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**ENVIRONMENT DIRECTORATE  
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PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY****CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR  
TESTING AND ASSESSMENT FOR 90-DAY RAT ORAL REPEATED-DOSE  
TOXICITY OF CHLOROBENZENE-RELATED CHEMICALS**

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AND ASSESSMENT FOR 90-DAY RAT ORAL REPEATED-DOSE TOXICITY OF  
CHLOROBENZENE-RELATED CHEMICALS**

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## Forward

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 Kao Corporation (BIAC) for illustrating practical use of IATA and submitted to the 2019 review cycle of the IATA Case Studies Project. This case study was reviewed by the project team. The document was endorsed at the 4<sup>th</sup> meeting of the Working Party on Hazard Assessment in June 2020.

The following case study was also reviewed in the project in 2019:

1. CASE STUDY ON USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT (IATA) AND NEW APPROACH METHODS TO INFORM A THEORETICAL READ-ACROSS FOR DERMAL EXPOSURE TO PROPYLPARABEN FROM COSMETICS, ENV/JM/MONO(2020)16.
2. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR SYSTEMIC TOXICITY ARISING FROM COSMETIC EXPOSURE TO CAFFEINE, ENV/JM/MONO(2020)17.
3. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT TO INFORM READ-ACROSS OF P-ALKYLPHENOLS: REPEATED-DOSE TOXICITY, ENV/JM/MONO(2020)19.
4. CASE STUDY ON THE USE OF INTEGRATED APPROACHES TO TESTING AND ASSESSMENT FOR PREDICTION OF A 90 DAY REPEATED DOSE TOXICITY STUDY (OECD 408) FOR 2-ETHYLBUTYRIC ACID USING A READ-ACROSS APPROACH FROM OTHER BRANCHED CARBOXYLIC ACIDS, ENV/JM/MONO(2020)20.
5. CASE STUDY ON THE USE OF INTEGRATED APPROACHES TO TESTING AND ASSESSMENT FOR READ-ACROSS BASED FILLING OF DEVELOPMENTAL TOXICITY DATA GAP FOR METHYL HEXANOIC ACID, ENV/JM/MONO(2020)21.
6. CASE STUDY ON THE USE OF INTEGRATED APPROACHES TO TESTING AND ASSESSMENT FOR IDENTIFICATION AND CHARACTERISATION OF PARKINSONIAN HAZARD LIABILITY OF DEGUELIN BY AN AOP-BASED TESTING AND READ ACROSS APPROACH, ENV/JM/MONO(2020)22.

7. CASE STUDY ON THE USE OF INTEGRATED APPROACHES TO TESTING AND ASSESSMENT FOR MITOCHONDRIAL COMPLEX-III-MEDIATED NEUROTOXICITY OF AZOXYSTROBIN - READ-ACROSS TO OTHER STROBILURINS, ENV/JM/MONO(2020)23.

These case studies are illustrative examples, and their publication as OECD monographs does not translate into direct acceptance of the methodologies for regulatory purposes across OECD countries. In addition, these cases studies should not be interpreted as official regulatory decisions made by the authoring member countries.

In addition, a considerations document summarising 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) -Fifth Review Cycle (2019) -, ENV/JM/MONO(2020)24.

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

## Abstract

Read-across is a useful concept for non-animal toxicological predictions. However, due to the lack of experience for the standard of regulatory acceptance regarding read-across, its use for chemical risk assessment to meet the regulatory acceptance is limited. Therefore, case studies may provide novel insights into the standard of regulatory acceptance. In addition, the Integrated Approaches to Testing and Assessment (IATA) seems to enhance the accuracy and robustness of conclusions. Thus, this study aimed to clarify regulatory acceptance and improve OECD guidance on the IATA that could be useful for non-animal toxicological prediction. We selected 90-day rat oral repeated-dose toxicity of chlorobenzene-related chemicals as targets. The adverse outcome pathway (AOP) of these chemicals was examined. The data showed that active metabolites produced by P450 induce oxidative stress, glutathione downregulation, and protein binding in the target organs. These key events might lead to the observed adverse outcomes. In the repeated dose toxicity tests of these chemicals, a negative correlation was observed between no-observed-adverse-effect-level (NOAEL) and logKow based on toxicological investigation. Considering the accessibility of the P450 of these chemicals based on logKow, it is considered that this trend is reasonable. This study shows that read-across can be applied for hazard assessment of chlorobenzene-related chemicals.

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## 1. Introduction

Repeated-dose toxicity is one of the most difficult endpoints in the chemical risk assessment of cosmetic ingredients without using an animal test. Due to lack of animal testing data, several candidate cosmetic ingredients have not been adopted as final products. For chemical risk assessment under the EU Cosmetics Testing and Marketing Bans, the Integrated Approach to Testing and Assessment (IATA) using *in vitro/in silico/in chemico* technologies is considered one of the appropriate approaches for chemical risk assessment of repeated-dose toxicity (OECD, 2016a). Read-across can be useful for more valid prioritisation under the animal test ban. In fact, Scientific Committee on Consumer Safety (SCCS) suggests that these approaches are important for the safety assessment of cosmetic ingredients (SCCS, 2018). Furthermore, Read-Across Assessment Framework (RAAF, ECHA, 2017) and Guidance on Grouping of Chemicals (OECD, 2014a) provide details of these approaches for chemical risk assessment.

Chlorobenzene-related chemicals have various industrial applications such as herbicides, pigments, and agricultural chemicals. Due to concerns about exposure to these chemicals, several toxicological tests have been conducted. However, most of these assessments have not included a detailed examination of the structure–toxicity relationships. In addition, some chemicals do not have no-observed-adverse-effect level (NOAEL) for 90-day rat oral repeated-dose toxicity data. Thus, in this case study, we estimated these point of departures (PoDs) with read-across based on the IATA. We focused on repeated-dose toxicity endpoints with *in vitro/in silico* non-guideline test methods (Cell viability and Metabolite prediction) generated for category members, and then integrated them into the assessment (OECD, 2014b). With this case study, we intended to address how read-across can be applied for hazard assessment without using an animal test under regulations by each country.

## 2. Purpose

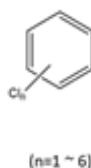
### 2.1. Purpose of use

The general purpose of this case study is to improve the OECD guidance on the IATA. The specific purpose of this case study is to demonstrate the use of read-across approach including in *in vitro/in silico* methods to estimate the NOAEL for 90-day rat oral repeated-dose toxicity data for some chlorobenzene-related chemicals to waive these data in regulatory context in each country.

### 2.2. Target chemical(s)/category definition

It is well established that chlorinated benzenes are metabolised to a quinone group and glutathione (GSH)-conjugated metabolites, which mainly lead to liver and kidney toxicities. The target chemicals in this study were chlorobenzene-related chemicals that have a similar metabolism and well known adverse outcome pathway (AOP). For a strict evaluation, these chemicals were defined as chlorinated benzenes with only chlorine atoms ( $n=1\sim6$ ) as the functional group. The basic chemical structure is shown in Figure 1.

**Figure 1. The structure of chlorobenzene-related chemicals**



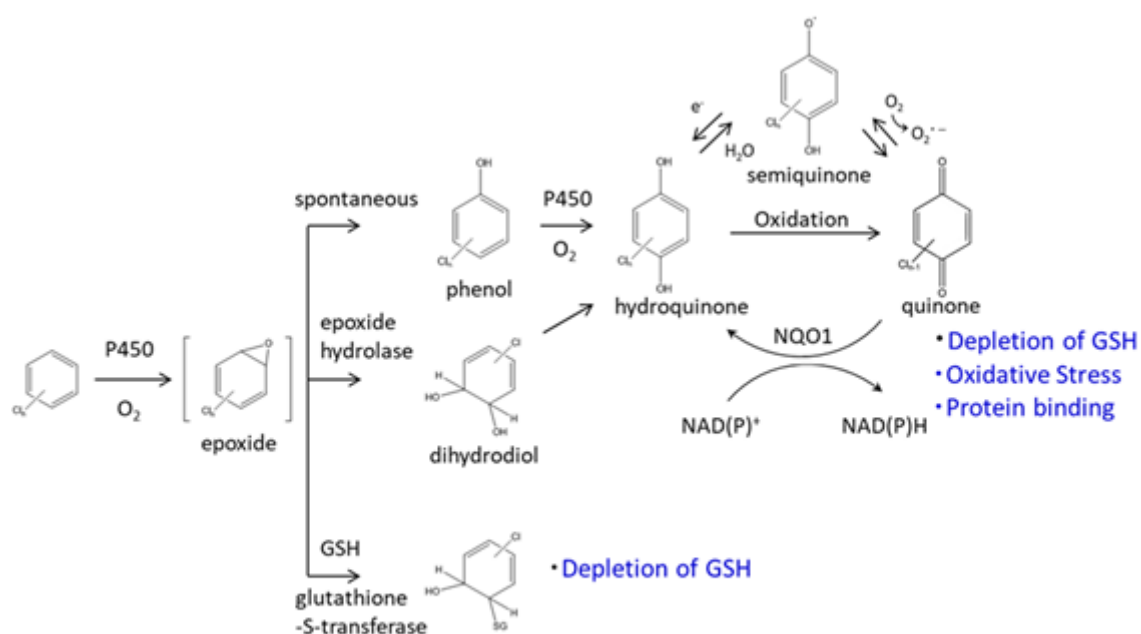
### 2.3. Endpoint(s)

The NOAEL for 90-day rat oral repeated-dose toxicity was the endpoint of this case study. The primary target organs of chlorobenzene-related chemicals are the liver and kidney. The NOAEL was mainly characterised by histopathological changes, hypertrophy, degeneration, vacuolation, relative organ weight increase, which are defined as toxicological effects, and blood biochemistry related to toxicological findings.

### 3. Hypothesis for the analogue approach/category

Chlorinated benzenes metabolised by P450 undergo oxidation in the liver to an epoxide form of metabolite (den Besten *et al.*, 1994). After the first P450 oxidation, the epoxide is transformed to three types of metabolite (Chan *et al.*, 2007). It is hypothesised that each type of metabolite induces a specific key event (Figure 2).

