable ___ Not Actionable without additional data Explanation / Comments: Although there is some degree of uncertainty associated with the justification of similarity between the analogue and target chemical, it is not expected to invalidate the read across for the intended purpose which is to derive an MOE based on system effects for a screening level risk assessment under Canada's Chemical Management Plan.
Based on the analogue data set considered, the identified point-of-departure from the critical study is:	NOAEL = 15 mg/kg/day LOAEL = 150 mg/kg/day <u>Liver</u> - Centriolobular hepatocyte enlg. / Lipid vacuolation (150mf)

	Haematological - ↑APTT (150m/500f) ↑PT (150m/500f)↓haemoglobin and haematocrit (500mf) ↓ MCH/MCHC (150f) Clincal biochem para - ↓ plasma protein ↓ALB (500m/150f); ↑AST (500mf)↑ALT (500mf) ↑ALP (150m/150f) ↓creatinine (500m) ↑phosphorus and triglyceride (150f) ↓Ca (150m/500f) ↑total bilirubin (500mf) Spleen - ↑pigment accumulation (probably haemosiderin) (150f) 28 day gavage study (Rat) (Japan MHW Guideline 1986 GLP) (SafePharm Laboratories 1999)
The degree of uncertainty in the read-across is judged to be:	_X_ Low ___ Low/Moderate ___ Moderate ___ High (i.e. not actionable)
Uncertainty description(s) that apply from Appendix D:	Read across data (of sufficient quality for risk assessment) is contributed by at least 1 'suitable' analogue for the target.

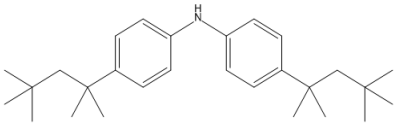
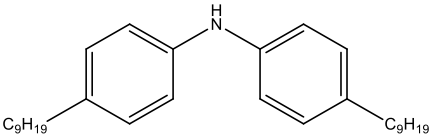
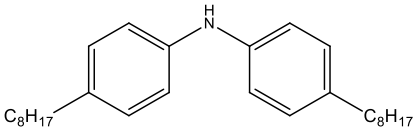
Target Chemical(s) CAS: 26603-23-6  CAS: 68608-79-7  CAS: 68608-77-5 	**Source Chemical (Analogue)** CAS: 36878-20-3 
Questions	**Responses by Endpoint**
Section I. Think about the chemical similarity between target and source chemicals:	
What is the "similarity rating" of the analogue(s) or category members?	_X_ Suitable (CAS 26603-23-6; 68608-79-7) ___ Suitable with precondition _X__ Suitable with interpretation (CAS 68608-77-5) ___ Not Suitable ** Stop questionnaire
Are there any differences in functional groups and associated reactivity expected to be in the conservative direction (i.e.	___ Yes _X_ No (CAS 26603-23-6 / 68608-79-7 / 68608-77-5) ___ Unknown Explanation/Notes (if any):

analogues would be expected to be more reactive than target?)	
Are there any differences in predicted metabolic pathways/rate expected to be in the conservative direction (e.g. where target and analogues are predicted to undergo activating metabolism and analogue metabolism to active/toxic species is more rapid than the target OR where the target and analogues are predicted to undergo deactivating metabolism and analogue metabolism to detoxification products proceeds more slowly than the target)? *Note: some knowledge of MOA will be necessary to answer.*	___ Yes ___ No _X_ Unknown (CAS 26603-23-6 / 68608-79-7 / 68608-77-5) ___ No Differences Explanation/Notes (if any): The metabolic pathway is expected to be similar. Differences in rates are unknown.
Are there any differences in predicted metabolites expected to be in the conservative direction (i.e. data on analogue metabolites is more toxic than the predicted metabolites of the target)?	___ Yes ___ No _X_ Unknown ___ No Differences Explanation/Notes (if any): Both are expected to yield hydroxylated derivatives of the parent structure. Differences in toxicity between metabolites are not known but not expected.
Are there any differences in physical-chemical properties expected to be in the conservative direction (i.e. bioavailability of the analogue would be expected to be greater than or equal to the target)?	_X_ Yes (CAS 68608-77-5) ___ No ___ Unknown _X__ No Differences (CAS 26603-23-6 / 68608-79-7) Explanation/Notes (if any): Predicted oral bioavailability was comparable for CAS 26603-23-6 and 68608-79-7. Predicted oral bioavailability for 68608-77-5 is expected to be *lower* than the analogue. Thus the analogue is expected to be conservative in this regard.
Are there structural characteristics that would be expected to decrease toxicity of target relative to analogues (e.g. structural hindrances for metabolism or receptor binding)?	___ Yes ___ No _X__ Unknown (CAS 68608-79-7 / 68608-77-5) __ No Differences Explanation/Notes (if any):
Section II. Think about the quality and consistency of the data being contributed by different analogues to the endpoint read across:	
Did the endpoint data set include more than one study on the same analogue?	___ Yes _X__ No Was the single study of sufficient quality? _X__Yes

	___ No (stop questionnaire) Explanation/Notes: Data for read across originates for a guideline GLP study considered of sufficient quality for risk assessment.
Were data from multiple analogues considered in the read across assessment for the endpoint?	_X_ Yes ___ No Explanation/Notes (if any): The read-across in this subgroup is one to one. However, a broader group of SDPA substances was also looked at in parallel within the subgroup and similarities in liver histopathology and related clinical chemistry effects were seen across the group.
Were most of the studies generally of sufficient quality to serve as a basis for risk assessment?	_X_ Yes ___ No Explanation/Notes (if any): Data for read-across originates from a guideline GLP study. All other studies within the subgroup were GLP guideline studies.
Was there <u>any</u> evidence in the entire analogue data set of a specific toxic effect(s) for the endpoint (i.e. some target organ toxicity; some reproductive toxicity; or some developmental toxicity)? For example, answer no if there was only general toxicity such as reduced body weight and no specific target for the repeat dose toxicity endpoint.	_X_ Yes (continue questionnaire) ___ No (STOP questionnaire) Explanation/Notes (if any): The liver was the target organ within this subgroup.
If multiple analogues contributed to the endpoint data set, was there concordance in endpoint-specific adverse effects across analogues, suggesting they have same targets of toxicity? Examples: Repeat Dose Toxicity: liver toxicity, renal effects, heme changes etc. Reproductive Toxicity: repro organ weight change, estrous cycle change, % resorptions, etc. Developmental Toxicity: pup body weights, litter sizes, malformations, etc.	_X__ Yes ___ No Explanation/Notes (if any): Histopathological findings included hepatocyte enlargement, vacuolation or minimal hypertrophy with associated changes in clinical biochemical parameters related to the liver across multiple SDPAs within subgroup.
If multiple analogues contributed to the endpoint data set, was there concordance in endpoint-specific adverse effects and/or PODs (at	_X_ Yes ___ No Explanation/Notes (if any): Potency comparison based on LOAEL was difficult to assess due to dose spacing differences across