**Figure 2. Summary of the metabolic pathway and key events of chlorobenzene-related chemicals**



Adapted from K. Chan et al. Chem Biol Interact. 2007; 65(3): 165-74

1) The epoxide transforms to a hydroxyl group spontaneously. Furthermore, the secondary oxidation of phenols by P450 forms a hydroquinone, which is subsequently oxidised to a quinone. Quinone induces the alkylation of cellular proteins and leads to the formation of reactive oxygen species (ROS) (Bolton *et al.*, 1999). These reactions result in depletion of GSH, oxidative stress, and protein binding which lead to cellular damage.

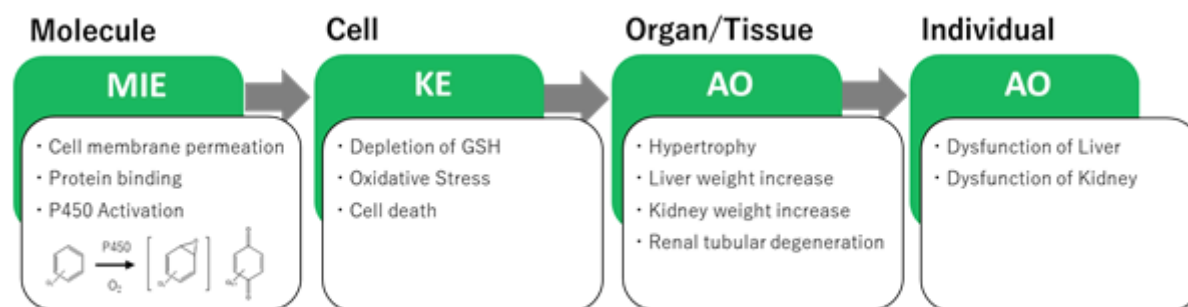
2) The epoxide is converted to hydroquinone via dihydrodiols by epoxide hydrolase. Furthermore, chlorobenzene oxide reacts with cellular protein, which accounts for the most observed toxicity among chlorobenzene analogues (EPA, 2003).

3) The epoxide is conjugated with GSH by glutathione-S-transferase. The metabolic reaction leads to a deficiency of GSH in the liver and kidney. This process induces key event in the cells (Reed, 1990).

It has been reported that the depletion of GSH along with oxidative stress induce liver damage through cell death and fatty liver formation (Yuan L and Kaplowitz N., 2009). In fact, depletion of GSH was detected in a single ip administration of some category members such as chlorobenzene and 1,2-dichlorobenzene in rats (den Besten C., 1991a). Furthermore, these key events may lead to hepatotoxicity or nephrotoxicity in the liver and

kidney due to the high metabolic activities of these organs. Liver and kidney dysfunction were a final observed adverse outcome. The overview of the adverse of outcome pathway of chlorobenzene-related chemicals is shown in Figure 3.

**Figure 3. The adverse outcome pathway of chlorobenzene related chemicals.**



MIE: Molecular initiation event, KE: Key event, AO: Adverse outcome

## 4. Source chemicals/Category members

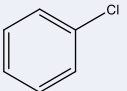
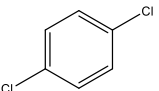
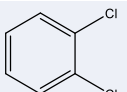
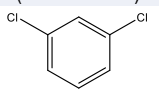
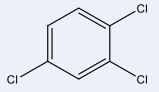
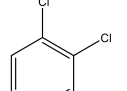
### 4.1. Identification and selection of source chemicals/category members

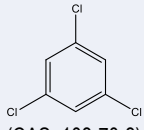
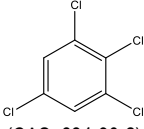
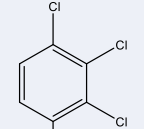
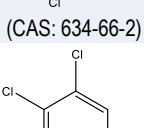
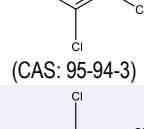
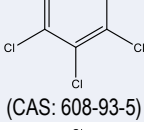
Hazard Evaluation Support System (HESS) is an available free-access software for performing read-across and grouping chemicals with the same toxicological properties (<http://www.nite.go.jp/en/chem/qsar/hess-e.html>) (Sakuratani *et al.*, 2013). HESS was used to select category and candidates of category members in this study. To minimise the effects of some functional groups on the initiation and key events, we have selected simple chemical structure, containing only chlorine atoms as chlorinated benzenes (n=1~6). The OECD QSAR Toolbox version 4.2 was then used to confirm the formation of active metabolite in each candidate using the metabolite prediction system (<http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.html>). Finally, 12 substances were identified as listed in Table 1.

### 4.2. List of source chemicals/category members

A list of the selected category members is shown in Table 1.

**Table 1. List of chlorobenzene-related category members**

No.	Substance structure (CAS No.)	Chemical name	Molecular formula	Molecular weight	Endpoint data
1	 (CAS: 108-90-7)	Chlorobenzene	C <sub>6</sub> H <sub>5</sub> Cl	112.56	Not determined
2	 (CAS: 106-46-7)	1,4-Dichlorobenzene	C <sub>6</sub> H <sub>4</sub> Cl <sub>2</sub>	147.01	Not determined
3	 (CAS: 95-50-1)	1,2-Dichlorobenzene	C <sub>6</sub> H <sub>4</sub> Cl <sub>2</sub>	147.01	Determined
4	 (CAS: 541-73-1)	1,3-Dichlorobenzene	C <sub>6</sub> H <sub>4</sub> Cl <sub>2</sub>	147.01	Not determined
5	 (CAS: 120-82-1)	1,2,4-Trichlorobenzene	C <sub>6</sub> H <sub>3</sub> Cl <sub>3</sub>	181.45	Determined
6	 (CAS: 87-61-6)	1,2,3-Trichlorobenzene	C <sub>6</sub> H <sub>3</sub> Cl <sub>3</sub>	181.45	Determined

7	 (CAS: 108-70-3)	1,3,5-Trichlorobenzene	C <sub>6</sub> H <sub>3</sub> Cl <sub>3</sub>	181.45	Determined
8	 (CAS: 634-90-2)	1,2,3,5-Tetrachlorobenzene	C <sub>6</sub> H <sub>2</sub> Cl <sub>4</sub>	215.89	Determined
9	 (CAS: 634-66-2)	1,2,3,4-Tetrachlorobenzene	C <sub>6</sub> H <sub>2</sub> Cl <sub>4</sub>	215.89	Determined
10	 (CAS: 95-94-3)	1,2,4,5-Tetrachlorobenzene	C <sub>6</sub> H <sub>2</sub> Cl <sub>4</sub>	215.89	Determined
11	 (CAS: 608-93-5)	Pentachlorobenzene	C <sub>6</sub> HCl <sub>5</sub>	250.34	Determined
12	 (CAS: 118-74-1)	Hexachlorobenzene	C <sub>6</sub> Cl <sub>6</sub>	284.8	No data

## 5. Justification of data gap filling

### 5.1. Data gathering

Publicly available repeated-dose toxicity data and related toxicological and metabolism information were gathered from toxicity database and literature search for the all category members. The OECD QSAR Toolbox version 4.2 (<http://www.oecd.org/env/ehs/risk-assessment/oecd-qsar-toolbox.htm>), Hazard Evaluation Support System Integrated Platform (HESS) version 3.8, PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), ECHA database (<http://echa.europa.eu/>), and Google (<https://www.google.co.jp>) were used for finding chemical structure, repeated-dose toxicity data, and metabolism information. Metabolite simulation using the OECD QSAR Toolbox version 4.2 was utilised for predicting metabolites in rat *in vivo* and liver S9 (Annex III). EPI-Suite TM version 4.1 was used for finding and calculating physico-chemical properties. ChemMine tools (<https://chemminetools.ucr.edu/>) was used for calculating MCS Tanimoto. All the 90-day repeated-dose toxicity studies and data are summarised in Annex I.

### 5.2. Justification

Based on the on the putative AOP (Figure 1), metabolic activation was found to be a key point for adverse outcome in category members. The formation of GSH conjugates and metabolism of reactive intermediate form dichlorobenzenes (DCBs) were detected in rat liver slices (Fisher *et al.*, 1995). In addition, trichlorobenzenes (TCBs), especially 1,2,4-TCB, was metabolised to trichlorophenol and trichlorohydroquinone (den Besten *et al.*, 1991b). Pentachlorobenzene (PCB) was administrated orally to the rats. Pentachlorophenol was a major metabolite in their plasma, although reactive intermediates (tetrachlorobenzoquinone) could covalently bind to internal protein (den Besten *et al.*, 1989; Umegaki *et al.*, 1989). Furthermore, hexachlorobenzene (HCB) was metabolised to oxidative HCBs, such as pentachlorothiophenol and pentachlorophenol, decreasing the hepatic GSH level in isolated perfused rat liver (Ingebrigtsen *et al.*, 1986). Furthermore, despite hexachlorobenzene not showing the epoxide, other members showed epoxide formation in the metabolite prediction (Annex III). In these studies, almost all chlorobenzenes underwent a similar metabolic pathway (Figure 1)—epoxidation and GSH conjugation that are related to molecular initiation event.

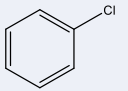
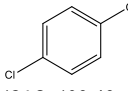
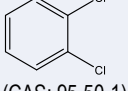
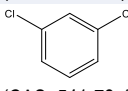
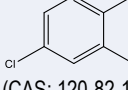
Indeed, chlorobenzenes mainly showed similar toxicological findings such as increased relative organ weight, hypertrophy, and histological changes in the liver and kidney. For chlorobenzene (member 1), a decrease in the relative spleen weight was observed at 60 mg/kg, besides liver and kidney toxicities. In this study, the NOAEL was not decided because there was toxicological effect (spleen) at the lowest dose (60 mg/kg). Liver toxicity was observed at 125 mg/kg (NTP, 1985a). Furthermore, dichlorobenzenes (members 2, 3, and 4) mainly induce hepatocellular and renal cellular damages. However, there are two isomers (members 2 and 4), whose NOAEL was not decided due to kidney toxicity at the lowest dose and parameter alteration in blood biochemistry, respectively (ECHA, 1988; NTP, 1985b; McCauley *et al.*, 1995). The histological change in the liver mainly appeared after 90-day oral toxicity study at 82 (member 5), 78 (member 6), and 82 mg/kg (member 7) in trichlorobenzenes (Côté *et al.*, 1988). For tetrachlorobenzenes, histological changes in the kidney and liver were observed at 34 and 41 mg/kg (member 8), histological changes in liver was observed at 34 mg/kg (member 9) histological changes in the kidney and liver

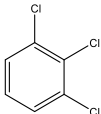
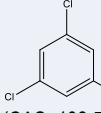
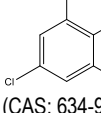
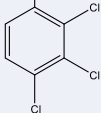
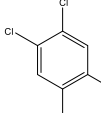
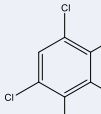
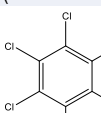
were observed at 3.4, 34, 41 mg/kg (member 10). Especially, similar toxicological findings such as liver and kidney weight increase appeared only for member 10. After dosing with pentachlorobenzene, the relative kidney weight increase, renal lesions, and hepatocellular hypertrophy were observed. In addition to these toxicological effects in the liver and kidney, thyroid toxicity was detected (NTP, 1991).

It is strongly suggested that these toxicological findings were caused by active metabolites, following the induction of oxidative stress and depletion of GSH as subsequent key events on the cellular level (Chan *et al.*, 2007; den Besten *et al.*, 1994). With respect to metabolism, the main target organs are reasonable because P450 is abundantly expressed in the liver and kidney. Overall, it is considered that these toxicological effects were induced by a similar molecular initiation event and following key event for members 1~12. The 90-day rat oral repeated-dose toxicity data of chlorobenzene-related chemicals are shown in Annex I.

The summary data and integrated conclusion of the NOAEL for 90-day rat oral repeated dose toxicity and logKow are shown in Table 4. In the relationship between the NOAEL and logKow, the NOAEL decreased with increase in logKow. Taken together, the NOAEL of chlorobenzene-related chemicals highly negative correlated with the physico-chemical properties, especially the logKow value. It is inferred that more hydrophobic chlorobenzenes readily permeate the cell membrane and access the P450 hydrophobic active site for bioactivation of reactive metabolites. The toxicological hypothesis is supported by several toxicological studies. For dichlorobenzenes, although logKow values of members 2 and 3 are close, the rank order of dichlorobenzene hepatotoxicity was 1,3-DCB > 1,2-DCB > 1,4-DCB in human liver slices (Fisher *et al.*, 1991). Similarly, the same trend was examined for trichlorobenzenes (Chu *et al.*, 1984).

**Table 2. A data matrix for chlorobenzene-related chemicals**

No.	Substance structure (CAS No.)	Target organ (LOAEL*)	LOAEL*	Study information	LogKow**	Data source
1	 (CAS: 108-90-7)	Spleen	60 mg/kg (NOAEL was Not determined)	F344/N, rat, 91 days, Gavage, Male, Female: 0, 60, 125, 250, 500, 750 mg/kg, GLP, Purity: >99%	2.84	NTP, 1985a
2	 (CAS: 106-46-7)	Kidney	75 mg/kg (NOAEL was Not determined)	Fischer 344 rat, 91 days, Gavage, Male, Female: 0, 75, 150, 300, 600 mg/kg, GLP, Purity: 99.9%	3.44	ECHA, 1988
3	 (CAS: 95-50-1)	Liver	125 mg/kg	F344/N rat, 91 days, Gavage, Male, Female: 0, 30, 60, 125, 250, 500 mg/kg, GLP, Purity: > 99%	3.43	NTP, 1985b
4	 (CAS: 541-73-1)	Liver	9 mg/kg (NOAEL was Not determined)	SD rat, 90 days, Gavage, Male, Female: 0, 9, 37, 147, 588 mg/kg, Purity: > 99.99%	3.53	McCauley <i>et al.</i> , 1995
5	 (CAS: 120-82-1)	Liver, Kidney, Thyroid	82 mg/kg	SD rat, 91 days, Feed, Male: 0.07, 0.78, 7.8, 82 mg/kg, Female: 0.11, 1.4, 15, 101 mg/kg, Purity: > 99%	4.02	Côté <i>et al.</i> , 1988

6	 (CAS: 87-61-6)	Liver, Kidney, Thyroid	78 mg/kg	SD rat, 91 days, Feed, Male: 0.08, 0.78, 7.6, 78 mg/kg, Female: 0.13, 1.3, 12, 113 mg/kg, Purity: > 99%	4.05	Côté <i>et al.</i> , 1988
7	 (CAS: 108-70-3)	Liver, Kidney, Thyroid	82 mg/kg	SD rat, 91 days, Feed, Male: 0.08, 0.81, 7.7, 82 mg/kg, Female: 0.13, 1.5, 17, 146 mg/kg, Purity: > 99%	4.19	Côté <i>et al.</i> , 1988
8	 (CAS: 634-90-2)	Kidney	34 mg/kg	SD rat, 90 days, Feed, Male: 0, 0.034, 0.34, 3.4, 34 mg/kg, Female: 0, 0.042, 0.42, 4.2, 41 mg/kg, Purity: > 99.5 %	4.56	Chu <i>et al.</i> , 1984
9	 (CAS: 634-66-2)	Liver	34 mg/kg	SD rat, 90 days, Feed, Male: 0, 0.034, 0.34, 3.4, 34 mg/kg, Female: 0, 0.042, 0.42, 4.2, 41 mg/kg, Purity: > 99.5%	4.6	Chu <i>et al.</i> , 1984
10	 (CAS: 95-94-3)	Liver	3.4 mg/kg	SD rat, 90 days, Feed, Male: 0, 0.034, 0.34, 3.4, 34 mg/kg, Female: 0, 0.042, 0.42, 4.2, 41 mg/kg, Purity: > 99.5%	4.64	Chu <i>et al.</i> , 1984
11	 (CAS: 608-93-5)	Kidney	6.7 mg/kg	F344/N rat, 91 days, Feed, Male: 2.4, 6.7, 22, 73, 159 mg/kg, Female: 2.2, 6.9, 24, 69, 164 mg/kg, GLP, Purity: > 99%	5.17	NTP, 1991
12	 (CAS: 118-74-1)	-	-	-	5.73	-

\*Lowest observed adverse effect level: LOAEL,

\*\* LogKow is based on experimental data

## 6. Strategy for and integrated conclusion of data gap filling

### 6.1. Uncertainty

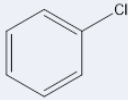
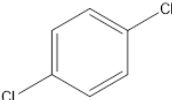
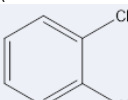
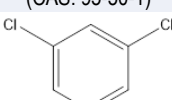
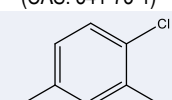
**Table 3. Uncertainty of category justification and prediction by read-across**

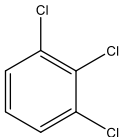
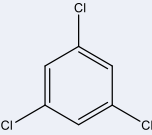
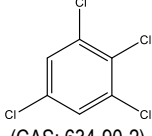
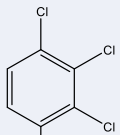
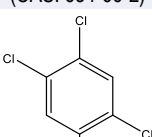
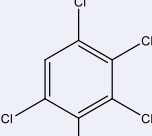
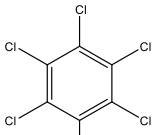
Factor	Uncertainty (low, medium, high)	Comment
Hypothesis used for the read across	Low	Chlorobenzene-related chemicals induce hepatotoxicity and renal toxicity after repeated oral dosing in rat. Metabolites cause oxidative stress, GSH downregulation, protein binding, leading to toxicological effects. Thus, the chemicals that have the same metabolic pathways are expected to show similar key events. Furthermore, NOAEL of some chlorinated benzenes correlated with water solubility, because of the accessibility to P450 and cell permeability (molecular initiation events).
Structural similarity	Low	Chlorobenzene-related chemicals are defined as benzenes having one to five chlorine atoms, and they are oxidised to an epoxide form which is further metabolised. The structure similarity factor (MCS Tanimoto) is shown in Annex II.
Similarity of physico-chemical properties	Low	LogKow was measured or calculated for all members and could be correlated with the chemical structure. The number and position of chlorine atoms affected the logKow which decreased with increasing number of chlorine substituents.
Similarity of toxicokinetics data	Medium	Based on <i>in silico</i> , <i>in vivo</i> , and <i>in vitro</i> data, all members might undergo similar metabolism, namely, epoxide hydrolysis and GSH conjugation. However, it was difficult to find experimental data regarding the detection of metabolites such as epoxide and quinone for all members. Furthermore, Hexachlorobenzene did not show active metabolites in metabolite prediction. In terms of adverse effects of each metabolite, it is difficult to explain the toxicological effects of all predicted metabolites.
Similarity of other supportive data (e.g., data related to key event)	Medium	The negative correlation between NOAEL and logKow has been reported for some chlorobenzenes (see Table 4). However, NOAEL values are not available for all members.
Number of analogues used for the read-across	Low-Medium	The number of chemicals used in this study was sufficient, since the category included all chemicals selected according to the definition of this study without any exception. However, it remains a few uncertainties since only one chemical which has NOAEL was available in the group of dichlorobenzenes.
Quality of the endpoint data used for the read-across	Medium	Well documented 90-day repeated dose toxicity data based on the NOAEL were available for 8 chemicals (members 3, 5, 6, 7, 8, 9, 10, and 11). The logKow was calculated and investigated using EPI-Suite TM version 4.1 (data matrix). However, non-GLP data were used for predicting the NOAEL of members 1, 2, 4, and 12.
Similarity of the endpoint data (among source chemicals)	Medium	The NOAEL was negatively correlated with physico-chemical properties. All available studies consistently showed similar metabolite. These results showed there is a negative correlation between the NOAEL and physico-chemical property based on initiation event. However, the target organ varied between the liver and kidney. Furthermore, NOAEL for member 1 was not decided by hepatotoxicity or nephrotoxicity.
Concordance and weight of evidence of all data used for justifying the hypothesis	Medium	The existing data including <i>in vivo/vitro/silico</i> showed concordance with the hypothesis of toxicological pathway and negative correlation between NOAEL and logKow. However, in terms of target organ, member 1 showed no hepatotoxicity or nephrotoxicity. Furthermore, in terms of negative correlation, member 11 was the exception to the trend observed.
Overall uncertainty of the read-across	Medium	We concluded that the uncertainty correlated with read-across is Medium. All chemicals are expected to have similar active metabolites which lead to a key event. Although we could examine each endpoint; the existing data could not cover the all endpoints with some exceptions, such as target organs, in repeated dose toxicity test. Furthermore, the simple logKow may not be sufficient to perfectly explain all of the repeated dose toxicity data.