least where dose spacing was comparable) across analogues, suggesting they have similar potency for the critical effects in the endpoint?	multiple studies. However, NOAELs are considered comparable across multiple SDPAs for the effect. Alkylated SPDAs had a NOAEL between 5 and 25 mg/kg bw/day for the liver effects.
Severity of effect. Does the endpoint-specific analogue data set demonstrate very low/no toxicity (e.g. very high dose point of departure or free-standing NOAELs or no selective repro/devo effects seen)?	_X_ Yes ___ No Explanation/Notes (if any): The effects related to the liver and changes in clinical chemistry parameters are considered of toxicological significance. However, the effect is considered adaptive in many toxicity studies and there is some evidence that the hepatic effects are reversible once dosing is ceased. The severity of this effect is considered low.
Is the most conservative point of departure in the endpoint-specific analogue data set being used as the read-across for that endpoint? If not, is there a robust rationale to support why (i.e. clear rationale for selecting nearest neighbour approach to account for differences?)	___ Yes _X_ No Explanation/Notes (if any): Sub-groups the SDPAs were formed based on differences in side chains, phys-chem properties, predicted oral bioavailability and differences in observed effects outside of the liver toxicity. Based on these differences it is more defensible to select the closest neighbour within each subgroup to apply read-across.
Section III. Think about consistency between analogue and COI data across all endpoints:	
For the analogues used in the endpoint data set, do these analogues have data on another endpoint (anchor data) for which there are also direct data on the target, are the data concordant?	_X_ Yes ___ No
Section IV: For Read Across based on metabolites and/or 'suitable with precondition' analogues requiring a metabolic precondition to be met: N/A	
<u>Conclusions from Read-Across Uncertainty Evaluations</u> Use questionnaire responses above and weight of evidence of available data to select the appropriate uncertainty category for each endpoint (see uncertainty descriptions in Appendix D)	
Based on the analogue data set considered, read across is:	_X_ Actionable ___ Not Actionable without additional data Explanation / Comments: Although there is some degree of uncertainty associated with the justification of similarity between the analogue and target chemical, it is not expected to invalidate the read across for the intended purpose which is to derive a margin of exposure based on system effects for a screening level risk assessment under Canada's Chemical Management Plan.
Based on the analogue data set considered, the identified point-of-departure from the critical study is:	LOAEL = 100 mg/kg/day* Liver - centriloular hypertrophy (grade 1) (100mf), fatty change (100m / 300f), single cell nercrosis (300m) Haematology - ↑PTT (300m) (secondary to liver effects)

	Clinical biochem para - ↓ALB (1000mf) ↑ALP (100f / 300m) ↑Triglyceride (300f), ↑ Glucose (1000f) ↓Bile acid (100mf) Thyroid effects - hypertrophy / hyperplasia (grade 1) (100mf) (secondary to liver effects) 90 day gavage study (Rat) OECD TG 408 (ECHA 2015a)*lowest dose tested
The degree of uncertainty in the read-across is judged to be:	_X_ Low ___ Low/Moderate ___ Moderate ___ High (i.e. not actionable)
Uncertainty description(s) that applies from Appendix D:	For CAS 26603-23-6 and 68608-79-7 read across data (of sufficient quality for risk assessment) is contributed by at least 1 'suitable' analogue for the target. Highly concordant toxicity effects and potency (indicating low/moderate hazard) in across data set in subgroup. The analogue has structural features that collectively bracket the structural features of the target. For CAS 68608-77-5 read across data (of sufficient quality for risk assessment) is thought to be from a 'worst-case' analogue based on higher oral bioavailability of the comparative components. Highly concordant toxicity effects and potency (indicating low/moderate hazard) in a read across data set.

Target Chemical(s)	**Source Chemical (Analogue)**
CAS: 15721-78-5  CAS: 24925-59-5  	CAS: 101-67-1 
Questions	**Responses by Endpoint**
Section I. Think about the chemical similarity between target and source chemicals:	
What is the "similarity rating" of the analogue(s) or category members?	_X_ Suitable (all) ___ Suitable with precondition ___ Suitable with interpretation ___ Not Suitable ** Stop questionnaire
Are there any differences in functional groups and associated reactivity expected to be in the conservative direction (i.e.	___ Yes _X_ No (all) ___ Unknown Explanation/Notes (if any):

analogues would be expected to be more reactive than target?)	
Are there any differences in predicted metabolic pathways/rate expected to be in the conservative direction (e.g. where target and analogues are predicted to undergo activating metabolism and analogue metabolism to active/toxic species is more rapid than the target OR where the target and analogues are predicted to undergo deactivating metabolism and analogue metabolism to detoxification products proceeds more slowly than the target)? *Note: some knowledge of MOA will be necessary to answer.*	___ Yes ___ No _X_ Unknown (all) ___ No Differences Explanation/Notes (if any): The metabolic pathway is expected to be similar. Differences in rates are unknown.
Are there any differences in predicted metabolites expected to be in the conservative direction (i.e. data on analogue metabolites is more toxic than the predicted metabolites of the target)?	___ Yes _X__ No ___ Unknown ___ No Differences Explanation/Notes (if any): All are expected to yield hydroxylated derivatives of the parent structure. Differences in toxicity between metabolites are not expected.
Are there any differences in physical-chemical properties expected to be in the conservative direction (i.e. bioavailability of the analogue would be expected to be greater than or equal to the target)?	__ _Yes ___ No ___ Unknown _X_ No Differences (all) Explanation/Notes (if any): Predicted oral bioavailability was comparable for all targets and the source.
Are there structural characteristics that would be expected to decrease toxicity of target relative to analogues (e.g. structural hindrances for metabolism or receptor binding)?	___ Yes ___ No _X__ Unknown _ _ No Differences (all) Explanation/Notes (if any): Differences in substitution pattern and branching of the side chains has an unknown effect on toxicity.
Section II. Think about the quality and consistency of the data being contributed by different analogues to the endpoint read across:	
Did the endpoint data set include more than one study on the same analogue?	___ Yes _X__ No Was the single study of sufficient quality? _X__Yes ___ No (stop questionnaire) Explanation/Notes: Data for read across originates for a guideline GLP study considered of sufficient quality for risk