## 6.2. Integrated conclusion

First, all members presented a similar metabolic pathway, leading to similar toxicological effects in the category members. In this category, it was apparent that there is negative correlation between logKow and the NOAEL. It was verified that water solubility is associated with cell membrane permeability and accessibility to P450 hydrophobic active site (Chan *et al.*, 2007). The data gaps for each member (members 1, 2, 4, and 12) were filled with the relevant data of other chlorobenzenes, having lower water solubility than the target compound. Furthermore, source compounds were selected in terms of the target organ. Regarding member 1, the *in vivo* data showed that the lowest observed adverse effect level (LOAEL) was 60 mg/kg in spleen toxicity, a NOAEL was not determined. However, the NOAEL of member 3 is same value as LOAEL of member 1 (60 mg/kg). Furthermore, the target organ of member 1 was neither the liver nor kidney, thus a NOAEL for member 1 was not decided. Member 2 did not show liver toxicity, rather kidney toxicity was observed in the toxicological study. Considering similarity of target organ, the NOAEL of member 5, which was decided by i.e. kidney toxicity, was used for data gap filling of member 2 in a conservative manner. Moreover, concerning the group of dichlorobenzenes, a NOAEL is only available for member 3, therefore the group of trichlorobenzenes with lower water solubility and lower NOAELs were used for read-across. Member 4 showed various toxicities such as liver and kidney. Considering the similarity of target organs, the NOAEL of member 5 (7.8 mg/kg) was used for data gap filling of member 4. In addition, member 12, which has the highest logKow, could not be applied with the NOAEL of any other compound. Thus, we concluded that the NOAEL of member 12 is under 0.34 mg/kg, based on that of member 10 in a conservative manner. The summary of integrated conclusion is shown in Table 4.

**Table 4. Summary of integrated conclusion**

No.	Substance structure (CAS No.)	Chemical name	LogKow	PoDs (Conclusion)
1	 (CAS: 108-90-7)	Chlorobenzene	2.84	NOAEL Not determined
2	 (CAS: 106-46-7)	1,4-Dichlorobenzene	3.44	NOAEL 7.8 mg/kg (RA from 1,2,4-trichlorobenzene)
3	 (CAS: 95-50-1)	1,2-Dichlorobenzene	3.43	NOAEL 60 mg/kg
4	 (CAS: 541-73-1)	1,3-Dichlorobenzene	3.53	NOAEL 7.8 mg/kg (RA from 1,2,4-trichlorobenzene)
5	 (CAS: 120-82-1)	1,2,4-Trichlorobenzene	4.02	NOAEL 7.8 mg/kg

6	 (CAS: 87-61-6)	1,2,3-Trichlorobenzene	4.05	NOAEL 7.7 mg/kg
7	 (CAS: 108-70-3)	1,3,5-Trichlorobenzene	4.19	NOAEL 7.6 mg/kg
8	 (CAS: 634-90-2)	1,2,3,5-Tetrachlorobenzene	4.56	NOAEL 3.4 mg/kg
9	 (CAS: 634-66-2)	1,2,3,4-Tetrachlorobenzene	4.6	NOAEL 3.4 mg/kg
10	 (CAS: 95-94-3)	1,2,4,5-Tetrachlorobenzene	4.64	NOAEL 0.34 mg/kg
11	 (CAS: 608-93-5)	Pentachlorobenzene	5.17	NOAEL 2.4 mg/kg
12	 (CAS: 118-74-1)	Hexachlorobenzene	5.73	NOAEL 0.34 mg/kg> (RA from 1,2,4,5-tetrachlorobenzene)

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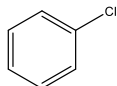
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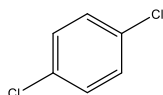
## Annex I. Summary of 90-day rat oral repeated-dose toxicity data

- Member 1: Chlorobenzene (CAS No. 108-90-7)



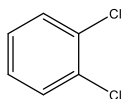
Reference	NTP, 1985a
Study type	Repeated dose toxicity test
Species	Rat (F344/N)
Sex	Male, Female
Route	Oral (Gavage)
Exposure period	91-days
GLP	YES
Dose level	0, 60, 125, 250, 500, 750 mg/kg (Male, Female)
Purity	>99%
LOAEL	60 mg/kg
NOAEL	Not determined
Findings	GGTP, ALP ↑(female 500, 750 mg/kg), Relative liver weight ↑(female, 125, 250, 500, 750 mg/kg), Relative kidney weight ↑(female, 750 mg/kg), Relative spleen weight ↓ (male, 60, 125, 250, 500, 750 mg/kg), Liver lesion (male, female 250, 500, 750 mg/kg), Nephropathy (female, 750 mg/kg, male, 500, 750 mg/kg), Lymphoid depletions of thymus and spleen (male, female, 750 mg/kg)

- Member 2: 1,4-Dichlorobenzene (CAS No. 106-46-7)



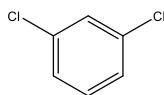
Reference	ECHA, 1988
Study type	Repeated dose toxicity test
Species	Rat (Fischer 344)
Sex	Male, Female
Route	Oral (Gavage)
Exposure period	91-days
GLP	YES
Dose level	0, 75, 150, 300, 600 mg/kg (Male, Female)
Purity	99.9%
LOAEL	75 mg/kg
NOAEL	Not determined
Findings	Urinary epithelial cell excretion and exacerbation of hyaline droplet accumulation in renal cortical cells ↑ (male, 75, 150, 300, 600 mg/kg), Tubular single cell necrosis, dilated tubules with granular cast formation (male, 150, 300, 600 mg/kg)

- Member 3: 1,2-Dichlorobenzene (CAS No. 95-50-1)



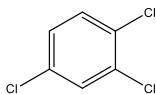
Reference	NTP, 1985b
Study type	Repeated dose toxicity test
Species	Rat (F344/N)
Sex	Male, Female
Route	Oral (Gavage)
Exposure period	91-days
GLP	YES
Dose level	0, 30, 60, 125, 250, 500 mg/kg (Male, Female)
Purity	>99%
LOAEL	125 mg/kg
NOAEL	60 mg/kg
Findings	Renal tubular degeneration (male, 500 mg/kg), Histopathological change: Centrolobular necrosis of the liver (male, female, 500 mg/kg), Hepatocellular degeneration, lymphocyte in the thymus and spleen↓ (male, female, 500 mg/kg), Hepatocellular necrosis (male, female, 250, 500 mg/kg), Relative liver weight↑ (male, female, 125, 250, 500 mg/kg), Relative lungs, kidney and brain weight↑ (male, 500 mg/kg)

- Member 4: 1,3-Dichlorobenzene (CAS No. 541-73-1)



Reference	McCauley <i>et al.</i> , 1995
Study type	Repeated dose toxicity test
Species	Rat (SD)
Sex	Male, Female
Route	Oral (Gavage)
Exposure period	90-days
GLP	NO
Dose level	0, 9, 37, 147, 588 mg/kg (Male, Female)
Purity	>99.99%
LOAEL	9 mg/kg
NOAEL	Not determined
Findings	Relative liver weight↑ (male, female 147, 588 mg/kg), Relative kidney weights↑ (male, 147, 588 mg/kg, female 588 mg/kg), Cholesterol and calcium level in serum↑ (male, 9, 37, 147, 588 mg/kg, female, 37, 147, 588 mg/kg), Histopathological changes: Thyroid (male, female, 37, 147, 588 mg/kg) Liver (male, female, 588 mg/kg), Pituitary (male, 147, 588 mg/kg)

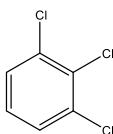
- Member 5: 1,2,4-Trichlorobenzene (CAS No. 120-82-1)



Reference	Côté <i>et al.</i> , 1988
Study type	Repeated dose toxicity test
Species	Rat (SD)
Sex	Male, Female
Route	Oral (Feed)
Exposure period	91-days
GLP	NO
Dose level	0.07, 0.78, 7.8, 82 mg/kg (Male), 0.11, 1.4, 15, 101 mg/kg (Female)
Purity	>99%
LOAEL	82 mg/kg
NOAEL	7.8 mg/kg
Findings	Liver and kidney weights↑ (male, 82 mg/kg), Hepatic AH ↑ (male, 82 mg/kg), Hepatic ADPM↑ (male, 82 mg/kg, female, 101 mg/kg), Histological change: Liver, Thyroid (male, 82 mg/kg)

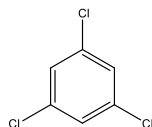
AH, Aniline hydroxylase; ADPM, Aminopyrine demethylase

- Member 6: 1,2,3-Trichlorobenzene (CAS No. 87-61-6)



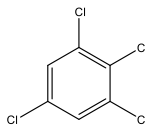
Reference	Côté <i>et al.</i> , 1988
Study type	Repeated dose toxicity test
Species	Rat (SD)
Sex	Male, Female
Route	Oral (Feed)
Exposure period	91-days
GLP	NO
Dose level	0.08, 0.78, 7.6, 78 mg/kg (Male), 0.13, 1.3, 12, 113 mg/kg (Female)
Purity	>99%
LOAEL	78 mg/kg
NOAEL	7.7 mg/kg
Findings	Liver and kidney weights↑ (male, 78 mg/kg), Histological changes: Liver, Thyroid (male, 78 mg/kg)

- Member 7: 1,3,5-Trichlorobenzene (CAS No. 108-70-3)



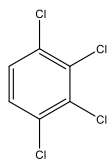
Reference	Côté <i>et al.</i> , 1988
Study type	Repeated dose toxicity test
Species	Rat (SD)
Sex	Male, Female
Route	Oral (Feed)
Exposure period	91-days
GLP	NO
Dose level	0.08, 0.81, 7.7, 82 mg/kg (Male), 0.13, 1.5, 17, 146 mg/kg (Female)
Purity	>99%
LOAEL	82 mg/kg
NOAEL	7.6 mg/kg
Findings	Liver and kidney weights↑ (male, 82 mg/kg), Histological changes: Liver, Thyroid, Renal (male, 82 mg/kg)