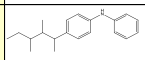
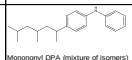
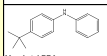
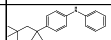
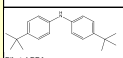
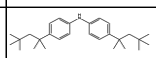
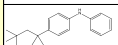
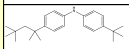
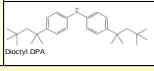
	assessment.
Were data from multiple analogues considered in the read across assessment for the endpoint?	___ Yes _X_ No Explanation/Notes (if any): The read-across in this subgroup is one to one. However, a broader group of SDPA substances was also looked at in parallel and similarities in haematological effects were noted. The source chemical in this subgroup did not show histopathological findings in the liver compared to the other SDPAs in other subgroups.
Were most of the studies generally of sufficient quality to serve as a basis for risk assessment?	_X_ Yes ___ No Explanation/Notes (if any): Data for read-across originates from a guideline GLP study.
Was there <u>any</u> evidence in the entire analogue data set of a specific toxic effect(s) for the endpoint (i.e. some target organ toxicity; some reproductive toxicity; or some developmental toxicity)? For example, answer no if there was only general toxicity such as reduced body weight and no specific target for the repeat dose toxicity endpoint.	_X_ Yes (continue questionnaire) ___ No (STOP questionnaire) Explanation/Notes (if any): Subgroups of SDPA substances were also looked at in parallel and similarities in haematological effects were noted. The source chemical in this subgroup did not show histopathological findings in the liver compared to the other SDPAs in other subgroups. Therefore, the closely related substances were placed in their own group.
If multiple analogues contributed to the endpoint data set, was there concordance in endpoint-specific adverse effects across analogues, suggesting they have same targets of toxicity? Examples: Repeat Dose Toxicity: liver toxicity, renal effects, heme changes etc. Reproductive Toxicity: repro organ weight change, estrous cycle change, % resorptions, etc. Developmental Toxicity: pup body weights, litter sizes, malformations, etc.	___ Yes (see notes) _X_ No Explanation/Notes (if any): Liver effects were not observed with the source chemical but other common effects were noted including effects on blood clotting. However, these were considered to be secondary to liver toxicity in most cases for other SPDAs. They occur in the absence of liver toxicity for this particular SDPA. Concordance with other SDPAs is lacking and therefore, these substances are placed in their own subgroup.
If multiple analogues contributed to the endpoint data set, was there concordance in endpoint-specific adverse effects and/or PODs (at least where dose spacing was comparable) across analogues, suggesting they have similar potency for the critical effects in the endpoint?	_X_ Yes ___ No Explanation/Notes (if any): Potency comparison based on LOAEL was difficult to assess due to dose spacing differences across multiple studies. Where effects on blood clotting occur LOAELs range from 75 to 150 mg/kg bw/day.

Severity of effect. Does the endpoint-specific analogue data set demonstrate very low/no toxicity (e.g. very high dose point of departure or free-standing NOAELs or no selective repro/devo effects seen)?	_X_ Yes ___ No Explanation/Notes (if any): The effects related to blood clotting were found to be reversible in a recovery group for the source chemical. The severity of this effect is considered low.
Is the most conservative point of departure in the endpoint-specific analogue data set being used as the read-across for that endpoint? If not, is there a robust rationale to support why (i.e. clear rationale for selecting nearest neighbour approach to account for differences?)	___ Yes _X_ No Explanation/Notes (if any): Sub-groups for the SDPAs were formed based on differences in side chains, phys-chem properties, predicted oral bioavailability and differences in observed effects outside of the liver toxicity. Based on these differences it is more defensible to select the closest neighbour within each subgroup to apply read-across.
Section III. Think about consistency between analogue and COI data across all endpoints:	
For the analogues used in the endpoint data set, do these analogues have data on another endpoint (anchor data) for which there are also direct data on the target, are the data concordant?	_X_ Yes ___ No
Section IV: For Read Across based on metabolites and/or 'suitable with precondition' analogues requiring a metabolic precondition to be met: N/A	
<u>Conclusions from Read-Across Uncertainty Evaluations</u> Use questionnaire responses above and weight of evidence of available data to select the appropriate uncertainty category for each endpoint (see uncertainty descriptions in Appendix D)	
Based on the analogue data set considered, read across is:	_X_ Actionable ___ Not Actionable without additional data Explanation / Comments: Although there is some degree of uncertainty associated with the justification of similarity between the analogue and target chemical, it is not expected to invalidate the read across for the intended purpose which is to derive a margin of exposure based on system effects for a screening level risk assessment under Canada's Chemical Management Plan.
Based on the analogue data set considered, the identified point-of-departure from the critical study is:	NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day Liver - no histopath findings (250mf) Haematological - ↑PTT (75m) ↑APTT (75m) Clinical biochem para - no changes (250mf) 42 day gavage comb. rep dose/repro/devo study OECD TG422 (Rat) (Japan NITE 2008)

The degree of uncertainty in the read-across is judged to be:	_X_ Low ___ Low/Moderate ___ Moderate ___ High (i.e. not actionable)
Uncertainty description(s) that applies from Appendix D:	For all targets read across data (of sufficient quality for risk assessment) is contributed by at least 1 ‘suitable’ analogue for the target.

ANNEX - DATA MATRIX

Chemical ID

	Group 1 Member 1	Group 1 Member 2	Group 2 Member 1	Group 2 Member 2
CAS	4175-37-5	27177-41-9	184378-08-3	68411-46-1
Domestic Substance List Name	Benzenamine, 4-octyl-N-phenyl-	Benzenamine, ar-nonyl-N-phenyl-	Benzenamine, N-phenyl-, reaction products with isobutylene and 2,4,4-trimethylpentene (UVCB)	Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (UVCB)
Representative Structure 1	 Monooctyl DPA	 Monononyl DPA (mixture of isomers)	 Monobutyl DPA	 Monooctyl DPA
Representative Structure 2			 Dibutyl DPA	 Dioctyl DPA
Representative Structure 3			 Monooctyl DPA	
Representative Structure 4			 Monooctyl Monobutyl DPA	
Representative Structure 5			 Dioctyl DPA	

Summary of data gap filling

		Member 2	Member 7	Member 8	Member 8
Short-term Oral repeat dose toxicity	Experimental result		NOAEL = 15 mg/kg/day LOAEL = 150 mg/kg/day Clinical - ↑salivation/staining (150mf) fur loss (500mf) Liver - Centrilobular hepatocyte enlg. / Lipid vacuolation(150mf) (slight) Haematological - ↑APTT (150m/500f) ↑PT (150m/500f) ↓haemoglobin and haematocrit (500mf) ↓ MCH/MCHC (150f) Clincal biochem para - ↓ plasma protein ↓ALB (500m/150f); ↑AST (500mf)↑ALT (500mf) ↑ALP (150m/150f) ↓creatinine (500m) ↑phosphorus and triglyceride (150f) ↓Ca (150m/500f) ↑total bilirubin (500mf) Spleen - ↑ pigment accumulation (probably haemosiderin) (150f) 28 day gavage study (Japan MHW Guideline 1986 GLP) (SafePharm Laboratories 1999)	NOAEL = 5 mg/kg/day LOAEL = 25 mg/kg/day Liver - Centriolobular hepatocyte enlg. (125m/25f) Haematological - ↑PTT ↓ platlet count (125mf) Clincal biochem para - ↓ plasma protein (125m/25f); ↓ALB ↓ALB/G ↑AST ↑ALP (125m/25f) Repro - ↓ gestation length ↑ incident offspring death (125) upto 54 days gavage repro/devo study (Rat) (OECD TG 422) (SafePharm Laboratories 2006b cited in ACC 2006)	NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day Liver - Minimal hypertrophy and vacuolation (75mf) Haematological - not summarized in dossier Clincal biochem para - ↓ plasma protein (75f); ↓ALB ↑total bilirubin ↑ALP (75mf) Repro - ↓ viability index, ↑ postnatal loss/litter and ↓ mean number of living pups/litter (225) 28 (males) - 53 (female) day gavage study (Rat) OECD TG 422 (ECHA 2015b) LOEL = 125 mg/kg/day* Liver - Slight centrilobular hepatocyte hypertrophy and minimal single cell necrosis (125m) Haematological - ↓ red blood cell counts, hemoglobin and hematocrit (300m) Clincal biochem para - ↓↑total bilirubin, alubumin, total bile acids (300m) ↑ALP (125m) 28 day gavage (non-GLP) (Rat) (ECHA 2015b) *lowest dose tested in range finding study
Sub-Chronic Oral Repeat-dose toxicity	Experimental result				
Chronic Oral Repeat-dose toxicity	Experimental result				
Oral Repeat Dose Toxicity	**Integrated conclusion (eg. read-across)**	Read Across Nearest Neighbor based on structure/compostion, properties (MW, LogP, WS) and oral bioavailability **(CAS 27177-41-9)** NOAEL = 15 mg/kg/day LOAEL = 150 mg/kg/day Liver and spleen effects / clinical para and haematological para 28 day gavage study (Rat) OECD TG 408 (ECHA 2015a) *lowest dose tested	N/A	N/A	N/A

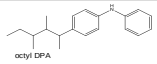
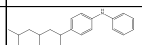
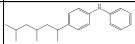
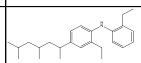
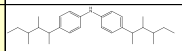
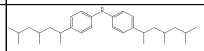
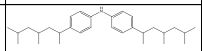
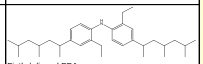
Molecular profiling related to the category hypothesis

Parent chemical	Rpeated Dose Profiler (HESS available in OECD Toolbox, v 2.6)	Not categorized	Not categorized	(All structures) Not categorized	Not categorized

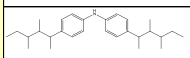
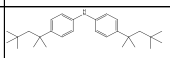
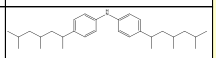
Physical-chemical data

Molecular Weight (Da)	Monooctyl DPA - 281	Mononnyl DPA - 295	Monobutyl DPA - 225 Dibutyl DPA - 281 Monooctyl DPA - 281 Monobutyl monooctyl DPA -338 Dioctyl DPA - 394	Monooctyl DPA - 281 Dioctyl DPA - 394
Melting Point (°C) - measured			10 (US EPA 2009)	-31 OECD TG 102 (ECHA 2015b)
Boiling Point (°C)- measured				>300 OECD TG 103 (ECHA 2015b)
Boiling Point (°C)- modelled EPI Suite v4.11 - MPBPVP v1.43	364.18	375.79	Range of Components 326.04 - 431.62	Range of Components 361.98 - 431.62
logPow - measured				
logPow - modelled EPI Suite v4.11 - KOWWIN v1.68	7.06	7.55	Monobutyl DPA - 5.2 Dibutyl DPA - 7.11 Monooctyl DPA - 7.05 Monobutyl monooctyl DPA - 8.96 Dioctyl DPA - 10.8	Monooctyl DPA - 7.05 Dioctyl DPA - 10.8
Water Solubility (mg/L) - measured			5.93×10^{-5} - 9.09×10^{-2} (US EPA 2009)	2 @ 20°C; pH 5.6 OECD TG 105 (ECHA 2015b)
Water Solubility (mg/L) - modelled Epi Suite V4.11 - WSKOWWIN v1.42	1.48×10^{-2}	4.68×10^{-3}	Monobutyl DPA - 1.17 Dibutyl DPA - 0.0133 Monooctyl DPA - 0.0149 Monobutyl monooctyl DPA -1.63 x 10^{-4} Dioctyl DPA - 1.93 x 10^{-5}	Monooctyl DPA - 4.36 x 10^{-2} Dioctyl DPA - 1.71 x 10^{-5}
Vapour pressure (Pa) - measured			9.5×10^{-5} (US EPA 2009)	1.5×10^{-3} @20°C (ECHA 2015b)
Vapour pressure (Pa) - modelled EPI Suite v4.11 - MPBPVP v1.43	1.27×10^{-3}	5.37×10^{-4}	Range of Components 6.73×10^{-6} - 1.52×10^{-2}	Monooctyl DPA - 2.07×10^{-3} Dioctyl DPA - 1.6×10^{-7}