- Member 8: 1,2,3,5-Tetrachlorobenzene (CAS No. 634-90-2)



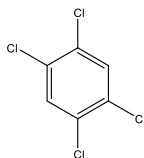
Reference	Chu <i>et al.</i> , 1984
Study type	Repeated dose toxicity test
Species	Rat (SD)
Sex	Male, Female
Route	Oral (Feed)
Exposure period	90-days
GLP	NO
Dose level	0, 0.034, 0.34, 3.4, 34 mg/kg (Male), 0, 0.042, 0.42, 4.2, 41 mg/kg (Female)
Purity	>99.5%
LOAEL	34 mg/kg
NOAEL	3.4 mg/kg
Findings	Histopathological changes: Kidney (male, 34 mg/kg), Liver (female, 41 mg/kg)

- Member 9: 1,2,3,4-Tetrachlorobenzene (CAS No. 634-66-2)



Reference	Chu <i>et al.</i> , 1984
Study type	Repeated dose toxicity test
Species	Rat (SD)
Sex	Male, Female
Route	Oral (Feed)
Exposure period	90-days
GLP	NO
Dose level	Male: 0, 0.034, 0.34, 3.4, 34 mg/kg, Female: 0, 0.042, 0.42, 4.2, 41 mg/kg
Purity	>99.5%
LOAEL	34 mg/kg
NOAEL	3.4 mg/kg
Findings	Histopathological change: Liver (male, 34 mg/kg)

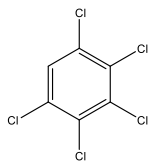
- Member 10: 1,2,4,5-Tetrachlorobenzene (CAS No. 95-94-3)



Reference	Chu <i>et al.</i> , 1984
Study type	Repeated dose toxicity test
Species	Rat (SD)
Sex	Male, Female
Route	Oral (Feed)
Exposure period	90-days
GLP	NO
Dose level	0, 0.034, 0.34, 3.4, 34 mg/kg (Male), 0, 0.042, 0.42, 4.2, 41 mg/kg (Female)
Purity	>99.5%
LOAEL	3.4 mg/kg
NOAEL	0.34 mg/kg
Findings	Liver and kidney weight ↑ (male, 34 mg/kg, female, 41 mg/kg), Serum cholesterol ↑ (male, 34 mg/kg, female, 41 mg/kg), Hepatic AH and Hepatic APDM ↑ (male, 3.4, 34 mg/kg, female 34 mg/kg), Histopathological changes: Kidney (male, 3.4, 34 mg/kg), Liver (male, 34 mg/kg, female, 41 mg/kg)

AH, Aniline hydroxylase; APDM, Aminopyrine demethylase

- Member 11: Pentachlorobenzene (CAS No. 608-93-5)



Reference	NTP, 1991
Study type	Repeated dose toxicity test
Species	Rat (F344/N)
Sex	Male, Female
Route	Oral (Feed)
Exposure period	91-days
GLP	YES
Dose level	2.4, 6.7, 22, 73, 159 mg/kg (Male), 2.2, 6.9, 24, 69, 164 mg/kg (Female)
Purity	>99%
LOAEL	6.7 mg/kg
NOAEL	2.4 mg/kg
Findings	Relative Kidney weight↑ (male, 6.7 mg/kg, female, 6.9 mg/kg), Free thyroxin and total thyroxin concentration in serum ↓ (male, 2.4, 6.7, 22, 73, 159 mg/kg, female, 6.9, 24, 69, 164 mg/kg) Thyrotropin concentration ↑ (male, 73, 159 mg/kg, female, 69, 164 mg/kg), Renal lesions (male, 6.7, 22, 73, 159 mg/kg, female, 69, 164 mg/kg), Hepatocellular hypertrophy (male, 22, 73, 159 mg/kg, female, 69, 164 mg/kg), Thyroid follicular cell hypertrophy (male, 73, 159 mg/kg, female, 69, 164 mg/kg)

## Annex II. Structure similarity between category members in MCS Tanimoto

Members	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12
No. 1		0.88	0.88	0.88	0.78	0.78	0.78	0.70	0.70	0.70	0.64	0.58
No. 2	0.88		0.78	0.78	0.89	0.70	0.70	0.80	0.80	0.80	0.73	0.67
No. 3	0.88	0.78		0.78	0.89	0.89	0.70	0.80	0.80	0.80	0.73	0.67
No. 4	0.88	0.78	0.78		0.89	0.89	0.89	0.80	0.80	0.80	0.73	0.67
No. 5	0.78	0.89	0.89	0.89		0.80	0.80	0.90	0.90	0.90	0.82	0.75
No. 6	0.78	0.70	0.89	0.89	0.80		0.80	0.90	0.90	0.73	0.82	0.75
No. 7	0.78	0.70	0.70	0.89	0.80	0.80		0.90	0.73	0.73	0.82	0.75
No. 8	0.70	0.80	0.80	0.80	0.90	0.90	0.90		0.82	0.82	0.91	0.83
No. 9	0.70	0.80	0.80	0.80	0.90	0.90	0.73	0.82		0.82	0.91	0.83
No. 10	0.70	0.80	0.80	0.80	0.90	0.73	0.73	0.82	0.82		0.91	0.83
No. 11	0.64	0.73	0.73	0.73	0.82	0.82	0.82	0.91	0.91	0.91		0.92
No. 12	0.58	0.67	0.67	0.67	0.75	0.75	0.75	0.83	0.83	0.83	0.92	

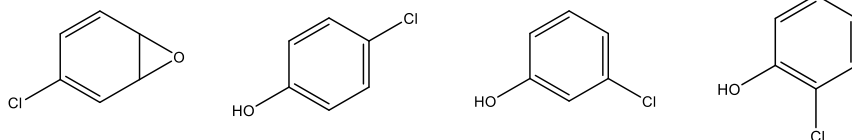
### Annex III. Summary of metabolite prediction data in OECD QSAR Toolbox version 4.2

- Member 1: Chlorobenzene (CAS No. 108-90-7)

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*In vivo* rat metabolism simulator

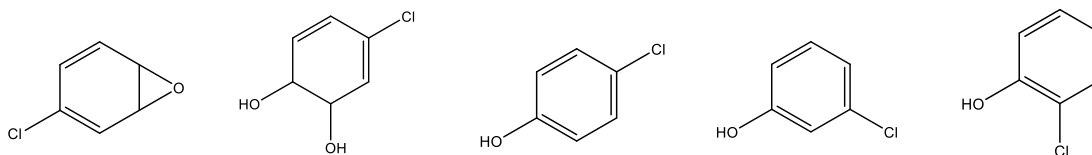
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Rat liver S9 metabolism simulator

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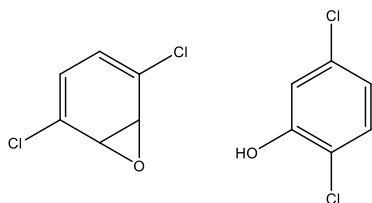


- Member 2: 1,4-Dichlorobenzene (CAS No. 106-46-7)

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*In vivo* rat metabolism simulator

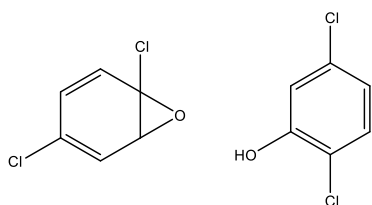
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Rat liver S9 metabolism simulator

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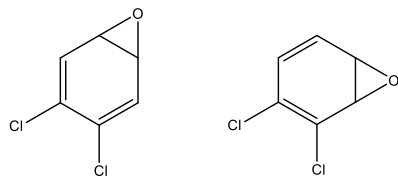


- Member 3: 1,2-Dichlorobenzene (CAS No. 95-50-1)

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*In vivo* rat metabolism simulator

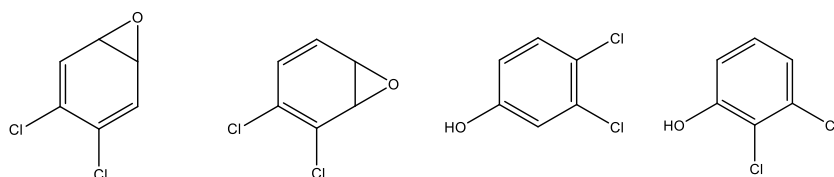
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Rat liver S9 metabolism simulator

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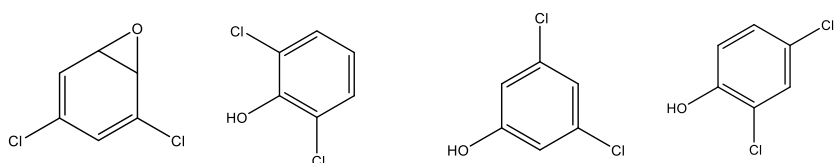


- Member 4: 1,3-Dichlorobenzene (CAS No. 541-73-1)

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*In vivo* rat metabolism simulator

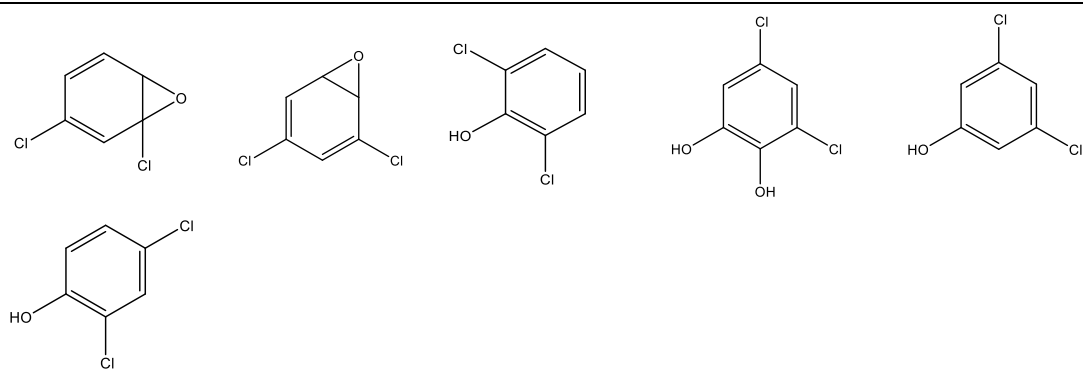
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Rat liver S9 metabolism simulator