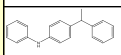
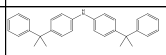
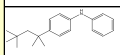
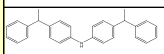
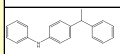
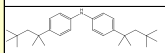
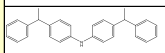
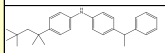
Chemical ID						
		Group 1 Member 1	Group 1 Member 2		Group 2 Member 1	Group 2 Member 2
CAS		4175-37-5	27177-41-9		184378-08-3	68411-46-1
Kinetics						
Toxicokinetics (ADME) Empirical Summary						
Toxicokinetic Parameters - measured	Cmax[µg/ml]					
	T_{max}[h]					
	AUC_{0-inf}[µg.h/ml]					
	k_{el} [1/h]					
	MRT [h]					
	$t_{1/2-el}$ [h]					
	CL [ml/h]					
	V_{ss} [ml]					
	V_{el}[ml]					
Toxicokinetic Parameters - modelled ACD Percepta 2012 (v2076) - PK Explorer Modelled @ 5mg/kg (70kg human)	Oral bioavailability (%F)	23.64	21.53		Monobutyl DPA - 39.35 Dibutyl DPA - 28.88 Monooctyl DPA - 21.8 Monobutyl monooctyl DPA -11.04 Dioctyl DPA - 0.43	Monobutyl DPA - 39.35 Dibutyl DPA - 28.88 Monooctyl DPA - 21.8 Monobutyl monooctyl DPA -11.04 Dioctyl DPA - 0.43
	Cmax (ug/ml)	0.16	0.13		Monobutyl DPA - 0.43 Dibutyl DPA - 0.20 Monooctyl DPA - 0.15 Monobutyl monooctyl DPA -0.07 Dioctyl DPA - 0.002	Monobutyl DPA - 0.43 Dibutyl DPA - 0.20 Monooctyl DPA - 0.15 Monobutyl monooctyl DPA -0.07 Dioctyl DPA - 0.002
	Tmax (h)	6.46	6.26		Monobutyl DPA - 5.87 Dibutyl DPA - 6.33 Monooctyl DPA - 6.46 Monobutyl monooctyl DPA -8.05 Dioctyl DPA - 9.6	Monobutyl DPA - 5.87 Dibutyl DPA - 6.33 Monooctyl DPA - 6.46 Monobutyl monooctyl DPA -8.05 Dioctyl DPA - 9.6
	AUC_{0-inf} (ug.h/ml)	2.34	1.62		Monobutyl DPA - 5.71 Dibutyl DPA - 2.88 Monooctyl DPA - 2.17 Monobutyl monooctyl DPA -1.67 Dioctyl DPA - 0.06	Monobutyl DPA - 5.71 Dibutyl DPA - 2.88 Monooctyl DPA - 2.17 Monobutyl monooctyl DPA -1.67 Dioctyl DPA - 0.06
Supporting data related to the target endpoint(s)						
In vivo	Mechanistic Studies					Dysregulations in the liver cell metabolism of rats can be assumed because of lower urea and amino acid levels, indicating a decreased protein metabolism. In addition, creatine, creatinine, phosphocreatine and urea were significantly decreased whereas citrulline, uric acid, ornithine and phosphate (inorganic and from organic phosphates) were significantly increased indicating a slight functional effect on the kidneys or an alteration on the urea cycle. In all dosed rats increased levels of complex lipids and degradation products indicated an altered lipid metabolism. Higher levels of complex lipids like phosphatidylcholines particularly in the 300 mg/kg dose group could be due to an increased degradation of cell membranes. The correlation analyses of the whole plasma metabolite profile of benzenamine, Nphenyl-, reaction products with 2,4,4-trimethylpentene against the metabolite profiles of the reference compounds and the patterns available in the MetaMap®. No matches in database were found to indicate a certain mode of action. 29-day oral study in male rats (ECHA 2015b)
In Silico (Q)SAR	Expert system (Derek Nexus, v. 3.0.1)	No Alert	No Alert		No Alert	No Alert

Chemical ID					
		Group 2 Member 3	Group 2 Member 3	Group 2 Member 4	Group 2 Member 5
CAS		26603-23-6	36878-20-3	68608-79-7	68608-77-5
Domestic Substance List Name		Benzenamine, ar-octyl-N-(octylphenyl)- (UVCB)	Benzenamine, ar-nonyl-N-(nonylphenyl)- (UVCB)	Benzenamine, N-phenyl-, (tripropenyl) derivs. (UVCB)	Benzenamine, 2-ethyl-N-(2-ethylphenyl)-, (tripropenyl) derivs. (UVCB)
Representative Structure 1		 octyl DPA	 Mononnyl DPA	 Mononnyl DPA	 Diethyl mononnyl DPA
Representative Structure 2		 Dioctyl DPA	 Dinonyl DPA	 Dinonyl DPA	 Diethyl dinonyl DPA
Representative Structure 3					
Representative Structure 4					
Representative Structure 5					
Summary of data gap filling					
		Member 6	Member 8	Member 8	Member 8
Short-term Oral repeat dose toxicity	Experimental result				
Sub-Chronic Oral Repeat-dose toxicity	Experimental result		LOAEL = 100 mg/kg/day* Liver - centrilouar hypertrophy (grade 1) (100mf), fatty change (100m / 300f), single cell nercrosis grade 1 (100m) Haematology - ↑PTT (1000m) (secondary to liver effects) Clinical biochem para - ↓ALB (1000mf) ↑ALP (100f / 300m) ↑Triglyceride (300f), ↓Bile acid (100mf) Thyroid effects - hypertrophy / hyperplasia (grade 1) (100mf) (secondary to liver effects) 90 day gavage study (Rat) OECD TG 408 (ECHA 2015a) *lowest dose tested		
Chronic Oral Repeat-dose toxicity	Experimental result				
Oral Repeat Dose Toxicity	**Integrated conclusion (eg. read-across)**	**Read Across** Nearest Neighbor based on structure/compostion, properties (MW, LogP, WS) and oral bioavailability **(CAS 36878-20-3)** LOAEL = 100 mg/kg/day* Liver and thyroid (secondary) effects / clinical biochem para. 90 day gavage study (Rat) OECD TG 408 (ECHA 2015a) *lowest dose tested	N/A	**Read Across** Nearest Neighbor based on structure/compostion, properties (MW, LogP, WS) and oral bioavailability **(CAS 36878-20-3)** LOAEL = 100 mg/kg/day* Liver and thyroid (secondary) effects / clinical biochem para. 90 day gavage study (Rat) OECD TG 408 (ECHA 2015a) *lowest dose tested	**Read Across** Nearest Neighbor based on structure/compostion, properties (MW, LogP, WS) and oral bioavailability **(CAS 36878-20-3)** LOAEL = 100 mg/kg/day* Liver and thyroid (secondary) effects / clinical biochem para. 90 day gavage study (Rat) OECD TG 408 (ECHA 2015a) *lowest dose tested
Molecular profiling related to the category hypothesis					
Parent chemical	Rpeated Dose Profiler (HESS available in OECD Toolbox, v 2.6)	Not categorized	Not categorized	(All structures) Not categorized	(All structures) Not categorized
Physical-chemical data					
Molecular Weight (Da)		Monooctyl DPA - 281 Dioctyl DPA - 394	Mononnyl DPA - 295 Dinonyl DPA - 421	Mononnyl DPA - 295 Dinonyl DPA - 421	Diethyl mononnyl DPA - 351 Diethyl dinonyl DPA - 477
Melting Point (°C) - measured			-43 OECD TG 103 (ECHA 2015a)		
Boiling Point (°C)- measured			>300 OECD TG 103 (ECHA 2015a)		221 (US EPA 2009)
Boiling Point (°C)- modelled EPI Suite v4.11 - MPBPVP v1.43		436.03	Range of Components 375.79 - 459.23	Range of Components 375.79 - 459.23	Range of Components 422.19 - 505.64
logPow - measured					
logPow - modelled EPI Suite v4.11 - KOWWIN v1.68		Dioctyl DPA - 10.82	Mononnyl DPA- 7.55 Dinonyl DPA - 11.8	Mononnyl DPA- 7.55 Dinonyl DPA - 11.8	Diethyl mononnyl DPA - 9.62 Diethyl dinonyl DPA - 13.88
Water Solubility (mg/L) - measured			$< 5 \times 10^{-3}$ @ 20°C; pH 6.1, 6.2 OECD TG 105 (ECHA 2015a)		
Water Solubility (mg/L) - modelled Epi Suite V4.11 - WSKOWWIN v1.42		1.92×10^{-6}	Mononnyl DPA - 4.68×10^{-3} Dinonyl DPA - 1.87×10^{-7}	Mononnyl DPA - 4.68×10^{-3} Dinonyl DPA - 1.87×10^{-7}	Diethyl mononnyl DPA - 3.66×10^{-5} Diethyl dinonyl DPA - 4.7×10^{-7}
Vapour pressure (Pa) - measured			< 1 @ 25°C (ECHA 2015a)		
Vapour pressure (Pa) - modelled EPI Suite v4.11 - MPBPVP v1.43		6.51×10^{-6}	Mononnyl DPA - 5.37×10^{-4} Dioctyl DPA - 1.29×10^{-6}	Mononnyl DPA - 5.37×10^{-4} Dinonyl DPA - 1.29 x 10-6	Diethyl mononnyl DPA - 1.53×10^{-5} Diethyl dinonyl DPA - 3.93×10^{-8}

Chemical ID					
		Group 2 Member 3	Group 2 Member 3	Group 2 Member 4	Group 2 Member 5
CAS		26603-23-6	36878-20-3	68608-79-7	68608-77-5
Kinetics					
Toxicokinetics (ADME) Empirical Summary					
Toxicokinetic Parameters - measured	Cmax[µg/ml]				
	T_{max}[h]				
	AUC_{0-inf}[µg.h/ml]				
	k_{el} [1/h]				
	MRT [h]				
	$t_{1/2 el}$ [h]				
	CL [ml/h]				
	V_{el} [ml]				
	V_{ss}[ml]				
Toxicokinetic Parameters - modelled ACD Percepta 2012 (v2076) - PK Explorer Modelled @ 5mg/kg (70kg human)	Oral bioavailability (%F)	Dioctyl DPA - 0.27	Monononyl DPA - 21.53 Dinonyl DPA - 0.06	Monononyl DPA - 21.53 Dinonyl DPA - 0.06	Diethyl monononyl DPA - 2.74 Diethyl dinonyl DPA - 0.002
	Cmax (ug/ml)	Dioctyl DPA - 0.0012	Monononyl DPA - 0.1279 Dinonyl DPA - 0.0002	Monononyl DPA - 0.1279 Dinonyl DPA - 0.0002	Diethyl monononyl DPA - 0.014 Diethyl dinonyl DPA - 0.0
	Tmax (h)	Dioctyl DPA - 9.06	Monononyl DPA - 6.26 Dinonyl DPA - 9.38	Monononyl DPA - 6.26 Dinonyl DPA - 9.38	Diethyl monononyl DPA - 8.56 Diethyl dinonyl DPA - 9.63
	AUC_{0-inf} (ug.h/ml)	Dioctyl DPA - 0.03	Monononyl DPA - 1.62 Dinonyl DPA - 0.006	Monononyl DPA - 1.62 Dinonyl DPA - 0.006	Diethyl monononyl DPA -0.34 Diethyl dinonyl DPA - 0.0001
Supporting data related to the target endpoint(s)					
In vivo	Mechanistic Studies				
In Silico (Q)SAR	Expert system (Derek Nexus, v. 3.0.1)	No Alert	No Alert	No Alert	No Alert