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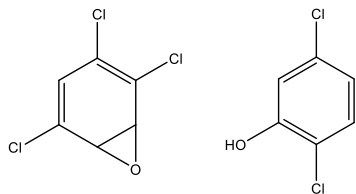


- Member 5: 1,2,4-Trichlorobenzene (CAS No. 120-82-1)

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*In vivo* rat metabolism simulator

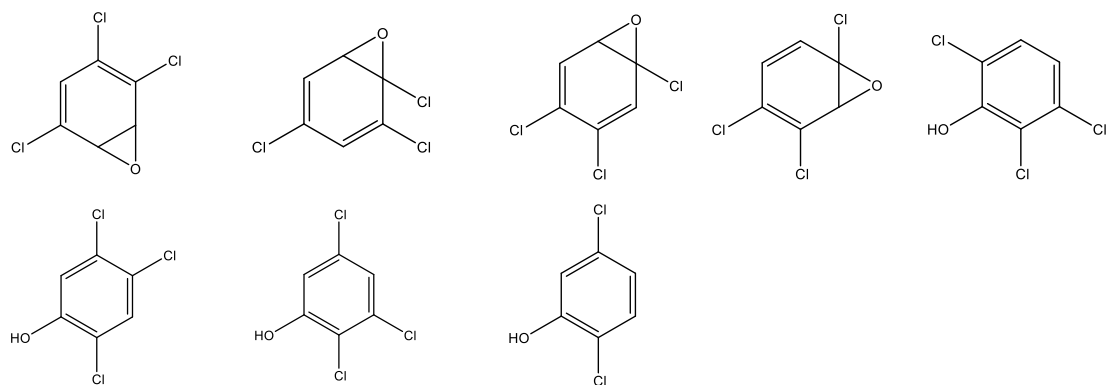
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Rat liver S9 metabolism simulator

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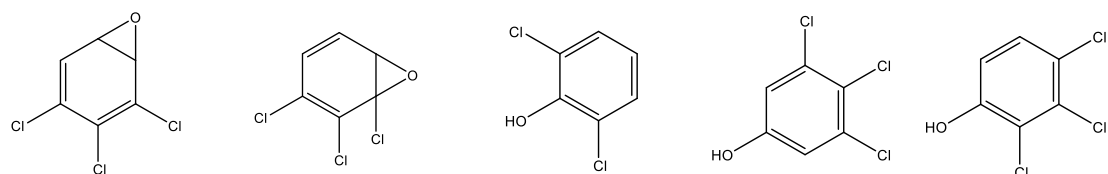


- Member 6: 1,2,3-Trichlorobenzene (CAS No. 87-61-6)

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*In vivo* rat metabolism simulator

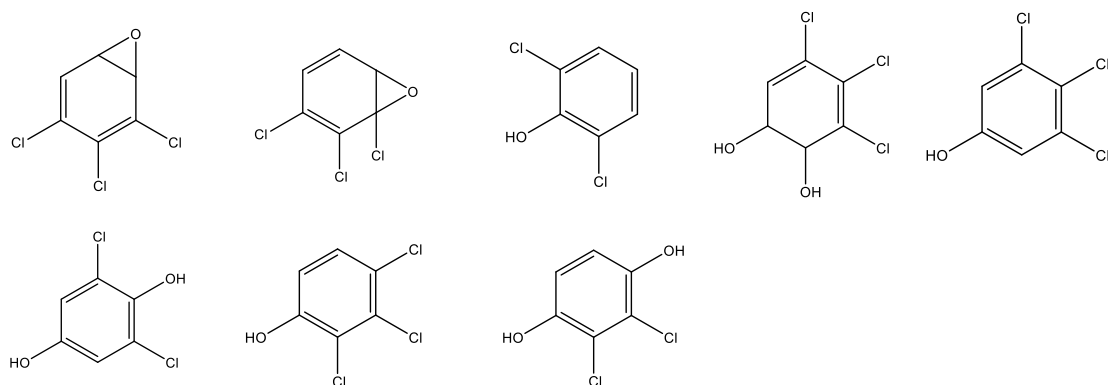
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Rat liver S9 metabolism simulator

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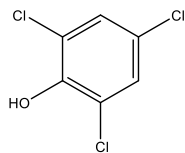


- Member 7: 1,3-Dichlorobenzene (CAS No. 108-70-3)

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*In vivo* rat metabolism simulator

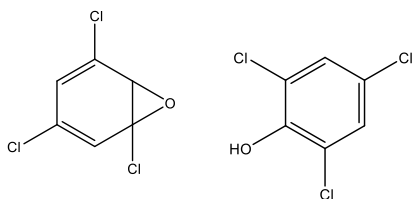
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Rat liver S9 metabolism simulator

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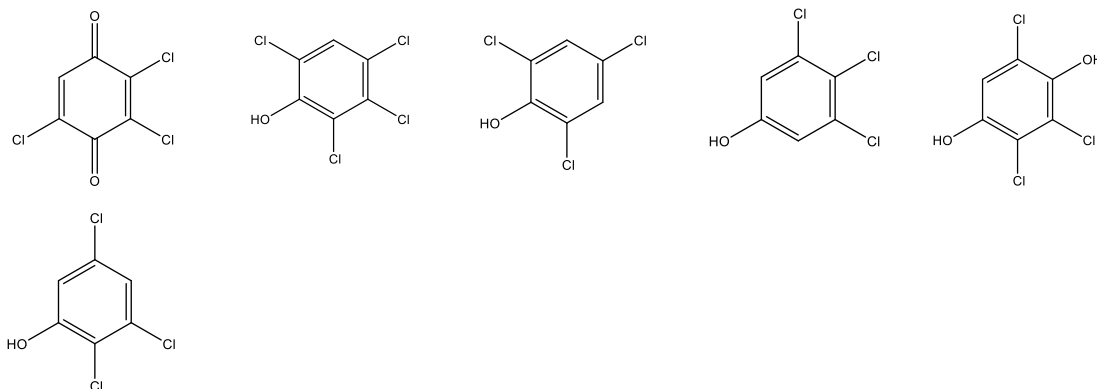


- Member 8: 1,2,3,5-Tetrachlorobenzene (CAS No. 634-90-2)

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*In vivo* rat metabolism simulator

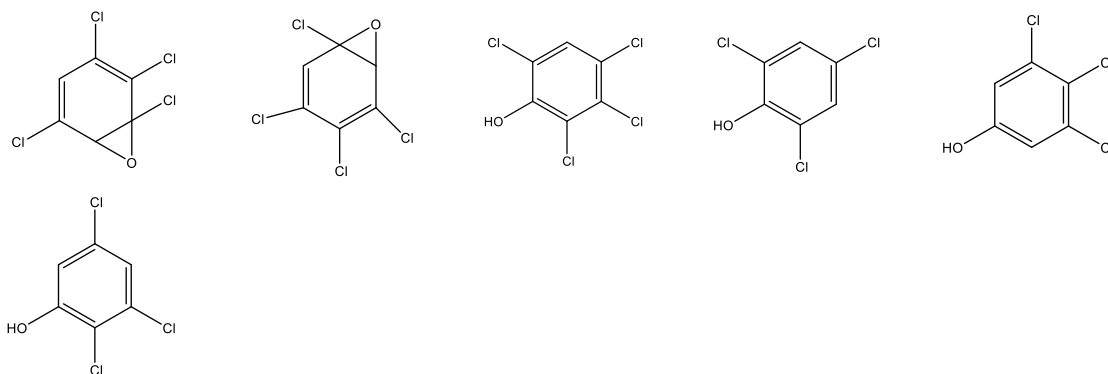
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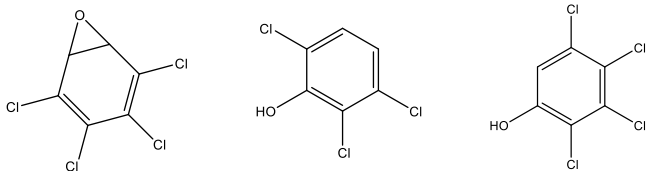
Rat liver S9 metabolism simulator

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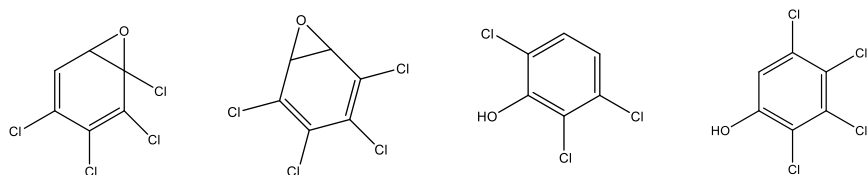


• Member 9: 1,2,3,4-Tetrachlorobenzene (CAS No. 634-66-2)

*In vivo* rat metabolism simulator

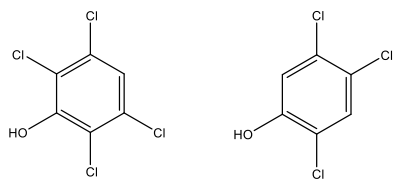


Rat liver S9 metabolism simulator

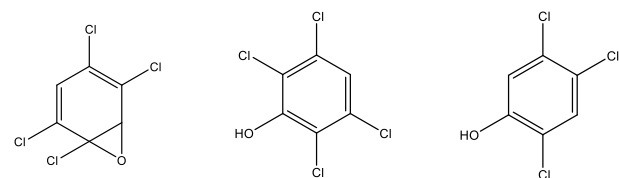


• Member 10: 1,2,4,5-Tetrachlorobenzene (CAS No. 95-94-3)

*In vivo* rat metabolism simulator



Rat liver S9 metabolism simulator

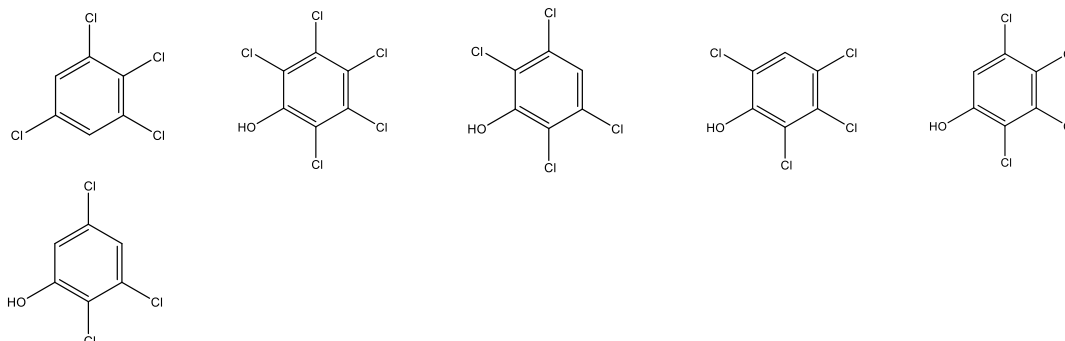


- Member 11: Pentachlorobenzene (CAS No. 608-93-5)

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*In vivo* rat metabolism simulator

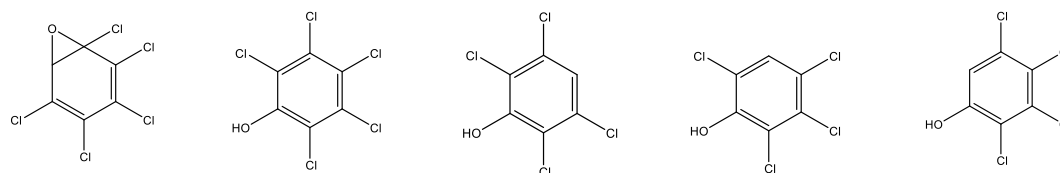
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Rat liver S9 metabolism simulator

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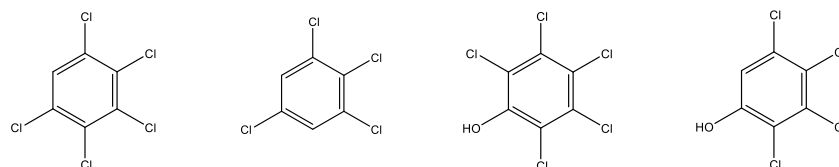


- Member 12: Hexachlorobenzene (CAS No. 118-74-1)

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*In vivo* rat metabolism simulator

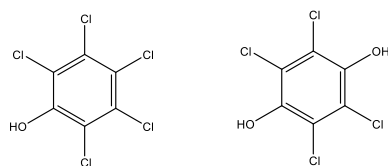
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
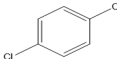

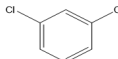
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Rat liver S9 metabolism simulator

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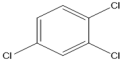
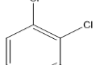
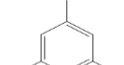
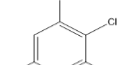
## Appendix I. Data matrix, IATA for "Case Study on the Use of Integrated Approaches for Testing and Assessment for 90-Day Rat Oral Repeated-Dose Toxicity of Chlorobenzene-Related Chemicals"

Chemical ID					
No	1	2	3	4	
CAS	108-90-7	106-46-7	95-50-1	541-73-1	
Name	Chlorobenzene	1,4-Dichlorobenzene	1,2-Dichlorobenzene	1,3-Dichlorobenzene	
Structure					
Summary of data gap filling					
Target endpoint1	Experimental condition	F344/N rat, 91days, Gavage, Male, Female: 0, 60, 125, 250, 500, 750 mg/kg, GLP, Purity: >99%	Fischer 344 rat, 91 days, Gavage, Male, Female:0, 75, 150, 300, 600 mg/kg, GLP, Purity: 99.9%	F344/N rat, 91 days, Gavage, Male, Female: 0, 30, 60, 125, 250, 500 mg/kg, GLP, Purity: > 99%	SD rat, 90 days, Gavage, Male, Female: 0, 9, 37, 147, 588 mg/kg, Purity: > 99.99%
	Experimental result	GGTP, ALP ↑(female 500, 750 mg/kg), Relative liver weight ↑(female, 125, 250, 500, 750 mg/kg), Relative kidney weight ↑(female, 750 mg/kg), Relative spleen weight↓ (male, 60, 125, 250, 500, 750 mg/kg), Liver lesion (male, female 250, 500, 750 mg/kg), Nephropathy (female, 750 mg/kg, male, 500, 750 mg/kg), Lymphoid depletions of thymus and spleen (male, female, 750 mg/kg),	Urinary epithelial cell excretion and exacerbation of hyaline droplet accumulation in renal cortical cells ↑(male, 75, 150, 300, 600 mg/kg), Tubular single cell necrosis, dilated tubules with granular cast formation (male, 150, 300, 600 mg/kg)	Renal tubular degeneration (male, 500 mg/kg), Histopathological change: Centrolobular necrosis of the liver (male, female, 500 mg/kg), Hepatocellular degeneration, lymphocyte in the thymus and spleen↓ ( male, female, 500 mg/kg), Hepatocellular necrosis ( male, female, 250, 500 mg/kg), Relative liver weight↑ (male, female, 125, 250, 500 mg/kg), Relative lungs, kidney and brain weight↑(male, 500 mg/kg)	Relative liver weight↑ (male, female 147, 588 mg/kg), Relative kidney weights↑ (male, 147, 588 mg/kg, female 588 mg/kg), Cholesterol and calcium level in serum ↑ (male, 9, 37, 147, 588 mg/kg, female, 37, 147, 588 mg/kg), Histopathological changes: thyroid (male, female, 37, 147, 588 mg/kg) liver (male, female, 588 mg/kg), Pituitary (male, 147, 588 mg/kg)
	Integrated conclusion (eg. read-across)	Not determined	derived result from member 5	NOAEL: 60 mg/kg	derived result from member 5
	Reference	NTP, 1985a	ECHA, 1988	NTP, 1985b	McCauley <i>et al.</i> , 1995
Molecular profiling related to the analogue approach hypothesis					
Parent chemical	Profiler 1 (name, version)	-	-	-	-
	Expert system 1 (name, version)	-	-	-	-
		-	-	-	-
Metabolite*	Profiler 1 (name, version)	OECD QSAR Toolbox Ver 4.2	OECD QSAR Toolbox Ver 4.2	OECD QSAR Toolbox Ver 4.2	OECD QSAR Toolbox Ver 4.2

	Expert system 1 (name, version)	<i>In vitro</i> rat metabolism simulator	<i>In vitro</i> rat metabolism simulator	<i>In vitro</i> rat metabolism simulator	<i>In vitro</i> rat metabolism simulator
	Metabolites	4 metabolites	2 metabolites	2 metabolites	4 metabolites
	Expert system 2 (name, version)	Rat liver S9 metabolism simulator	Rat liver S9 metabolism simulator	Rat liver S9 metabolism simulator	Rat liver S9 metabolism simulator
	Metabolites	5 metabolites	2 metabolites	4 metabolites	6 metabolites
Physical-chemical data					
	Melting point	-45.78	-14.33	-14.33	-14.33
	Boiling point	139.63	174.69	174.69	174.69
	Density	-	-	-	-
	logKow (measured value)	2.84	3.44	3.43	3.53
	logKow (calculated value)	2.64	3.28	3.28	3.28
Kinetics**					
	Absorption ( <i>in silico</i> )	-	-	-	-
	Distribution	-	-	-	-
	Metabolism ( <i>in vivo</i> )	-	-	-	-
	Metabolism ( <i>in vitro</i> )	-	Glutathione/Cysteine conjugates	Glutathione/Cysteine conjugates	Glutathione/Cysteine conjugates
	Excretion	-	-	-	-
Supporting data related to the target endpoint(s)					
<i>In vivo</i>	Cell viability	Rat primary Hepatocyte (SD) MTT assay Ref: K. Chan <i>et al.</i> , 2007	Rat primary Hepatocyte (SD) MTT assay Ref: K. Chan <i>et al.</i> , 2007	Rat primary Hepatocyte (SD) MTT assay Ref: K. Chan <i>et al.</i> , 2007	Rat primary Hepatocyte (SD) MTT assay Ref: K. Chan <i>et al.</i> , 2007
	Results (LC50 (µM))	1500 ± 150	1182.5 ± 118.25	700 ± 150	552 ± 55.2
<i>In vitro</i>	Toxicogenomics	-	-	-	-
	Results	-	-	-	-
Classification	GSH	Composition 3	Composition 7	Composition 2, 3	Composition 1, 2
	Acute toxicity - oral	Acute Tox. 4	conclusive but not sufficient for classification	Acute Tox. 4	Acute Tox. 4
	Specific target organ toxicity - repeated	conclusive but not sufficient for classification	conclusive but not sufficient for classification	conclusive but not sufficient for classification	conclusive but not sufficient for classification
	Carcinogenicity	conclusive but not sufficient for classification	Carc. 2	conclusive but not sufficient for classification	inconclusive

\* More relevant metabolite such as toxicant

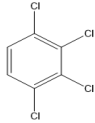
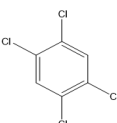
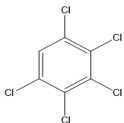
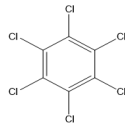
\*\*General outline of relative comparative kinetics