Chemical ID				
		Group 3 Member 1	Group 3 Member 3	Group 3 Member 3
CAS		101-67-7	15721-78-5	24925-59-5
Domestic Substance List Name		Benzenamine, 4-octyl-N-(4-octylphenyl)- (Isomer mixture)	Benzenamine, 4-(1,1,3,3-tetramethylbutyl)-N-[4-(1,1,3,3-tetramethylbutyl)phenyl]-	Benzenamine, 4-nonyl-N-(4-nonylphenyl)- (Isomer mixture)
Representative Structure 1		 Dioctyl DPA	 Dioctyl DPA	 Dinonyl DPA
Representative Structure 2				
Representative Structure 3				
Representative Structure 4				
Representative Structure 5				
Summary of data gap filling				
		Member 1	Member 4	Member 5
Short-term Oral repeat dose toxicity	Experimental result	NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day Liver - no histopath findings (250mf) Haematological - ↑PTT (75m) ↑APTT (75m) Clinical biochem para - no changes (250mf) Repro/Devo - no effects 42 day gavage comb. rep dose/repro/devo study OECD TG422 (Rat) (Japan NITE 2008)		
Sub-Chronic Oral Repeat-dose toxicity	Experimental result		Planned OECD TG408 90 - day gavage (ECHA 2014b)	
Chronic Oral Repeat-dose toxicity	Experimental result			
Oral Repeat Dose Toxicity	**Integrated conclusion (e.g. read-across)**	N/A	**Read-across** Nearest Neighbor based on structure/compostion, properties (MW, LogP, WS) and oral bioavailability **(CAS 101-67-7)** NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day haematological effects 42-53 day gavage comb. rep dose/repro/devo study OECD TG422 (Rat)	**Read-across** Nearest Neighbor based on structure/compostion, properties (MW, LogP, WS) and oral bioavailability **(CAS 101-67-7)** NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day haematological effects 42-53 day gavage comb. rep dose/repro/devo study OECD TG422 (Rat)
Molecular profiling related to the category hypothesis				
Parent chemical	Rpeated Dose Profiler (HESS available in OECD Toolbox, v 2.6)	Not categorized	Not categorized	Not categorized
Physical-chemical data				
Molecular Weight (Da)		Dioctyl DPA - 394	Dioctyl DPA - 394	Dioctyl DPA - 394
Melting Point (°C) - measured			98.9 OECD TG 102 (ECHA 2014b)	
Boiling Point (°C)- measured			>400 OECD TG 103 (ECHA 2014b)	
Boiling Point (°C)- modelled EPI Suite v4.11 - MPBPVP v1.43		436.03	431.62	459.23
logPow - measured			8.8 @ 40°C, pH 8 OECD TG 117 (ECHA 2014b)	
logPow - modelled EPI Suite v4.11 - KOWWIN v1.68		Dioctyl DPA - 10.82	Dioctyl DPA - 10.82	11.8
Water Solubility (mg/L) - measured			< 0.1 @ 25°C; pH 7 OECD TG 105 (ECHA 2014b)	
Water Solubility (mg/L) - modelled Epi Suite V4.11 - WSKOWWIN v1.42		1.92×10^{-6}	1.94×10^{-6}	1.87×10^{-7}
Vapour pressure (Pa) - measured		9.40×10^{-5} (US EPA 2009)	$< 1.1 \times 10^{-5}$ @ 20°C EU Method A.4 (ECHA 2014b)	
Vapour pressure (Pa) - modelled EPI Suite v4.11 - MPBPVP v1.43		6.51×10^{-6}	6.73×10^{-6}	1.29×10^{-6}

Chemical ID					
		Group 3 Member 1	Group 3 Member 3	Group 3 Member 3	
CAS		101-67-7	15721-78-5	24925-59-5	
Kinetics					
Toxicokinetics (ADME) Empirical Summary					
Toxicokinetic Parameters - measured	Cmax[ug/ml]				
	T_{max}[h]				
	AUC_{0-inf}[ug.h/ml]				
	k_{el} [1/h]				
	MRT [h]				
	$t_{1/2\ el.}$[h]				
	CL [ml/h]				
	$V_{el.}$[ml]				
	V_{ss}[ml]				
Toxicokinetic Parameters - modelled ACD Percepta 2012 (v2076) - PK Explorer Modelled @ 5mg/kg (70kg human)	Oral bioavailability (%F)	Dioctyl DPA - 0.27	Dioctyl DPA - 0.43	Dinonyl DPA - 0.06	
	Cmax (ug/ml)	Dioctyl DPA - 0.0012	Dioctyl DPA - 0.002	Dinonyl DPA - 0.0002	
	Tmax (h)	Dioctyl DPA - 9.06	Dioctyl DPA - 9.6	Dinonyl DPA - 9.38	
	AUC_{0-inf} (ug.h/ml)	Dioctyl DPA - 0.03	Dioctyl DPA - 0.06	Dinonyl DPA - 0.006	
Supporting data related to the target endpoint(s)					
In vivo	Mechanistic Studies				
In Silico (Q)SAR	Expert system (Derek Nexus, v. 3.0.1)	No Alert	No Alert	No Alert	