Chemical ID					
No	5	6	7	8	
CAS	120-82-1	87-61-6	108-70-3	634-90-2	
Name	1,2,4-Trichlorobenzene	1,2,3-Trichlorobenzene	1,3,5-Trichlorobenzene	1,2,3,5-Tetrachlorobenzene	
Structure					
Summary of data gap filling					
Target endpoint1	Experimental condition	SD rat , 91 days, Feed, Male: 0.07, 0.78, 7.8, 82 mg/kg, Female: 0.11, 1.4, 15, 101 mg/kg, Purity: > 99%	SD rat, 91 days, Feed, Male: 0.08, 0.78, 7.6, 78 mg/kg, Female: 0.13, 1.3, 12, 113 mg/kg, Purity: > 99%	SD rat, 91 days, Feed, Male: 0.08, 0.81, 7.7, 82 mg/kg, Female: 0.13, 1.5, 17, 146 mg/kg, Purity: > 99%	SD rat, 90 days, Feed, Male: 0, 0.034, 0.34, 3.4, 34 mg/kg, Female: 0, 0.042, 0.42, 4.2, 41 mg/kg, Purity: > 99.5 %
	Experimental result	Liver and kidney weights↑(male, 82 mg/kg), Hepatic AH ↑ (male, 82 mg/kg), Hepatic ADPM↑ (male, 82mg/kg, female, 101 mg/kg), Histological change Liver: thyroid (male, 82 mg/kg)	Liver and kidney weights↑ (male, 78 mg/kg), Histological change: Liver thyroid (male, 78 mg/kg)	Liver and kidney weights↑ (male, 82 mg/kg), Histological change: Liver, thyroid, renal (male, 82 mg/kg)	Histopathological change: Kidney (male, 34 mg/kg), Liver (female, 41 mg/kg)
	Integrated conclusion (eg. read-across)	NOAEL: 7.8 mg/kg	NOAEL: 7.7 mg/kg	NOAEL: 7.6 mg/kg	NOAEL: 4.1 mg/kg
	Reference	Côté <i>et al.</i> , 1988	Côté <i>et al.</i> , 1988	Côté <i>et al.</i> , 1988	Chu <i>et al.</i> , 1984
Molecular profiling related to the analogue approach hypothesis					
Parent chemical	Profiler 1 (name, version)	-	-	-	-
	Expert system 1 (name, version)	-	-	-	-
		-	-	-	-
Metabolite*	Profiler 1 (name, version)	OECD QSAR Toolbox Ver 4.2	OECD QSAR Toolbox Ver 4.2	OECD QSAR Toolbox Ver 4.2	OECD QSAR Toolbox Ver 4.2
	Expert system 1 (name, version)	<i>In vitro</i> rat metabolism simulator	<i>In vitro</i> rat metabolism simulator	<i>In vitro</i> rat metabolism simulator	<i>In vitro</i> rat metabolism simulator
	Metabolites	2 metabolites	5 metabolites	1 metabolites	6 metabolites
	Expert system 2 (name, version)	Rat liver S9 metabolism simulator	Rat liver S9 metabolism simulator	Rat liver S9 metabolism simulator	Rat liver S9 metabolism simulator
	Metabolites	8 metabolites	8 metabolites	2 metabolites	6 metabolites
Physical-chemical data					

Melting point	16.44	16.44	16.44	39.40
Boiling point	207.39	207.39	207.39	237.75
Density	-	-	-	-
logKow (measured value)	4.02	4.05	4.19	4.56
logKow (calculated value)	3.93	3.93	3.93	4.57
Kinetics**				
Absorption ( <i>in silico</i> )	-	-	-	-
Distribution	-	-	-	-
Metabolism ( <i>in vivo</i> )	Trichlorophenol, Trichlorohydroquinone		-	-
Metabolism ( <i>in vitro</i> )	-	-	-	-
Excretion	-	-	-	-
Supporting data related to the target endpoint(s)				
<i>In vivo</i>	Cell viability	Rat primary Hepatocyte (SD) MTT assay Ref: K. Chan <i>et al.</i> , 2007	-	-
	Results (LC50 (µM))	280 ± 28	-	-
<i>In vitro</i>	Toxicogenomics	-	-	-
	Results	-	-	-
Classification	GSH	Composition 2	Composition 1	-
	Acute toxicity - oral	Acute Tox. 4	conclusive but not sufficient for classification	Acute Tox. 4
	Specific target organ toxicity - repeated	conclusive but not sufficient for classification	conclusive but not sufficient for classification	data lacking
	Carcinogenicity	conclusive but not sufficient for classification	conclusive but not sufficient for classification	data lacking

\* More relevant metabolite such as toxicant

\*\*General outline of relative comparative kinetics


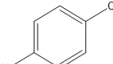

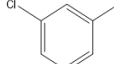
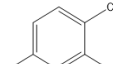

Chemical ID					
No	9	10	11	12	
CAS	634-66-2	95-94-3	608-93-5	118-74-1	
Name	1,2,3,4-Tetrachlorobenzene	1,2,4,5-Tetrachlorobenzene	Pentachlorobenzene	Hexachlorobenzene	
Structure					
Summary of data gap filling					
Target endpoint1	Experimental condition	SD rat, 90 days, Feed, Male: 0, 0.034, 0.34, 3.4, 34 mg/kg, Female: 0, 0.042, 0.42, 4.2, 41 mg/kg, Purity: > 99.5%	SD rat, 90 days, Feed, Male: 0, 0.034, 0.34, 3.4, 34 mg/kg, Female: 0, 0.042, 0.42, 4.2, 41 mg/kg, Purity: > 99.5%	F344/N rat, 91 days, Feed, Male: 2.4, 6.7, 22, 73, 159 mg/kg, Female: 2.2, 6.9, 24, 69, 164 mg/kg, GLP, Purity: > 99%	-
	Experimental result	Histopathological change: Liver (male, 34 mg/kg)	Liver and kidney weight ↑ (male, 34 mg/kg, female, 41 mg/kg), Serum cholesterol ↑ (male, 34 mg/kg, female, 41 mg/kg), Hepatic AH and Hepatic APDMH↑ (male, 34, 3.4 mg/kg, female 34 mg/kg), Histopathological change: Kidney (male, 3.4, 34 mg/kg), liver (male, 34 mg/kg, female, 41 mg/kg)	Relative Kidney weight↑ (male, 6.7 mg/kg, female, 6.9 mg/kg), Free thyroxin and total thyroxin concentration in serum ↓ (male, 2.4, 6.7, 22, 73, 159 mg/kg, female, 6.9, 24, 69, 164 mg/kg) Thyrotropin concentration ↑ (male, 73, 159 mg/kg, female, 69, 164 mg/kg), Renal lesions (male, 6.7, 22, 73, 159 mg/kg, female, 69, 164 mg/kg), Hepatocellular hypertrophy (male, 22, 73, 159 mg/kg, female, 69, 164 mg/kg), Thyroid follicular cell hypertrophy (male, 73, 159 mg/kg, female, 69, 164 mg/kg)	-
	Integrated conclusion (eg. read-across)	NOAEL: 3.4 mg/kg	NOAEL: 0.34 mg/kg	NOAEL: 2.4 mg/kg	derived result from member 10 (0.34 mg/kg > NOAEL)
	Reference	Chu <i>et al.</i> , 1984	Chu <i>et al.</i> , 1984	NTP, 1991	-
Molecular profiling related to the analogue approach hypothesis					
Parent chemical	Profiler 1 (name, version)	-	-	-	-
	Expert system 1 (name, version)	-	-	-	-
	-	-	-	-	-
Metabolite*	Profiler 1 (name, version)	OECD QSAR Toolbox Ver 4.2	OECD QSAR Toolbox Ver 4.2	OECD QSAR Toolbox Ver 4.2	OECD QSAR Toolbox Ver 4.2

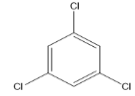
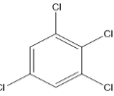
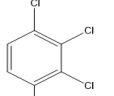
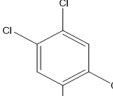
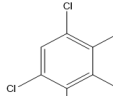
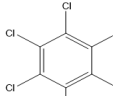
	Expert system 1 (name, version)	<i>In vitro</i> rat metabolism simulator	<i>In vitro</i> rat metabolism simulator	<i>In vitro</i> rat metabolism simulator	<i>In vitro</i> rat metabolism simulator
	Metabolites	3 metabolites	2 metabolites	6 metabolites	4 metabolites
	Expert system 2 (name, version)	Rat liver S9 metabolism simulator	Rat liver S9 metabolism simulator	Rat liver S9 metabolism simulator	Rat liver S9 metabolism simulator
	Metabolites	4 metabolites	3 metabolites	5 metabolites	2 metabolites
Physical-chemical data					
Melting point		39.40	39.40	64.45	80.57
Boiling point		237.75	237.75	265.77	291.43
Density		-	-	-	-
logKow (measured value)		4.60	4.64	5.17	5.73
logKow (calculated value)		4.57	4.57	5.22	5.86
Kinetics**					
Absorption ( <i>in silico</i> )		-	-	-	-
Distribution		-	-	-	-
Metabolism ( <i>in vivo</i> )		-	-	Tetrachlorobenzoquinone	-
Metabolism ( <i>in vitro</i> )		-	-	-	Pentachlorothiophenol, Pentachlorophenol
Excretion		-	-	-	-
Supporting data related to the target endpoint(s)					
<i>In vivo</i>	Cell viability	-	-	-	-
	Results (LC50 (µM))	-	-	-	-
<i>In vitro</i>	Toxicogenomics	-	-	-	-
	Results	-	-	-	-
Classification	GSH	-	-	-	-
	Acute toxicity - oral	-	-	-	-
	Specific target organ toxicity - repeated	-	-	-	-
	Carcinogenicity	-	-	-	-

\* More relevant metabolite such as toxicant

\*\*General outline of relative comparative kinetics

## Appendix II. Summary of metabolic activation

Chemical ID						
No	1	2	3	4	5	6
CAS	108-90-7	106-46-7	95-50-1	541-73-1	120-82-1	87-61-6
Name	Chlorobenzene	1,4-Dichlorobenzene	1,2-Dichlorobenzene	1,3-Dichlorobenzene	1,2,4-Trichlorobenzene	1,2,3-Trichlorobenzene
Structure						
Summary of Metabolic activation data						
<i>In silico</i>	Epoxide	Epoxide	Epoxide	Epoxide	Epoxide	Epoxide
<i>In vitro</i>	-	Glutathione/ Cysteine conjugates	Glutathione/ Cysteine conjugates	Glutathione/ Cysteine conjugates	-	-
<i>In vivo</i>	-	-	-	-	Trichlorophenol, Trichlorohydroquinone	-

Chemical ID						
No	7	8	9	10	11	12
CAS	108-70-3	634-90-2	634-66-2	95-94-3	608-93-5	118-74-1
Name	1,3,5-Trichlorobenzene	1,2,3,5-Tetrachlorobenzene	1,2,3,4-Tetrachlorobenzene	1,2,4,5-Tetrachlorobenzene	Pentachlorobenzene	Hexachlorobenzene
Structure						
Summary of Metabolic activation data						
<i>In silico</i>	Epoxide	Epoxide	Epoxide	Epoxide	Epoxide	-
<i>In vitro</i>	-	-	-	-	-	Pentachlorothiophenol, Pentachlorophenol
<i>In vivo</i>	-	-	-	-	Tetrachlorobenzoquinone	-