Chemical ID					
		Group 4 Member 1	Group 5 Member 2		Standalone
CAS		68442-68-2	10081-67-1		68921-45-9
Domestic Substance List Name		Benzenamine, N-phenyl-, styrenated (UVCB)	Benzenamine, 4-(1-methyl-1-phenylethyl)-N-[4-(1-methyl-1-phenylethyl)phenyl]-		Benzenamine, N-phenyl-, reaction products with styrene and 2,4,4-trimethylpentene (UVCB)
Representative Structure 1		 Monostyrenated DPA	 Dimethylstyrenated DPA		 Monooctyl DPA
Representative Structure 2		 Distyrenated DPA			 Monostyrenated DPA
Representative Structure 3					 Dioctyl DPA
Representative Structure 4					 Distyrenated DPA
Representative Structure 5					 Monooctyl monostyrenated DPA
Summary of data gap filling					
					Member 8
Short-term Oral repeat dose toxicity	Experimental result	NOAEL = 50 mg/kg/day LOAEL = 250 mg/kg/day* Liver - centrilobular hepatocyte enlargement (250m / 50f) Haematology - no effects Clinical biochem para - ↓ cholesterol (250m); ↑ALP (600m) Thyroid - Follicular cell hypertrophy (250m) Repro - pre-implantation loss (600f) 43-54 days gavage repro/devo study OECD TG 422 (Rat) (SafePharm Laboratories 2006a cited in ACC 2006; US EPA 2009) *LOAEL - as interpreted by Health Canada	NOEL = 40 mg/kg/day LOEL = 80 mg/kg/day Liver - no significant histopath findings at high dose (80mf) Clinical biochem para -↑Bilirubin (80m) ↓ cholesterol (80m) ↑ALP (80mf) ↑Triacylglycerols (80m) ↓Creatine (80m) Kidney - degenerative nephrosis in proximal tubles (10f) 28 day gavage rep. dose OECD TG407 (Rat) (ECHA 2014a)		
Sub-Chronic Oral Repeat-dose toxicity	Experimental result		Planned OECD TG408 90 - day gavage (ECHA 2014a)		
Chronic Oral Repeat-dose toxicity	Experimental result				LOAEL = 125 mg/kg/day (2500 ppm in diet)* Liver - degenerative changes (fatty change, diffuse cloudy swellings) Haemetological - no data Clinical Biochem para - no data 64 week diet study (non-GLP) (Rat) (Treon et al. 1957 report cited in ACC; US EPA 2009; ECHA 2014c) *lowest dose tested
Oral Repeat Dose Toxicity	**Integrated conclusion (eg. read-across)**	N/A	N/A		N/A
Molecular profiling related to the category hypothesis					
Parent chemical	Rpeated Dose Profiler (HESS available in OECD Toolbox, v 2.6)	Hepatotoxicity alert: 50% Dice structural similarity to Tamoxifen (Hepatotoxicity) (Structure 2) Hepatotoxicity alert: 50% Dice structural similarity to Oxyphenistain (Hepatotoxicity)			Hepatotoxicity alert: 50% Dice structural similarity to Tamoxifen (Hepatotoxicity) (Structure 4) Hepatotoxicity alert: 50% Dice structural similarity to Oxyphenistain (Hepatotoxicity)
Physical-chemical data					
Molecular Weight (Da)		Monostyrentated DPA - 273 Distyrenated DPA- 377	405		Monooctyl DPA - 281 Monostyrentated DPA - 273 Distyrenated DPA- 377 Dioctyl DPA - 394 Monooctyl monostyrenated DPA - 385
Melting Point (°C) - measured		~6 (US EPA 2009)	95 (US EPA 2009)		
Boiling Point (°C)- measured			>400 OECD TG 103 (ECHA 2014a)		>198 (ECHA 2014c)
Boiling Point (°C)- modelled EPI Suite v4.11 - MPBPVP v1.43		Range of Components 392.71 - 507.08	507.08		Range of Components 392.71 - 431.62
logPow - measured		4.64 @ 22°C (US EPA 2009)	7.9 OECD TG 117 (ECHA 2014a)		5.2 (ECHA 2014c)
logPow - modelled EPI Suite v4.11 - KOWWIN v1.68		Monostyrentated DPA - 5.45 Distyrenated DPA- 7.61	8.51		Range of Components 5.45 - 10.82
Water Solubility (mg/L) - measured		2.06 x 10-2 to <5.88 x 10-2 (US EPA 2009)	< 6.7 x 10-3 @ 20°C; pH 7.04, 7.67 EU Method A.6 (ECHA 2014a)		0.706 @ 20°C; pH 6.9-7 OECD TG 105 (ECHA 2014c)
Water Solubility (mg/L) - modelled Epi Suite V4.11 - WSKOWWIN v1.42		Monostyrenated DPA - 3.8 x 10^{-1} Distyrenated DPA - 1.5 x 10^{-4}	1.52 x 10-4		Range of Components 1.93 x 10^{-6} - 3.8 x 10^{-1}
Vapour pressure (Pa) - measured			667 @20°C (ECHA 2014a)		1.5 x 10^{-3} @ 20°C OECD TG 104 (ECHA 2014c)
Vapour pressure (Pa) - modelled EPI Suite v4.11 - MPBPVP v1.43		Monostyrenated DPA - 1.3 x 10-4 Distyrenated DPA - 2.32 x 10-8	2.32 x 10-8		Range of Components 6.73 x 10^{-6} - 1.3 x 10^{-4}

Kinetics							
Toxicokinetics (ADME) Empirical Summary					**Adsorption**: maximal plasma level occured after 7h (10.81 µg/mL at 80mg/kg) and were considered low and consistant with no high or rapid adsoprtion. **Metabolism**: parent compound identified in plasma with an additional unknown metabolite (likely hydroxylated derivatives). **Distribution:** mainly to liver and spleen. Test substance also distributed to lung, kidney, adipose fat, muscle and skin. **Excretion:** mostly eliminated in feces; low levels in urine OECD TG 417 (ECHA 2014a)		
Toxicokinetic Parameters - measured	Cmax[µg/ml]				10.81 @ 80mg/kg (Rat) OECD TG 417 (ECHA 2014a)		
	T_{max}[h]				6.8 @ 80mg/kg (Rat) OECD TG 417 (ECHA 2014a)		
	AUC_{0-inf}[µg.h/ml]				122.13 @ 80mg/kg (Rat) OECD TG 417 (ECHA 2014a)		
	k_{el} [1/h]				0.034 @ 80mg/kg (Rat) OECD TG 417 (ECHA 2014a)		
	MRT [h]				28.62 @ 80mg/kg (Rat) OECD TG 417 (ECHA 2014a)		
	$t_{1/2\ el.}$ [h]				20.61 @ 80mg/kg (Rat) OECD TG 417 (ECHA 2014a)		
	CL [ml/h]				116 @ 80mg/kg (Rat) OECD TG 417 (ECHA 2014a)		
	$V_{el.}$ [ml]				3456.4 @ 80mg/kg (Rat) OECD TG 417 (ECHA 2014a)		
	V_{ss} [ml]				3327.7 @ 80mg/kg (Rat) OECD TG 417 (ECHA 2014a)		
Toxicokinetic Parameters - modelled ACD Percepta 2012 (v2076) - PK Explorer Modelled @ 5mg/kg (70kg human)	Oral bioavailability (%F)			Monostyrenated DPA - 24.31 Distyrenated DPA - 2.5	Dimethylstyrenated DPA - 2.34		Monooctyl DPA - 2.81 Monostyrentated DPA -24.31 Distyrenated DPA- 2.5 Dioctyl DPA - 0.43 Monooctyl monostyrenated DPA - 4.41
	Cmax (ug/ml)			Monostyrenated DPA - 0.181 Distyrenated DPA - 0.014	Dimethylstyrenated DPA - 0.01		Monooctyl DPA - 0.15 Monostyrentated DPA - 0.181 Distyrenated DPA- 0.014 Dioctyl DPA -0.002 Monooctyl monostyrenated DPA - 0.022
	Tmax (h)			Distyrenated DPA - 8.81 Monostyrenated DPA - 6.36	Dimethylstyrenated DPA - 8.9		Monooctyl DPA - 6.46 Monostyrentated DPA - 6.36 Distyrenated DPA- 8.81 Dioctyl DPA - 9.6 Monooctyl monostyrenated DPA - 8.78
	AUC_{0-inf} (ug.h/ml)			Distyrenated DPA - 0.35 Monostyrenated DPA - 2.48	Dimethylstyrenated DPA - 0.28		Monooctyl DPA - 5.71 Monostyrentated DPA - 2.48 Distyrenated DPA- 0.35 Dioctyl DPA - 0.06 Monooctyl monostyrenated DPA - 0.621
Supporting data related to the target endpoint(s)							
In vivo	Mechanistic Studies						
In Silico (Q)SAR	Expert system (Derek Nexus, v. 3.0.1)			No Alert	No Alert		No Alert